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Editorial

The role and future impacts of Nuclear Magnetic Resonance at the National Medical Research Centre

Abdul M. Gbaj
National Medical Research Center, Zawia, Libya

Today, nuclear Magnetic Resonance (NMR) has become a sophisticated and powerful analytical technology that has found a variety of applications in different disciplines of research and medicine. Modern NMR spectroscopy was emphasizing the application in biomolecular systems and plays important role in structural biology. With developments in methodology and instrumentation over the time, NMR has become one of the most powerful and versatile spectroscopic techniques for analysis of biomacromolecules, allowing characterization of biomacromolecules and their complexes up to 100 kDa. Together with X-ray crystallography, NMR spectroscopy is one of the two leading technologies for the structure determination of biomacromolecules at atomic resolution.

NMR provides unique and important molecular motional and interaction profiles containing pivotal information on protein function. NMR is an analytical chemistry technique used in the quality control and research to determine the content and purity of a sample and its molecular structure. NMR can quantitatively analyze mixtures containing known compounds. For unknown compounds, NMR can be used to match against spectral libraries or to infer the basic structure directly. Once the basic structure is known, NMR can be used to determine molecular conformation in and studying physical properties at the molecular level such as conformational exchange, phase changes, solubility and diffusion. The principle behind NMR is that many nuclei have spin and are electrically charged. If an external magnetic field is applied, an energy transfer is possible between the base energy to a higher energy level (generally a single energy gap). The energy transfer takes place at a wavelength that corresponds to radio frequencies and when the spin returns to its base level, energy is emitted at the same frequency. The signal that matches this transfer is measured in many ways and processed in order to yield an NMR spectrum for the nucleus concerned.

The NMRC offers a high resolution (solution state) NMR service to external users. The NMR experiments are available at frequencies of 400 MHz, including routine 1- and 2-D proton and carbon, fluorine and phosphorus experiments. The NMRC is equipped with a 60 position sample changer. The DRX400 spectrometer is primarily for research purposes. There are 3 different probes available; however, the majority of spectra are obtained using the QNP probe which is controlled/accessed using the ICONNMR software. Non-routine use (for variable temperature spectra for example) of the DRX400 can be booked by contacting Dr Salah Bensaber to arrange a time. For biological studies, NMRC is unique among the methods available for three-dimensional structure determination of proteins and nucleic acids at atomic resolution, since the NMR data can be recorded in solution. Considering that biological fluids (blood, stomach fluid, saliva, etc) are protein solutions where these molecules perform their physiological functions, knowledge of the molecular structures in solution is highly relevant. In addition to protein structure determination, NMR applications include investigations of dynamic features of the molecular structures and studies of structural, thermodynamic, kinetic aspects of interactions between proteins and other solution components which may either be other macromolecules or low molecular weight ligands.

Review article

Structure and function of A_1 adenosine receptor

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Abstract: The adenosine A_1 receptor is one member of the adenosine receptor group of G-protein-coupled receptors with adenosine as endogenous ligand. Adenosine regulates several physiological functions through specific cell membrane receptor. On the basis of studies related to pharmacology and molecular cloning, four distinct adenosine receptors have been identified and classified as A_1, A_{2A}, A_{2B}, and A_3. These adenosine sub-receptors are members of the G-protein coupled receptor family. The A_{2A} and A_{2B} receptors stimulate adenyl cyclase with a consequent increase of cAMP levels, while the A_1 and A_3 receptor subtypes produce the opposite effect. Intense efforts made over the last three decades have led to synthesis of a variety of selective adenosine agonists and antagonists. The A_1 receptor is usually found on the working cells tissues (e.g., neurons and cardiomyocytes) and mediates a decrease in oxygen demand. Thus, for these reasons, A_1 agonists could be useful for the treatment of renal failure, arrythmias, diabetes type II and myocardial ischaemia as well as certain neurodegenerative disorders. Therefore, the recent developments on structure-activity relationships at A_1 receptor and its possible use as therapeutic agents are reviewed in this article, with a particular emphasis on the recent patent literature.

Key words A_1 adenosine receptors, adenosine, agonists, G-protein, diseases

Introduction

The adenosine receptors, hence their name, are subject to activation by the endogenous ligand adenosine. In extracellular space adenosine is formed by the break-down of adenosine triphosphate (ATP, Figure 1). There are four categories of adenosine receptor, the A_1, A_{2A}, A_{2B} and A_3 which all of these sub-classes are G-protein coupled receptors. The A_{2A} and A_{2B} receptors preferably interact with members of the Gs family of G-proteins while the A_1 and A_3 receptors preferably interact with G_i-proteins. However, other G protein interactions have also been described (1).

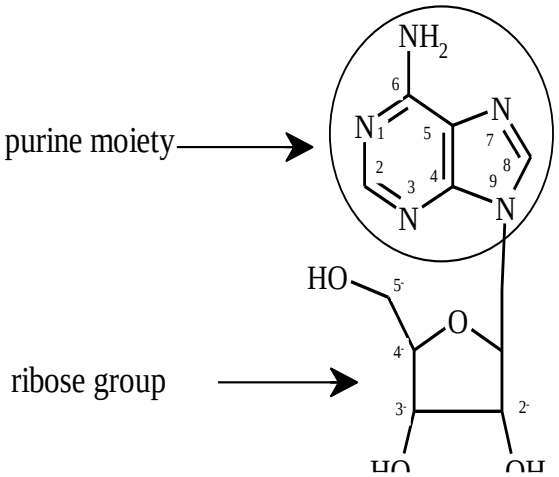


Figure 1. Chemical structure of adenosine

Adenosine receptors are not sensitive to nucleotides such as ATP, ADP and AMP. When adenosine binds to A_1 or A_3 receptors, the intra-cellular level of cyclic AMP is decreased; however, A_2 receptor acts through increasing the level of cyclic AMP concentration in the cell (1). Adenosine nucleoside and nucleotide exert a variety of effect on numerous cell types. This action derived from the activation of specific membrane receptors, generally referred to as purinoceptors. There are two major classes of purinoceptors, P_1 (adenosine) and P_2 (ATP/ADP) purinoceptors were recognized at the first time and named latterly as A_1 and A_2 (1).

It is well known that adenosine receptors exert its effect through the specific cell surface receptors. These receptors have further been classified into four subtypes A_1, A_{2A}, A_{2B} and A_3 (2). Adenosine is an endogenous substance present in all the cells and body fluids. Concentrations of adenosine reflect the metabolic state and regulate biological processes throughout the body (2). Under the physiological conditions, generation of adenosine occurs both intracellularly and extracellularly. The intracellular and extracellular pools of adenosine are tightly regulated by bidirectional equilibrative and concentrative nucleoside transporters. Adenosine is produced intracellularly by either dephosphorylation of adenosine 5'-phosphates (ATP, ADP and AMP) by 5´-nucleotidase or by hydrolysis of S-adenosyl homocysteine (3). Extracellular adenosine can be formed by rapid hydrolysis of nucleotides such as ATP *via* dephosphorylation of adenine nucleotides by ecto-nucleotidases and ectophosphodiest-erases (3).

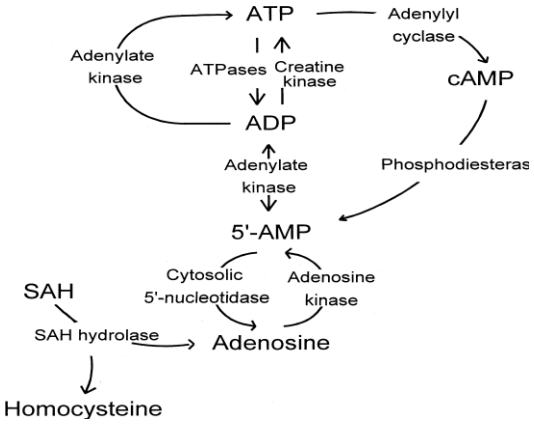
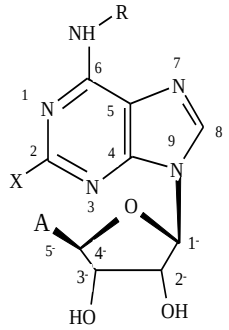


Figure 2: The primary intracellular pathways of adenosine. Adenosine is formed from 5-AMP and is converted back to 5-AMP

A_1 Adenosine receptors: All four adenosine receptors subtypes have been cloned from several species including humans (4). The A_1 receptor sequences are well known and there are some differences among different species. The cloned A_1 adenosine receptors, including the non-mammalian, show > 90% identity in the transmembrane regions (5). The ability of the A_1 receptor to inhibit adenylate cyclase activity in several systems strongly suggested that the receptor interacted directly with the G_i proteins (6). In addition, A_1 adenosine receptors mediated activation channels in the cardiovascular system. In atrial cells, agonist-occupied A_1 adenosine receptors produce an inward-rectifying outward potassium current *via* activation of a subset of K^+ channels. Also the inositol - 1, 4, 5 - triphosphate (IP_3) pathways *via* stimulation of A_1 receptor subtypes. This receptor subtypes is distributed in most regions of the CNS where adenosine act as neuromodulator inhibitor *via* prejunction A_1 receptor which lead the release of several neurotransmitters including excitatory amino acids (7, 8).

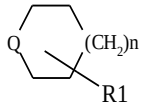
In the cardiovascular system, A_1 adenosine receptors are located on atrial and ventricular myocytes at this level, adenosine exerts cardiodeprssive effects *i.e.*, chronotropic, dromotropic and anti-arrhythmic action *via* this receptor subtype (9, 10). The activation of the A_1 receptor in the kidneys mediates vasoconstriction reducing the glomerular filtration rate (the rate at which the blood is filtered) and thus resulting in fluid retention in the patient. This receptor subtype is also present in lower concentrations in other systems as respiratory, gastrointestinal and urogenital tracts. On this basis, A_1 adenosine receptor agonists could be useful as therapeutic agents in disorders of the CNS or diseases of the cardiovascular system (11).

A_1 Adenosine receptor agonists: Among the adenosine rceptor agonists, there are few examples of compounds where the ribose moiety of the adenosine is chemically modefied. However, minor modefications at the 3^- and 5^- positions appear to be allowed. Morever, there are various examples of N6-substituted A_1 selective adenosine analogues have previously been reported (11). These compounds have relatively high lipophilicity especially when compared to adenosine analogues which are not substituted on the 6-amino group or at the purine 2-position. The highly lipophilic property makes these compounds suitable for passage across the blood brain barrier. The compounds are also substrates for nucleoside-specific active transport system into the CNS across the blood brain barrier. The compounds are purine derivatives of general formula (**1,** Figure 3).

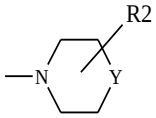


1

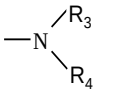
R =



Q = N, CH; n=1, 2, 3;
R1 = alkyl, alkenyl, alkynyl, phenoxy, phenylsulonyl, phenylsulfinyl,phenylthio,hydroxy,phenyl and alkoxy



Y = O, S, NZ ., where Z=H, alkyl, phenyl
R2=alkyl, alkenyl, alkynyl, phenoxy, phenyl and alkoxy



or

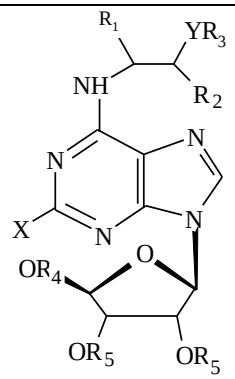
Y= O; R_3 = Alkyl; R_4 =Phenyl, alkyl; R_5=alkyl, cycloalkyl

X= halogen,amino, perhalomethyl, cyano, alkoxy, alkylthio, and alkyamine
A = methyl,halomethyl, cyanomethyl,aminomethyl, vinyl, methylthiomethyl and methoxymethyl

2 X = Cl; A=Cl;R=1-piperidinyl; K_i =6.4 nM A_2A/A_1 428
3 X = Cl; A=F;R=1-piperidinyl; K_i =5.3 nM A_{2A}/A_1 457
4 X = Cl; A= CH_2OH; R= 4-Phenoxy-1-piperidinyl; K_i =1.4 nM A_{2A}/A_1 857

***Figure 3**: General structure of N6-substituted A_1 selective adenosine analogues*

Evaluation of these compounds in established animal models has indicated that the compounds possess desirable CNS properties and they can act as anticonvulsant agents; are effective in animal model of pain and show cerebro-protective effects in animals subjected to stimulated cerebral ischaemia. The more interesting compounds of this series resulted from 2',5'-dichloro –5' deoxy-N-[1-pipeidi-nyl] adenosine (**2**) and the 2-chloro-5'-deoxy-5'-fluoro-N-[1-piperid-inyl] adenosine (**3)** exhibited greater affinity for A_1 receptor in the nanomolar range [K_i = 6.4 nM and K_1 = 5.3 nM respectively] and high affinity and selectivity for A_{2A} receptor ($A_{2A}/A_{1=}$ 428 and 457, respectively); unfortunatly they are slightly less potent than the two reference compounds N^6 –Cyclopentyl adenosine (CPA) with K_i = 1.2 nM and *R*-phenylisopropyladenosine (*R*-PIA) with K_i = 1.9nM but they are much more selective *vs* A_{2A} receptor subtype (A_{2A}/A_1) =77 and 61, respectively) (12).



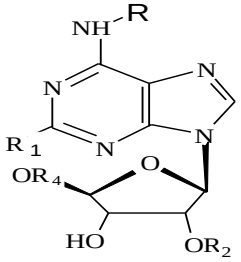
5

X= amino, alkylamino, di-alkylamino
R_1 =H, stright or branched alkyl, trifluromethyl
R_2 =H, stright or branched alkyl
R_1 and R2 togther from a cyclobutyl, cyclopentyl, cyclohexyl ring
Y = O, S, SO_2, NH, Nalkyl
R_3 = heterocyces
R_4 = H, benzoyl, alkanoyl
R_5 = H, benzoyl, alkanoyl

6 X = F; R_1= methyl; R_2 =H; Y=S; R_3 =2-benzothiazolyl K_i = 3.4 nM $_2A/A_1$ 756
$R_4 = R_5$ = H

Figure 4*: General structure of adenosine derivatives with heteroaryl substitutents at N^6-position*

The 2-chloro-N-[4-Phenoxy-1-piperidinyl]-adenosine (**4**) displayed an affinity slightly higher than the reference compound CPA, but all of them are more selective *vs* A_{2A} receptot (**4** K_i = 1.4 nM A_{2A}/A_1 = 857; CPA K_i = 1.6 nM , A_{2A}/A_1 = 108). Other compounds in the same series showed lower affinity for A_1 receptor respective to the reference compound but all of them are more selective *vs* A_{2A} (13). It was presented adenosine derivatives of general formula (**5**, Figure 4) bearing substitutents with heteroaryl at N^6, especially those which contain sulphur and nitrogen, differently substituted. The affinity for A_1 receptor was no better than the reference compound CPA, but the selectivity was highly enhanced (N -[1 (*R)* - 1- (2 -benzothiazolyl) thio – 2 - propyl] – 2 - fluro-adenosine (**6**) (K_i = 3.4 nM, A_{2A}/A_1 = 756). The compounds have a relatively high lipophilicity especially when compared to adenosine analogue; a property which makes these compounds suitable for passage across the blood brain barrier; a remarkable property for compounds which should be used in CNS pathologies (12). The compounds of general formula **(7,** Figure 5), which bear substituents at N^6, C_2 and 2' positions and focused attention on N^6-cyclohexyl-2'-O-methyl- adenosine (**8**) which shows a good affinity for A_1 receptor subtype and a high selectivity vs A_{2A} receptor (K_i = 23 nM, A_1/A_{2A} = 1090).



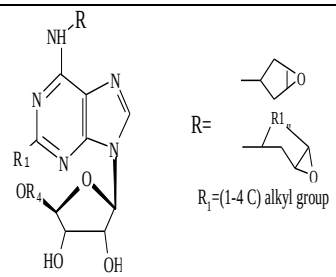
R= cycloalkyl substituted by a hydroxy group
R_1 = H, alkyl, amino, cycloalkyl and halogen
R_2 = alkyl

7

8 R= cyclohexyl; R_1= H; R_2 = methyl Kd = 23 nM A_{2A}/A_1= 1090
9 R= *(trans) 4*-hydroxycyclohexyl; R_1 = H; R_2 = methyl
10 R = (*trans*)2-hydroxycyclopentyl; R_1= H; R_2 = H

***Figure 5**: General structure of adenosine derivatives with cyclohexyl moiety at N^6-position*

The known compounds of general formula **7** are know as antihypertensive agents inhibitors of thrombocyte aggregation and activation of leukocytes; they lower blood lipid levels and are useful in the treatment of neurodegenerative diseases (14, 15). Moreover, these authors claimed the present compounds as particularly interesting analgesics for the treatment of acute or chronic pain. For example, compound (**8**) shows a significant analagesic activity in a dose-dependent manner, the antinociceptive potency of adenosine agonists was comparable to that of morphine. Interestingly, the authors found that the administeration of compound **8** leads to the production of hydroxycyclohexyl-2'-O-methyladenosine (**9**) which is probably the real active agent for the therapeutic application (K_i for N^6-hydroxycycloalkyl-2'-alkyladenosine from 1 - 500 nM) (15). The N-[(trans) -2-hydroxycyclopentyl] adenosine (**10**) is a useful compound in the treatment of severe myocardial ischaemia, when it was planned or expected, for cardiac surgery, heart attack and unstable angina (16). The substitution at N^6- amino group of adenosine derivative containing an epoxide moiety of general formula (**11**, Figure 6) exhibited greater selectivity for A_1 receptor, which can be used as A_1 agonists and for cardiac tissue.



11

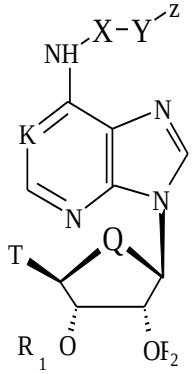
12 R= exo-5,6-epoxynorbom-2yl EC_{50} = 1.1 nM
13 R= endo-5,6-epoxynorbom-2yl EC_{50} = 1.0 nM

***Figure 6**: General structure of adenosine derivatives with modefication at 2', 3', 5' ,and N^6-position*

These adenosine agonists can be used as functional β-blockers, anti-arrhythmic agents and cardioprotectors. The dosage administered will be dependent upon the anti-arrhythmic response desired, the agonist compounds were tested for their potency to inhibit isoproternol stimulated cyclic AMP accumulation in DDT_1 MF-2 (DDT) cells, and they were compared to the reference compound (CPA). The N^6 - (*exo*-5, 6 – epoxynorborn – 2 - yl) aden-osine (**12**) and N^6- (*endo*- 5, 6- epoxynorborn - 2 - yl) adenosine (**13**) showed an activity for A_1 receptor comparable to the reference

compound (EC_{50} = 1.1 nM and EC_{50} = 1.0 nM, respectively; CPA EC_{50} = 1.7 nM) (17).

A series of compounds related to adensine of general formula (**14**, Figure 7) were modified at 2', 3', 5' and N^6 positions. The N^6- [2- (2'- (aminobenzothiazolyl) ethyl]- adenosine- 5-N- ethyl carboxamide (**15**) and the N^6- [1-methyl – 2 (3- chlorothien- 2-yl) ethyl] adenosine (**16**) showed a higher affinity and selecivity for A_1 receptor subtype than the reference compound CPA (**15** IC_{50} = 0.32 nM, A_{2A}/A_1 = 2800 ; **16** IC_{50} = 0.049 nM, A_{2A}/A_1 = 900; CPA IC_{50} = 0.72 nM, A_{2A}/A_1 = 2200). These compounds are useful in the treatment of high blood pressure. They are increase coronary blood flow and are accordingly useful in the treatment of myocardial ischaemia and they are useful as anti-lipolytic agents for the treatment of hyperlipidemia and hyperch-olesterolemia (18).



14

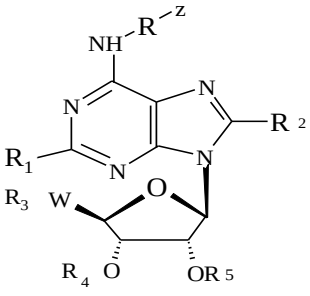
K= N, N-O, CH
Q= O, CH_2
T=R_3 , R_4= NCO, R_5 = OCH_2
X= stright or branched alkylene,cycloalkylene, and cycloalkenylene
Y= N, R_6 = O, S
Z=substituted or unsubstituted heterocycles containing N, O or S
R_1 and R_2 =H, alkyl,aralkyl, carbamoyl and alkylcarbamoyl, dialkylcarbamoyl, acyl, alkoxycarbonyl, aralkoxycarbonyl, and aryloxy carbonyl
R_3,R_4,R_5,R_6 = H,alkyl,aryl and heterocyle

15. K = N ; Q =O T = C_2H_5NHCO; X= CH_2 CH_2; Y= NH; Z = 2-benzothiazolyl; R_1 = R_2= H, IC_{50} =0.32 nM A_{2A}/A_1 = 2800

16. K = N ; Q =O T= $HOCH_2$; X= (CH_3) $CHCH_2$; Z =3-chlorothien-2-yl; R_1= R_2 =H, IC_{50} = 0.049 nM A_2A/A_1 = 900

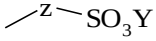
***Figure 7**: General structure of adenosine derivatives with modefication 2',3',5' and N^6-position*

It was found that a series of sulfohydro-carbon derivatives of adenosine of general formula (**17,** Figure 8) useful in the treatment of ischaemia or hypoxia (19). The sulfohydrocarbon derivatives exhibit lower affinity and selectivity for A_1 receptor subtype than the reference compound CPA (*e.g* N^6-phenyladenosine, **18**, K_i = 3.5 nM, N^6-p-sulfophenyl adenosine, **19**, K_i = 74 nM) (20).



17

W= OCH_2, SCH_2, NHCO
R, R_1, R_2, R_3, R_4, R_5, are collectively known as 'R groups and at least one is sulfohydrocarbon radical;



Z= alkyl;Y= H, monovalent cation

18 R= phenyl; R_1 = R_2=R_3 = R_4=R_5= H; W=OCH_2 , K_i =3.5 nM
19 R = 4-sulophenyl;R_1=R_2=R_3=R_4=R_5=H; W = OCH_2 , Ki =74 nM

***Figure 8**: General structure of sulfohydrocarbon derivatives of adenosine.*

Several N^6-substituted and / or 2-Chloro substituted-1-deazadenosine showed a several folds loss in A_1 adenosine receptor affinity but a greater loss in A_2 subtypes affinity compared to the reference compound CPA (*e.g.* 2- Chloro - N^6 - cyclopentyl-1-deazadenosine (**20**, Figure 9) which displayed a potent and highly A_1-selective adenosine receptor agonist (21).

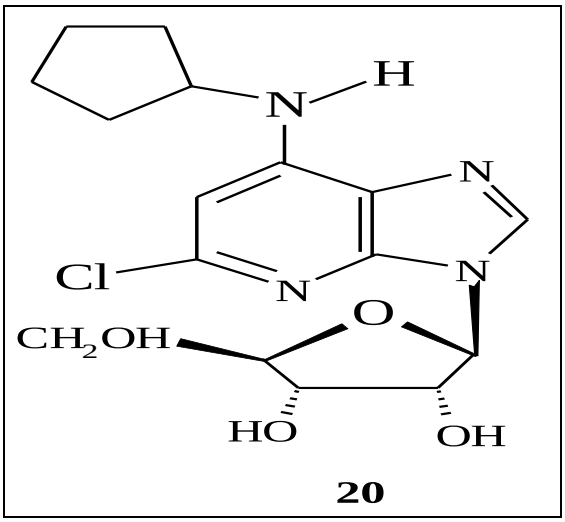


Figure 9*: General structure of substituted deazadenosine at N^6 position.*

Therapeutic applications: Over the last years, the discovery of potent and selective adenosine agonists and antagonists eluidated physiological roles of adenosine, the receptor in different parts of the body and consequently the possible therapeutic use. The A_1 adenosine receptor agonists can be useful in the treatment of hypertension through their action at different levels, myocardial ischaemia as cardioprotective agents and as antilipolytic agents (22). The A_1 adenosine receptor agonists reduce the extent of tissue damage that is observed following the interruption of blood flow to the heart (22). The anti-lipolytic activity is due to the reduction of intracellular cyclic AMP concentration in adipocytes with consequent reduction of the activity of lipoprotein lipase and, therefore, the hydrolysis of triglycerides (23). The A_1 adenosine agonists are also known for the treatment of neurodegenerative diseases, peripheral neuropathy (diabetic neuropathy) and protect the heart against infraction (24).

Conclusion

The A_1 adenosine receptor is the best characterized of the widely distributed purinergic receptor family. A_1 receptors can be clearly distinguished from A_2 (A_{2a} and A_{2b}) and A_3 adenosine receptors on the basis of structure activity relationships with selective ligands. A_1 receptor-mediated responses are coupled *via* GTP binding proteins (G proteins) to many different effectors in various tissues: adenylate cyclase, phospholipase C, Na^+, Ca^{2+} exchange, Ca^{2+} channels, Cl^- channels, and K^+ channels. Potent and selective A_1-adenosine receptor (AR) antagonists (derivatives of xanthine or other heterocyclic structures) have been developed during the past ten years. In fact, only weak and not very selective agonists and antagonists are available; the releation between A_1 and other adenosine receptors (A_2 and A_3) subtypes complicated the comprehension of the results reported till now.

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Review article

Umbilical cord and stem cells

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Abstract: Cord blood is the blood which remains in the umbilical cord after delivery of the newborn. It contains many cells, among them special cells called stem cells which can give rise to all of the components of human blood and bone marrow. For many years, cord blood was discarded along with the placenta after the delivery of a baby. However, it has now become an established alternative to bone marrow transplantation, especially in the haematological, immunological and metabolic storage disorders in children and young adults. On the other hand, storage of cord blood for therapeutic purpose will require a license from the human authority under the human tissue act.

Key words: Cord blood, stem cell, banks, gene, therapy

Introduction

Worldwide interest in the cord blood began in 1972 when Ende and Ende published the article of *"Hematopoietic transplantation by means of fetal (cord) blood: a new method."* In this article, they raised the possibility of exploiting the im-mature immune system present in the cord blood to avoid the occurrence of GVHD, one of the transplantation's most common problems. In 1988, the first cord blood transplant was performed on a boy with Fanconi anemia at the St. Louis Hospital in Paris with cells collected in North Carolina, USA. The donor was the boy's newborn sister. The cord blood stem cells were shipped overnight to the laboratory of Broxmeyer who with other doctors proceeded to carry out what was then a revolutionary method of treatment for cancer.

Today, the recipient is alive and cured of the hematologic manifestations of Fanconi anemia. As of today, more than 6000 cord blood transplants have taken place around the world. In 2006, the New England Journal of Medicine stated that cord blood banks in 21 countries currently store about 170,000 units of cord blood (1).

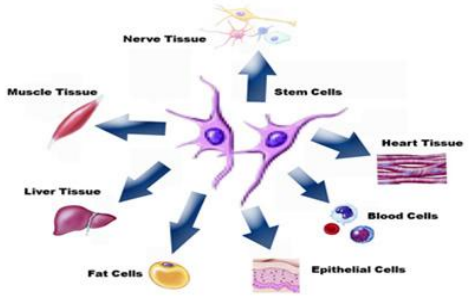

Figure 1: Stem cells differentiate into many cell types

Stem cells: Stem cells are cells found in the human body that have the unique ability to divide continuously, often throughout the life of a human. They are the "master" cells that transforms into cells that form the brain, nerves, muscles and other parts. Stem cell research is generating strong evidences about how healthy stem cells, when under the right conditions or signals, can give rise to differentiated cells as skin, neurons and hematopoietic cells. These cells can be used to replace damaged or diseased tissues in a particular human organ (2).

