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Research Article

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Prophylactic and Curative Effect of Selenium on Infertility Induced by Formaldehyde Using Male Albino Mice

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ABSTRACT

Infertility is a source of psychological, and sometimes social, stress on parents who desire to have children. Formaldehyde is used chiefly as disinfectant, preservative and in chemical synthesis. The medical uses of formaldehyde are limited, but focused especially on laboratory use. Selenium is an essential trace mineral element for human; it is essential for sperm function and male fertility. Selenium deficiency has been linked to reproductive problems in animals.

The main objectives of this study is to investigate the prophylactic and curative effect of selenium on male infertility induced by formaldehyde using male albino mice.

Forty male albino mice were used, weight 25-30gm divided into five groups of mice (n=8). Group 1 was daily administered (i.p.) water for injection (5ml/kg) for five days, group 2 was daily administered selenium (100 µg/kg) for five days, group 3 was daily administered formaldehyde (30 mg/kg) for five days, group 4 (prophylaxis) was daily administered a combination of formaldehyde and selenium for five days, while group 5 (curative) was daily administered formaldehyde for five days followed by daily administration of selenium for the next five days. At the end of administration, seminal fluid was collected from vas deferens. Sperm count, morphology and motility were scored; Histopathological screening of genital system was carried out. SPSS software was applied for comparing groups.

It was found that formaldehyde toxicity did not change the sperm count and percentage of motile sperm. Formaldehyde produces degeneration/damage to the male mice testis and sperm parameters. Selenium alone resulted in an increase in sperm count, volume of seminal fluid and the percentage of motile sperm. Selenium has prophylactic and curative effects against formaldehyde-induce testis and sperm parameters toxicity. Future work is recommended to find out if selenium protective effect is through antioxidant or other mechanisms.

Key words- *Male infertility; Selenium; Formaldehyde.*

INTRODUCTION

Male infertility defined when men are not able to conceive a child even though they have had frequent sexual intercourse[1]; also defined as the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year.[2,3]

Infertility has increased, some studies pin the blame for this increase on social phenomena, including the tendency for marriage to occur at later age and trying to start families at this later age.[2] Infertility has increased by 4% since the 1980s, mostly from problems with fecundity due to an increase in age.[4]

Male infertility is due to low sperm production, misshapen or immobile sperm, or blockages that prevent the delivery of sperm; also the illnesses, injuries, chronic health problems (e.g Hypertension, Hyperthyroidism and Diabetes Mellitus), lifestyle (e.g Sleeplessness, Diet and Smoking), some prescribed drugs e.g Corticosteroids, Sulfasalazine, Phenytoin and other factors can play a role in causing male infertility; being not able to conceive a child can be stressful and frustrating.[1]

In male infertility, four basic characteristics are usually evaluated under the microscope (semen analysis)[5]: a} Sperm count refers to the number of sperm present in a semen sample. The normal number of sperm present in just one milliliter (ml) of semen is more than 20 million. An individual with only 5-20 million sperm per ml of semen is considered sub fertile, an individual with less than 5 million sperm per ml of semen is considered infertile; b} Volume of the semen sample is important. An abnormal amount of semen could affect the ability of the sperm to successfully fertilize an ovum in the female; c} Not all sperm within a specimen of semen will be perfectly normal. Some may be immature, and some may have abnormalities of the head or tail. A normal semen sample will contain no more than 25% abnormal forms of sperm; d} Sperm are also examined to see how well they swim (sperm motility) and to be sure that most have normal structure.

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Other causes considered a risk factors of male infertility[6]: *Overweight* – Obesity can cause hormone changes that reduce male fertility; *Alcohol abuse* – Drinking alcohol can lower testosterone levels, cause erectile dysfunction and decrease sperm production, also liver disease caused by excessive drinking may lead to fertility problems; *Tobacco smoking* – Men who smoke may have a lower sperm count than do those who do not smoke, also the negative smoking may affect male fertility; *Exposed to toxins* – Some toxins can cause liver disorders and may affect male fertility; Using certain *illegal drugs* – Anabolic steroids taken to stimulate muscle strength, can cause the testicles to shrink and sperm production to decrease. Use of cocaine or marijuana may temporarily reduce the number and quality of sperm as well. *Emotional stress* - Stress can interfere with certain hormones needed to produce sperm.