Stem cells can typically be broken into four types: *embryonic stem cells-stem cells taken from human embryos, fetal stem cells – stem cells taken from aborted fetal tissue, umbilical tem cells-stem cells taken from umbilical cord and placental blood and tissues and adult stem cells- stem cells taken from bone marrow, adult peripheral blood and other body tissues.*

The baby's umbilical cord blood is a rich and valuable source of stem cells, which are genetically unique to the baby and his/her family. It is an alternative source of stem cell transplantation where traditionally bone marrow or peripheral blood stem cells have been used. Umbilical cord blood stem cells are less prone to develop GVHD than either bone marrow or peripheral blood stem cells. This is because the umbilical cord stem cells have not yet develop into features that could be recognized by our own body cells and tissues (3).

Storing cord blood: Cord blood collection is a simple, quick and painless process that neither interferes nor complicates the delivery process. Cord blood stem cells are generally preferred in stem cells transplantation, because they offer a lower complication rate compared to bone marrow transplantation. The baby's cord blood stem cells that are stored with a private cord blood bank, can be readily made available, thus, should the need arises. This eliminates the time needed to search for a matching donor's stem cells and allows treatment to begin promptly. The baby's cord blood stem cells guarantee a 100% perfect match for the baby and can potentially increase the odds of having a match for their siblings and parents. Cord blood stem cells are recognized as one of the most promising medical treatments for the future. Currently, expecting parents can store their baby's cord blood which can then be used to treat over 80 different types of diseases.

Collections of cord blood stem cells: To assure that cord blood will be safe for future use, the following tests are done: cord, blood: sterility, cell, ount, ABO blood, grouping and maternal blood: Hepatitis, HIV, cytomegalovirus, alanine trans-aminnase, and syphilis. Upon receipt of the enrollment forms and enrollment fee, service Provider Company will send a collection kit. The kit contains all the materials that the medical professional delivering the baby will need for the collection of the child's cord blood. The kit also contains all the shipping materials needed to safely send the blood directly to the laboratory. Cord blood can be collected regardless of the birthing manner (naturally or cesarean section) after the delivery of the newborn and before or after the delivery of the placenta. The portion of the umbilical cord still attached to the placenta is clamped about 4-8 inch area and cleaned. The needle from the collection bag is inserted into the umbilical vein the minimum acceptable collection volume is 40 ml with an ideal collection consisting of between 100 and 140 ml and the blood is allowed to drain into the collection bag. The collection bag contains an anticoagulant, which prevents the blood from clotting (4). It takes approximately ten minutes for all the

blood to be collected. Once the collection is complete; the specimen is packaged and within 24 hours of the delivery is sent to the laboratory (5).

Banking process: There are basically two types of cord blood banks: public and private. Public cord blood banks store cord blood stem cells that are donated by baby's parents which are then made available to anyone in immediate need of stem cells transplantation. Private cord blood banks provide families with the option to collect and store their cord blood cells for their exclusive use, if the need ever arises, Banking Process steps as follows: as soon as the blood sample arrives in the laboratory it is assigned a unique identification number.

The sample is accessioned into a computerized system, carefully measured and tested for sterility, viability, cell count and blood type. Cryopreservation process the stem cells are separated and protected with a cell protectant which allows the cells to withstand very low temperatures. The cells are stored in liquid nitrogen at -196 ^{o}C (6).

Figure 1: Clinical use of umbilical cord blood in medicine

Transplantation

Stem cells are primarily used in transplant medicine to regenerate a patient's blood and immune system after they have been treated with chemotherapy and/or radiation to destroy cancer cells. At the same time the chemotherapy and radiation destroys the cancer cells in a patient. They also destroy stem cells. Therefore, an infusion of stem cells or a stem cell transplant is performed after the chemotherapy and/or radiation treatment. The stem cells then migrate to the patient's bone marrow where they multiply and regenerate all of the cells to create a new blood and immune system for the patient.

CURRENT TREATABLE DISEASES WITH STEM CELLS

Acute Leukemias
Acute Biphenotypic Leukemia
Acute Lymphocytic Leukemia (ALL)
Chronic Leukemias
Chronic Lymphocytic Leukemia (CLL)
Chronic Myelogenous Leukemia (CML)
Myelodysplastic Syndromes
Amyloidosis
Chronic Myelomonocytic Leukemia (CMML)
Stem Cell Disorders
Aplastic Anemia (Severe)
Congenital Cytopenia
Myeloproliferative Disorders
Acute Myelofibrosis
Agnogenic Myeloid Metaplasia (Myelofibrosis)
Lymphoproliferative Disorders
Hodgkin's Disease
Non-Hodgkin's Lymphoma
Prolymphocytic Leukemia
Phagocyte Disorders
Chediak-Higashi Syndrome
Chronic Granulomatous Disease
Neutrophil Actin Deficiency
Reticular Dysgenesis
Liposomal Storage Diseases
Adrenoleukodystrophy
Gaucher's Disease
Hunter's Syndrome (MPS-II)
Hurler's Syndrome (MPS-IH)
Krabbe Disease
Maroteaux-Lamy Syndrome (MPS-VI)
Wolman Disease

Histiocytic Disorders
Familial Erythrophagocytic Lymphohistiocytosis
Hemophagocytosis
Histiocytosis-X
Langerhans' Cell Histiocytosis
Inherited Erythrocyte Abnormalities
Beta Thalassemia Major
Blackfan-Diamond Anemia
Pure Red Cell Aplasia
Sickle Cell Disease
Congenital (Inherited) Immune System Disorders
Absence of T & B Cells SCID
Absence of T Cells, Normal B Cell SCID
Ataxia-Telangiectasia
Bare Lymphocyte Syndrome
Other Inherited Disorders
Cartilage-Hair Hypoplasia
Ceroid Lipofuscinosis
Inherited Platelet Abnormalities
Amegakaryocytosis / Congenital Thrombocytopenia
Plasma Cell Disorders
Multiple Myeloma
Plasma Cell Leukemia
Waldenstrom's Macroglobulinemia
Other Malignancies
Brain Tumors
Breast Cancer
Ewing Sarcoma
Neuroblastoma
Ovarian Cancer
Autoimmune Diseases
Evan Syndrome
Multiple Sclerosis (Experimental)
Rheumatoid Arthritis (Experimental)

Gene therapy

Scientists have acknowledged that hematopoietic stem cells, readily isolated from cord blood, are a delivery cell of choice in gene therapy. Gene therapy is basically the introduction of a therapeutic transgene (the new genetic material) into the patient's body to alter or supplement the function of an abnormal (or faulty) gene. Stem cells have been acknowledged as an important vector in gene therapy for several reasons: stem cells are a self-renewing population of cells and reduce the need for repeated administrations in gene therapy. Stem cells are easily obtained bynon-intrusive methods such as cord blood collection. They are easily identified and manipulated in the laboratory and can be returned to patients by a simple infusion.

Hematopoietic stem cells are able to give rise to many different types of blood cells. In May 2005, Escolar et al. have reported that successful umbilical cord blood transplants in babies with infantile Krabbe's disease. Infantile Krabbe's disease results in progressive neurologic deterioration and death in early childhood. The extensive study was carried out on 25 newborns with infantile Krabbe's disease using umbilical cord blood from unrelated donors. It was found that transplantation of umbilical cord blood from unrelated donors in newborns with infantile Krabbe's disease favorably altered the natural history of the disease. Infants who underwent transplantation before the development of symptoms showed progress in developmental skills as well as cognitive function and receptive language skills equivalent to that of normal unaffected infants.

In the future, Potential treatable diseases with stem cells believe in the ability to replicate tissue could lead to development of ways to replace organ treat life threathing disease such as: Alzheimer's disease, diabetes, heart disease, liver disease, muscular dystrophy, Parkinson's disease, spinal cord injury and stroke. Such in diabetes, success using stem cells to regenerate pancreas function and insulin production, may help insulin dependent patients become free of adjunctive therapy (7 - 9).

Legal and ethical issues: Legal implications of parental requests to take cord blood. It would be wise for hospitals providing obstetric services to clear policy on this issue and to make it available for patients. Hospitals believe that they will be to provide this service safely those who demand it. It is suggested that they should make it clear to prospective patents that this agreement will be conditional upon clinical and logistical demands on the service locally at the time (10).

Conclusion

Use of haemopoietic stem cells obtained from umbilical cord blood has become an established alternative to bone marrow transplantation, especially in haematological, immunological and metabolic storage disorders in children and young adults. Storage of cord blood for therapeutic purpose will require a license from the human authority under the human tissue act.

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Short communication

Chemical compositions of some Libyan medicinal plants

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Abstract: A total of twenty-one folklore medicinal plant were collected from various parts of Libya. The plant extracts were screened for the presence of alkaloids, saponins, flavones, coumarins, terpenes, anthraquinones and tannins. The number of positive tests obtained was 26 for alkaloids (18.05%), 23 for saponins (15.97%), 20 for flavones (13.88%), 12 for coumarins (8.33%), 31 for terpenes (21.52%), 03 for anthraquinones (2.08%) and 30 for for tannins (20.83%). Thus, the plants can be utilized as a potential source of useful drugs.

Keywords: Alkaloids, tannins, flavanoids, saponins, terpenes, coumarins, anthraquinones.

Introduction

Traditional medicine was practiced over the past several thousands of years in one form or another and is wide spread through out the world for the past 50 centuries (1). Its practices are based on beliefs that were in existence before the development and spread of modern scientific medicine and are still prevalent today. The folklore medicinal plants which are used for therapeutic purpose are precursors for the synthesis of some useful drugs (2). The medicinal value of these herbals lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive principles of these aromatic medicinal plants are alkaloids, tannins, flavanoids, saponins, terpenes, coumarins anthraquinones and phenolic compounds (3). Over the last two decades, the pharmaceutical industry has made massive investments on chemical, pharmacological and clinical researches all over the world in an effort to discover still more potent herbal drugs for incurable diseases like AIDS, HIV, cancer and diabetes (4, 5). As the time passed, this branch of indigenous medicinal treatment has undergone radical changes in many developing countries and investigations are conducting in this field (6 - 8). The World Health Organization (WHO) estimates that 4 billion people, 80% of the world population, presently use folklore medicinal plants for

some aspect of primary health care (9). This is not surprising since only 4% of the estimated 350,000 floral species have been screened or investigated for biologically active principles. Phytopharmacological screening surveys have been conducted for the investigation of their active principles by various researchers. A few of preliminary data obtained from certain flora are considered significant and encouraging as their extracts were shown to be remedial for tumor inhibitors in cancer chemotherapy (10, 11).

Materials and methods

Collection/identification of plant materials

The leaves and stems of the plants were collected from different regions of Libya as shown in Table 1. All the twenty-one collected plant materials were identified by botanists and their herbarium sheets were deposited at the medicinal chemistry and pharmacognosy research laboratory, faculty of pharmacy, 7th April University, Zawia.

The plant samples were air-dried and ground into uniform powder using a milling machine. The aqueous extract of each sample was prepared by soaking 100 g of dried powdered samples in 200 ml of distilled water and the ethanol extract by dissolving 25 g of the grinded fresh leaves in 250 ml of 95% ethanol. The extracts were filtered using Whatman filter paper No 42 (125 mm) and subjected to chemical investigation methods.

Phytochemical screening

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as it has previously been described (12, 13).

Alkaloids: Two methods are applied for the investigation of alkaloids:

A. Color reaction test by using Mayor's and Wagner's reagent. Thus, 2.5 g alcoholic extract of powdered plant extract and (25 ml) of ethanol were evaporated to dryness and the residue was heated with 2N HCl (5 ml) and cooled, the mixture was filtered and the filtrate was divided into two equal portions. One portion was treated with a few drops of Mayer's reagent and the other with similar amounts of Wagner's reagents. The samples were then observed for the presence of turbidity or precipitation. A (+) reaction was recorded if only a slight opaqueness was observed. A (++) reaction was recorded for a definite turbidity but no flocculation was recorded. A (+++) reaction was recorded for a definite heavy precipitate or flocculation produced (14).

B. TLC: The alcoholic plant extracts are spotted on TLC and then eluted with chloroform. After drying, the TLC was sprayed with Dragendrorff's reagent. The presence of alkaloids is detected by formation of orange –red color.

Test for saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and then filtered. 10 milliliters of the filtrate was mixed with 5 ml of distilled water and shaked vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for flavanoids: Three methods were used to determine the presence of flavanoids in the plant sample. Five milliliters of dilute ammonia solution were added to a portion of

the aqueous filtrate of each plant extract followed by addition of concentrated H_2S0_4. A yellow coloration observed in each extract indicated the presence of flavanoids. The yellow coloration disappeared on standing. Few drops of 1% aluminum solution were added to a portion of each filtrate. A yellow coloration was observed indicating the presence of flavanoids.

A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration was observed indicating a positive test for flavanoids.

Test for steroids: Two milliliters of acetic anhydride was added to 0.5 g ethanol extract of each sample with 2 ml H_2S0_4. The color changed from violet to blue or green in some samples indicating the presence of steroids.

Test for terpenoids (Salkowski test): Five milliliters of each extract was mixed with 2 ml of chloroform and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids.

Test for cardiac glycosides (Keller-Killani test): Five millilitres of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for Anthraquinones: A five gram of plant extract was boiled with 10 ml of (1%) HCl and filtered, shaken the filtrate with (5 ml) of benzene. The benzene layer was removed and then (10%) NH_4OH was added. Formation of pink, violet or red color indicated the presence of anthraquinones **(**15, 16).

Test for Coumarins: One gram of plant extract was taken in a boiling tube and covered with filter paper moistened with dil.NaOH. The test tube was placed in boiling water for few minutes; the filter was removed and examined it under UV light. Yellow fluorescin indicated the presence of coumarins **(**15, 16).

Test for Tannins: About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration (17, 18).

Table 1: Phytochemical screening results of some Libyan medicinal plants

n	Family and Species	Local name	Plant parts	Alkaloids			A*	C*	F*	S*	T*	Tannins		Used as (14)
				DR	MR	WR						Gel	$FeCl_3$	Anti fungal, anti-bacterial, anti viral agents, analgesic,
1	Myrtaceae *Eucalyptus*	Sarval	L	--	-	-	+	+	-	+	+	++	+++	Heart muscle toner used in nausea and arthersclersis
2	Rutaceae *Citrus lemon*	Limcas	L	-	-	-	-	-	++	-	++	-	-	
3	Cactaceae *Opuntiaficusindica*L	Al-hindi	L	+	+	+	-	-	++	+	++	-	-	Alzheimer's, Parkinson's ischemia, or cardiovascular diseases
4	Globulariaceae *Globularia vulgarisL*	Al-zrega	L	++	+	+	-	-	+	-	++	-	++	Anti rheumatic, laxative and stimulant
5	Asteraceae *Artemesia compestrisL*	Shaal	L	+	-	+	+	+	+	+	+	+	-	Treatment of wounds, burns, cramped muscles, bronchitis
6	Apocynaceae *Nerium oleander* L	Defla	L	+	+	-	-	+	-	-	++	-	-	Poisonous shrubmedicine for cardiac, cancer
8	Rutaceae *Haplophyllum tuberculatum*	Sisutria	L	+	+	+	-	+	+	++	+	-	-	Antidiabetic, antimalarial, bitter tonic, febrifuge, used in nausea
9	Coniferae *Cupressus arizonica*	Sarvah	w	+	+	-	-	-	+	+	+	+	+	Ornamental, good wind break plant
10	Solanaceae *Hyoscymus albus*	Gageeth	L	+	+	-	-	+	+	+	++	+	+	Sedative, analgesic and antispasmodic
11	*Moraceae Ficus benjamina*	Feeks	w	+	+	-	+	+	++	+	++	++	+++	Ulcers, skin diseases, liver enlargement and spleen, diabetes, dysentry, Diarrhea and leprosy

No	Family and Species	Local name	Plant parts	Alkaloids			A*	C*	F*	S*	T*	Tannins		Used as (19)
				DR	MR	WR						Gel	$FeCl_3$	
11	Anacardiaceae *Schinus terebenthefolius*	Filfilared	L	-	-	-	-	-	-	_	+	-	-	Astringent, antibacterial, digestive stimulant, diuretic antiviral and wound healer.
12	Laminaceae Marrubium vulgare	Robia	W	+-	+	+	-	-	+	+	+	+	-	Heart tonic, sore throat, cold cough, laryngitis and bronchitis.
13	Geraniacea *Geranium robertianum*	Atr	W	-	-	-	-	-	++	+	++	+	+	Astringent, haemostatic, sedative, diarrhea and wounds.
14	Labiatae *Mentha piperita*	Mentha	W	+	-	-	-	++	+	++	++	-	+	Chills, colic, fever, nausea, diarrhea heart trouble, oral dyspepsia, brain injuries rheumatism, convulsions.
15	Asteraceae *Erigeron Canadensis*	Hashish ataljaba	W	-	-	-	-	+	-	+	+	+	-	Tonic effect on muscles, mucous, stop bleeding and astringent.
16	Rutaceae *Rutagraveolen* L	Faigel	L	+	-	+	-	-	-	+	-	+	-	Antihelmintis, anti, anti parasitic, for gout sprains.
17	Lamiaceae *Ocimumbasilicu m* L	Hbag	L	-	-	-	-	+	-	+	+	-	-	Expectorant, bronchial, catarrh, diuretic and snakebite.
18	Lamiaceae *Rosamariaoffin alis* L	Ekleel	W	-	-	-	-	-	+	+	++	++	-	Culinary herb, for strengthening memory. Used in poultry.
19	Asteraceae *Artemisia absinthium* L	Sheeh roomy	L	-	-	-	-	-	+	+	+	+	+	Gastric pain, antiseptic and febrifuge.
20	Lmiaceae Origanum majorana L	Mardigu - Sha	L	-	-	-	-	-	+	+	+	+	-	Antispasmodic, stomachic, diaphoretic, rheumatism, expectorant and carminative. Used for tooth and ear aches.

W: Whole plant, L: Leaves, A*: Anthra quinone, C*: Coumarins, F*: Flavones, S*: Saponinns, T*: Terpenes
DR: Dragendroff reagent, M: Mayer's reagent, WR: Wagner's reagent, $FeCl_3$: Ferric chloride.

Results and discussion

The present study carried out on the plant samples revealed the presence of medicinally active principles like alkaloids, flavanoids, saponins, anthraquinones, coumarins, tannins and terpenes. The phytochemical characters of the twenty medicinal plants investigated are given in Table 1. The phytochemical screening results of the plants showed 18.05% alkaloids, 13.88 % flavanoids, 15.97% saponins, 21.83% terpenes, coumarins 8.33% and 20.83% tannins. Anthraquinone (1.94 %) was present in less quantity. These plants were known to exhibit both medicinal and physiological activities due to the presence of above active principles (20).

It has been found that some of these investigated plants contained steroidal compounds. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones (21). The plants which were used for phytochemical investigations can be utilized as a potential source of useful drugs. Further studies are going on these plants in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds. The antimicrobial activities of these plants for the treatments of the diseases as claimed by traditional healers are also to be investigated.

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Short communication

Accidental poisoning in children in El-Fateh Children Hospital in Benghazi

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Abstract: Poisoning is a qualitative term used to define the potential of a substance producing damage or dysfunction in the body by chemical activity. The pattern of child poisoning rapidly change and the occurrence of vary in different parts of the world depending on several factors. Thus, estimation of the magnitude of poisoning cases in Benghazi (the second largest city in Libya) and identification the causes of acute poisoning were the aims of this study. Medical records of poisoning admission in 2008 to El-Fateh Children Hospital in Benghazi were studied. Thus, out of 144 patients, 21.52% of admissions were admitted with no signs or symptoms, meanwhile 59.03% had mild symptoms. The most common cause of admission was due to ingestion of medications (56.9%). Food poisoning (27.2%) was the second causes of admission while 16% were due to house hold products exposure. Detergent was the leading cause of poisoning in the later group. Almost all the admitted cases were accidental and medicines were the most consumed substances. Improper storage of toxic agents was the major risk factor of poisoning.

Introduction

Poisoning is a qualitative term used to define the potential of a substance producing damage or dysfunction in the body by chemical activity. Poisoning of children is a common problem and a major cause of morbidity in developing and developed countries. Toxicants ingestion ranged from 500,000 to 2,000,000 per year in children under five years of age in the world. Globally, the pattern of child poisoning rapidly change and the occurrence of poisoning including the type and the agent vary in different parts of the world depending on the education status, local attitude, customs, current availability of the drugs and chemicals. There were about 1.5 million potentially poisoning exposures in children under 19 years of age reported in the united states in 1999, the majority of children being under age of six (1). Although death due to accidental poisoning in children is rare it is still major cause of ill health in young children (2). Thus, the aim of this study was to estimate the magnitude of poisoning cases in Benghazi, Libya in children and to identify the causes of acute poisoning over one year.

Materials and methods

A retrospective review of medical records of poisoning admission during a period of one year from January to December 2008 was conducted at the main children hospital (El-Fateh Children Hospital) in Benghazi,

Libya. Microsoft Excel in was used as a statistical data program. Variables such as age, gender, cause of toxicity and seasons of admission as well as severity of cases were collected.

Results and discussion

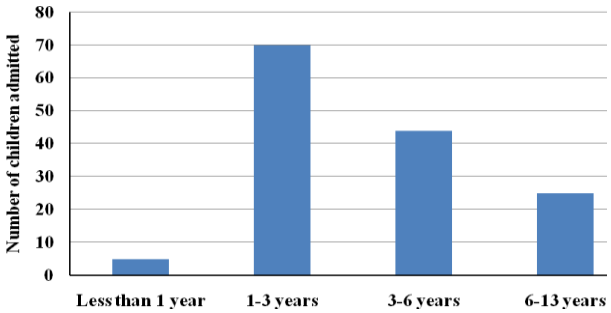
During one year period from January to December 2008, there were 144 admissions accounted as an accidental poisoning admission in children aged between one year and 13 years old. The data collected were divided according to the clinical status into three groups as shwon in Table 1.

Table1: The clinical status and the percentage

Clinical states	No. of admission (%)
Without symptoms	31 (21.52%)
Mild symptoms	85 (59.03%)
Severe symptoms	28 (19.44%)
Total	144

Thus, out of the 144 young patients, 21.52% of admission was admitted with no signs or symptoms, meanwhile 59.03% had mild symptoms which include fever, vomiting, diarrhea, abdominal pain and lethargy. These patients were linked to the food poisoning. Furthermore, the admission with severe symptoms (19.44%) was either due to house hold products exposure or because of drugs ingestion. Only few cases admitted to intensive care unit and there was no mortality recorded over the period of study. Most of the cases were discharged within two days. The majority of the cases (55.56%) were boys; male to female ratio was 1.25 : 1.00. The total number of admitted children was divided according to age groups into four subgroups; the highest frequency was occurred at the age group of 1 - 3 years and the present finding similar to that mentioned on the report of New York City (3). According to the collected data the number of admissions decreased as their age increased as given in Figure 1 which indicated that as children grown up their awareness getting better.

Figure 1: The distribution of children according to the age group



As illustrated in Table 2, the highest percent of poisoning admission found to be during the summer (36.11%) then spring (31.94%). For the duration of winter, the total admission cases were 24.31% while the lowest percent was throughout autumn (7.6%).

Figure 2: Percentage of admission due to different causes

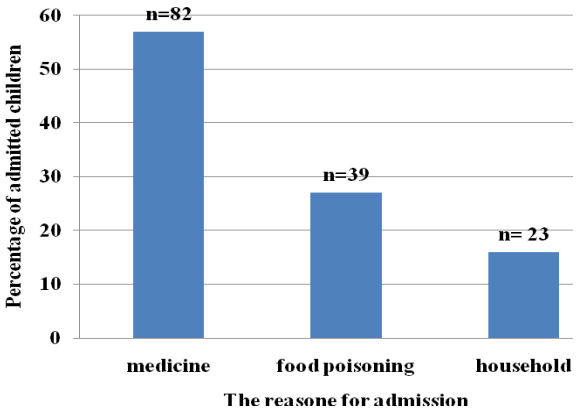


Table 2: Distibution of admissions throughout the study period

Season of admission	n
Winter	**35**
Spring	**46**
Summer	**52**
Autumn	**11**

The finding obtained in Figure 2 indicates that the most common cause of admission was due to ingestion of medications (56.9%). This finding was similar to that of the study carried out in Victoria which confirms that the most poisoning admitted cases were due to pharmaceutical ingestions (4). Food poisoning (27.2%) was the second causes of admission while 16.0% of admissions were due to house hold products exposure. Detergents were the leading cause of poisoning in the later group. In term of food poisoning, fast food was responsible for most of the cases which were among age group 6 - 13 years old. The most frequent medications taken by children were found to be oral contraceptive pills, antihypertensive drugs and tricyclic antidepression drugs.

Conclusion

Almost all the admitted cases were accidental and medicines were the most consumed substances. In addition, improper storage of the toxic agents was the major risk factor of poisoning. The present findings indicate that children poisoning seems to be a common paediatric care problem which need to be controlled.

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Short Communication

Faecal examination of occult blood: evaluation of the situation

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Abstract: Bleeding can be as a result of erosion in the lining of the gastrointestinal tract e.g., stomach or duodenum. Cancer or polyps in the colon and rectum can commonly lead to bleeding. The aim of this study was to determine the percentage of positive cases tested for occult blood and to compare the performance characteristics of the two different reagents. Two-hundred and eighty-three specimens collected from Central Hospital, Tripoli, Libya were tested for occult blood in feces. The results obtained showed a confliction which may be due to poor or inadequate patient preparation for the test that lead to occurrence of false positive or negative results.

Key words: Peptic ulcers, colorectal cancer, faecal occult blood

Introduction

Gastrointestinal tract (GIT) bleeding may be acute, chronic, massive, slight obvious, occult and may originate anywhere from the gingiva to the rectum. Therefore, it should never be ignored. Bleeding often results from minor pathology such as hemorrhoids and anal fissures. Previous studies showed that some of the patients with GIT bleeding were found to have malignant tumors and others have benign peptic ulcer (1). The major forms of common peptic ulcers are duodenal ulcer (DU) and gastric ulcer (GU). DU may cause acute GI hemorrhage. GU is similar to that of DU and it deeply penetrates beyond the mucosa of the stomach in many cases.

Although not all blood in the stool is caused by cancer but blood in the stool may be the only symptom of colorectal cancer. The small amounts of blood can only be detected by chemical testing. So, stool occult blood test may be used to diagnose colorectal cancer. However, this test can be considered as the first tool for the diagnosis of colorectal cancer. Other facilities such ascolonoscopy or flexible sigmoidoscopy are needed to locate the source of bleeding (2).

Materials and methods

This study was conducted at the Medical laboratory, Central Hospital, Tripoli, Libya. Specimens were received from hospitalized patients and outpatient clinics over the period of June 2007 to March 2009. The majority of the patients were older than 35 years. Two different reagents were used; commercially available reagent kits (CARK) and Inter-laboratory preparation (ILP) by dissolving O-tolidine in methanol.

Results and discussion

Examination of the feces is sometimes approached with reluctance of the offensive nature of material, yet simple observations may give important diagnostic clues. Testing for occult blood may lead to early detection of carcinoma. Thus, stool examination should be a part of any routine physical evaluation in adults older than 40 years. The development of commercial reagent kits has greatly facilitated the potential to detect occult fecal blood but even when used optimally it still has a major limitations as a screening technique due to interfering factors (3).

Table 1 shows a considerable variation in analysis outcome that can not be ignored. Out of the 283 specimens tested only 76 samples were positive for faecal occult blood test. Both reagents were positive for the 53 samples (18.73%), meanwhile only 11 samples (3.89%) were positive by CARK and only 12 samples (4.24%) were positive by ILP only.

Table 1: The results of the two techniques used to detect occult blood in feces

Groups	**I**	**II**	**III**	**IV**
Results	Both reagents (-)	Both reagents (+)	Only (CARK) (+)	Only (ILP) (+)
No.	207	53	11	12
(%)	73.14	18.73	3.89	4.24

It is beyond controversy that the conflicting results were due to inadequate or poor patient preparations for the test concerning diet and medicine restrictions. Educate the patient when and how to collect specimens is an important part of the process to minimize the false positive and negative results. Otherwise another technique (4, 5) called faecal immunochemical test (FIT) has to be introduced and employed. The FIT test has the advantage of being specific, requires only one specimen and avoids changing of diet or medication to perform the test.

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Short Communication

Evaluation of anti-inflammatory activity of Libyan traditional plants

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Abstract: Long time ago man has tried to treat their illness by natural remedies due to less of side effects and more safe and effective agents. Thus, the aim of this study was to evaluate the anti-inflammatory activity of some traditional plants in Libya. The studied methanolic plant extracts were investigated for its activity against inflammation. There is a significantly inhibition of the paw oedema induced by carrageenan compared with that of the reference drug (aspirin). The findings suggest that the screened plants might be used in the treatment of inflammation and it supports the traditional use of these plants in relieving inflammation.

Key words: Medicinal plants, methanolic extracts, anti-inflammatory activity, Libya

Introduction

Over the past time, man has tried to treat their illness by natural remedies due to less of side effects and more safe and effective agents than others. Medicinal plants and herbs are considered as a national wealth due to so many strategic industries depend on them and the research activities on the traditional plants growing searching for new active agents and to confirm the traditional uses for treatment of diseases. Industrial anti-inflammatory drugs are quite potent but they present a number of problems of cost and unwanted side effects. On the contrary, some of natural remedies obtained from traditional plants reach good activity at low cost and low side effects (1). The prototype drug of NSAIDs is aspirin (acetylsalicylic acid), a salicylate derivative obtained from Willow bark which acts to inhibit the activity of the cyclo-oxygnase enzyme that leads to decrease prostaglandins synthesis reducing fever, pain and inflammation (2). Other types of NSAIDs such as derivatives of acetic acid (indomethacin) and propionic acid (ibuprofen) are also commonly used (3).

As a part of the program on screening of traditional Libyan plants, an investigation was undertaken on six plants among them are Globularia alypum, Myrtus communis, Asphodelus microcarpus and Lonicera erusca. Thus, the main aim of this study was to evaluate the anti-inflammatory activity of these commonly used plants.

Materials and methods

Plant materials: The aerial parts of the Globularia alypum, Myrtus communis, Asphodelus microcarpus and Lonicera erusca were collected from Aljabal Alakdar region, east part of Libya, in April 2010. Voucher specimens were identified by the botanist Dr. M. Abouhadra, Department of Botany, Faculty of Science, University of Alfatah and deposited at the herbarium of Faculty of Pharmacy, University of Alfatah, Tripoli, Libya and J. 125/2010 was given as a reference.

Extractions and preparations of extracts: Shade-dried powdered materials with certain weight were extracted by soxhlet apparatus using methanol as a solvent at a temperature of 40 - 60 ^{o}C. The filtered solvent was evaporated in vacuum to obtain the crude extract.

Animals: Female adult Albino mice with a body weight of 20 - 25 g were used for screening of anti-inflammatory activity. The animals were maintained according to the International Environmental Conditions at a room temperature of 25 ± 0.5 ^{o}C and at a humidity of 55 ± 0.5%. Mice were fasted for four hours before and during experimental hours and had a freely access to water before the plant extracts intake. All mice were obtained from the local animal house of National Medical Research Centre, Zawia, Libya.

Inflammation induced by carrageenan: This study was done according to the guidelines for Care and Use of Laboratory Animals published by the Greek Government 160/1999 based on E.U. regulations 86/609 with modifications. Mice were divided into ten groups (n = 5 in each group). Acute inflammation was induced by intradermal injection of 0.02 µl of 2% carrageenan (v/w) (sigma-aldrich) in the right hind paw of mice (20 - 25 g) in water. **Group 1** given water intraperitoneal (i.p.) only and served as a control, **groups 2 - 9** were injected with methanolic extracts of the studied plants 500 mg/kg, i.p., before 30 min from the injection of carrageenan solution. **Group 10** given aspirin as a reference (100 mg/kg, i.p.). The oedema volume was measured after 3 hrs from the injection of carrageenan by means of plethysmometer (Ugo, Basile). The difference in weight between the two paws was calculated and taken as oedema volume for all the groups and compared with the control group and percentage of inhibition was calculated using D_o-D_t/ D_ox100 where D_o was the average inflammation (hind paw oedema) of the control group of mice at 3 hrs and D_t was the average inflammation of the drug treated (extracts or reference drug) mice at the same time.

Statistical analysis: Data were analysed using Student-*t* test and expressed as a mean ± S.E. $P < 0.05$ was considered statitically significant.

Results and discussion

Carrageenan oedema is a multimediated phenomenon that liberates diversity of mediators. It is believed to be a biphasic; the first phase (within 1 hr) involves the release of serotonine and histamine while the second phase of oedema (after 1 hr) which it is attributed to prostaglandins synthesis (4). Since carrageenan induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation (5). In this study, the mice carrageenan induced paw edema assay was employed as a model for acute inflammation and aspirin was included as a reference drug. As presented in Table 1, one

of the plant extract gave a percentage of inhibition higher than that of the reference ($p < 0.05$). The inhibition of inflammation such as Globularia alypum show the most percentage of inhibition of inflammation after 3 hrs (56.2%) compared with that of aspirin (54.6%) as it has previously been reported (6). Other plants such as Myrtus communis, Asphodelus microcarpus and Lonicera erusca were given an inhibition of 39.9%, 28.1% and 26.8%, respectively.

Table 1: Effect of plant extracts on carrageenan induced inflammation model in mice

Plant extract and reference	Oedema volume µl	% inhibition after 3 hrs
Control	**64**	**-**
Globularia alypum	**28**	**56.2**
Myrtus communis	**38**	**39.9**
Asphodelus microcarpus	**46**	**28.1**
Lonicera erusca	**60**	**26.8**
Aspirin	**29**	**54.6**

Conclusion

The findings of the present study suggested that the studied medicinal plants might be used in the treatment of inflammations which were compared with that of aspirin as a reference drug. In addition, this study seems to support the traditional use of this plant in relieving inflammation. Isolation of active compounds could also be of a great interest for searching a lead compounds.

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Short communication

Influence of greenhouse on antioxidant activity of cucumber in Libya

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Abstract: In Libya, cucumbers traditionally and largly consumed among people all over the year growing under various conditions. Thus, cucumbers of greenhosue are tested for free radical scavenging activity using 1, 1-diphenyl-2-picrylhydrazil radical screening assay. Methanolic cucumber extracts of two different sources (greenhouse and natural, 4.5 g each) showed a significant difference in the free radical scavenging activity. The HPLC results showed that the quantity of ascorbic acid is more in organic cucumber than that in the greenhouse cucumber. The difference in antioxidant activity between the two types of cucumber could be related to the quantity difference of ascorbic acid.

Key words: Cucumber, antioxidant, ascorbic acid, free radical scavenging activity.

Introduction

Vegetables and fruits are important sources for antioxidants like vitamin C, vitamin E, carotenes, phenolic acids and they have been recognized as having the potential to reduce disease risk. Oxidation, the use of oxygen by the body, results in oxygen molecules that are highly unstable, called free radicals. These free radicals, which either carry an extra electron, or lack an electron, attack neighbouring cells to either release or steal the extra electron and, therefore, stabilise. While free radicals occur naturally in the body, they are also produced by chemicals, smog, cigarette smoke and other pollutants, causing a free-radical overload.

Antioxidant compounds in food play an important role to reduce the risks for chronic diseases including cancer and heart disease. While the body produces some antioxidants, nutrients from food play an important role in keeping free radicals in check, especially when the body is subjected to environmental stress. Cucumber (*Cucumis sativus* L.) is a vegetable from the *Cucurbitaceae* family. Cucumber requires a temperature between 25 and 29 °C and plenty of sunlight (1). Cucumber is a source of vitamins and minerals for human body but its caloric and nutritional value is very low (2).

Materials and methods

Preparation of extract: The fruits of the cucumber were thoroughly washed by distilled water then dried at a room temperature and 4.5 g was taken from it and divided into small parts and then macerated

with 10 ml 100% methanol and then the supernatant was filtered using Whatman No. 1 sheet and finally the filtrate was used in the assay as a crude extract. The cucumbers used in this method were grown in Libya, freshly obtained from a National Medical Research Centre, Zawia and were in a good condition.

The free radical scavenging activity of cucumber using DPPH assay: The DPPH (1, 1-diphenyl-2-picrylhydrazil radical) radical scavenging activity of cucumber was determined using the method given by Von Gadow et al. (3). An aliquote of 50 µl tested samples were added to 950 µl of 6×10^5 M methanolic solution of DPPH radical. The decrease or increase in absorbance at 517 nm was determined after 16 min for all samples. Methanol was used as a blank sample in the experiment.

Determination of ascorbic acid content using HPLC method: The HPLC (Gilson) consists of 234 autoinjector and 306 pump. The detector was set in the range between 200 - 350 nm. The experiment was done on 243 nm, the type of column was pimade Π C18, 5µm, 150 × 4.6 mm. The mobile phase was methanol : acetonitril : tetrahydrofuran (75: 20: 5 v/v/v) respectively. The flow rate was 1.2 ml/min and the injection loop was 20 µl.

Calibration curves: Standard solutions of ascorbic acid were prepared in different concentrations ranged from 20 – 160 µg/ml and the peak area values were plotted against the concentrations of ascorbic acid.

Analysis of sample: After dilution of the sample with water an aliquot of sample was injected into HPLC in triplicate and then the peak area values of ascorbic acid peak obtained by injecting the two types of cucumber and the concentrations were interpolated on the calibration curve to determine the quantity of ascorbic acid in the two types of cucumber.

Results and discussion

Free radical scavenging activity: It is well known that organic cucumber rich in both vitamin C and vitamin B, in addition to thiamine, riboflavin and many others of mineral contents as K^{+1} , Na^{+1} , Ca^{+2} and Mg^{+2} (4). On one hand, the organic cucumber showed a significant decrease in the absorbance of DPPH radical (Figure 1) which may be related to the antioxidant property of the ascorbic acid. On the other hand, the greenhouse cucumber showed an increase in the absorbance of DPPH radical which attributed to the small quantities of ascorbic acid or no ascorbic acid is careful (Figure 2).

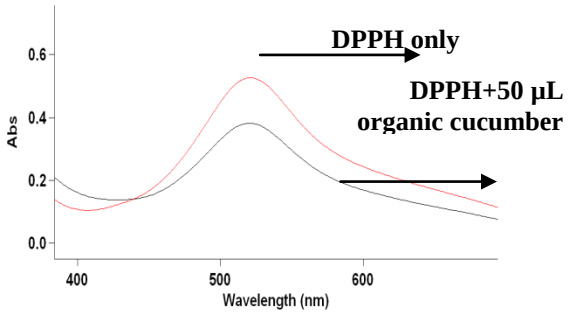

Figure 1: The absorbance of DPPH in the presence of 4.5 g/10 ml organic cucumber at a room temperature in methanolic solution

HPLC method: The results of HPLC showed that the quantity of ascorbic acid in the organic cucumber was more than that in greenhouse cucumber.

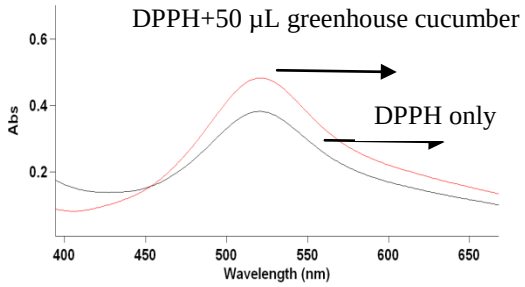

Figure 2: The absorbance DPPH in the of presence 4.5 g/10 ml greenhouse cucumber at a room temperature in methanol solution

Conclusion

The results of the present study demonstrate that the effects of greenhouse on the cucumber agriculture in Libya and having a deleterious effect on antioxidant activity of the cucumber. However, the literature resources indicated that the cucumber contains ascorbic acid which has an antioxidant activity (5). This study confirms that the decrease in antioxidant activity of the greenhouse cucumber was due to the amount of ascorbic acid available in the plant. The present study also indicates that the organic cucumbers are a strong radical scavengers and can be considered as a good sources of natural antioxidants for side dishes, medicinal and commercial uses.

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Original article

Post-marketing surveillance of enalapril brands in Libyan market

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Abstract: Quality control assessment was carried out on six brands of commercially available enalapril (10 mg) brands tablets in the Libyan market with the aim of selecting brands that are interchangeable with one another and with the innovator brand, Renitec®. The in-vitro parameters (e.g., weight uniformity, hardness, friability and disintegration time) employed for characterization of these brands were as indicated by United State and European Pharmacopeias. The findings indicated that all the tested brands comply with United State Pharmacopoeia limits. Comparison of the six brands with the innovator brand showed that two brands were the closer in their physical properties to the innovator. This study highlights the importance of post-marketing surveillance of the available drug products in Libyan market.

Key words: Enalapril, disintegration, innovator, brand, hypertension, Libya.

Introduction

A cardiovascular disease (e.g., hypertension) is estimated to affect one billion individuals worldwide and is a major risk factor for stroke, coronary heart disease, heart failure, and end-stage renal disease. The discovery of the renin-angiotensin system (RAS) as a master regulator of blood pressure and cardiovascular function provided numerous targets for pharmacologic intervention. Angiotensin-converting enzyme (ACE) plays a crucial role in the RAS by the production of the vasoconstrictive peptide angiotensin II. Enalapril is an ACE inhibitor widely used in the treatment of hypertension and congestive heart failure (1, 2). Enalapril was discovered at Merck in the 1970s and supplied as 2.5 mg, 5 mg, 10 mg and 20 mg tablets for oral administration. In addition to the active ingredient (enalapril maleate), each tablet contains, in addition, the following inactive ingredients: lactose monohydrate, magnesium stearate, sodium bicarbonate and pregelatinized starch. The 2.5 mg and 5 mg tablets also contain iron oxide yellow and the 10 mg and 20 mg tablets also contain iron oxide red. Enalapril is available in different brands. In the United States, more than 50 brands of Enalapril tablets are available in the market. In the Libyan market, ten brands of enalapril are available at the time of study. In addition to the innovator brand Renitec®, bioavailability is a measurement of the extent of a therapeutically active drug that reaches the systemic circulation and is available at the site of action (3). Bioavailability of drug is

largely determined by the properties of the dosage form which depend partly on its design and manufacture rather than by the drug's physicochemical properties which determine absorption potential. Differences in the bioavailability among formulations of a given drug can have clinical significances; thus, knowing whether drug formulations are equivalent is very essential (3). Bioequivalence is a term used when comparing a brand name and generic drugs. Pharmaceutical equivalence implies the same amount of the same active substance(s) in the same dosage form for the same route of administration and meeting the same or comparable standards (4). Excipients such as diluents, binders, lubricants, disintegrant and granulating agents are used with the formulation to satisfy certain pharmaceutical function (5). Diluents are commonly added to tablet and capsule formulations to produce the necessary bulk. Drug-diluent interaction can occur and resulting in poor bioavailability (6). Binders and granulating agents are used to hold powders together to form granules or promote cohesive compacts for directly compressible materials and to ensure that the tablet remains intact after compression. However, the proportion of strong binders in the tablet formulation is very critical. Large amounts of such binders increase hardness and reduce disintegration / dissolution rates of the tablets (7). Lubricants / antifrictional agents are added to tablet terminations to aid flow of granules to reduce interparticle friction and sticking or adhesion of particles to dies and punches (8, 9). The commonly used lubricants are metallic stearates and waxes and known to inhibit wettability, penetration of water into tablet and their disintegration and dissolution. There has been an interest in characterization and comparison of enalapril brands. Some of these studies indicated the bioequivalence of the enalapril brands when tested on animals (10) and on human's volunteers (11). Thus, this study was aimed to investigate the physical characterizations of different enalapril tablet brands comercially available in the Libyan market in comparison to the innovator brand.

Materials and methods

The six brands of enalapril (10 mg) tablets assigned as **A, B. C. D. E and F** and the innovator **R** were bought from private pharmacies located in different areas of Tripoli city. Table 1 presents the detailed specifications as appeared on the package for each brand. Apparatus used in physical characterizations are also mentioned with experimental procedures.

Table 1: Specifications as appeared on the package of different brands of enalapril tablets

Brands		Batch number	Manufacture and Expiry dates	Manufacturer country
code	name			
A	Enalapril maleate	0803066	3 / 2008 3 / 2011	Almus pharmaceuticals England
B	Enapril	11635	1 / 2008 1 / 2011	ASIA pharmaceutical industries- Syria
C	Prilenap	2805127	6 / 2008 6 / 2010	HemofamA.D Serbia
D	Korandil	36867	5 / 2008 5 / 2011	Remedica Ltd Cyprus
E	Enapril	E1	08/2008 08 / 2010	Framex company s.r.l. Romania
F	Lapril	080839B	08 / 2008 08/ 2011	Middle East Pharmaceutical Co.midpharma-Jordan
R	Renitec	NL02530	06/2009 06/2011	Merk Sherp & Dohme B.V. Haarlem-Netherlands

Tablet visual inspection: Tablets from each brand were randomly visualized for the general appearance, size, shape, texture, colour, colour uniformity and odour.

Tablet average weight: Twenty tablets from each brand were individually weighed using analytical balance (Sartorious, 19304645, FDCC/TPI /P/12, Germany) and the average weight then was calculated. The weight variationin percentage for the tablets was calculated using the following equation:

$$\% \text{ weight variation } = \frac{W - W1}{W1} \times 100$$

Where W is the weight of each tablet taken from the brand and W_1 is the average weight of tablets from the brand.

Tablet thickness, diameter and hardness: The thickness, diameter and hardness for 20 tablets from each brand were measured using hardness tester (Erweka, 118392, FDCC/TPI/P/11, Germany). The mean values, standard deviation and the relative error were calculated with the software instilled with the tester.

Tablet friability test: Ten tablets from each brand freed of dust, weighed and were then subjected to abrasion using a friabilator (Erweka, 115446.07od, FDCC/TPI/P/17, Germany) set to run for 4 min at 25 r.p.m. Thereafter, tablets were removed, dusted and reweighed: the percentage of friability (%F) was calculated using the equation:

$$\%Friability = [\frac{W_i - W_f}{W_i}] \times 100$$

Where W_i is the initial weight and W_f the final weight of the tablets.

A maximum loss of mass (obtained from a single test or from the mean of three tests) not greater then 1.0% is considered to be acceptable.

Tablet disintegration time: Disintegration time for six tablets from each brand was determined in distilled water at 37 ± 0.5 ^{o}C with the disintegration apparatus (Erweka, 15331.0645, FDCC/TPI/P04, Germany) and the medium was taken. Six tablets were used for the determination. The time taken for the tablets to disintegrate until no particle remained on the basket of the system and all the granules to go through the wire mesh was taken as the disintegration time.

Statistical analysis: Data of the weight, thickness, diameter and hardness of tablets were presented as the mean and standard deviation. Student t-test was used to compare each brand with the innovator brand. P value less than or equal to 0.05 was considered to be significant.

Results

In this study, enalapril tablets visualized were found to vary in size and generally circular with either flat or biconvex faces with white or pink colour. The average weights for the tablet were ranged from 149.07 ± 4.17 mg for brand **F** to 241.26 ± 1.84 mg for brand **C** (Table 2 and Figure 1).

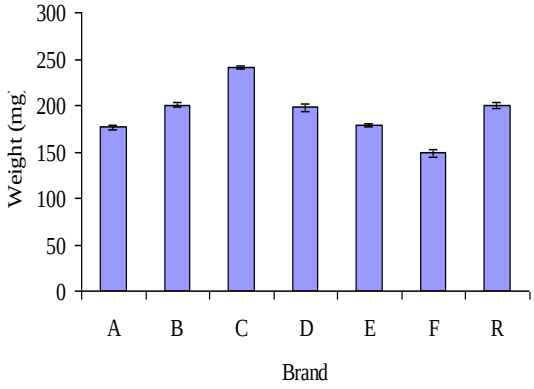

Figure 1: The average weight of tablet for different brands of enalapril where A: Enalapril maleate, B: Enapril, C: Prilenap, D: Korandil, E: Enapril and R: Renitec

Table 2: Physical characterization findings for the various brands of enalapril (10 mg) tablets

Test	The data are presented as the mean ± SD						
	A	**B**	**C**	**D**	**E**	**F**	**R**
Colour	pink	white	white	pink	white	white	pink
Shape	Biconvex/ quadrisectd	Heart shaped/ bisected	Biconvex/ bisected	Oplanged/ bisected	Heart shaped/bisected	Round/ bisected	Plunged/ bisected
Weight variations (mg)	176.7±2.2	201±1.8	241±1.8	197.9±4.2	178.7±1.9	149±4.17	200±2.7
Thickness (mm)	3.36±0.02	3.7±0.05	4.1±0.05	3.5±0.05	4.41±0.04	2.86±0.08	3.7± .0
Diameter (mm)	7.95±0.02	7.9±0.02	9.0±0.01	7.2±0.04	7.05±0.06	6.98±0.07	8.0± 0.1
Hardness (N)	58.2±24.2	155±13.8	83.1±15	65±28.1	90.5±9.28	94±18.2	205±27
Friability (%)	0.045	0.144	0.075	0.376	0.347	0.072	0.025
CSFR	1300	1076	1107	172	261	1301	8186
Disintegration time (min)	5	4	1	9	2	3	7

CSFR: Crushing strength friability ratio, A: Enalapril maleate, B: Enapril, C: Prilenap, D: Korandil, E: Enapril and R: Renitec

Generally the average weight for different brands was in agreement with the official reference specifications for weight uniformity as none of the brands tablet weight deviated by more than 5 % from the mean value. Thickness of tablet was varied from 2.86 ± 0.08 mm for brand **F** to 4.41 ± 0.04 mm for brand **E**. The diameter for the different brands was ranged from 6.92 ± 1.62 mm for brand **D** to 8.99 ± 0.01 mm for brand **C**. Figure 2 illustrates the relationship between the thickness and diameter for the different brands of enalapril tablets tested in this work.

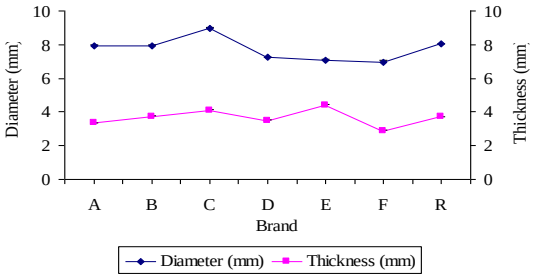

Figure 2: Thickness and diameter variation among brands of enalapril tablet A: Enalapril maleate, B: Enapril, C: Prilenap, D: Korandil, E: Enapril and R: Renitec,

A parallel change in the thickness and diameter of tablets with the change of brand was observed. Results of the hardness and friability values are shown in Table 2. The highest and least hardness values were recorded by brand **B** (154.9 ± 13.84 N) and brand **A** (58.15 ± 24.23 N). Friability of brand **D** was 0.376% (the highest friability value) while brand **A** had the least friability value of 0.045% among other tested brands. Disintegration time recorded for different brands (Table 2) were less than 15 minutes, the official specification for uncoated tablets. Brand **D** had the longest

disintegration time of nine minutes while brand **C** was with the shortest time of one minute. According to the disintegration time, brands can be ranked in descending order as brand **D** > brand **A** > brand **B** > brand **F** > brand **E** > brand **C**.

Discussion

In this study, the six brands of enalapril tablets and the innovator brand examined were within their shelf life at the time of study. Assessments of tablets for each brand involved both qualitative and quantitative methods of evaluation. Thus, the qualitative methods of evaluation were tablet description (colour, size and shape) which were carried out by visual inspection. The quantitative evaluation methods were uniformity of weight, friability, hardness, and disintegration time. Colour consistency and a smooth texture are important for easy identification and consumer acceptance of tablets. The colour of tablets usually affects the compliance of patient. Therefore, the colour and texture should be uniform through out the tablet and from tablet to tablet. The physical appearance test of tablets showed that brands **A** and **D** were with the pink colour as the inventor brand **R**. The other brands were having white colour. The shape of the tablets are adjusted by the design of the tablating machine and usually used as character of the manufacturer.

In the present study, different shapes were observed for the brands. Comparison of the shape of tablet for different brands to the shape of innovator brand revealed no matching with the innovator brand shape (Plunged/bisected). A tablet designed to contain a specific amount of drug in a specific amount of tablet formula. The weight of the tablet being made is routinely measured to ensure that a tablet contains the proper amount of drug. It is desirable that all the tablets of a particular batch should be uniform in weight. If any weight variation is there that should fall within the prescribed limits. The weight variation test would be a satisfactory method of determining the drug content uniformity of tablets if the tablets were all or essentially all active ingredients or if the uniformity of the drug distribution in the granulation or powder from which the tablets were made perfect. The test is considered correct if not more than two tablets fall outside the range if tablets are taken for the test and not more than one tablet falls outside this range. The difference in weight variation for the tablets can lead to variation in doses. The weight determination for all the brands showed compliance with the official specifications (12). One can conclude from the finding that the weights of each brand tablet are within the expected official specifications as none of the brands deviated by more than 5% from their mean (Table 2). The average weight of tablets for brands **B** and **D** was not significantly different from the innovator tablet average weight. According to the similarity of the weight of tablet of different brands to the weight of the innovator brand, brands can be ranked as brand **B** > **D** > **E** > **A** > **C** > **F**. The variation associated with the tablet weight among different brands could be due to the bulk density in the formulation of the brand (13). Thickness and diameter are non-pharmacopoeial requirements but naturally they have an effect on packaging. For uniformity of diameter of tablets, the requirements apply to the tablets which are not sugar coated, enteric-coated or film coated. A deviation of ± 5% from the stated diameter is allowed except that for diameters exceeding 12.5 mm the deviation allowed is ± 3 mm (12). The thickness and diameter of a tablet can vary without any change in weights. This study revealed no matching between the change in weight and the dimensions of tablets. This could be

explained by the variation in the density of tablets due to variation in the excipients used by different manufacturers. The difference of density of granules, pressure applied for compression, the speed of compression, the die and punch selected for making the tablets. Comparing the dimensions (thickness and diameter) of the tablet for different brands with dimensions for the innovator brand revealed that only brand **B** was similar in the diameter to the innovator **R**. Tablet requires certain amount of strength or hardness to withstand mechanical shocks of handling in manufacture, packaging and shipping. In addition, tablet should be able to withstand reasonable abuse when in the hands of consumer. Adequate tablet hardness and resistance to powdering are requisite for customer acceptance. The hardness or crushing strength for brand **B** was quite high, as high as to have resulted in low friability but not less friable then brand **A**. The high crushing strength for brand **B** is attributed to a high compression force, high binder concentration or excess volume of granulating fluid. Brand **A** with the least value of crushing strength may be due to the reverse of the aforementioned reasons. Brand **D** was the most friable compared to other brand and low crushing strength but not less then brand **A**. So, whenever crushing strength increases the friability value decreases. According to British Pharmacop-oeia, a maximum loss of weight of tablets tested for friability not greater then 1% is considered acceptable (14). Brands tested were considered acceptable in terms of friability. When the hardness, friability and disintegration time values of tablets for different brands were compared with the innovator **R**, the findings showed that all brands were different from the innovator. The ratio of crushing strength to friability is an index of measuring the mechanical properties of tablets; the higher the stronger the tablet (15). In the present study, the CSFR was ranged from 172 for **D** to 1301 for **F**. The innovator **R** recorded the highest CSFR of 8186 when compared to values recorded by other brands. The disintegration time for the innovator brand was 7 min. Disintegration test was carried according to the method of USP; this test checks whether a tablet disintegrates within a fixed time limit when placed within a liquid medium. Brand **D** had the highest value for disintegration (9 min) among other tested brands. USP disintegration allowance for uncoated tablets is within 15 min; this means that all brands tested in the present study were with acceptable values. Selecting the best brand close to the innovator would be brand **B** based on weight variation and thickness of tablet and **D** based on the weight variation and disintegration time. Nevertheless, all brands were with limits of USP for weight variation, hardness and disintegration time. Obviously dissolution and in-vivo studies are worth doing to substantiate to the in-vitro results.