Formaldehyde is an organic compound with the formula CH_2O or HCHO, it is described as the simplest aldehyde. Formaldehyde is a gas at room temperature (Flammable Gas), colorless and has a characteristic pungent, irritating odor. It is important precursor to many other materials and chemical compounds. Formaldehyde was formerly used as disinfectant and for preservation of biological specimens, also used commonly in nail hardeners and/or nail polish.[7]

Formaldehyde is used industrially in manufacturing of other chemicals, pesticides, industrial fungicides, germicides, fertilizers, disinfectant, preservatives, latex rubber and photographic films.[8] The medical uses of formaldehyde are limited due to its toxicity, but focused especially on laboratory use.[9]

Reproductive system toxicity caused by formaldehyde

Harmful effects of formaldehyde injection on testicular tissue, are quite well documented. However, detailed studies on the effects of formaldehyde on testis functions are quite limited.[9] Administration of high dose of formaldehyde resulted in pathological changes in seminiferous tubules (STs) and sperm parameters of adult rats and mice.[10]

Long-term exposure to formaldehyde can cross the blood testes barrier and induce lipid peroxidation and oxidative stress by increasing the reactive oxygen species (ROS). These may cause decreased seminiferous tubules diameters and degeneration of Leydig cells resulted from oxidative damage from formaldehyde vapor; the degeneration of Leydig cells leads to decrease in testosterone levels, which affect sperm parameters of sperm progressive count, motility and normal morphology.[11]

Selenium is an essential trace mineral element for humans and animals. It is found in soil, rocks and consequently may then accumulate in plants.[12] Selenium is taken into the body in water and foods. Selenium naturally present in some foods, such as: Fish, Shellfish, Red meat, Offal, Chicken, Liver, Garlic, Eggs, Brazil Nuts and Grains.[13,14] The crab, liver, fish, poultry and wheat are generally good selenium sources.[15]

The body requires small amounts of selenium in daily diet. Selenium is incorporated in a small cluster of important proteins, each of which play critical role in body health; the scientists named these selenium-containing proteins "Selenoproteins".[16] Selenoproteins are necessary for synthesis of glutathione peroxidase enzyme (antioxidant and detoxifier); this enzyme plays a role in preventing cell damage caused by free radicals in tissues.[17]

Selenium and male infertility: The supplementation with selenium has been reported to improve reproductive performance in mice, rats and sheep.[18] Selenium is essential for sperm function and male infertility; its deficiency has been linked to reproductive problems in rats, mice, pigs and other animals[19]; Selenium has an important role in normal testicular development and spermatogenesis.[14] High selenium intake has been associated with impaired semen quality.[20] Selenium is essential in energy transfer reactions as in the production of sperm cells. It is thought that male infertility can be the result of selenium deficiency as the absence of selenium in the testicular tissues induces degeneration, which results in the active impairment of sperm motility as the first indication of impending infertility.[21]

The aim of this study is to investigate the effect of selenium on male infertility induced by formaldehyde using male albino mice. Histopathology, sperm count, sperm motility and sperm morphology were scored for the different treated groups.

MATERIALS AND METHODS

Materials

Selenium supplement was obtained from Jamieson Company - Canada; Formaldehyde, NaCl and Eosin Y were purchased from Red El-De Haen AG Seelze-Hannover - Germany.

Methods

Strain and care

Male albino mice were inbred in animal house of Faculty of pharmacy, University of Tripoli. They were kept until they reached an age of 8-12 weeks (weight ranged from 25-30gm). Mice were housed in plastic cages containing wooden flakes of ships (8 mice per group/cage) in an air-conditioned room. Day length was 12 hours, and temperature approximately 23°C. Mice were fed Purina lab chow ad libitum. The animals were healthy and free of any external parasites or skin diseases.

Experimental design

Forty albino male mice were divided into five groups of male mice (n=8). Group 1, daily received water for injection (5ml/kg) for five days; group 2, daily received selenium (100 µg/kg) for five days; group 3, daily received formaldehyde (30mg/kg) for five days[22]; group 4, (as prophylaxis) daily received a combination of formaldehyde and selenium for five days; while group 5, (as curative) received daily formaldehyde for five days followed by daily administration of selenium for the next five days. Intraperitoneal administration was adopted. Mice were weighed at the beginning of the experiment, before injection and before killing. The animals were killed by cervical dislocation, mice were processed and evaluated

for sperm count, sperm motility and sperm morphology (abnormalities). Histopathological screening was carried out for mouse testes.