Conclusion

This work has shown that the physical characterizations data of the six brands were within the limits set in USP. Brands B and D could be interchangeable with the innovator brand in weight and dimensions. The results also highlighted the importance of post-marketing surveillance of enalapril brands. There is a need, however, to carry out in vivo studies to further substantiate the in vitro predictions.

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Original article

Effects of tamoxifen on acetaminophen-induced hepatotoxicity in rats

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Abstract: Acetaminophen is a mild analgesic and antipyretic drug that is known to cause centrilobular hepatic necrosis at toxic doses which may be due to a direct interaction of reactive acetaminophen metabolites with hepatocyte proteins. Tamoxifen, the anti-estrogen is most widely used in the chemotherapy and chemoprevention of breast cancer also known to cause hepatic damage. The aim of this study is to evaluate the effect of pretreatment with tamoxifen on acetaminophen-induced hepatotoxicity in rats. Liver damage was induced by acetaminophen in a dose of 2 g/kg administered by gavage to fasting female rats as a single dose. Tamoxifen was pre-administered in a dose of 0.5 mg/kg/day for 5 consecutive days. Assessment of liver toxicity was based on determination of the activity of liver enzymes glutamic pyruvic acid transaminase, glutamic oxaloacetic acid transaminase and alkaline phosphatase as well as the ratio of liver weight to the total body weight and histopathology. The results showed that acetaminophen produced a significant elevation in liver enzymes, tamoxifen produced less significant elevation and the combined treatment with acetaminophen/tamoxifen, unexpectedly, resulted in an amelioration of liver histology, decreased enzyme activity and protected the liver from injury induced by acetaminophen. This protective effect may be related to tamoxifen scavenging activity of free radicals produced by acetaminophen.

Key words: hepatotoxicity, acetaminophen, tamoxifen, free radicals, scavenging activity, rats.

Introducion

The liver is the principal organ that is capable of converting drugs into metabolic forms that can be readily eliminated from the body. Given the diversity in use today and the complex burden they imposed upon the liver, it is not surprising that a broad spectrum of adverse drugs effects on liver function and structure has been documented ranging from mild and transient changes in the liver function to complete liver failure with potential death of the host (1). Many drugs may affect the liver adversely in more than one way, some are known to cause hepatic damage (such as nitrofurantoin, statins, niacin, isoniazid, acetaminophen, amidarone, methotrexate, disulfiram, allopurinol, sulfasalzin, ketoconazol and phenytoin) (2). Therefore, the use of these drugs requires a careful monitoring of their effects on the liver during the entire course of treatment (1).

Acetaminophen or paracetamol (N-acetyl-para-aminophenol, APAP) is one of the most commonly administered analgesic and antipyretic drug in children and adults worldwide. Despite the fact that it is generally regarded as a very safe drug, it may lead to severe hepatic necrosis and death when used in large doses intentionally or accidentally (2). In acute overdose, or when the maximum daily dose is exceeded over a prolonged period, the normal conjugative pathways of metabolism become saturated. Excess APAP is then oxidatively metabolized in the liver via the cytochrome oxidase P_{450} system to a toxic metabolite, *N* -acetyl-p-benzoquinone-imine (NAPQI) which has an extremely short half-life and rapidly conjugated with glutathione, a sulfhydryl donor and is renally excreted. Under conditions of excessive NAPQI formation or reduced glutathione stores, NAPQI binds to and interacts with vital cellular proteins and the lipid bilayer of hepatocyte membranes. An ensuing cascade of oxidative and inflammatory damage can result in hepatocellular death and centrilobular liver necrosis (3). Cytotoxic drugs are associated with many side effects including: pain, diarrhea, constipation, mouth sores, hair loss, nausea and vomiting, as well as blood-related side effects such as *neutropenia, anemia, thrombocytopenia and* bone marrow suppression) (4). In addition, the use of cytotoxic drugs is linked to hepatic toxicity such as methotrexate (hepatic veno-occlusive disease; VOD); busulphan (cholestatic and oxidative stress due to hepatic gluthathione depletion); dacarbazine (mild elevation in aminotrans-ferases and VOD); melphalan (transient elevation of tranasaminases and VOD in high doses); methotrexate (elevation of transaminases in large doses and steatosis, fibrosis and cirrhosis with maintenance therapy); 6-mercaptopurine (hepatocellular or cholestatic liver disease); fluorod-eoxyuridine (sclerosing cholangitis); l-asparaginase (steatosis and hepatocellular necrosis); actinomycin D (VOD in synergy with abdominal irradiation); tamoxifen (which is the subject of this study), is also implicated in hepatic toxicity (5). Tamoxifen (TMX) is a non-steroidal anti-estrogen that has successfully been used for a decade as post-operative adjuvant therapy for breast cancer (6). This drug lengthens the disease free interval as well as improves survival. So, it is used in the treatment and prevention of all stages of hormone dependent breast cancer (7). In addition, TMX reduces the level of estrogen and estrogen receptor with no change in progesterone contents (8).

Despite all the beneficial effects of TMX in the treatment of breast cancer, it can not totally ignore its side effects which may be some times troublesome such as: hot flashes, increased bone and tumor pain, vaginal discharge, nausea, vomiting, irregular menustrals, fatigue / asthenia and mood disturbances (9). Hepatic side effects of TMX are also common including: elevation in liver enzymes, jaundice, peliosis hepatitis, steatohepatitis, cholestasis, massive hepatic necrosis and even carcinoma which is the most serious (10). It has been documented that several drugs and chemicals can enhance APAP-induced hepatic toxicity including ethanol (11), acrabose (12), isoniazid (13) and antiepileptic drugs (14). However, no previous reports have been published concerning the combined effect of TMX/APAP in particular the effect of TMX on the hepatic toxicity induced by large dose of APAP. Therefore, the present study was aimed to examine the effect of pretreatment with TMX on the hepatic toxicity induced by APAP in rats.

Materials and methods

Animals: Female Albino adult Wistar rats (bred at the Animal Care Unit, Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Al-Fateh University, Tripoli, Libya) weighing 160 - 210 g were used in this study. They were housed in an ambient temperature of 23 °C with a 12/12 hrs light-dark cycle. Animals were fed a balanced diet with a free access of water. The study was approved by the Faculty (2009) and the experiments were done according to the ethics guidelines of Al-Fateh University (2009).

Chemicals: Tamoxifen (TMX) citrate from Sigma was dissolved at a concentration of 1 mg/ml in sesame oil containing 1% benzyl alcohol (17), APAP was a kind gift from (Al-maya Pharmaceutical Factory, Al-maya, Libya) and all the other chemicals were of an analytical grade and obtained from Merck, F.R., Germany and BDH Chemicals Ltd., Poole, England.

Experimental protocol: Rats were randomly divided into four groups (n = 5 - 6 for each group) as follows: **Group I:** Control group treated with subcutaneous injection of the vehicle (sesame oil) for 5 consecutive days. **Group II:** TMX-treated rats: Rats were treated with TMX in 0.5 mg/kg/day for 5 consecutive days, injected subcutaneously (15). **Group III:** APAP-intoxicated rats: rats were treated with a single dose of APAP (2 g/kg) given by gastric gavage (16). **Group IV:** TMX-treated/APAP intoxicated rats: treated as in group II then treated with 2 g/kg of APAP given on day 6 by gavage. Rats were killed on day 7.

Biochemical analysis: At the end of treatments in all the groups, rats were weighed and anesthetized by ketamine (80 mg/kg, i.p.) and blood sample was collected by cardiac puncture (rats in groups 3 and 4 were killed 24 hours following the administration of APAP). Blood samples (4 ml) were allowed to clot and the serum was obtained by centrifugation at 3000 rpm for 10 min and serum stored at - 20 °C until assayed for glutamic pyruvic acid transaminase (GPT), glutamic oxaloacetic acid transaminase (GOT) and alkaline phosphatase (ALP) (Vitalab Selectra C, The Netherlands). Determination of sGOT and sGPT based on the fact that phenylhydrazone that produced after incubation the substrate with the enzyme, was measured spectrophotometrically. The amount of phenylhydrazone formed was directly proportional to the enzyme quantity (18). Measurement of ALP depends on reaction of paranitrophenyl phosphate that hydrolyzed by alkaline phosphatase at pH 10.5 and 37 ^{o}C to form free paranitrophenol which is coloured yellow. The addition of NaOH stops the enzyme activity and the final colour shows maximum absorbance at 410 nm (18).

Histopathological analysis: The liver from each rat was carefully dissected, blotted free of blood and weighed. Samples from each liver were placed in 10% formalin; paraffin-embedded samples were sliced and studied under the light microscopy after staining with haematoxylin and eosin (19). At least 3 slides were studied from each specimen in a blinded fashion for any histological changes.

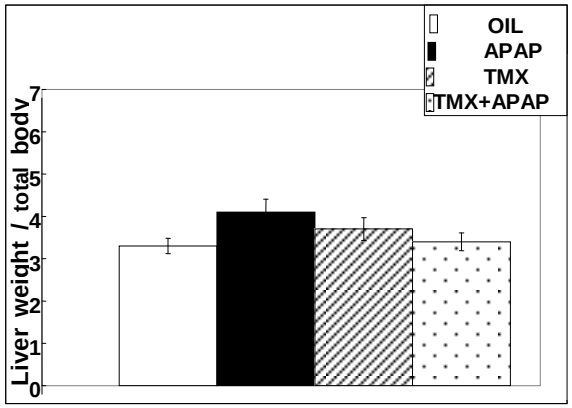
Statistical analysis

Data are expressed as mean $\pm$ S.E.M. One-way analysis of variance (ANOVA) was performed and sequential differences among the means were calculated at the level of $p < 0.05$ using Student-t test.

Results

Effect of treatment drugs on liver weight/ total body weight ratio: Administration of 0.5 mg TMX per kg/day for 5 consecutive days produced a slight but not a significant elevation of liver weight/body weight ratio compared with the negative control group. The liver index (liver weight/body weight X 100%) was increased from 3.3 ± 0.18 to 3.7 ± 0.27. The liver index was less in the combination treatment group in comparison to APAP or TMX administered singularly (3.4 ± 0.21; 4.1 ± 0.31 and 3.7 ± 0.27, respectively). However, the difference was not statistically significant (Figure 1).

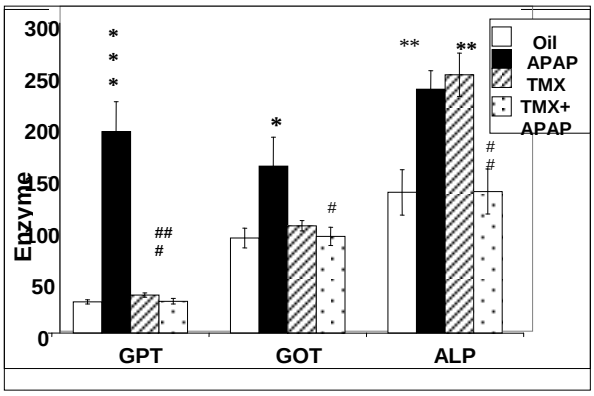
Figure 1: Effect of different treatments on liver weight/body weight ratio (LW/BW)



Values are mean ± S.E.M., n = 5

Effect of treatment drugs on liver enzymes: Treatment with APAP at a dose of 2 g/kg orally significantly increased the serum levels of ALP, GOT and GPT compared to the control group ($p < 0.01$; $p < 0.05$ and $p < 0.0001$, respectively, Figure 2). The increase in liver enzymes by APAP compared with TMX-treatment groups was significant ($p < 0.001$) for SGPT and unsignificant for the SGOT and ALP. Compared to the control group, treatment of TMX only significantly increased the levels of ALK ($p < 0.01$). In addition, GOT or GPT levels were slightly increased but did not reach the significantly level (Figure 2). However, the level of GPT was significantly lower than that of the APAP-treated group ($p < 0.001$). The level of ALK was significantly lower in the group treated with a combination of APAP and TMX compared with only treated with APAP ($p < 0.01$). The levels of GOT in the group of animals treated with APAP/TMX combination were significantly less than in those rats treated with APAP ($p < 0.05$) and for GPT by $p < 0.0001$ with less significant differences compared to TMX for GOT and GPT, results are summarized in Figure 2.

Figure 2: Effect of treatment with (TMX, APAP and TMX+APAP) on serum levels of ALP, GOT and GPT



Values are mean ± S.E.M., n=5. ***p<0.001 **p<0.01, *p<0.05, respectively in comparison to control and ##p<0.001, ###p<0.0001 in comparison to APAP

Histopathological findings: The histopathological observations of the liver showed normal structural and architectural intactness without any apparent damages or disruptions (Figure 3A). Rats treated with APAP exhibited damage to the hepatic architecture characterized by central vein congestion, hemorrhage, inflammatory cells collection causing mainly cetrilobular inflammation and moderate to severe central necrosis

(Figure 3B). The hepatic damage in TMX treated rats was mild portal tract inflammation with no significant necrosis and there is a distinct preservation of structural and architectural frame (Figure 3C). In the group of rats treated with combination of APAP/TMX, the liver exhibited surprisingly improved picture with less central and portal inflammation compared to the only treated APAP or TMX groups (Figure 3D).

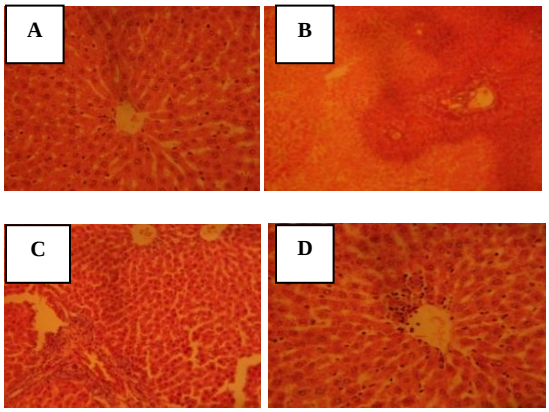


Figure 3: Photomicrographs of liver tissues from control rats treated with sesame oil. (A) shows normal hepatic cells with normal architecture. (B) shows liver treated with APAP 2 g/kg showing severe degree of necrosis, congestion and inflammatory cell collections. (C) shows liver from rats treated with TMX 0.5 mg for 5 days, indicating mild inflammation of the portal vein and no inflammation in the central vein with distinct preservation of architecture of hepatocytes. (D) shows livers from rats treated with combination of TMX and APAP, showing less inflammation damage with distinct preservation of structure and architecture of hepatocytes (H&E, 200X).

Discussion

This study presents that the pretreatment with a small dose of TMX before induction of APAP hepatotoxicity decreased the liver injury due to APAP regardless the fact that TMX is also a hepatotoxic drug. This protection might be due to free radical scavenging activity of TMX and its antioxidant action (20). Since several studies have reported that TMX or its active metabolite 4-OH-TMX possesses a free radical-scavenging and antioxidant activity in vitro and in vivo (21, 22). Zhao et al. (23) have reported that TMX improves mitochondrial respiratory function and enhances superoxide-scavenging activity of mitochondria in the heart. TMX is not a typical chain-breaking antioxidant compound as compared with vitamin E. Additionally OH-TMX is a more powerful intramembraneous inhibitor of lipid peroxidation as compared with TMX; this effectiveness may be due to the presence of a hydrogen-donating HO-group and its preferential location in the outer bilayer regions where it can donate the hydrogen atom to quench free radicals capable of initiating the membrane oxidative degradation (20). In addition, TMX is known to have cardioprotective effects which most likely due to elevation levels of manganese superoxide dismutase (MnSOD), a mitochondrial antioxidant enzyme, in cardiac tissues and cardiomyocytes (24). TMX treatment induced MnSOD expression in vitro and in vivo. Almost all hypothesis explaining APAP hepatotoxicity is related to the free radical formation after metabolic activation by cytochrome P_{450} enzymes to a more reactive metabolite which is (N - acetyl-p-benzoquinone-imine (NAPQI) that is capable of depleting glutathione (GSH) and covalently binds to protein (specifically cysteine groups on protein) (25). Other possible mechanism of acetaminophen-mediated hepatocellular death is that loss of mitochondrial or nuclear ion balance which can lead to increases in cytosolic Ca^{2+}

concentrations; mitochondrial Ca^{2+} cycling, activation of proteases and endonucleases, and DNA strand breaks (26). Oxidative stress is another suggested mechanism in the development of APAP toxicity. Thus, increased formation of superoxide would lead to hydrogen peroxide and peroxidation reactions. It has been shown that NAPQI reacts very rapidly with GSH (27). Other factors related to covalent binding to critical proteins. These factors include nitrotyrosine formation inflammatory cytokines (28). The biochemical data of APAP obtained in this study showed a significant elevation in measured liver enzymes compared to sesame oil-treated group which is in agreement with the studies by Somchit et al. (29). From the histopathological findings, an obvious sign of hepatic injury is the leakage of cellular enzymes into the plasma in APAP-intoxicated rat livers that is in concurrence with other studies (30).

Conclusion

This study indicates that the pretreatment with a small dose of TMX can attenuate the APAP hepatic injury which probably caused by interacting with NAPQI free radical and, therefore, preventing its binding to critical cellular proteins.

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Original article

Prostate specific antigen in amniotic fluid

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Abstract: Recent studies have demonstrated the presence of prostate specific antigen in amniotic fluid. It has conclusively been proven that prostate specific antigen is expressed by several non prostatic cell lines under the steroid hormone stimulation. The massive increase in the levels of placental hormones during pregnancy stimulates prostate specific antigen secretion and expression by placenta and endometrium. This antigen probably acts as a growth regulator by virtue of its IGFBP-3 protease activity, hydrolysis of insulin chains and interleukin-2, activation of latent transforming growth factor-beta (TGF-β), inactivation of protein C-inhibitors and regulation the hormonal bioactivity of PTH-related protein. Thus, this study was planned to investigate the effect of gestation, type of delivery, birth weight and sex of the baby on the levels of prostate specific antigen in amniotic fluid of Asian women. The mean prostate specific antigen level in amniotic fluid was found to be 0.966 ± 0.17 ng/ml. Prostate specific antigen level in amniotic fluid was higher in case of higher gestational age, higher birth weight, male babies and operative deliveries.

Key words: Prostate specific antigen, amniotic fluid, insulin like growth factor-I, binding-protein-3 (IGFBP-3).

Introduction

Prostate specific antigen (PSA) has been demonstrated in females for the first time in 1984. Initially, the source of this PSA was thought to be Skene's glands (1) but later studies have demonstrated that normal breast tissue, endometrium and salivary glands are the sources of serum PSA in healthy females (2). Recent studies have demonstrated the presence of PSA in amniotic fluid (3 - 5). PSA from amniotic fluid, breast tissue, placenta**,** endometrium, lungs, ovaries and Skene's glands contribute to the PSA in serum during pregnancy (6 - 8). Prostate specific antigen expression is regulated by the steroid hormones and their receptors in prostatic as well as in non prostatic cells (7, 9 - 11). There is a remarkable endocrinal alteration during pregnancy in women: 15 to 20 mg of estradiol and 250 to 600 mg of progesterone are produced per day near term. These placental hormones are responsible for PSA synthesis by breast, endometrium, placenta etc. (7, 8).

Somatomedins [insulin like growth factor-I (IGF-I), IGF-II, somatomedin-A and -C] are growth stimulators involved in the control of skeletal growth and presumably in fetal growth (12, 13). Serum levels of IGF-I and IGF-II rise during pregnancy correlating well with gestational age (14). IGF-binding-protein-3 (IGFBP-3) is the major binding

protein for IGF-I and IGF-II in plasma and it regulates their biological effects by forming a 150 KD complex with them (15, 16). The IGFBP-3 levels in serum fall markedly from six weeks of gestation till term and return to normal five days postpartum (17). This fall in the IGFBP-3 levels has been attributed to a significant increase in IGFBP-3-protease activity of PSA during pregnancy. PSA degrades IGFBP-3 complex releasing IGF's and thereby reverses the inhibitory effect of IGFBP-3 on the IGF induced mitogenesis (16 - 20). Yu and Diamandis analyzed 115 amniotic fluid samples and found that PSA in all the samples. The concentration of PSA was found to increase with gestational age from 0.022 µg/l at 11 weeks to 2.400 µg/l at 21 weeks and then decrease to 0.130 µg/l at delivery. PSA in amniotic fluid is present predominantly in free form (21) and a minor fraction of it is present bound to Alfa-anti-chymotrypsin. PSA concentration in amniotic fluid was found to be very low before the gestational age of 12 - 13 weeks (0.022 - 0.059 µg/l) (22).

In another study, the amniotic fluid PSA concentration was found to be < 0.005 µg/l at gestation ≤ 12 weeks rose to about 1 µg/l at 14 - 22 weeks and fluctuated at 0.3 - 0.6 µg/l until the delivery (23). Wolffa and others reported median amniotic fluid PSA to be 0.193 ng/ml at weeks of 16 - 18 and 0.39 ng/ml after 36 weeks. No significant association was seen between amniotic fluid PSA and fetal sex. The median amniotic fluid PSA level was 0.233 ng/ml for boys and 0.222 ng/ml for girls (4). Baxter et al. have reported a negative correlation between fetal maturity and the IGFBPs levels in amniotic fluid (24). Other studies have also reported that IGFBP-3 levels are negatively correlated with fetal maturity whereas the IGF levels are positively correlated with fetal maturity (14, 15). PSA depresses IGF-I and IGF-II by degrading IGFBP-3 (16). PSA hydrolyzes insulin chains and Interleukin-2 and activates Latent human transforming growth factor-beta (TGF-β) but inactivates protein C-inhibitors and regulates the hormonal bioactivity of PTH-related protein. The proteolytic activity of PSA on these different biological substrates, detected in term placenta, could in part, explain the potential role of placental PSA as an initiator of protease cascade which is an important biological mechanism for tissue remodeling (8, 25).

It has been suggested that high levels of PSA may be associated with fetal Down's syndrome and with Rhesus incompatibility (22). However, Wald et al. have reported similar PSA levels in amniotic fluid of pregnancies affected by Down's syndrome and in normal pregnancies in both the first and second trimester of pregnancy (26).

To the best of our knowledge, no previous studies have been reported on correlating PSA in amniotic fluid with gestational age in Asian women or correlating amniotic fluid PSA with type of delivery or birth weight of the baby. Therefore, this study was planned to measure PSA in amniotic fluid at different gestations and to study the relationship of these levels with type of delivery and with gender and birth weight of the baby.

Materials and methods

Fifty three samples of amniotic fluid were collected during artificial rupture of membranes (ARM), vaginal deliveries, midterm abortions and caesarian sections. Women below 18 years or above 45 years were excluded from the study. No sample was taken from women having antenatal complications including threatened abortion or malignancy. The data sought from these women included the date of delivery, type of delivery, sex of the baby, birth weight of the baby and the gestation at time of delivery. All the amniotic fluid samples were analyzed for PSA levels.

The samples were collected in sterile Kidney Dish or an Asepto Syringe transferred to clean plain vials and transported to the biochemistry laboratory immediately where the samples were centrifuged at 3000 rpm for 5 min to remove the particulate matter. The clear fluid was transferred to well-labeled clean plain vials and frozen till analysis. All the samples collected for this study were analyzed for PSA using 'Active PSA DSL-9700 ultra sensitive' kit. The procedure employs a two-site immune-radiometric assay (IRMA) principle (27).

Results

A total of 53 amniotic fluid samples were analyzed for PSA levels. The mean PSA concentration ± SEM was found to be 0.966 ± 0.17 ng/ml. The values ranged from 0.000 - 4.600 ng/ml.

Table 1: Effect of gestation at delivery on PSA levels in amniotic fluid

Gestation at delivery (weeks)	(n)	PSA ± SEM (ng/ml)
< 28	13	0.423 ± 0.051
> 36	40	1.143 ± 0.218
		p = 0.354

PSA concentration was found to increase with increasing the gestation. PSA levels at 36 weeks of gestation were nearly 2.5 times the levels at gestation less than 28 weeks. The mean PSA concentration in amniotic fluid at 12 - 14 weeks of gestation was 0.360 ± 0.175 ng/ml and at 22 - 24 weeks was 0.370 ± 0.068 ng/ml.

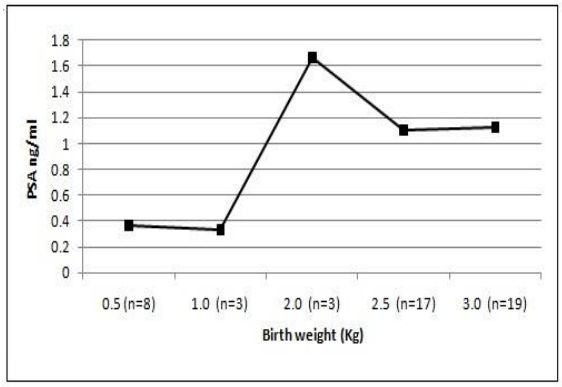

Figure 1: PSA levels in amniotic fluid and its relationship with birthweight of the baby

PSA levels in amniotic fluid of babies weighing less than 2000 gm were remarkably lower than the levels for babies weighing more than 2000 gm. This trend was not statistically significant.

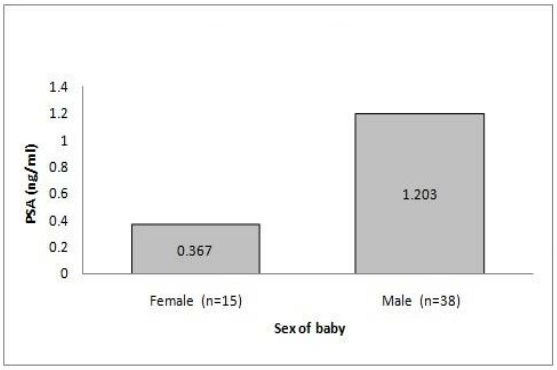

Figure 2: PSA levels in amniotic fluid and its relationship with sex of the baby

In this study, the PSA level in amniotic fluid of males is nearly four times that of the female babies.

Table 2: PSA in amniotic fluid against type of delivery

Type of delivery	(n)	PSA ± SEM (ng/ml)
Normal vaginal delivery (NVD)	18	0.387 ± 0.048
Caesarian section (LSCS)	36	1.280 ± 0.241
Total	53	0.966 ± 0.170
		p = 0.380

Furthermore, PSA in the amniotic fluid was found to be four times higher in caesarian sections as compared to the normal deliveries.

Discussion

In this study, in table 1, PSA concentration was found to increase with increasing gestation. Thus, Wolffa et al. (4) have found median amniotic fluid PSA 0.193 ng/ml at gestational 16 - 18 weeks and 0.39 ng/ml after 36 weeks (p = 0.1). On the other hand, Yu and Diamandis (7, 22) found a very low PSA in amniotic fluid before 12 - 14 weeks with a peak at 14 weeks of gestation, then a decrease followed by a second peak at 18 weeks of gestation and then a leveling off or decrease in levels till term. Filella et al. (21) have found PSA in amniotic fluid ranging from 0.050 to 1.000 ng/ml. Melegos et al. (28) have reported PSA concentrations less than 0.05 ng/l at less than or at 12 weeks of gestation which quickly increased to nearly 1.000 ng/l at 14 - 22 weeks and then fluctuated between 0.3 and 0.6 ng/ml until delivery. The values found in this study were higher than those quoted by others (7, 22, 28). PSA levels in amniotic fluid of babies weighing less than 2000 gm were remarkably lower than the levels for babies weighing more than 2000 gm. This trend was not statistically significant. None of the previous studies have analyzed PSA in amniotic fluid against birthweight of the baby. Yu and Diamandis did not find any significant difference in the PSA levels of amniotic fluid in the two sexes (29). Wolffa et al. (4) have reported no significant association between amniotic fluid PSA and fetal sex. The median amniotic fluid PSA level was 0.233 ng/ml, n = 21 for boys and 0.222 ng/ml, n 27 for girls investigated (p = 0.72). In this study, PSA in the amniotic fluid was found to be four times higher in caesarian sections as compared to normal deliveries. Further, no previous studies have reported the amniotic fluid PSA in different types of deliveries.

Conclusion

This study reports that PSA levels in amniotic fluid were found to be higher in case of higher gestational age, higher birth weight, male babies and operative deliveries. PSA levels in amniotic fluid were found to be (0.966 ± 0.17 ng/ml). During pregnancy, the presence of increased levels of PSA in amniotic fluid, placenta and endometrium suggests that PSA plays an important role in the fetal growth and development probably by virtue of its IGFBP-3-protease activity as well as activation of latent TGF-beta. Its homology with Gamma-nerve growth factor, epidermal growth factor binding protein and alpha-nerve growth factor supports the argument. Alos, it is plausible that PSA acts as a mediator of implantation and/or parturition.

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Original article

Prostate specific antigen in breast milk

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Abstract: Prostate specific antigen has long been used as a biological marker for prostatic cancer. Recent studies have demonstrated that prostate specific antigen synthesis can be induced in both sexes by steroid hormones including progesterone. Prostate specific antigen is present in the breast milk and other body fluids and tissues of women in health and in various disease conditions. The massive amounts of steroid hormones produced by the placenta during pregnancy stimulate prostate specific antigen synthesis in the breast tissue. Prostate specific antigen in breast milk probably plays an important role in post-natal growth and development of the infant by virtue of its IGFBP 3 protease activity liberating IGF-I, its very cationic nature, the inactivation of the protein C inhibitors and extensive homology of prostate specific antigen with tissue kallikrein, γ nerve growth factor and epidermal growth factor binding protein. In this study, prostate specific antigen levels in breast milk were found to be reduced with increasing the time of post-partum. The milk prostate specific antigen levels were higher in case of lesser gestation at delivery, smaller birth weight of the baby and for a male baby.

Key words: Prostate specific antigen, serine protease, kallikrein, breast milk, binding protein, insulin like growth factor

Introduction

Prostate specific antigen (PSA) is a serine protease of human glandular kallikrein family used in the clinical practice as marker for the prostatic cancer (1, 2). Recent studies have, however, demonstrated the presence of PSA in body fluids of women including breast milk, serum, amniotic fluid, saliva, urine, cord blood, CSF, pleural fluid and ascitic fluid as well as in tissues including mammary glands, kidney, placenta, salivary glands, sweat glands and pituitary gland (3 - 16). Yu and Diamandis reported breast milk PSA concentrations ranging from less than 0.010 to 350 µg/l and it did not show any relationship to maternal age and sex of the baby. PSA concentration was found to decrease with time post delivery: the highest PSA concentrations were reported on 3rd and 4th post-partum day, it was still detectable 17 - 18 days post delivery but seemed to be declined with time. The dominant molecular form of PSA in milk is free PSA. 10 - 20% of PSA is complexed with α-Anti-chymotrypsin (17). Filella et al. reported detecting PSA in 88% of all milk samples, 100% of breast cyst fluids and 100% nipple aspirates (18). In nipple aspirate fluid of non-pregnant healthy females, the PSA concentration was between 0 and 50 µg/l and in cystic disease it varied between less than 0.01 and 82 µg/l. In both conditions, very high concentrations of PSA have been reported in breast tissue of patient receiving

progestin containing oral contraceptives (17). The presence of PSA and of PSA mRNA was reported in breast cancer associated with the presence of steroid hormone receptors (17, 19 - 22) and was considered a favorable prognostic indicator. (23). High amounts of PSA have also been found in cystic fluid of intracystic carcinoma of breast and in tumour cytosols (24, 25).

The PSA gene (HKLK-3) was spans a 6 kilo base long region on chromosome 19 has a signal peptide encoded in it allowing PSA to be actively secreted by the cells and it contains steroid hormone binding sequence at - 170 position (11). It is 70 - 80% homologous to the human glandular kallikrein and tissue kallikrein of human kallikrein (HKLK) family (15, 16, 26). Human glandular kallikrein can regulate the proteolytic activity of PSA through processing and activating pro-PSA (27). Normal female breast can produce PSA (17, 27) under the conditions of stimulation by steroid hormones and this PSA is secreted into the milk during lactation (17) and is present in nipple aspirates (3). During pregnancy, the presence of increased levels of PSA in normal breast tissue, breast milk, amniotic fluid, placenta and endometrium suggests that PSA plays an important role in pre-natal, post-natal and fetal growth (12, 17, 28) and development (4). In-vitro studies have shown that PSA can be induced by progesterone, androgens, vitamin D3 (11, 17) and estrogens (11). PSA mRNA appears two hours after the hormonal stimulation and PSA protein appears after four to eight hours (29). IGFBP-3 is the major IGFBP in serum and it regulates the mitogenic effects of IGF's by binding to them (30, 31). IGFBPs control the bioavailability of IGFs through regulation of their transportation and binding to IGF receptors. Proteolysis of IGFBP-3 by PSA results in the liberation of IGF peptides from the binding protein and this result in the increased availability of IGF's for fetal and neonatal growth (32). IGF-I in turn increases production of PSA by activating androgen or progesterone receptors and thereby resulting in steroid hormone receptor mediated PSA gene transcription. PSA is very cationic molecule and may exert non-specific stimulatory effects on cells. Proteolytic action of PSA on fibronectin which is growth inhibitor, results in increased proliferation (11). PSA inactivates the protein C inhibitors (17), activates latent transforming growth factor-β (TGFβ) (33) and cleaves parathyroid hormone related peptide, insulin chains and interleukin-2 and has extensive homology with tissue kallikrein (57%), γ-nerve growth factor (56%), tonin (54%), epidermal growth factor binding protein (53%) and α-nerve growth factor (51%). It was also implicated in translational or post translational protein regulation of mammalian tissues (34, 35). The proteolytic activity of PSA on these different biological substrates could explain the potential role of PSA as an initiator of protease cascade, which is an important biological mechanism for tissue remodeling in the breast (12, 13) and in neonates. The massive amounts of the steroid hormones produced by the placenta during pregnancy stimulate PSA production in the breast tissue and this PSA in breast milk plays a significant role in postnatal growth and development. Its deficiency leads to dwarfism (17, 36). Total proteins in milk decrease rapidly between 6 hours pre-partum and 72 hours postpartum **(**37, 38) as colostrum changes to milk (38 - 40): from 2.4 gm% in colostrum to 1.22% in milk (41, 42). There is a decline in the total protein levels with increasing time postpartum (43 - 47). This study is planned as no previous studies have been conducted on Asian women correlating PSA in breast milk to gestation at delivery, birth weight of the baby or dilution of milk with increasing time post partum.

Materials and methods

Subjects: In this study, subjects were women undergoing normal, instrumental or operative deliveries without any antenatal complications. 51 samples of breast milk were collected during 0 - 15 days post-partum period. Women with age below 18 years or above 45 years were excluded from this study. Women having ante-natal complications or malignancy were excluded. The data sought from these women included age, date of delivery, type of delivery, sex of the baby, birth weight of the baby, gestation at the time of delivery and the number of days postpartum. The breast milk samples were analyzed for PSA and total proteins.

Collection of breast milk: Ten milliliters of breast milk was collected aseptically from each woman during first fifteen days postpartum in clean plain vials and shifted immediately to the biochemistry laboratory, where it was frozen till analysis. For analysis, the colostrum sample was thawed and centrifuged at 3500 rpm for 10 min. After removing the top lipid layer, the clear supernatant was analyzed. Other samples did not require centrifugation.

Estimation of PSA: All the samples collected were analyzed for PSA using active PSA DSL - 9700 ultra sensitive kit. The procedure employs a two-site immune-radiometric assay (IRMA) principle (48).

Estimation of total proteins in milk: The total proteins in the milk samples were estimated by as described by Lowry et al. (49).

Results

Fifty-one milk samples were collected from postpartum women within 15 days of parturition. The relationship of PSA levels in the breast milk to the postpartum period was studied and the relationship between PSA levels and protein content of milk was also analyzed.

In the analysis, the difference in values was considered significant (S) if $p < 0.05$. $P < 0.01$ is considered highly significant (HS) and $p > 0.05$ is considered nonsignificant (NS). PSA concentrations in milk were found to be decreased with increasing the time of postpartum. The highest values were found in the samples belonging to the first three days postpartum (8.915 ± 2.907 ng/ml). At days of 6 – 9 postpartum, it was (3.830 ± 2.506) ng/ml and at days of 12 – 15 postpartum, the PSA was 0.250 ± 0.250 ng/ml ($p = 0.397$, Figure 1).

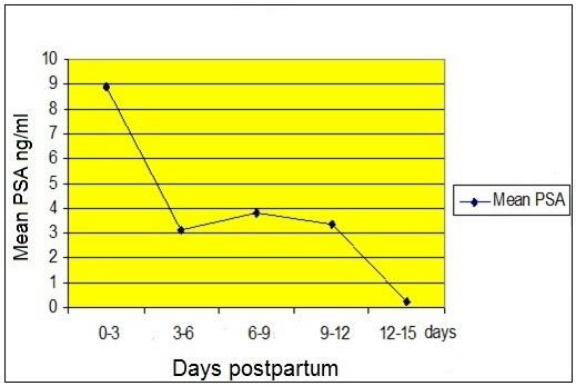
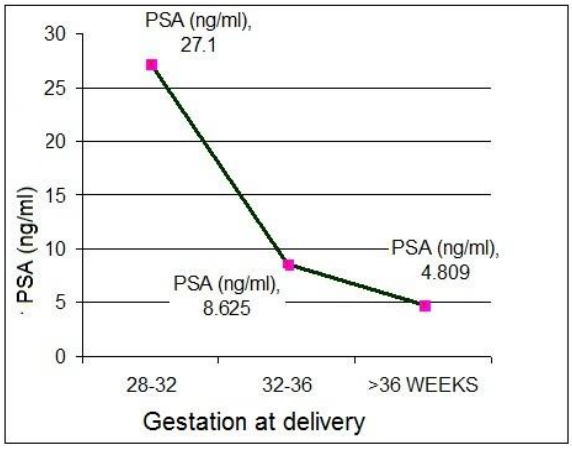


Figure 1: Mean PSA and days of postpartum

Women delivering at 28 - 32 weeks had a higher PSA levels in the breast milk as compared to those delivering later. Milk PSA concentrations for delivery at gestation of 28 - 32 weeks were 27.10 ± 0.0 ng/ml for

delivery at 32 - 36 weeks of gestation, it was 8.625 ± 3.55 ng/ml and for delivery after 36 weeks of gestation, the mean PSA was 3.809 ± 1.10 ng/ml. These changes in the PSA levels of milk were statistically significant (p = 0.021, Figure 2).

Figure 2: Mean PSA in breast milk against gestation at delivery



Highest PSA was seen in milk samples from mothers of babies weighing 1500 - 2000 g. No study comparing PSA levels in milk for babies of different birth weights have been previously reported, Table 1.

Table 1: PSA in milk against birth weight of the baby

Birth weights (gm)	Observations (n)	Mean PSA ± SEM (ng/ml)
1000 – 1500	2	0.6550 ± 0.450
1500 – 2000	5	14.833 ± 6.113
2000 – 2500	14	2.921 ± 1.919
2500 – 3000	19	3.311 ± 1.411
3000 – 3500	9	6.344 ± 3.774
> 3500	3	6.100 ± 2.551
		p = 0.503

The PSA level in milk for male babies was more than four times the PSA levels for females. In case of male babies, the range was from 0.00 to 27.06 ng/ml and for female babies the range was from 0.00 to 14.14 ng/ml (p = 0.091, Figure 3).

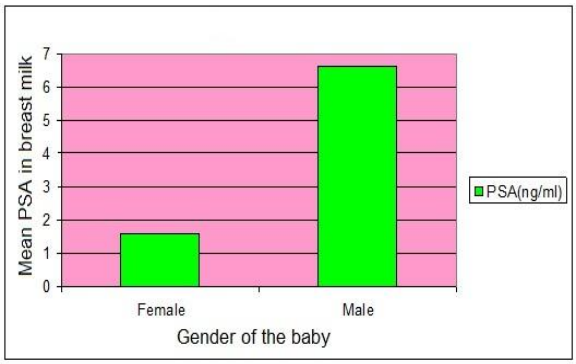


Figure 3: Mean PSA in breast milk against of the baby

Highest values of proteins were found in the milk collected on the first 3 days postpartum (5.78 ± 0.73 gm %). The protein levels of the milk were found to be decreased with an increasing of the time postpartum. Pattern of the change in protein levels was highly statistically significant (p = 0.00, Table 2).

Postpartum (days)	Observation (n)	Mean Protein ± SEM, (gm%)
0 - 3	13	5.78 ± 0.73
3 - 6	23	2.69 ± 0.30
6 - 9	10	1.59 ± 0.57
9 - 12	3	1.40 ± 0.29
12 - 15	2	1.06 ± 0.03
		p = 0.000 (HS)

Table 2: Postpartum and mean protein levels

PSA had been analyzed per gram of protein to rule out dilution as a reason for decrease in the PSA levels with increasing time postpartum. PSA per gm of protein was highest in the first three days postpartum; it was lower at 3 - 6 days (71% of the value at 0 - 3 days). At 6 - 9 days postpartum PSA per gm of protein rose by 60% at 9 - 12 days postpartum it was double the levels at 6 - 9 days and at 12 - 15 days it is only 12% that of the levels at 9 - 12 days, Table 3.

Table 3: PSA/gm of protein in milk against postpartum day

Postpartum day (days)	Number (n)	Mean PSA per gm Protein ± SEM (ng/gm)
0 - 3	**13**	**150.3 ± 49.8**
3 - 6	**23**	**107.4 ± 41.8**
6 - 9	**10**	**185.1 ± 101.2**
9 - 12	**3**	**351.5 ± 200.0**
12 - 15	**2**	**21.0 ± 21.0**
p = 0.805		

Table 4: Mean PSA per gm of protein in milk vs sex of the baby

Sex of the baby	Number (n)	Mean PSA per gm Protein ± SEM (ng/gm)
Female	**20**	**68.275 ± 33.135**
Male	**31**	**193.768 ± 52.118**
P = 0.080		

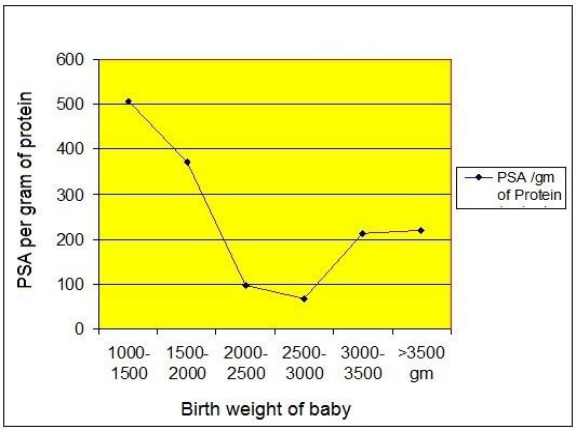


Figure 4: PSA per gram of protein against birth weight of baby.

PSA per gm of protein was much higher in the milk for smaller babies (< 2000 g) as compared to that of weighing more than 2000 g, however, this difference was not significant statistically (p = 0.074, Figure 4). The levels of PSA per gm of protein in milk meant for male babies are 2.8 times the levels in milk of female babies (p = 0.080, Table 4). Consistently higher PSA values have been found for male babies in all the samples including pre-partum and post-partum blood, amniotic fluid, cord blood and milk, Figure 5.

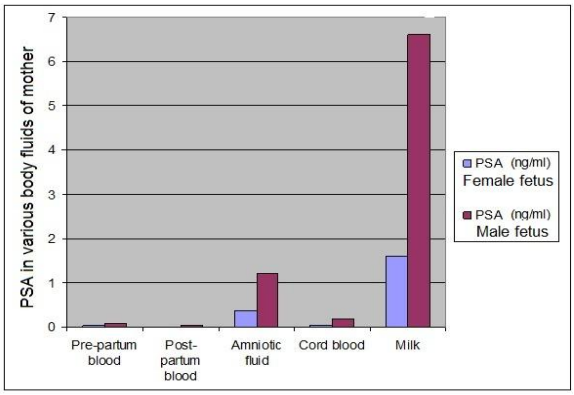


Figure 5: Mean PSA in various body fluids of mother against sex of the baby

Discussion

The PSA concentration in milk was found to be reduced with increasing time postpartum. This finding is in line with the findings of Yu and Diamandis (17). Women delivering at 28 - 32 weeks had higher PSA in the breast milk as compared to those delivering later. No previous study comparing the milk PSA levels in women delivering at different gestations has been reported. Highest PSA was seen in milk samples from mothers of babies weighing 1500 - 2000 g. Further, no study comparing PSA levels in milk for babies of different birth weights was found in the literature. The statistical analysis of this study showed that the relationship was not statistically significant. Probably this requires a bigger study since, the range of PSA in milk is very wide (0.000 - 27.060 ng/ml). Another possibility can be that PSA being a growth factor and a mitogenic and anabolic molecule, the high PSA in the milk of mothers whose babies were born with small birth weight could be a compensatory mechanism as this PSA may be necessary to promote growth. The PSA level in milk for male babies was more than four times the PSA levels for females however because of the wide range of PSA this difference was not statistically significant. ($p = 0.091$). This finding corroborates with Yu and Diamandis (17) findings who did not find statistically significant difference between the milk PSA concentration in case of male and female babies. Highest values of proteins were found in the milk collected on first three days postpartum and decreased with increasing time post-partum. Pattern of change in protein levels was highly significant statistically. Several studies have found a similar trend in milk protein levels (37, 41, 43 - 46).

The PSA per gm of protein was highest in the first three days postpartum when PSA as well as total proteins in milk were high. Protein levels at 3 - 6 days were nearly half the value of that at 0 - 3 days. PSA per gm of protein was low at 3 - 6 days (71% of the value at 0 - 3 days) while PSA in milk during this period was only 33% the values at 0 - 3 days. Therefore, it may be concluded that PSA production is significantly reduced at 3 - 6 days and this decrease is more than the decrease in protein levels. At 6 - 9 days postpartum, the milk proteins were nearly 60% the values at 3 - 6 days. The rate of fall of PSA was lesser than the rate of fall of protein. Therefore, PSA per gm of protein has been higher by 60% in spite of dilution factor. At 9 - 12 days postpartum, the total protein was 88% that of proteins at 6 - 9 days. But the PSA per gm of protein was double the levels at 6 - 9 days showing that PSA synthesis is not decreased though PSA per ml of milk is decreased (87% of the value at 6 - 9 days) due to dilution. At 12 - 15 days postpartum milk protein was lesser than the proteins at 9 - 12 days (76%). PSA per gm of proteins was only 12% that of the levels at 9 - 12 days showing that PSA synthesis is markedly decreased by this time much more than the decrease in protein levels.

PSA per ml of milk is therefore, markedly decreased (0.237 ng/ml) which can not be explained on the basis of dilution alone. To conclude, the PSA synthesis in breast tissue and secretion into milk is maximum in first 3 days postpartum, decreases precipitously over next 3 days and then gradually between day 6 and 9. The amount secreted per day is static over the next 3 days and after 12 days postpartum it undergoes a sharp reduction. A few samples collected beyond 15^{th} day postpartum (but not included in the study) suggest that PSA is not expressed in milk after 20^{th} day postpartum. No previous study has analyzed PSA per gm of protein against time postpartum. PSA per gm of protein was

much higher in milk for smaller babies (< 2000 g) as compared to ones weighing more than 2000 gm, however, this difference was not statistically significant (p = 0.074). No previous studies have analyzed PSA per gm of protein in breast milk against birth weight of the baby. The levels of PSA per gm of protein in milk meant for male babies are 2.8 times the levels in milk female babies. The difference, however, was statistically nonsignificant (p = 0.08). PSA per gm of protein in milk has not been analyzed according to sex of the baby in any of the previous studies.

Conclusion

The breast milk PSA levels were higher in case of lesser gestation at delivery, a smaller birth weight of the baby and for a male baby. PSA per gm of protein behaved in a fashion identical to PSA per ml of milk in relation to gestation at delivery, birth weight and sex of the baby. PSA levels were found to decrease with increasing time postpartum. Analysis of the PSA per gm of protein against the time postpartum showed that PSA and proteins in the milk behave independent of each other. It may be inferred that PSA synthesis and secretion are induced in breast by massive amounts of progesterone and estrogen produced by placenta during pregnancy. This PSA may play the role of growth factor or growth factor regulator in the infant by virtue of its IGFBP-3 protease activity as well as by activation of latent TGF-β. Its homology with γ-Nerve growth factor, epidermal growth factor binding protein and α-nerve growth factor supports the argument.

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Original article

Ferritin and erythrocyte lipid peroxidation in acute myocardial infarction in Libyan diabetics

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Abstract: Iron sequestration in transport and storage proteins may contribute to antioxidant defences. Oxidants can cause the release of catalytic iron from the storage proteins and this promotes the formation of more reactive free radicals. The catalytic iron was implicated in ischemic myocardial damage and in the genesis of apoptosis of beta cells of langerhans of pancreas. This study inclined to investigate the status of body iron and the lipid peroxidation and their correlation in acute myocardial infarction in diabetics. Thirty acute myocardial infarction patients with or without diabetes were subjected to analysis for ferritin, a marker of body iron status, total iron, total iron binding capacity in serum and lipid peroxidation activity in the erythrocytes along with haemoglobin and hematocrit values and compared these parameters with that of thirty age and sex matched healthy controls. Diabetic and non-diabetic patients of acute myocardial infarction showed significant increase in ferritin, total iron and erythrocyte lipid peroxidation activity with a decrease in total iron binding capacity. A statistical positive correlation was observed among ferritin, total iron and lipid peroxidation activity and significant negative correlation with total iron binding capacity. Strikingly elevated levels of ferritin and total iron and concomitant decrease in total iron binding capacity might be contributing the availability of the catalytic iron which is postulated to be capable of generating more reactive oxygen species causing lipid peroxidation that ultimately culminating into the acute myocardial infarction and the pancreatic damage. The markedly increased lipid peroxidation in this study supports cellular biochemical perturbations.

Key words: Acute myocardial infarction, diabetes, free radicals, apoptosis, lipid peroxidation, atherosclerosis

Introduction

Diabetes is a multi-faceted disorder that is consequent to the relative or absolute deficiency of insulin. In humans, the diabetogenic process appears to be caused by immune destruction of the beta cells; part of this process is apparently mediated by the white cell production of active oxygen species. Alloxan and streptozotocin are drugs that produce diabetes in animals by the mechanism involving production of active oxygen species. The free iron, too, is noxious to cells because it catalyses the generation of hydroxyl radicals, superoxide and hydrogen peroxide via the Fenton reaction. The reactive hydroxyl radical subsequently causes lipid peroxidation leading to cell damage (1). Formation of hydroxyl radicals catalysed by iron may play

an important role in the development of diabetes since the cells that produce insulin are extraordinarily sensitive to damage from oxidation and insulin secretion is impaired. Oxygen radicals involved in the cause of diabetes also appear to play a role in the development of some of the complications seen in long standing diabetes (2). Higher iron stores, reflected by an elevated ferritin concentration and a lower ratio of transferrin receptors to ferritin are associated with an increased risk of type 2 diabetes in healthy women independent of known diabetes risk factors (3). In fact, it is the heme iron in diet (4) particularly heme-iron intake from red meat sources is positively associated with the risk of type 2 diabetes (5). In prospective cohort study, total iron intake, heme-iron intake from non–red meat sources and blood donations are not related to the risk of type 2 diabetes (5). In another cohort study, low plasma concentrations of vitamin E were associated with an increased incidence of diabetes (6) while randomized trial data showed no significant overall effects of vitamin C, vitamin E and β-carotene on risk of developing type 2 diabetes in women at high risk of cardiovascular diseases (7). The role tissue iron and elevated body iron stores play in causing type 2 diabetes and or the pathogenesis of its important complications, particularly diabetic nephropathy and cardiovascular diseases continues to remain inconclusive and is, therefore, lately under extensive investigations. Viewing the hypothesis promulgating the participation of the free radicals in potentiating the pathogenesis of diabetes and complications such as cardiovascular diseases, it was fascinating to investigate the body iron status and lipid peroxidation activity in type 2 diabetics who suffered acute myocardial infarction (AMI).

Material and methods

Subjects: This study included 30 patients (21 men and 9 women) who were admitted to Jamahiriya hospital, Benghazi, Libya during the period of June to December, 2007 with acute myocardial infarction. The age of the patients was between 29 - 79 years with a mean value and S.D. of 55 ± 13 years. The patients were subjected to clinical examination. The detailed medical history, blood pressure and ECG tracing were recorded. These patients were divided into two subgroups: non-diabetic (17 patients) of the age group between 38 - 79 years old with a mean value ± S.D., 60 ± 11 years and diabetic (13 patients) of the age-matched group.

The glucose levels above 126 mg/dl in overnight fasting and/or 200 mg/dl or above after two hours of a meal intake or on random checking were the criteria for diabetes mellitus.

The control group included 30 healthy subjects (21 men and 9 women) of the age group between 28 - 78 years with a mean value ± S.D. of 55 ± 13 years. They were normotensive with normal ECG tracing. Hypertension was defined as resting systolic blood pressure more than 140 mm Hg and diastolic blood pressure more than 90 mm Hg and anaemia was defined using world health organization (WHO) criteria as a hematocrit value at initial presentation less than 39% for men and less than 36% for women. The present study was conducted after obtaining the written informed consent of the participating subjects. The study was approved by the ethical committee of the hospital and procedures were followed in accordance with the ethical standards laid down on human experimentation (institutional and national) and with the Helsinki Declaration of 1975.

Exclusion criteria

None of the subjects in the study groups and control group had a previous history of myocardial infarction, angina, coronary artery surgery, transient ischemic attack and peripheral arterial diseases, liver, kidney and thyroid related diseases. None of the study subjects had undergone blood transfusions/ donations throughout their life time. For selecting the subjects for the present study, a care was taken to exclude the subjects with habbits like smoking, tobacco chewing and those with chronic inflammatory diseases like tuberculosis, rheumatoid arthritis and malignancy, all of which play a role in contributing to oxidative stress injury.