Seminal fluid collection

At the end of experiment, mice were killed by cervical dislocation. Sperm of each mouse were obtained by squeeze the vasa deferentia gently into 1ml normal saline in small dish. The specimen was mixed gently by a special dropper to distribute the seminal fluid. Sperm suspension was incubated for 15 minutes at 32 ºC to allow sperm separation.[23]

Determination of sperm count

Sperm count was made using the method by Yokoi et al.[24] The sperm were counted by charging both chambers of improved neubauer hemocytometer with sperm suspension. The number of spermatozoa in the squares of the hemocytometer was counted under the microscope at 400X magnification. Two samples of each were counted to ensure accurate data. Sperm count was expressed in millions per milliliter.

Examination of sperm morphological abnormality

For sperm morphology test, two smears were made from each mouse, and allowed to dry in air. Smears were stained with 1% eosin Y in water for 10 minutes. Slides were randomly read with regard to slides from individual control or treated groups. From each mouse 500 sperms were examined at 400 magnification for morphological abnormalities. The result were expressed as percentage of abnormal sperm.[25]

Determination of sperm motility

Sperms from treated mice were examined according to Ficsor and Ginsberg[26] using the improved neubauer hemocytometer (American optical Co., Buffalo. N.Y.). A drop of diluted sperm suspension, was taken up by fine pipette; the mouth of the pipette was held near the edge of the cover slip where it lied above that part of the slide containing graticules (ruling). The numbers of motile and non-motile sperms of treated mice were counted under the X40.

Histological study

At the end of the administration, mice were sacrificed, testes of treated mice were removed and then were fixed in formalin for 24 hours. The specimens were washed twice with 70% alcohol. The fixed tissues were dehydrated in an ascending series of alcohol ranging from 70% to 100% (absolute). The dehydrated tissues were cleared in xylene (twice), infiltrated and then were embedded in paraffin wax. The testes were sectioned on a rotary microtome, sections were 5µm in thickness. The prepared sections were stained by routine methods using Hematoxylin-eosin method. The stained sections were examined under the microscope and the different cell types were carefully studied and photographed.[27]

Testis sections from each study group were evaluated for structural changes, blind by a histologist. Light microscopy (Leica , Germany) was used for the evaluations.

RESULTS

The mean value of sperm count increased significantly ($P = 0.049$) in the group treated with selenium compared to the group injected with water (control group); also sperm count was significantly higher in group treated with selenium compared to the group with combined treatment of selenium and formaldehyde (prophylactic) at $P = 0.063$ (Table 1).

Sperm count was increased significantly in the group treated with selenium when compared to groups injected with water, formaldehyde or combined treatment of formaldehyde and selenium (prophylactic) at $P \leq 0.05$. Combined treatment of formaldehyde and selenium (curative) showed significant increase in the total number of sperm compared to the group injected with water (control) at $P \leq 0.05$.

The number of motile sperm in selenium treated group was significantly higher than the water injected group, formaldehyde treated groups or prophylactic group by administration of formaldehyde and selenium ($P \leq 0.05$) (Table 1).

The number of normal sperm morphology was decreased in formaldehyde treated group compared to the control, selenium, or combined treatment of formaldehyde and selenium (prophylactic and curative); while the number of abnormal sperm morphology in formaldehyde treated group was significantly increased compared to the control, selenium, or combined treatment of formaldehyde and selenium (prophylactic and curative) at $P \leq 0.05$ (Table 2).

The body weight of mice did not change significantly with different treatments ($P > 0.05$).

Histological studies

Group I:

Sections of the testes of the control group showed the normal histological features of seminiferous tubules; closely packed seminiferous tubules, separated from each other by narrow interstitial spaces containing interstitial cell of Leydig, surrounded by blood capillaries (Figure 1,a). These seminiferous tubules containing spermatogenic cells and Sertoli cells; Sertoli cells have pale cytoplasm which were observed among the spermatogenic cells. The spermatogenic cells were formed of spermatogonia, primary spermatocytes and spermatids. The spermatogonia appeared as small cells with dark, ovoid nuclei. These cells are located basally in the epithelium next to the basement membrane; lumen of seminiferous tubules are occluded by many spermatozoa (Figure 1,b).