Blood specimen collection

Blood specimen were drawn from all study subjects and the controls after overnight fasting. One part of blood was placed into plain tube and kept for 30 minutes at room temperature. Samples were foroozing out the serum. The serum was separated by centrifugation at 3500 rpm for 10 minutes and stored at -30 ^{o}C till analysis. Another part of blood from healthy controls and patients was collected in EDTA-containing tubes. The tubes were centrifuged and plasma was separated for routine laboratory analysis. The packed cells were washed with an equal volume of physiological saline and centrifuged again. The supernatant was removed and cells were analyzed for lipid peroxidation activity.

Methods

The complete blood count, haemoglobin, serum iron (8), ferritin (9), total iron binding capacity (TIBC) (10) and lipid peroxidation activity (11) in erythrocytes were analysed by employing authentic methods.

Stastistical analysis

All the biochemical parameters were statistically analysed using software SPSS version 11 (statistical package for social sciences) for calculation of Student 't' test for obtaining the p values and pearson's correlation coefficient 'r'.

Results

The non-diabetic AMI patients showed significant increase in the levels of serum iron ($p < 0.03$) and serum ferritin ($p < 0.001$) and a significant decrease in the level of serum total iron capacity (TIBC) ($p < 0.03$) in comparison to the controls while there were non-significant changes in the levels of hemoglobin, hematocrit and erythrocytes lipid peroxidation between the non-diabetic AMI patients and control group. The diabetic AMI patients showed significant increase in the levels of serum iron ($p < 0.02$), serum ferritin ($p < 0.001$) and erythrocytes lipid peroxidation ($p < 0.03$) when compared to control group while serum TIBC, hemoglobin and hematocrit changed non-significantly from that of controls.

There were no significant changes between diabetic and non-diabetic AMI patients in all these parameters (Table1). The correlation coefficients (r, p.) between the laboratory parameters in diabetic acute myocardial infarction patients are shown in Table 2.

Table 1: Mean values ± SD of serum iron, TIBC, ferritin, blood hemoglobin, hematocrit, and erythrocyte lipid peroxidation in diabetic and non-diabetics with acute myocardial infarction and controls

Parameters	Controls (n = 30)	Non-diabetic AMI (n = 17)	Diabetic AMI (n = 13)
Serum iron (μg/dl)	62 ± 18	81 ± 23 P < 0.003	77 ± 22 P < 0.02
Serum TIBC (mg/dl)	341 ± 47	308 ± 46 P < 0.03	320 ± 54
Serum ferritin (ng/ml)	72 ± 33	190 ± 58 P < 0.001	180 ± 59 P < 0.001
Hemoglobin (g/dl)	13 ±1.6	13 ± 1.8 P > 0.05	12 ± 0.99
Hematocrit (%)	38 ± 5.3	37.8 ± 2.5	36 ± 3.40
Erythrocyte lipid peroxidation (absorptive value)	0.36 ± 0.14	0.49 ± 0..03	0.52 ± 0.32 P < 0.03

AMI: acute myocardial infarction; TIBC: total iron binding capacity; n: number of subjects, Significant at P < 0.05; P versus controls; P1 versus non-diabetic AMI patients

Table 2: Correlation coefficients between various parameters in diabetic acute myocardial infarction patients

	Serum Iron	Serum TIBC	Serum Ferritin	Blood Hemo-globin	Hematocrit	R.B.C. Lipid per oxidation	Age	Sex
S. Iron **r** **P**		- 0.75 0.003	0.70 0.01	- 0.39 0.18	- 0.29 0.33	0.62 0.02	- 0.30 0.31	- 0.09 0.76
S. TIBC **r** **p**			- 0.71 0.01	0.29 0.36	0.11 0.72	- 0.73 0.01	0.66 0.01	- 0.17 0.58
S. Ferritin **r** **p**				- 0.30 0.32	- 0.21 0.50	0.59 0.04	- 0.50 0.08	0.04 0.90
Hb **r** **p**					0.93 0.001	- 0.21 0.49	- 0.06 0.85	-0.60 0.03
Hematocrit **r** **p**						- 0.06 0.86	- 0.10 0.75	- 0.39 0.19

r: correlation coefficient; p: probability;Significant at $p \leq 0.05$; non-significant at $p \geq 0.05$.

The correlation of serum iron with other parameters: There were significant positive correlations between serum iron and serum ferritin ($r = 0.7$, $p < 0.01$) and erythrocyte lipid peroxidation ($r = 0.62$, $p < 0.02$), while there was a significant negative correlation with serum TIBC ($r = - 0.75$, $p < 0.003$). There was no significant correlation between serum iron and haemoglobin, hematocrit, age and sex.

Correlation of total iron binding capacity (TIBC) with other parameters: There were significant negative correlations between serum TIBC and serum ferritin ($r = - 0.71$, $p < 0.01$) and erythrocyte lipid peroxidation ($r = - 0.73$, $p < 0.01$), and a significant positive correlation between TIBC and age ($r = 0.66$, $p < 0.01$). There was no significant correlation between haemoglobin, hematocrit and sex.

Correlation of ferritin with other parameters: There were significant positive correlation between serum ferritin and erythrocyte lipid peroxidation ($r = 0.59$, $p < 0.04$). There was no significant correlation between ferritin and haemoglobin, hematocrit, age and sex.

Correlation of hemoglobin with other parameters: Hemoglobin showed significant negative correlation with sex ($r = - 0.6$, $p < 0.03$) and a significant positive correlation with hematocrit ($r = 0.93$, $p < 0.001$). There was no significant correlation between haemoglobin and other parameters studied. The correlation coefficients (r, p) between the laboratory parameters in non-diabetic acute myocardial infarction patients are shown in Table 3.

Table 3: Correlation coefficients between various parameters in non-diabetic acute myocardial infarction patients

	Serum Iron	**Serum TIBC**	**Serum Ferritin**	**Hemo-globin**	**Hematocrit**	**R.B.C. Lipid per oxidation**	**Age**	**Sex**
Iron								
r		- 0.54	0.80	- 0.14	- 0.07	0.78	0.14	0.08
P		0.03	0.001	0.59	0.80	0.001	0.58	0.76
TIBC			- 0.72					
r			0.001	0.15	0.06	- 0.56	- 0.33	-0.02
p				0.56	0.82	0.02	0.19	0.93
Ferritin								
r				- 0.19	- 0.09	0.85	0.12	-0.09
p				0.46	0.74	0.001	0.65	0.74
Hemo-globin					0.95	- 0.23	- 0.56	- 0.45
r					0.001	0.38	0.02	0.07
p								
Hematocrit								
r						- 0.18	- 0.58	- 0.50
p						0.49	0.01	0.04

Significant at $p \leq 0.05$; Non-Significant at $p \geq 0.05$; r: Correlation coefficients; p: probability

Similar trends in correlations amongst various parameters were observed in non-diabetic acute myocardial infarction patients as indicated in diabetic acute myocardial infarction patients.

Discussion

Body iron stores have been implicated in the development of diabetes and conversely, decreased iron stores improves insulin sensitivity and insulin secretion (5, 12). However, no interventional studies have yet directly evaluated the effect of reducing iron intake or body iron levels on the risk of developing type 2 diabetes. Such studies are required to prove the causal relationship between moderate iron overload and diabetes risk (13).

In the present study, an increased total iron and ferritin, an iron storage protein, were observed in acute myocardial infarction patients with or without having diabetes, Table 1. Elevated iron stores, reflected in elevated plasma ferritin levels, may induce baseline metabolic abnormalities that ultimately result in diabetes. Alternatively, elevated ferritin may be just one of several metabolic abnormalities related to the underlying process that ultimately results in diabetes, rather than a causal factor for diabetes. Longitudinal studies with repeated measurements of glucose and iron metabolism parameters are needed to establish the role of iron stores and plasma ferritin in diabetes development (14). Ferritin levels in patients with DM are reported to be high as compared to controls and serum ferritin may be a biomarker of insulin resistance (15). Literature reports opine that iron-catalyzed oxidative stress mediate apoptosis of pancreatic islets with a resultant decrease in insulin secretory capacity (16). It becomes significant in relevance of the investigation by which a high expression of divalent metal transporter predisposes pancreatic islets for more accumulation of iron than other cells (17). Moreover, pancreatic islets have an extreme susceptibility to oxidative damage owing to perhaps low expression of the antioxidant defence system (18). Iron induced oxidative stress in the form of increased generation of reactive oxygen species, in a series of fenton like reactions, not only makes the pancreatic islets but also the vascular endothelium vulnerable to dysfunctional injury leading to the development of diabetes and its associated or independent complication cardiovascular disease, an acute myocardial infarction. The present study also observed an enhanced lipid peroxidation activity in erythrocytes in patients with or without diabetes having acute myocardial infarction episode supporting the above conviction, Table 1.

Several studies have demonstrated a direct association between increased iron intake, body iron stores, and cardiovascular risk in the general population. Increased intake of heme iron is associated with increased cardiovascular events (19 - 22) and increased body iron stores are associated with myocardial infarction in a prospective epidemiological study (23).

The total iron binding capacity was found to be decreased in acute myocardial infarction patients with or without diabetes, Table 1. In such a situation tissues would experience more exposure to mobile reactive free form of iron which participates in generation of reactive free radicals. The results observed on correlations of various parameters in both the groups of diabetes and non-diabetes in acute myocardial infarction (Tables 2 and 3) lend support to events of alterations as shown in Table 1.

Pathologic mechanisms for iron in promoting vascular disease can be derived from cell culture studies, animal models, and human functional studies (vascular reactivity). In cell culture models, the addition of non-transferrin bound iron (NTBI) to human endothelial cell cultures increases surface expression of adhesion molecules (24, 25) and also increases monocyte adherence to the endothelium. These abnormalities can be corrected by the addition of iron chelators such as desferoxamine and dipirydyl which decrease expression of adhesion molecules and monocyte adherence (24 - 26). The beneficial effect of iron chelators on endothelial dysfunction suggests the role of iron in vascular disease. Impaired endothelial function as a result of increased NAD(P)H oxidase-dependent oxidant generation was restored by desferoxamine (27). Furthermore, desferoxamine has been shown to prevent diabetes-induced endothelial dysfunction (27) and deficits in endoneural nutritive blood flow in streptozotocin-induced diabetic rats (28, 29). Iron chelation therapy may present a novel way to interrupt the cycle of catalytic iron–induced oxidative stress and tissue injury and consequent release of catalytic iron in diabetes and to prevent diabetes-related complications (30).

Conclusion

The present study was conducted on diabetics who suffered acute myocardial infarction for assessing the relation and the body status of iron in potentiating coronary artery disease. There was an evidence of raised levels of serum iron and ferritin and the low levels of total iron binding capacity in both diabetics and non-diabetics at the onset of acute myocardial infarction episode. The elevated erythrocyte lipid peroxidation activity in both these groups further speculated the role of iron in generating free radicals which might trigger or aggravate the pathogenesis of diabetes and its associated complication, cardiovascular disease.

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Original article

Study of hydatid disease prevalence in north Libya

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Abstract: Hydatid disease is a common zoonotic disease in North Africa affecting human and animals transmitting from dogs through the environment contaminations causing serious cysts in their organs treated only by surgery causing morbidity and mortality among populations. Thus, the aim of this study was to find the prevalence of human hydatid disease among populations living in villages in north Libya**.** The study is a descriptive cross section study using ultrasound, serology to detect abdominal hydatid cysts. Two thousands and two hundred-twenty people were screened by portable ultrasound scanner in twelve areas, 339 were diagnosed as being hydatidosis giving an echotomographic prevalence of abdominal hydatid disease in the sample population of 1.7%. The prevalence of hydatid cysts increased with age. Females (2%) were affected more than males (1.3%). This prevalence was distributed among the different occupations, 3.2% of housewives examined, 1.3% of female students, 1.1% of male students, 2.3% of male civil servant and 2.6% of farmers. Sixty-nine percent (233) of those ultrasound positive hydatid cases gave positive results with ELISA serology. These results confirm that the disease is highly endemic among Libyan populations affecting all the ages and both sexes. Housewives appeared most at risk of infection followed by farmers. This study recommends the use of ultrasound as a mass screening technique for hydatid disease screening in other parts of Libya.

Key words: Hydatid, prevalence, Libya, ultrasound, ELISA, sex difference

Introduction

Echinococcus granulosus is one of the most geographically widespread of the pathogenic parasitic zoonoses (1) and is known to be endemic in all the countries bordering the Mediterranean. In North Africa, these are especially, Libya (2, 3), Tunisia **(**4**),** Algeria (5) and Morocco (6) were reoprted. Cystic echinococcosis (CE, or cystic hydatid disease) caused by infection with the larval (metacestode) stage of E. granulosus is one of the most important parasitic infections in Libya affecting human and domestic animal populations (7, 8). Prevalence rates in animals have been reported at 8.4% for sheep, 1.5% for goats, 5.4% for cattle and 31.9% for camels (9). The source of the infections and main definitive host in Libya is the domestic dog which has exhibited

prevalence rates of 60% in rural areas and in urban areas 31.5% of stray dogs and 10% of pet dogs were shown to be infected (10, 11).

Human CE is an important public health problem in Libya which is treated primarily by surgery (3, 12, 13). Human CE infection rates have only been quantified mainly by retrospective reviews of hospital surgical records. In northwest Libya, 0.85% of all admissions in major surgical departments over a six year period (1971 to 1976) were due to hydatid disease. In the northeast, between 1972 and 1979, 111 cases of hydatid disease were confirmed surgically out of 22,979 admissions giving a surgical prevalence of 0.48% (14 - 19). Two seroe-pidemiologic surveys for echinococcus antibody detection in humans have been undertaken in Libya. An ELISA using crude sheep hydatid cyst fluid was employed in the screening of 250 school children in the northeast (Benghazi) of which 10% were seropositive (7). In other study in Benghazi, 384 blood samples were examined by the indirect haemagglutination (IHA) test of which 13 persons were found positive giving a seroprevalence of 3.4%; 50% of the seroreactors were male (20). For these studies, however, there was no clinical follow-up of seroreactors. Serologic screening for human CE can identify asymptomatic cases (21 - 23) but both false negatives and false positives will occur. At the current time, the most effective clinical approach for mass screening is the use of portable ultrasound for abdominal scanning in the community (24). Confirmation of up to 60 - 90% of ultrasound detected cysts can then be made serologically (25 - 27). During the first pilot ultrasound - serological prevalence survey for human CE in five areas in northwest Libya, a total of 4103 people were screened and 57 were confirmed to have abdominal hydatid cysts giving an overall prevalence of 1.4% (2). This study was done to assist the burden of hydatid disease among Libyans. Thus, ehe aims of the present study was to find the ultrasound-serological point prevalence of hydatid disease in North of Libya, to find out which age, sex, occupation more affected and at risk, to obtain information about the possible routes of transmission of the disease and to confirm all hydatid cases with ELIZA serology using Libyan cyst fluids.

Materials and methods

Survey sampling method

Questionnaire: Individuals were registered as a self selected sample using a question-nnaire designed to provide information regarding knowledge and practices of population towards hydatid disease.

Ultrasound scanning: A portable ultrasound scanner (Siemens Sonoline SX, with a 3.5 MHz real time sector probe, Germany) was used throughout the survey which was adapted by Macpherson and others (24). The type of survey was a cross-sectional. First, all the villages in the north of Libya were located on the main map of the country and the numbers of villages needed for the study in each area were chosen at random. The population of each village was voluntarily screened, including all ages and both sexes by attending a central screening point.

Blood samples: Five milliliter venous blood sample was taken from each person who had any positive cystic image by ultrasound (hydatid or non-hydatid) as well as from all their immediate relatives.

Serological test: Serological confirmation of the ultrasound detected suspected hydatid cysts was done by ELISA test (field dot-Blot

ELISA and plate ELISA in the laboratory) using partially purified human antigen B (taken from Libyan surgically operated hydatid patients).

Statistical analysis: Study samples were analyzed by Statistical Package of Social Science (SPSS) and statistically tested using chi-square and Student t-test.

Results

Prevalence of cystic abdominal structures by ultrasound: During this study, 20220 subjects were screened by portable ultrasound in twelve localities in north Libya (36 villages). These comprised 5270 families with 10356 females (51.3%) and 9864 males (48.7%). 2.6% (530 persons) were found to have at least one ultrasound detectable abdominal cyst. This prevalence included all types of cysts which were diagnosed by ultrasonography including pathogonomic hydatid cysts and non-parasitic simple cysts found in any abdominal organ.

Ultrasound prevalence of abdominal hydatid disease: Among the 530 persons with different types of cysts detected in the 20220 population surveyed, 339 (65%) persons were ultrasonically diagnosed as having hydatid cysts because they showed one or more of the diagnostic criteria of hydatid images including laminated membranes, daughter cysts or both. Abdominal hydatid disease prevalence among the sample population was, therefore, 1.7% (339 hydatid cases) (Table 1). These hydatid cases were found among 311 families with 288 (93%) of these families having only one hydatid case, 22 (7%) of the families with hydatid diagnosis had two cases in each family. There was one family with more than 2 CE cases and in this family all seven members had liver hydatid cysts.

The overall prevalence of hydatid disease in North Libya: The above abdominal ultrasound prevalence of 1.7% represents only the approximately 70% of hydatid cysts in the human body which can be diagnosed by ultrasound (abdominal hydatid cysts), the remaining 30% require other imaging (or serological) techniques for diagnoses (these include lung, bone or brain hydatid disease). Hence, the prevalence rate detected by ultrasound was multiplied by a correction factor 1.42 to indicate the overall prevalence and to include the notional 30% of hydatid cases which can not detected by ultrasound. This 1.42 epidemiological index was used by Macpherson et al. (24) in Turkana, Kenya. Therefore, the predicated overall prevalence of hydatidosis in the Libyan communities surveyed was therefore 2.4%.

Hydatid prevalence according to age and sex: Age specific prevalence rates increased from 0.06% at age four years or less to 4% at above 50 years and an analysis for linear trend showed that CE increased significantly with increasing age for the sexes combined, Table 1.

The highest prevalence among age groups was 4% which found among those aged above 50 years. There was nearly a two-fold greater CE detection rate for females than for males that was significant for all age groups combined. The positive prevalence in the different sexes in the different age groups was higher in females in general in all the ages, except in the age group of 5 - 14 years old but was significantly higher among the age of 31 - 50 years (1% for males and 3.4% for females).

Table 1: Showing the ultrasound age and sex hydatid disease prevalence results obtained in people surveyed in north Libya

Age (years)	**Population screened by ultrasound**		**Hydatid cysts detected by ultrasound**		**Ultrasound hydatid cyst prevalence (%)**	
Sex	Male	Female	Male	Female	Male	Female
0 - 4	809	818	0	1	0%	0.1%
5 - 14	3445	3296	24	22	0.7%	0.7%
15 - 30	2730	3024	43	68	1.6%	2.2%
31 - 50	1732	2234	18	77	1%	3.4%
> 50	1148	984	43	43	3.7%	4.4%
total in each sex	9864	10356	128	211	1.3%	2%
Total	**20220**		**339**		**1.7%**	

Clinical manifestation of ultrasound detected hydatid cases: Almost all the US cases which were diagnosed in this survey were asymptomatic and no patients complained of any symptoms except for five persons where the cysts were very big (one patient had multiple cysts due to disease recurrence), however, these patients complained only from a swollen or protruded abdomen, Figure 1.

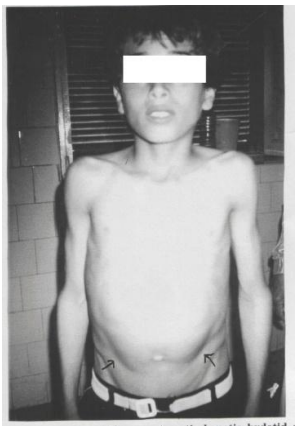

Figure 1: Showing hydatid Libyan patient complained only from a swollen abdomen

Ultrasound prevalence among different occupations: The prevalence of CE among all females was distributed between the housewives which was 3.2% (167/5178) and female students 1.4% (44/3431). Among males, CE was distributed between farmers 2.6% (14/544), civil servants 2.3% (64/2793) and male students 1.1% (50/4477) (Figure 2). The occupation most at risk was housewives and students.

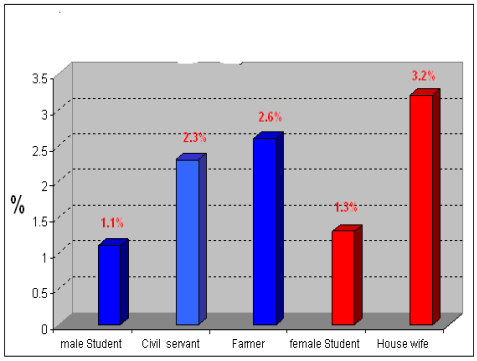

Figure 2: Ultrasound prevalence of hydatid disease among different occupations

Prevalence of ultrasound hydatid disease in north Libyan villages: This study was conducted in 36 villages in north Libya. The ultrasound prevalence among the villages is

ranging from 0 - 4.5%. The highest ultrasound prevalence was found among a population of one community living in a small village (485 pop.) in the Khmus area, which is located 200 km northwest of Tripoli. Ultrasound prevalence of abdominal CE among the human population of this community was 4.5% (22 cases per 485).

Common sites of abdominal hydatid disease: The abdominal organs which were affected by hydatid cysts were mainly the liver (89%), spleen (2.7%), abdominal wall (2.1%), ovaries (2.9%), uterus (1.2%) and muscle (0.9%). The size of the hepatic cysts ranged from 5 mm to 40 cm with an average diameter of 8.6 cm.

Ultrasound detected hydatid cases and serology: Sixty-nine percent (233 cases) of all ultrasound hydatid cyst cases were seropositive. The sensitivity among the cases which had the typical features of hydatid cysts like laminated membranes or daughter cysts or both was 91% (Figure 3) and most seronegative cases were small sized cysts (less than 50 mm in diameter) or degenerated or both. Liver hydatid cysts gave the highest seropositivity (73%).

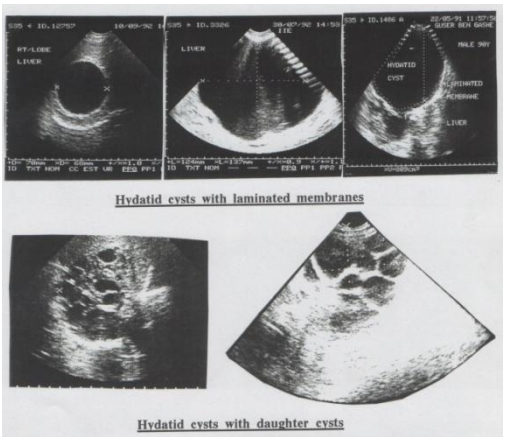

Figure 3: Showing ultrasound hydatid cysts with clear laminated membrane and daughter cysts

Attitude of the people towards dogs and livestock: Of the 5270 head of households questioned, 4151 (79%) owned one or more types of domestic animal (sheep, goats, etc.). These households owned a total of 69200 sheep, 13560 goats, 7870 cattle, 1506 horses and 182 donkeys. The average number of livestock per household was thirteen. Most people screened lived on farms and 23% of the whole population screened practiced slaughter of sheep in their houses at different times during the year, especially at Eid Al-Adha.

A total of 9919 dogs were kept on the farms or near the houses of the 5270 families of the surveyed population (i.e. approximately 0.5 dogs per person). Sixty-two percent (210/339) of newly diagnosed ultrasound hydatid cases kept dogs and this was significant compared to the negative ultrasound hydatid population were only forty-eight percent (9709/20220) of them kept dogs. Dog ownership may, therefore, be one of the important risk factors in disease transmission.

Discussion

Ultrasound had now been used extensively to diagnose human hydatidosis (28) and to monitor the response of hydatid cysts to chemotherapy (12, 13). It is also proved useful in the collection of field data for hydatid disease community surveys (24). Ultrasonography has been used in many communities to estimate the prevalence of cystic hydatid disease in different parts of the world (29, 30). Abdominal hydatid disease prevalence in this survey at 1.7% is one of a high prevalence rates in the world. In other parts of the world, cystic hydatid prevalence ranged from 0.4% to 3.6% in Tunisia, 0% to 5.6% in Kenya; in China from 0.3% to 2.9%, and 1.4% in Uruguay

and up to 8.6% in parts of Argentina (31 - 34). The US prevalence recorded in Libya was similar to that found among the 18565 population examined in East Africa (1.8%) (33). Increase in age prevalence of CE in Libya is also found in other parts of the world with an indication of greater risk among females. In Tunisia, the prevalence of cystic hydatid disease increased with age, up to 7.7% in the over 39 years of age group (25). In Kenya, the prevalence increased with age from 0.17% in 0 - 4 age group to 11.1% in more than 50 years of age group (24). This pattern of increasing prevalence with age in the present study was also reflected in hospital records reported from Libyan surgical departments which showed increase of hydatid surgery rate among older people (from 21 years to over 50 years age group) (35, 18). The ultrasound prevalence in this study was higher in females (2%) than males (1.3%). This result is similar to the results of other community studies, such as in Tunisia where females were more infected (4.3%) than males (2.5%) (25). In Kenya, females had about twice the prevalence of CE compared to males (5.6% versus 2.9% respectively) (24). Differences in the ultrasound hydatid prevalence rates between the sexes have been attributed to different behavioural attitudes to dogs (36). This study also showed that ultrasonography was overall superior to serology with only 69% sensitivity. The serological sensitivity, was, however, higher than that obtained in other studies. For example, in Kenya only 50% of ultrasound cases gave positive results with serology (24) and was only 14% sensitive when compared against ultrasound and chest x-ray in a CE study done in Israel (37).

The route of transmission of E. granulosus to humans may be via direct or indirect routes. The direct route requires direct contact between humans and dogs but is thought to be minimal in Libya and other North African countries because Islam forbids contact with dogs (38). However, the Islamic ruling applies to adults and not to children who play with dogs freely and therefore potential early transmission of the disease may occur (19). Probably the most important route of transmission in Libya is the indirect one, whereby dogs contaminate the environment especially open water sources and agricultural products like vegetables and salads (2, 11). The other important factors which facilitate spread of the disease among Libyans are human hygiene practices. In this area of the world, most adult males practice home slaughter. Studies in Libya showed that more than 90% of households practiced home slaughter; of which 23% recognized sheep hepatic hydatid cysts and admitted throwing such cysts to dogs, especially during at Eid Al-Adha (2). This practice and the potential for dogs to have access to sheep carcasses is probably one of the main sources of transmission of infection from sheep to dogs.

Conclusion

This study showed that the prevalence of cystic hydatid disease is high in different Libyan communities whatever the type of the land use or climate. It is high in arid, semi-arid, desert or agricultural areas in north Libya. Extensive studies are now required to investigate the epidemiology of the disease and serious consideration should be made regarding cost-benefit analysis of suitable regional or a national hydatid control programmes for Libya.

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Original article

Pre-formulation studies of tenoxicam with surface active agents and co-solvents as a matrix for softgels

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Abstract: Tenoxicam was reported a slightly lipophilic medicament. Effect of the change in pH, cosolvents and surface active agents were adopted as trials to enhance the solubility of the drug. Ethanol, polyethylene glycols (PEG) 400 and 6000 and propylene glycol were used as co-solvents. The finding revealed that the solubility of tenoxicam was generally enhanced to a reasonable extent. PEG $_{6000}$ proved to be superior, followed by PEG$_{400}$ to the other co-solvents tried in this study. Among the seven pH systems investigated, pH 7.5 proved to be superior by exhibiting high solubility (4.44 mg/ml) than the lower pH ranges. Inclusion of non-ionic surface active agents into polyethylene glycol 400 matrix resulted in a further improvement in the solubility of tenoxicam. The enhancement was more pronounced at concentrations of 5%. Surfactants used can be arranged according to their efficacy and solubility of tenoxicam in PEG$_{400}$ matrix as following: Tween 80 > Span 80 > Tween 40. The optimum concentration of PEG$_{400}$ was 5% in all the cases. The solubility and efficacy were decreased when the concentration of the PEG$_{400}$ higher or lower than 5%. Tween 80 of the tested surfactants comparatively offered the best result.