Group II:

Seminiferous tubules of selenium treated group contain normal spermatogenic cells. The spermatogenic cells are spermatogonia, primary spermatocytes and elongated spermatids; the interstitial spaces are clearly visualized and contain Leydig cells and blood vessel. Testicular architecture was close to normal morphology (Figure 2, a, b). There was no obvious great variation between group I and group II, where the histological structures of the seminiferous tubules in the selenium only-treated group was similar to that of the control group.

Group III:

The histological sections of the testes of formaldehyde

treated group showed variable degrees of the testicular tissue degenerative changes in some tubules, with marked intercellular and basal vacuolation (Figure 3,a), detachment of spermatogonia from the basement membrane and separation between germinative cells in the seminiferous tubules. Widened interstitial spaces were observed (Figure 3, b, c). In some seminiferous tubules there are slightly decrease in density of germinal cells. Some Leydig cells were degenerated (Figure 3, d).

Group IV:

The histological sections of the testicular tissue of selenium and formaldehyde treated group (prophylactic treatment) showed affections of some seminiferous tubules with more or less normal cellularity (normal spermatogenesis process), other tubules still show intercellular vacuolation with relatively normal spermatogenic cells and normal interstitial cells (Figure 4, a, b).

Group V, Selenium and formaldehyde treated group (curative treatment)

In this group, the structural changes is similar to that found in groups III and VI. Comparison to the control group, disorganization in some seminiferous tubules, cellular irregularity and vacuolization between spermatogenic cells and also a thickness in basement membrane of spermatogenic epithelium were observed in some tubules (Figure 5, a, b).

Table 1: Mean value of Sperm count and percentage of sperm motile and non-motile at different treated groups.

Treatments	Total count (10^6/ml)	Motile sperm	Non-motile sperm
Water for injection	9.87 ± 0.86	7.07 ±0.50 (71.6%)	2.8 ± 0.70 (28.36%)
Selenium (100µg/kg)	19.08 ± 0.44*	16.67 ± 0.17[a] (87.36%)	2.41 ± 0.22 (12.6%)
Formaldehyde (30mg/kg)	12.25 ± 0.15[b]	9.17 ± 0.37[b] (74.85%)	3.07 ± 0.40 (25.06%)
Formaldehyde + Selenium (Prophylactic)	11.7 ± 0.22[b]	9.96 ± 0.34[a] (85.12%)	1.73 ± 0.12 (14.78%)
Formaldehyde + Selenium (Curative)	14.87 ± 0.61*	13.61 ±0.93[a] (91.52%)	1.25 ± 0.88 (8.40%)

*, a, Significantly different compared to water injection at $P \leq 0.05$; b, Significantly different compared to selenium at $P \leq 0.05$

Table 2: Mean value and percentage of morphological abnormal sperm of different treated groups.

Treatment groups	Abnormal	Normal	Total
Water for injection	107 ± 7.06* (20%)	435.12 ±5.56 (80%)	542.12 ± 5.09
Selenium 100µg/kg	96.85 ±3.62* (18.17%)	436 ± 7.09* (81.82%)	532.85 ± 5.96
Formaldehyde 30 mg/kg	389.87 ± 8.00 (72.29%)	149.37 ± 6.08 (27.69%)	539.25 ± 8.58
Formaldehyde + Selenium (Prophylactic)	93 ± 3.50* (17.30%)	444.33 ± 4.19* (82.69%)	537.33 ± 7.07
Formaldehyde + Selenium (Curative)	107.14 ± 3.52* (20.36%)	419 ± 5.32*a (79.63%)	526.14 ± 7.20

*, Significantly different compared to formaldehyde at $P \leq 0.05$; a, Significantly different compared to selenium + formaldehyde prophylaxis at $P \leq 0.05$.