Key words: Tenoxicam, co-solvents, solubilization, surface active agents, softgels

Introduction

Many active pharmaceutical ingredients (APIs) have a limited bioavailability due to either the influence of the physiological factors associated with the structure and function of GIT or physicochemical properties of API and dosage form factors. As the API should be soluble to be absorbed, one of the methods to increase the API availability is to deliver it in solution dosage forms. Many of the old APIs and most of the outcome new molecules have limited solubility. The need to manipulate API`s poor solubility is essential to reach the desired solution concentration. Solubiliza - tion methods include: co-solvency, micelle-zation, complexation, pH control, hydrot-ropy, solid dispersion, chemical modific-ation, crystal modification and particle size reduction (1 - 10) were used. Physico-chemical properties of pharmaceutically active ingredients are much consid-ered during their formulation. Aqueous solubility is among the physical chemical characters that must completely be understood before designing any type of dosage forms. The correct rate of drug release from solid dosage forms; subsequent bio-absorption and therapeutic response are greatly depending on drug solubility. Weakly

soluble drugs may be brought into solution by incorporation of water-miscible co-solvents such as ethanol or propylene glycol. This would result in drug solubility by co-solvency as each solvent dissolving a different part of the molecule. Softgels or soft gelatin capsules (SGCs) are dosage forms mostly filled with liquids, may be pure API or API dissolved in oily or hydrophilic vehicle. SGCs demonstrated faster dissolution and bioavailability than tablets and hard gelatin capsules (HGCs), nevertheless, they retain the advantages of both solid and liquid dosage forms (11). Tenoxicam is a nonsteroidal anti-inflammatory drug used in the symptomatic management of osteoarthritis and rheumatoid arthritis (12).

It is an oxicam derivative, 4-hydroxy-2-methyl-N-(2-pyridyl)-2H-thieno [2, 3, e] [1, 2] thiazine-3-carboxamide 1, 1-dioxide ($C_{13}H_{11}N_3O_4S_2$). The solubility of tenoxicam in water is 0.45 mg/ml, in ethanol and methanol less than one mg/ml, in acetone two mg/ml. It dissolves in solutions of acids and alkalis (13). The partition coefficient (octanol /water) is 0.3 mg/ml at a pH 7 and 3.5mg/mL at pH 1.2, therefore, tenoxicam is slightly lipophilic (14 - 15). Liquid forms of polyethylene glycols (PEGs) are commonly used as solvents or co-solvents for APIs in preclinical or clinical studies (16). PEG_{400}, which exists as a liquid, is one of the most commonly used vehicles in SGCs (17). A potential problem with the use of PEG_{400} as a solvent is the precipitation of API as aggregates and phase separation on dilution with water. These can be minimized if surface active agents are used as a neat vehicle or as a mixture with another vehicle (18). Because of the surface activity of such vehicle, any API separation out of the aqueous solution can be re-dispersed or emulsified as fine particles or fine oily globules (16). The most widely used liquids in SGCs for human are vegetable oils (soya bean), mineral oils, non-ionic surfactants (polysorbate 80) and polyethylene glycols (400 and 600) either alone or in combination (19). In this study, an attempt was conducted in order to enhance the solubility of tenoxicam by the addition of pharm-aceutically suitable co-solvents and SAAs in preformulation studies for the formulation of such API in the form of SGCs.

Materials and methods

Materials

Tenoxicam (Chemi Iberica SR) was kindly supplied by EIPICO, Cairo, Egypt. PEG_{400} was purchased from (Huls, Germany). PEG_{6000} and propylene glycol purchased from Alec Lab., Cairo, Egypt. Tween 80, Tween 40 and Span 80 were purchased from Merck, Germany. All solvents used were of analytical grade.

Methods

Standard Calibration Curve of Tenoxica: Serial dilutions corresponding to 2, 4, 6, 8, 10 and 12 µg/ml from stock solution (30 µg/ml) of tenoxicam in deionized water were prepared. The absorbance of tenoxicam solutions were measured at 240 nm on spectrophotometer (Shimadzu, Kayato, Japan) of using deionized water as a blank. Beer`s-Lamberts law plot was applied to calculate the concentrations. The regression equation was $y = 0.03969x + 0.02543$, $R^2 = 0.9998$

Effect of Co-solvents on the Solubility of Tenoxicam: Excess amounts of powdered tenoxicam were added to stoppered flasks containing 5, 10, 15, 20 and 30 % (v/v) fractions of water co-solvent mixtures (10 ml in each flask). Flasks were shaken on a thermostatically controlled mechanical

shaker for 24 hrs. Following equilibrium was attained, aliquot samples were withdrawn, filtered (0.45 µm pore size, Gelman Sciences Inc., Germany) and diluted to the suitable concentration. Samples were then analyzed at 240 nm on spectrophotometer (Shimadzu, Kayato, Japan) using blanks of the same solution.

Effect of pH on the Solubility of Tenoxicam: The solubility of tenoxicam in citrate buffer solutions in pH range 3 - 7.5 was carried out using a pH-meter (G820, Schott, Germany). Different volumes of citric acid (0.2 M) and disodium phosphate (0.3 M) were mixed to obtain different solutions of appropriate pH. The reported procedures in the literature were used to analyse the solubility (20). Known Excess amounts of the drug in different buffer solutions in vials were shaken on a thermostatically controlled mechanical shaker for 24 hrs at 25 °C. After equilibrium, aliquots of the supernatant were withdrawn, filtered using 0.45 µm filter and measured spectrophtometrically after appropriate dilution using buffers of the same pH as blanks.

Effect of surface active agents on the solubility of Tenoxicam: Span 80, Tween 80 and Tween 40 with PEG_{400} in different concentrations (2.5, 5, 7.5 and 10% v/v SAA in deionized water) were used. The same analytical method was used employing excess amounts of tenoxicam in closed vials.

Results

Enhancement of the solubility of tenoxicam by co-solvency was carried out using mixtures of ethanol and glycols in water Figure 1.

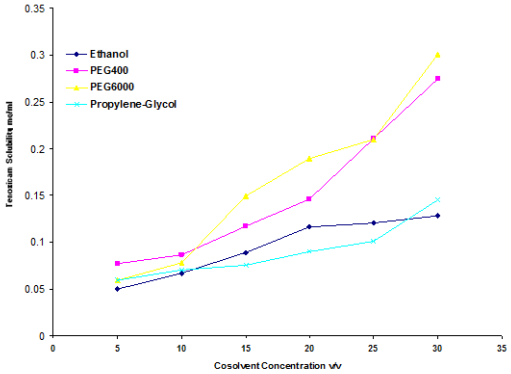

Figure 1: Effect of different co-solvents on the solubility of tenoxicam shows the increase in tenoxicam solubility by co-solvents.

In Figure 1, Tenoxicam solubility was improved with co-solvents used and as the concentration of co-solvents increased the solubility enhanced. Polyethylene glycol $_{400}$ and PEG_{6000} revealed the highest improvement in tenoxicam solubilization. While, ethanol and propylene glycol showed lower solubility improvement in comparison to the other co-solvents employed. Higher concentrations of PEG_{6000} gave higher viscous system in which unable to achieve acceptable tenoxicam solubility.Solubility determination of tenoxicam in citrate buffer at different pH ranges revealed an increase in the solubility as the pH increase from 3 to 7.5, which is suitable range for the matrix vehicle for SGCs. Data obtained was illustrated in Table 1 and Figure 2.

Table 1: Effect of the change in pH of citrate buffer solution on the solubility of tenoxicam

pH	Solubility µg/ml
3	47.2
4	45.56
5	54.32
6	121.23
6.5	451.4
7	962.8
7.5	4440

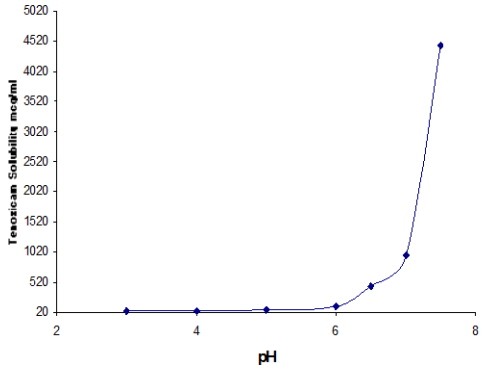

Figure 2: Effect of change in pH of citrate buffer solution on the solubility of tenoxicam

The solubility at pH 3, 6 and 7.5 at 25 °C were 47.2, 121.23 and 4440 µg/ml respectively. As PEG_{400} showed high improvement in the solubility of tenoxicam, further enhancement in the solubility was tried using different types and concentr-ations of non-ionic surfactants. Data are presented in Figure 3.

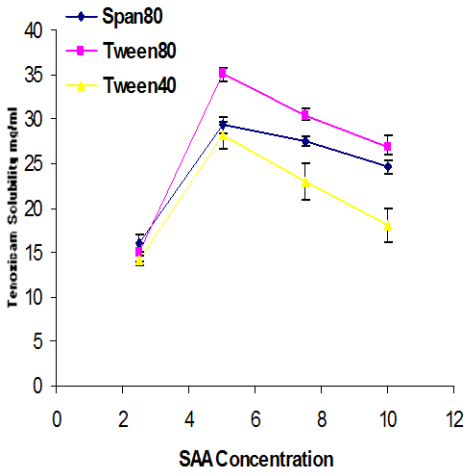

Figure 3: Effect of different SAA-PEG_{400} on the solubility of tenoxicam

Figure 3 shows the solubility of tenoxicam was increased as the concentration of SAA increased from 2.5 to 5%. The solubility values of tenoxicam at 5% concentration of span 80, tween 80 and tween4 0 were 29.3 ± 0.9, 35.1 ± 0.65 and 28.15 ± 1.5 mg/ml respectively. Further increases of SAA concentrations (7.5 and 10%) resulted in decrease in tenoxicam solubility.

Discussion

Encapsulation of drugs in soft gelatin capsules offers many advantages. It can improve the bioavailability of drugs by accelerating disintegration, dispersion and dissolution in the gastrointestinal tract as drugs are usually incorporated as water soluble non-aqueous solutions, oil solutions and suspensions (11). It can also protect drugs from deterioration by oxidation due to the poor permeability of the shell to the diffusion of oxygen. In order to design a suitable formulation of tenoxicam to be successively delivered by softgels, its solubility should be improved. One of the methods used to enhance the solubility of tenoxicam is co-solvency. As shown in Figure 1, the solubility of tenoxicam increased linearly with the increase of co-solvents concentration. Because co-solvents are organic compounds that are highly miscible with water and contain hydrocarbon regions that do not interact strongly with water. Therefore, reduce the ability of the aqueous systems to squeeze out non-polar compounds and more drug molecules are incorporated in the solvent system. PEG_{400} and PEG_{6000} demonstrated higher power in enhancement of the drug solubility than ethanol and propylene glycol because they are less polar solvents [2]. Citrate buffer system improved tenoxicam solubility up to pH 7.5. Incorporation of surface active agents to polyethylene glycol 400 solvent systems also improved the aqueous solubility of the drug, in particular at 5% concentration of SAAs (13). Higher synergistic effect in the solubility enhancement was obtained with Tween 80

in comparison to the other SAAs used. Surfactants in aqueous system aggregate and form micelles with non-polar inner regions and exterior polar regions. However, higher concentrations of SAA reduced the drug solubility. This would be due to the fact that higher concentrations of SAA increase the number and the diameter of micelles in which the API molecules were entrapped (21, 22) and may be due to the adsorption at the micelle-water interface (23).

Conclusion

The data obtained in this study revealed that the solubility of tenoxicam increased when the concentration of co-solvents increased. PEG_{400} and PEG_{6000} demonstrated the highest solubility improvement. Also, the solubility of the drug was pH dependent, of non-ionic surfactants to PEG_{400} system demonstrated further enhancement in the drug solubility used.

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Original article

Estimation of the mode of action of olive leaf extract against staphylococcus aureus

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Abstract: Olive (Olea europaea *L*., Oleaceae) leaf extract was obtained by using ethanolic extraction. Staphylococcus aureus was injured by treating the bacterium with a solution of 6.25% (w/v) ethanolic extract of the olive leaves for 30 min. Injury was shown by inability of the bacterium to tolerate 7.5% NaCl in tryptose agar and ability to grow on tryptose agar with no added salt. Colony-forming ability of the injured cells was restored when they recovered in tryptose broth containing sub-lethal amounts of metabolic or synthetic inhibitors. Rifampicn was the only inhibitor which effectively prevented recovery of injured cells. No changes occurred in the cell membrane that allowed soluble proteins or nucleotides to leak from cells during the course of injury.

Key words: Olive leaves; injury; mode of action; staphylococcus aureus.

Introduction

There is a considerable interest from the medical, food and pharmaceutical industries in the use of naturally occurring compounds with anti-microbial activity as an alternative additives or medications to prevent or delay the growth of pathogens (1). The olive leaf has widely been used to treat different microbial infections in traditional medicine in Libya. It is known to be active in the nature against microorganisms and can protect from insect attack and much research has focused on the antimicrobial activity of compounds contained in olive leaves (2, 3). Phenolic compounds including oleuropein have been shown to inhibit the growth of a range of microorganisms (4).

Olive leaf extract (OLE) is a dark brown, bitter-tasting liquid derived from the leaves of the olive tree (Olea europaea *L*., Oleaceae) (5). It did not show broad antibacterial action, rather, it was active against only Helicobacter pylori, Campylobacter jejuni, and S. aureus (6). Although the antimicrobial activity of OLE has previously been examined (7), no reports have described the intracellular activity of the extract (1). Therefore, the aim of this research was to investigate the mode of action of OLE against staphylococcus aureus and to provide data either to support or dispute the claims made for the product. Exposure of S. aureus to OLE is likely to cause injury of cells. Our experimentation

was designed to estimate the extent of such injury, determine the conditions for resuscitation of OLE-injured cells and characterize the mode of injury by OLE.

Materials and methods

Test organism: A clinical specimen of *S. aureus*, obtained from Zawia Teaching Hospital was used throughout the study. The stock culture was maintained through monthly transfers on slants of tryptose agar (TA) (Oxoid, UK) incubated aerobically for 24 hrs at 35 °C and stored at 4 °C until transferred for use in experiments.

Preparation of inoculums: Inocula from stock cultures were seeded to tryptose broth (TB) (Oxoid, UK) and incubated aerobically at 35 °C for 24 hrs. Culture activation was done by two subsequent transfers to sterile tubes of TB. After incubation, 40 ml of culture was centrifuged at 5000 g and 5 °C for 10 min. The spent medium was decanted and the cell pellet was washed and suspended in freshly made, phosphate buffered (0.01 M, pH 7.2) isotonic saline (0.85% NaCl) solution (SS). An initial cell population of approximately 2 x 10^6 cfu/ml was obtained spectrophotometrically at 600 nm (Pharmacia Ultraspace Plus, Model 80 - 2092 - 26, UK) using predetermined absorbance values.

Extraction of olive leaves: Olive leaves were gathered from olive trees in the summer of 2009 from the suburb of Zawia city, Libya. Leaves were used fresh and later broken into pieces using a household blender. The homogenized material (500 g) was air-dried and later extracted for 18 hrs with 1.9 l of ethanol (Fisher Sci., UK) at a room temperature (25 ^oC). This process is repeated twice and the drained supernatant extracts are combined, concentrated by evaporation and dried in drying oven till a brown paste stock of about 5 g was obtained. The concentrated crude extract was diluted in 5 ml SS, thoroughly mixed and autoclaved at 121 °C for 20 min labeled as 1:1 Stock, and stored at 4 °C until use.

Antimicrobial susceptibility assays: The antimicrobial activity of the extract was determined using the broth tube dilution assay following the method described by the Clinical and Laboratory Standards Institute for bacteria (8). OLE was tested in doubling dilutions ranging from 50% to 0.03% (v/v) for broth assays. The OLE was completely soluble in liquid growth media and as such no emulsifying agent was required. Minimum inhibitory concentrations (MICs) were determined as the lowest concentration of OLE resulting in an optically clear broth tube. Tests were repeated at least three times and model MIC values were selected.

Injury medium preparation: Concentrations of OLE at 0.12 and 0.06% (v/v) were prepared using deionized warm water as a dilutent; pH was adjusted to 7.0 using 0.1 N HC1 or 0.1 N NaOH. OLE concentrations were adjusted to the new volume. OLE solutions were filtered sterilized using a sterile filter apparatus containing a membrane with a pore size of 0.20 μm (Nalgene Disposable Filter Units, Type S CN, UK). Stock OLE solutions were kept refrigerated and used within two weeks.

*Injury procedure***:** One milliliter of the cell suspension, 2 x 10^6 cfu/ml was added to 9.0 ml of solutions of 0.12 or 0.06% (v/v) OLE. Controls were made by adding cells to SS. Tubes were incubated aerobically at 35 °C. After 15 and 30 min of incubation, 1 ml

samples were serially diluted in 9.0 ml of SS and plated in duplicate on TA and TA with 7.5% (w/w) NaCl (TAS). TA and TAS were adjusted to pH 7.1 with a predetermined amount of sterile 1 N NaOH immediately before pouring. Inoculated plates were incubated aerobically for 48 hrs at 35 °C. Colonies were counted using a colony counter (PBI Digicount, UK). Inability to form colonies on TAS was considered evidence of injury. The population of injured plus non-injured cells was obtained using TA, whereas the number of non-injured (or recovered) cells was measured with TAS. The difference in the initial colony counts on TA and TAS was considered to be the number of injured cells in the suspension. The extent of injury was determined as the ratio of colony counts on TAS to those on TA. The extent of death was determined as the ratio of colony counts on TA after various treatment times to initial TA colony counts.

*Recovery procedure***:** Samples (0.1 ml) of injured cells, exposed to 0.12% OLE for 30 min at 35 °C, were transferred to 125 ml Erlenmeyer flasks containing 98 ml of recovery medium, TB and 2 ml of deionized water in which 0.5 mg (600 units) of penicillin (Sigma) was dissolved before filter sterilization. Flasks were hand shaken for 5 min. Samples for all trials were incubated aerobically at 35 °C. Portions of the samples were taken periodically, serially diluted in 9.0 ml of sterile 0.1% peptone water, and 0.1 ml quantities were surface plated on TA and TAS plates which were incubated aerobically at 35 °C for 48 hrs; colony counts were determined as described earlier and percent recovery was calculated.

Calculation of recovery: Recovery (%) was obtained through application of the following formula to values of recovery trials:

$$\text{Recovery}\ (\%) = \frac{(S_1 - S_0)}{(T_0 - S_0)} \times 100$$

where S_0 = TAS colony count when just injured cells were inoculated into the recovery medium, T_0 = TA colony count when just injured cells were inoculated into the recovery medium, and S_1 = TAS colony count after injured cells were incubated in the recovery medium for one hr.

Effect of metabolic inhibitors on recovery: Low concentrations of different growth inhibitors were added to the TB recovery medium (penicillin at 10 & 100 µg/ml, chloramphenicol at 10 & 20 µg/ml and rifampicin at 5 & 10 µg/ml) to obtain information on synthetic processes involved in sorbate injury. The inhibitors were obtained from Sigma Chemical Company, UK. Solutions of inhibitors were filter-sterilized and aseptically added to sterile TB. Samples (0.1 ml) of injured cells exposed to 0.12% OLE for 30 min at 35 °C, were transferred to TB containing various inhibitors and incubated aerobically at 35 °C for two hrs. At zero time, and after each additional 30 min, 1 ml samples were withdrawn, serially diluted in 9.0 ml of sterile 0.1% peptone water and 0.1 ml quantities were surface-plated on both TA and TAS plates. Colonies were counted after incubation of plates at 35 °C for 48 hrs

.

Determination of nucleotide and protein leakage: Suspensions of cells treated with 0.03, 0.06 or 0.12% OLE for 30 min at 35 °C, were centrifuged for 15 min at 2000 rpm Supernatant was filtered through 0.20 µm filters (Nalgene) and examined spectroph-otometrically by scanning from 800 nm to 200 nm using a double beam spectroph-

otometer (Pharmacia Ultraspace Plus, Model 80-2092-26, UK). An equal amount of control cells was suspended in SS. Subsequently control and injured cells were washed with SS which was then filtered and scanned for leaked nucleotides or proteins.

Results

A concentration of 0.03% of OLE inhibited growth of S. aureus as seen by lack of turbidity in broth tube dilution assay. Cells of S. aureus which were not exposed to OLE formed colonies on TA and TAS with equal ease (Figure 1).

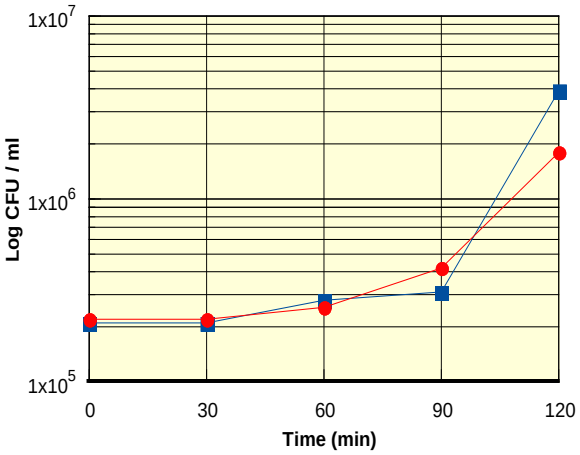

Figure1: Fate of untreated S. aureus after incubation in TB. Cells plated on TA (●) and TAS (■).

Differences in survival of S. aureus occurred when the OLE injured cells were plated on TA and TAS (Figure 2). TAS colony counts indicate 52% of cells were injured after exposure to the 0.12% OLE solution for 30 min. The lower OLE concentration (0.06%) gave less reproducible results (data not shown).

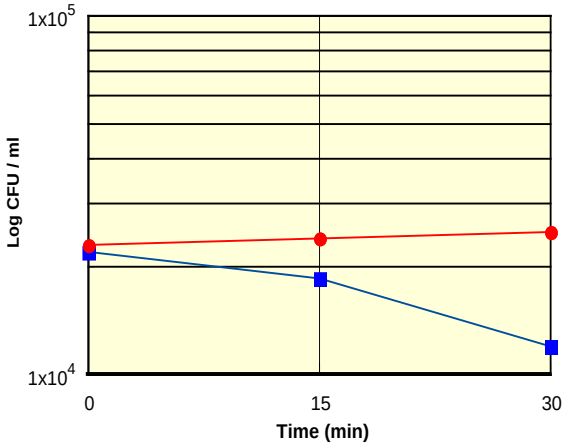

Figure 2: Injury of S. aureus treated with 0.12% (v/v) of olive leaf extract. Cells plated on TA (●) and TAS (■)

Estimation of recovery: Injured cells recovered their salt tolerance after incubation in TB (Figure 3).

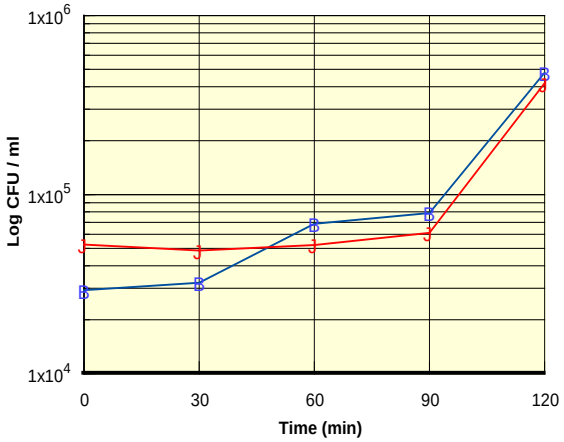

Figure 3: Count of *S. aureus* on TA (●) and TAS (■) after treating with 0.12% (v/v) of olive leaf extract

Complete repair as indicated by equal colony counts on TA and TAS was observed after approximately 50 min of incubation. Recovery was accompanied by a noticeable lag phase. The substantial growth observed upon complete recovery indicates inability of penicillin (5 µg/ml) to halt growth of uninjured cells and probably of injured cells. The population of treated cells was increased by 1.2 orders of magnitude after 2 hrs of incubation.

Recovery in the presence of inhibitors: Antibiotics or metabolic inhibitors to which gram-positive bacteria are sensitive were used to characterise cellular targets that may have been harmed by OLE (Table 1).

Table 1: Recovery of injured S. aureus after incubation in tryptose borth containing inhibitors

Inhibitor	Quantity (ug /ml)	% recovery in 1 hr
Penicillin	10	80
	100	80
Chloramphenicol	10	50
	20	50
Rifampicin	5	10
	10	10

No notable changes in TA colony count were noted in any antibiotic trial. However, recovery of cells injured after treatment with 0.12% OLE for 30 min at 35 °C was invariably suppressed by these inhibitors. The results indicate that addition of penicillin (10 or 100 µg/ml) to the medium reduced by one-fifth the number of OLE-injured cells which able to recover from the injury. Reduced recovery of injured cells in the presence of rifampicin indicates the need of RNA synthesis for recovery from injury. Less suppressive effect in recovery was caused by chloramphenicol suggesting a need of protein synthesis for recovery of OLE-injured cells at a moderate level.

Absence of leaked intra-cellular substances: There was no evidence of membrane damage since supernatant fluids of injured cells showed no leaked proteins or nucleotides when scanned spectrophoto-metrically from 800 to 200 nm. Supernatant liquids of control suspensions showed no absorbance in the tested range.

Discussion

To determine the primary cause of injury, the site first damaged by OLE, the total time allowed for injury was limited to the first 30 min. Secondary sites of damage may indirectly exist in the first generation of S. aureus which evolves after approximately 40 min of incubation at 35 °C in TB (9). To determine the specific action of OLE, effects of pH should be excluded. Hence, a neutral pH must be preserved. OLE was adjusted to pH 7 prior to incorporation in experiment. Incorporation of a growth - inhibiting substance like penicillin into the recovery medium is important to separate repair from sub-lethal damage and subsequent growth of S. aureus. Nevertheless, approximately 100% recovery after incubation for about 50 min and notable growth after 2 hrs were evident when injured cells were incubated in the presence of a low penicillin concentration (Figure 3). An increase in aerobic plate count in the presence of a low penicillin concentration was also observed during recovery of acid-injured (10) and heat-injured (9) cells of S. aureus. A penicillin concentration of only five µg/ml was used to inhibit growth during recovery of OLE- treated cells (Figure 3) because a higher concentration, 10 and 100 µg/ml prevented full recovery of these cells (Table 1). Cell-wall repair or synthesis may, to a very limited extent, be required for salt tolerance of S. aureus injured with OLE. There is no agreement in the literature on percentage of recovery that is considered a minimum for conclusion of growth inhibition by an antibiotic. For instance, chloramphenicol was concluded as inhibitory when there was 44% recovery of injured cells (11). In contrast, recovery of 41% in another study led to the conclusion that the antibiotic failed to inhibit its target organism (12). In our view, the percent

recovery should not exceed 10 for an antibiotic to be concluded as inhibitory. Higher than 10% recovery indicates the inhibitor was quite ineffective. Sub-lethal amounts of cycloserine did not inhibit recovery of injured cells. Chloramphenicol was used to inhibit peptide bond formation between newly synthesized amino acids inside 50S ribosomal subunits (13). Almost half of the injured cells of S. aureus managed to recover in 60 min after OLE injury and exposure to sublethal amounts of chloramphenicol. Protein synthesis may be a minor requirement for complete recovery of S. aureus injured with OLE. Rifampicin was used to inhibit initiation of RNA synthesis by non-covalently binding to the [3-subunit of the DNA dependent RNA polymerase (14). If OLE exerted a general inhibitory effect on different enzymes, including RNA polymerases, peptidoglycan building enzymes must have encouraged penicillin to completely disable cell wall synthesis but this was not observed in our case. Inhibition by rifampicin denotes involvement of RNA synthesis in recovery of S. aureus from OLE injury. Rifampicin inhibited Salmonella bareilly recovering from acid injury (15) as well. Injured cells frequently lose some of their cellular material through leakage into the surrounding medium. This leakage is primarily indicative of membrane damage, as that reported for mildly heated microbes (9). A small amount of OLE (0.03%) was used to avoid precipitation of cytoplasmic components and, hence, inability of likely-denatured macromolecules to leak through a damaged membrane. No leakage of 260-280 nm absorbing materials was noted in this work, nor has it been reported for acid injured S. aureus (10).

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Original article

Pre- and post-operative prophylactic use of local povidone-iodine in potentially contaminated surgery

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Abstract: Povidone-iodine is a complex of iodine with povidone. It is an iodophore which slowly liberate inorganic iodine in contact with the skin and mucous membranes. Since pre- and post-operative management of surgical patients require massive antibiotic treatment which is considered nowadays a very hard task due to high cost, misuse and non-availability of specific agents. A clinical trial on using 10% povidone-iodine solution locally was carried out on 20 selected patients who are candidates for elective and emergency surgical procedures. Povidone-iodine is used for local application on the surgical wound area instead of both pre-and post-operative antibiotic treatment compared with the use of 1.25 - 2.5% chloroxylenol solution wash associated with full pre- and post-operative antibiotic therapy in another 21 selected patients for comparison. The results showed no differences in healing time between the two groups was found while the percentage of occurrence of post-operative infection in povidone-iodine treated group became zero compared with 28.5% of post-operative infection observed in the comparison group. Concerning the cost of pre- and post-operative drug treatment, local treatment with povidone-iodine reduces the cost of drug therapy by 17 folds compared to the other group. The side effects and adverse reactions of the tested method of management are very mild indicating the safety of povidone-iodine in this respect. Thus, the findings strongly indicate that the high significance of local use of 10% povidone-iodine solution for pre- and post-operative treatment in elective clean and potentially contaminated surgery.

Key word: Povidone-iodine, surgical prophylaxis, antiseptic, prophylactic antibiotic.

Introduction

Povidone-iodine is a complex of iodine with poly povidone (1-vinyl-2-Pyrolidone). It is iodophore which slowly liberate inorganic iodine in contact with the skin and mucous membranes (1). The natural element iodine has been used for more than 150 years to prevent infections and treat wounds (2). Yet only due to the development of iodophores has it become possible to use this highly efficient microbicide in a wide range of medical applications (3). The antimicrobial spectrum is universal; its efficiency against clinically and epidemiologically significant new pathogens such as methicillin-resistant

staphylococcus aureus and enterococcus species was evaluated and proved (4). It seems likely that dressing with tulle gras - type medicated with povidone-iodine would provide a good topical antibacterial prophylaxis and may reduce the bacterial burden of colonized wound (5). Rikimaru et al. (6) demonstrated that the commercially available povidone-iodine products appeared to be useful agents as disinfectants against several mycobacterium species of microorganisms. Alcoholic solution of povidone-iodine is found to be reasonable and effective antibacterial agent for pre-operative skin preparation (7). Healing times and rate of post-treatment infection were found to be better in patients with superficial burn injury after treatment with povidone-iodine compared with patients treated with silver sulfadiazine (8). Application of povidone-iodine solution once a daily was proven to be effective in prevention of catheter associated urinary tract infection in male patients (9). However, rinsing with povidone-iodine reduces the incidence, severity and duration of oral mucositis during antineoplastic chemotherapy (10). Electron microscopic and biochemical observations support the conclusion that povidone-iodine act through interaction with cell walls of microorganisms causing pore formation or generating solid - liquid interfaces at the lipid membrane level which lead to loss of cytosol material in addition to enzyme denaturation (11).

In vitro experiments showed certain cytotoxicity yet in vivo investigations in animals and in humans could exclude cytotoxic effects of povidone-iodine, measured by the wound healing process (12). Only when administered in combination with detergents was obvious cytotoxicity seen in wound but not on intact skin when compared to frequently used antibiotic neomycin the sensitization rate of povidone-iodine is very low (13). Thus, the aim of this study was to evaluate the effectiveness of local povidone-iodine solution as a pre- and post-operative prophylaxis instead of routinely used pre- and post-operative antibiotic therapy in both clean and potentially contaminated surgical operations.

Patients and methods

Forty-one patients (21 males and 20 females) of different age groups and occupations who are candidates for elective and emergency surgical operations of various types were selected in the department of surgery in Baquba Teaching Hospital, Baquba, Iraq and divided into two groups: **Group A**: 20 patients (11 males and 9 females) to whom the types of surgical operations shown in Table 1 were done, in this group, the incision site was washed with 10% povidone-iodine before operation, before closure of the wound and after closure of the wound, a gauze soaked with povidone-iodine was applied on the wound after suture. No pre- and post- operative antibiotic treatment was given to patients of this group.

Table 1: Types of surgical operations performed on group A of patients

n	Type of surgery
5	Appendicectomy
1	Prostatectomy
3	Thyroidectomy
1	Renal stone
1	Hydatid cyst of liver
1	Sebaceous cyst of arm
2	Hydrocelectomy
1	Cholecystectomy
2	Obstructed inguinal hernia
3	Breast mass
20	Total

Group A: Patients treated with povidone-iodine locally and group B: Patients treated with antibiotics

Group B: 21 patients (10 males and 11 females) to whom the types of surgical operations shown in Table 2 were done in this group 1.25 - 2.5% chloroxylenol solution was used for pre-operative disinfection, in addition to pre- and post-operative antibiotic treatment according to the routine schedule followed in the department of surgery.

Table 2: Types of surgical operations performed on group B of patients

n	Type of surgery
9	Appendicectomy
	Prostatectomy
1	Renal stone
2	Cholecystectomy
1	Obstructed inguinal hernia
1	Breast mass
2	Ovarian cyst
1	Spleenectomy
1	Nephroctomy
1	Liver Biopsy
1	Liver Abscess
21	Total

Group A: Patients treated with povidone-iodine locally and Group B: Patients treated with antibiotics.

For the two groups of the patients, the following evaluation procedure was carried on:

A- Healing time was measured and reported as the time required for complete healing of the surgical wound without any sign of bacterial growth or infection until removal of the suture and follow up after that for at least three days.

B- Swabs for microbiological examinations and characterization of the invaded microorganisms, if present, were taken before and two min after disinfectant application and just before closure of the wound after finishing the surgery and each two days interval during follow up after surgery.

C- Clinical follow up and examination to report any sign of local skin irritation or abnormal appearance as a result of use of local povidone-iodine.

D- Cost of treatment with povidone-iodine was evaluated and compared with the pre- and post-operative antibiotic treatment according to the retail prices of the drugs, accessories and nursing charges.

Results and discussion

Despite advances in antibiotic prophylaxis, post-operative wound infection remains a major source of morbidity especially after potentially contaminated surgery, its prevention is challenging problem especially in high risk patients (14). Eason et al. (15) found that pre-operative vaginal povidone-iodine gel is safe and promising technique for reducing febrile morbidity after hysterectomy.

Pre-operative disinfection of conjunctival sac before cataract surgery using 9% povidone-iodine solution also found to be highly effective as pre-operative prophylaxis compared to benetonium chloride solution 30% and topical antibiotic use (16). In an attempt to use povidone-iodine solution as a pre-operative prophylactic treatment, Table 3 indicated no significant differences in healing time between group A and group B, but the skin manifestations in group A are milder, reflecting the safety of povidone-iodine solution when applied by extensive washing and dressing the surgical wound with povidone-iodine soaked gauze.

Table 3: Effects of pre-operative and post-operative use of povidone-iodine on healing time and skin manifestations

Group	n	Healing time (days)	Post-op. skin manifestations
A	**20**	**6**	**Skin discoloration disappear after 5 days**
B	**21**	**7**	**Skin irritation, Redness and eczematous reaction**

Group A: Patients treated with povidone-iodine locally and Group B: Patients treated with antibiotics.

The reaction of liberated iodine with injured tissue is thought to produce a possible delay in wound healing, however, Konig et al. (17) showed that povidone-iodine does not only kill a wide range of bacteria, but also inhibit generation and release of bacterial exotoxine, it also inactivates bacterial endotoxins, as well as granulocyte-derived tissue destructive enzymes and cytokines. The available data support the usefulness and efficiency of povidone-iodine as an effective therapeutic agent to combat infection. Povidone-iodine solution is proved as superior antiseptic for preparation of eyes and other parts of the body before surgical operations, compared to other topical broad spectrum antibiotics, to prevent post-operative complications, it is cheaper, caused minimal side effects, reduces bacterial counts to great extent, and eliminate fungi completely (18). Results of this study showed that pre-operative prophylaxis with topical povidone-iodine solution produces complete reduction in post-operative wound infection (0%) in group A patients, compared to (28.5%) wound infection in group B (table 4), where chloroxylenol solution was used with the ordinary antibiotic treatment pre-and post-operatively.

Table 4 also showed that post-operative bacterial growth was completely inhibited in group A patients compared to 26% in group B which show high sensitivity for contamination with microorganisms from hospital environments in this group. These results are compatible with those obtained by Jeng and Severin (19) who proved that povidone-iodine formulations delivered rapid and persistent antimicrobial activity against a broad spectrum of bacteria both in vitro and in vivo by a single step 30 second application.

Table 4: Effects of local pre-operative use of povidone-iodine on surgical wound infection and post-operative bacterial growth

Group	n	% wound infection	% pre wash bacterial growth	% post-operative bacterial growth	Types of Microorganisms isolated
A	20	0.0	18	0.0	Staphylococcus aureus
B	21	28.5	19	26	Staphylococcus aureus Escherichia coli Proteus species

Group A: Patients treated with povidone-iodine locally and Group B: Patients treated with antibiotics.

The effect of using topical povidone-iodine pre-operatively on the cost of antimicrobial therapy was investigated. Results presented in Table 5 showed the high differences in the cost between group A and B, where pre-operative povidone-iodine produces 17 folds reduction in the total cost of antimicrobial treatment.

Table 5: Cost effects of pre-operative prophylactic use of povidone-iodine solution locally compared to other regimen

Group	n	Cost of antimicrobial treatment		Total
		Pre-operative	Post-operative	
A	20	450	450	900
B	21	1500	14000	15500

Group A: Patients treated with povidone-iodine locally and Group B: Patients treated with antibiotics.

Povidone-iodine ophthalmic solution is found to be a good alternative to post-operative topical antibiotics because of its effectiveness in controlling conjunctival bacterial colony-forming units and species, its relatively low costs, and its availability (20).

Conclusion

This study indicates that local application of 10% povidone-iodine solution when used as a pre-and post-operative prophylactic measure was safe and may be more effective than the use of chloroxylenol solution with a full course of pre-and post-operative antibiotic treatment used for this purpose. This method is found highly economic where marked cost reduction was observed, that health care providers should take care of it, so they can reduce effectively the cost of both clean and potentially contaminated surgical operations. Further large scale clinical evaluation of this technique is required to establish its utility in the field of surgical practice.

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Original article

Libyan professional opinions on an extended role of the community pharmacist

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Abstract: To compare the opinions of the Libyan community pharmacists and gynaecologists about extending the role of the community pharmacist in medicines supply. This includes the supply without a prescription and the repeat dispensing according to an initial prescription. A focus was made on female hormonal products. A questionnaire was handed over to both study groups, 100 in each group. Participants were asked to tick the selected answers and to rate their attitudes on a 5-point Likert scale regarding the given statements. Libyan community pharmacists were in favour of allowing themselves to supply without a prescription some of the currently classified prescription-only medicines (PoMs). They also were in favour of the repeat dispensing. Opinions of the gynaecologists on the supply without a prescription were equally divided while on the repeat dispensing they were generally not supportive. 4/5 of the community pharmacists and 2/5 of the gynaecologists believed that oral contraceptives should be available for repeat dispensing. The competency of the community pharmacist was perceived differently by two groups. The community pharmacists were more positive to extend their roles in supplying some PoMs whilst the gynaecologists were generally not positive.

Key words: Opinions, community pharmacists, gynaecologists, medicines supply, Libya.

Introduction

The need for a greater involvement of the community pharmacist in health care has been further reinforced world-wide. It has recently been demonstrated that pharmacy should expand into new areas beyond those traditionally expected of the profession (1, 2). Pharmaceutical care in the USA goes further than providing pharmaceutical services and/or clinical pharmacy (3, 4). It includes, but not limited to patient assessment; patient education and counselling; patient - specific pharmacist care plans; drug adjustment; selection of the therapeutic alternatives; prescription authority; preventive services; and managerial services (5). The modern pharmacist professional roles include the supply of an increased range of medicines without a prescription; the repeat dispensing of medicines; the supplementary prescribing of medicines; and the management of the pharmacist clinics. The supply without a prescription involves extending the General Sales List (GSL) or Over the Counter (OTC) medicines as well as the Pharmacy (P) medicines (2, 6 - 9). At the pharmacist-managed clinic which is widely established

in the USA**,** the pharmacist may order laboratory tests, adjust dosages, determine treatment duration, schedule follow-up visits and may also write prescriptions. The pharmacist supplementary prescriber in the UK is able to vary the dosage, adjust the frequency or change the formulation of the medicine and prescribe a different medicine, according to a clinical management plan with the prescriber (7, 9). Nevertheless, studies on the opinions of health care professionals about these new roles are scarce. Moreover, up to the authors' knowledge, no previous studies on the repeat dispensing of hormonal contraceptives or hormone replacement therapy (HRT) are reported. This study was designed to document professional opinions about the community pharmacist's role to supply some of the currently classified prescription-only medicines (PoMs) in Libya without prescription (as P medicines) or according to an initial prescription (repeat dispensing). It was also to examine the perceptions about the competency of community pharmacists to supply some PoMs as P medicines or within the repeat dispensing approach.

Materials and methods

Local research ethical approval was obtained. Two similar forms of a closed-ended questionnaire were piloted to 10% of the targeted participants; the Libyan community pharmacists and gynaecologists. The questionnaire was then randomly distributed to both study groups until about 10% of returned completed forms (n = 100, each group). This was made personally and with the help of colleagues during one-to-one meetings in community pharmacies at the health facilities or during a medical conference. The study questionnaire consisted of three sections. The first section emphasised on attitudes towards allowing the Libyan community pharmacists to supply some of the currently classified PoMs without prescription (as P medicines) or to repeat the dispensing of some PoMs according to an initial prescription. The second section focused on the opinions about the existing competency of Libyan community pharmacists. In the final section, several categories of medicines were presented and participants were asked to tick these they thought should be available for repeat dispensing. The participants were asked to tick the selected answers and also to rate their attitudes on a 5-point Likert scale, rating from 'strongly agree' (+2) to 'strongly disagree' (-2) regarding the given statements.

Statsitical analysis: Descriptive statistics were used to present the data. These data were tested for normality using the Kolmogorv-Smirnov goodness-of-fit test. Comparisons between the two groups were tested with an independent *t* test. The χ^2 test was also used throughout this study and χ^2 test with contingency table analysis was performed. All data were analysed using the 'StatView SE+Graphics' Macintosh programme. A statistical significant difference was accepted throughout when $*p<0.05$, $**p<0.01$ and $***p<0.001$.

Results

Response rate was 90.9% for the community pharmacists and 87.7% for the gynaecologists. Forty-two percent of the community pharmacists and 65% of the gynaecologists were female. Eighty-six percent of the community pharmacists and 83% of the gynaecologists were aged between 25 and 44 years. Sixty-nine percent of the community pharmacists and 54% of the gynaecologists had duration of five to 24 years of practice. Figure 1 demonstrates the

opinions on the community pharmacist's role to supply some PoMs without a prescription and Figure 2 demonstrates the opinions on the role to repeat dispensing some PoMs according to an initial prescription. Figure 3 shows the perceptions on the knowledge of the community pharmacist to supply some PoMs without a prescription and Figure 4 shows the perceptions on the knowledge to repeat dispensing some PoMs according to an initial prescription. The perceptions on the skills of the community pharmacist to supply some PoMs without a prescription and those on the skills to repeat dispensing some PoMs according to an initial prescription are shown in Figures 5 and 6 respectively.

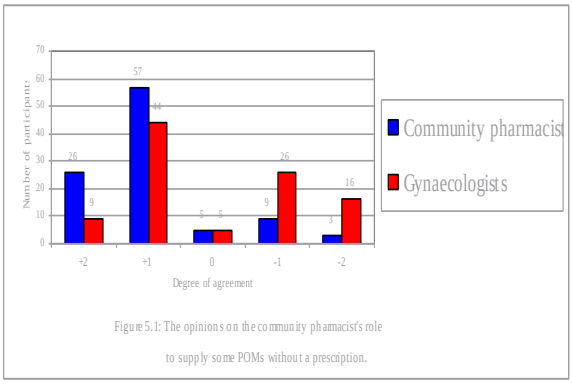

Figure 5.1: The opinions on the community pharmacist's role to supply some POMs without a prescription.

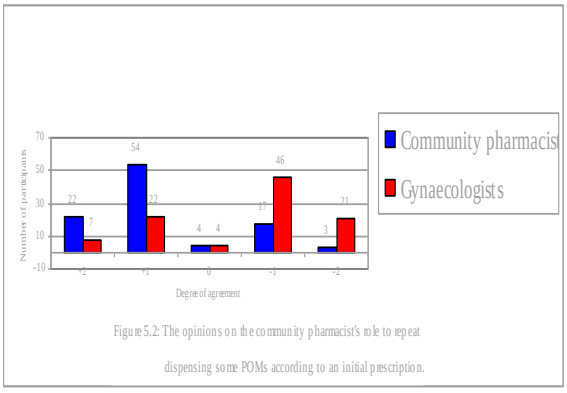

Figure 5.2: The opinions on the community pharmacist's role to repeat dispensing some POMs according to an initial prescription.

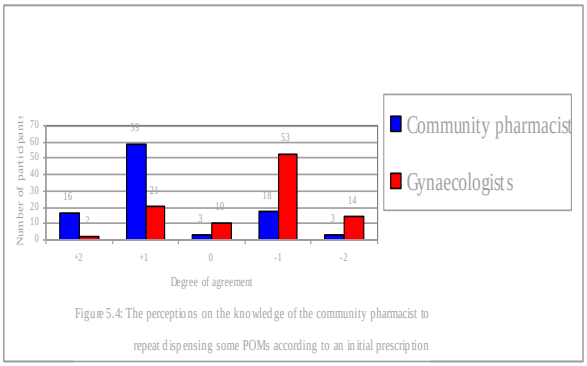

Figure 5.4: The perceptions on the knowledge of the community pharmacist to repeat dispensing some POMs according to an initial prescription

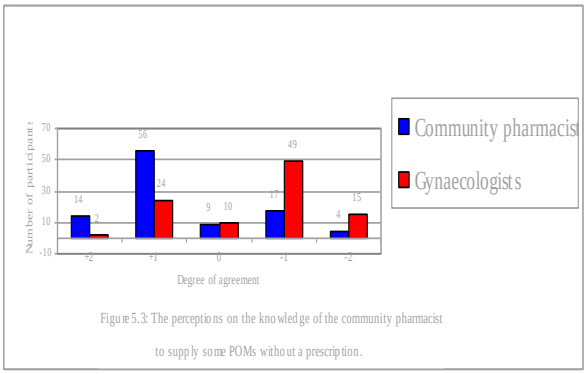

Figure 5.3: The perceptions on the knowledge of the community pharmacist to supply some POMs without a prescription.

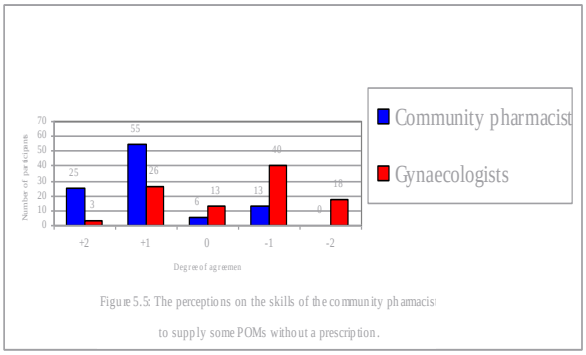

Figure 5.5: The perceptions on the skills of the community pharmacist to supply some POMs without a prescription.

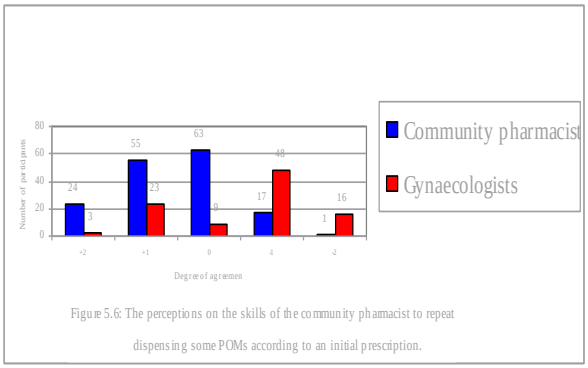

Figure 5.6: The perceptions on the skills of the community pharmacist to repeat dispensing some POMs according to an initial prescription.

A contingency table for the responses to allow the community pharmacist to repeat dispensing selected categories of medicines is given in Table 1. Opinions about the community pharmacist's knowledge on specific female hormonal medicines are given in a contingency Table 2.

Table 1: Contingency table for the responses (agreed/disagreed) to allow the community pharmacist to repeat dispensing selected categories of medicines

Category of medicines	Community pharmacists	Gynaecologists
Non-steroidal anti-inflammatory drugs	76/24	37/63***
Antihypertensive drugs	65/35	66/34
Hypoglycaemic drugs	64/36	58/42
Asthma medicines	67/33	44/56**
Antibiotics	39/61	6/94***
Ulcer healing drugs	56/44	22/78**
Antispasmodic medicines	71/29	43/57**
Antidiarrhoeal drugs	59/41	27/73**
Antiprotozoal & anthelmentic medicines	34/66	14/86*
Antifungal & antiviral topical medicines	56/44	24/76**
Vitamins & minerals	86/14	76/24
Oral contraceptives	80/20	41/59***
Injectable contraceptives	7/93	4/96
Hormone replacement therapies (HRTs)	11/89	14/86

Significantly different by *p < 0.05, **p < 0.01 and ***p < 0.001

Table 2: Contingency table for the opinions (agreed/disagreed) that the community pharmacists have sufficient knowledge about specific female hormonal medicines

Category of medicines	**Community pharmacists**	Gynaecologists
Oral Contraceptives:	80/20	60/40*
Effectiveness	68/32	38/62***
Safety Side effects	82/18	57/43**
Injectable Contraceptives:	49/51$_{\#\#\#}$	40/60$_{\#\#}$
Effectiveness	37/63$_{\#\#\#}$	21/79*$_{\#}$
Safety Side effects	49/51$_{\#\#\#}$	32/68*$_{\#\#}$
HRT:	52/48$_{\#\#\#}$	35/65*$_{\#\#\#}$
Effectiveness	33/67$_{\#\#\#}$	21/79*$_{\#}$
Safety Side effects	45/55$_{\#\#\#}$	32/68$_{\#\#}$

Significantly different by *p < 0.05, **p < 0.01 and ***p < 0.001 (between the groups) and $_{\#}$p < 0.05, $_{\#\#}$p < 0.01 and $_{\#\#\#}$p < 0.001 (reference is made to the first category of medicines)

Discussion

In the present study, the attitudes of both groups were more positive towards the supply without a prescription, than to the repeat dispensing according to an initial prescription. This is probably because the

participants perceived that it is an obligation to the community pharmacists to conduct the necessary assessment and investigations before supplying a medicine without a prescription. Thus, they take full responsibility for this action. However, allowing them to repeat dispensing these medicines according to an initial prescription, as per their opinion, lacks responsibility and encourages laxity and less assessment of the situation. Pharmacists in the UK are responsible for the continuing care of clients who have been clinically assessed by independent prescribers, by adhering to an agreed clinical management plan (1, 8).

Generally, the gynaecologists were more negative than the community pharmacists in all respects. Opinions about the community pharmacist's role were explained by the answers to the statements related to the community pharmacist's knowledge and skills. Some clinicians think prescription dispensing as the way for general health screenings (10), the way to discover any developed adverse effects (11, 12) and to increase user compliance (13). In the pharmacy, there is a limited record keeping and sharing. Pharmacies may lack client privacy or pharmacists some times are busy and not able to provide counselling before supplying medicines (14). Disease symptoms may be masked because of prior self-medication. Furthermore, pharmacists may become lax in supplying medicines or concerned with sales only. Non-prescription supply of medicines may encourage a lack of responsibility. It also can support misuse or abuse of medicines. However, concern about the re-regulation of medicines and pharmacist prescribing is not new and has its roots partly in the historical antipathy between the medical and pharmacy professions (15). Roberts (16) suggested that the antagonism between the professions is perhaps best symbolised by the long-standing conflict between dispensing doctors and pharmacists, who are potentially in competition for business. It may be argued that many health care clinics may be opposing OTC approval of birth control pills, in part because that would translate into lost revenue for them. Gynaecologists are probably over-evaluating the degree of monitoring and investigations that take place before prescribing oral contraceptives or they pretend that a very safe process is provided. A Survey was entitled 'What do family planning providers do before prescribing combined oral contraceptives?' revealed that out of 123 family planning doctors, general practitioners and gynaecologists, from the UK, other European countries, Australia, New Zealand and South Africa, 95.1% checked the blood pressure; 68.6% checked the weight of their clients; 32.5% did a breast examination; 33.1% did a pelvic examination; 31.7% took a cervical smear; 18.2% checked the urine before prescribing the pill; 10.7% did a blood test for glucose; 8.3% did a blood test for lipids; 3.3% did a blood test for clotting; 1.7% ordered renal function test; and mammography was arranged by only 0.8 % of them (17).

Although, the gynaecologists that participated in the present study were generally not supportive to extending the role of the community pharmacist to the repeat dispensing, a significant proportion of them viewed that some medicines can be repeat dispensed according to an initial prescription. Supporters believe that an extended role of the community pharmacist is more convenient for many people and provides easier, quicker and around the clock access with no need for an appointment with the doctor. Moreover, the pressure on doctors would be reduced. Non-prescription supply of medicines offers more choice, freedom and anonymity. It also represents an opportunity for pharmacists to

provide health education and health promotion. The self-purchase of medicines can reduce the health services's cost. It would be cheaper for the clients, because they would not have to pay the physician's fee. Furthermore, high dose oral contraceptives have generally been replaced by low dose preparations. Therefore, most of the initial concerns regarding health risks have been laid to rest. The pill today is low dosage and safer. Progestogen-only pills are even safer with an advantage that they can be used safely by many groups of women (13, 18). Since oral contraceptives are considered safe and do not require routine physical investigation (19), many of the arguments that could have initially been made as to why only a doctor should have the right to prescribe contraceptive items now do not apply.

Furthermore, oestrogens available in conventional HRT are described as 'natural', with lower hormone potency (20). Accordingly, minor side effects produced by synthetic oestrogens during oral contraceptives use are avoided in HRT. Therefore, the proposed transfer of oral contraceptives from PoMs to OTC or repeat dispensing status can be suggested to apply to HRT as well.

Communication between the pharmaceutical and medical professions in order to change the medical professionals' attitudes requires to be addressed. Further training and education for the community pharmacists may be recommended. Other studies to examine the reasons for positive and negative opinions on expanding the pharmacist's role within the community are also suggested.

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