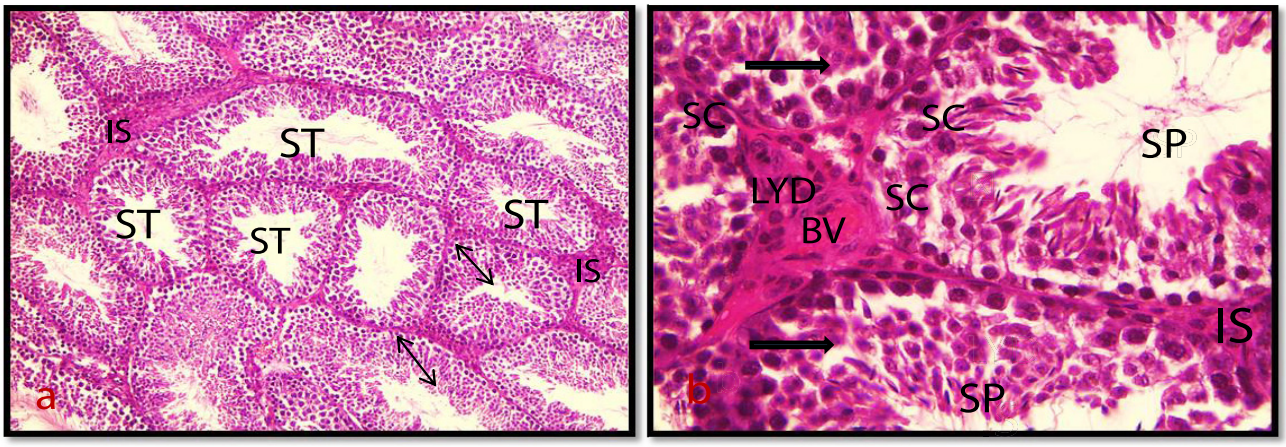

Figure 1 : A photomicrograph of a section of control group showed;
a) closely packed seminiferous tubules (ST), separated from each other by narrow interstitial spaces (IS) , lined by normal spermatogenic cells (⟷). **(H&E, 10x) .**
b) The interstitial spaces containing interstitial Leydig cells (LYD) around blood Capillaries (BV). The seminiferous tubules were lined by spermatogenic cells (⟶) and Sertoli cells (SC), many spermatozoa (SP) present in the seminiferous tubules, **(H&E, 40x).**

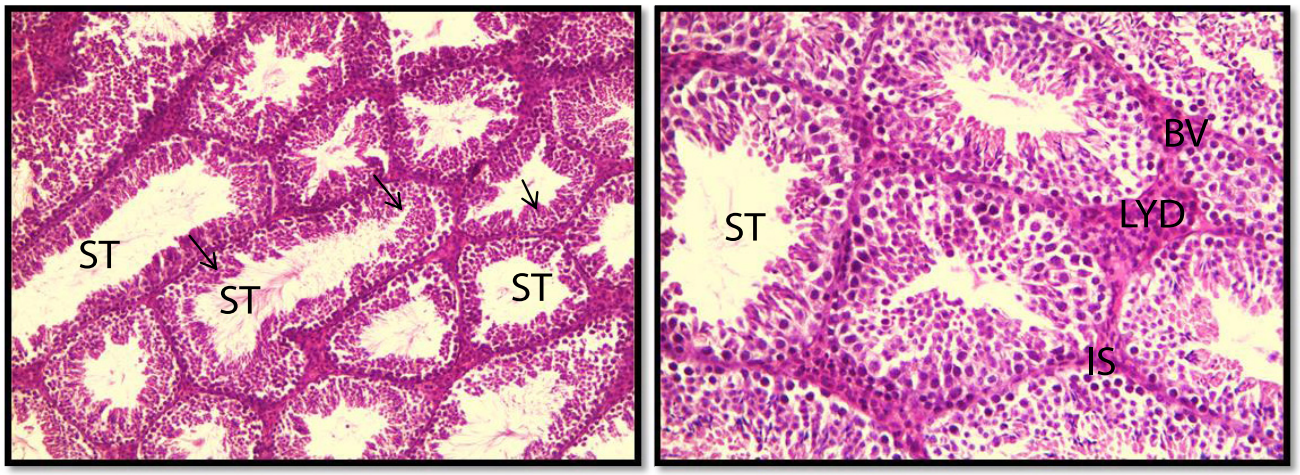

Figure 2 : A photomicrograph of a testis section of group II showed;
a) Seminiferous tubules (ST) lined by normal spermatogenic cells (⟶), **(H&E,10x)** .
b) The interstitial spaces (IS)are clearly visualized and contain Leydig cells (LYD) and blood vessels (BV), **(H&E,20x)**

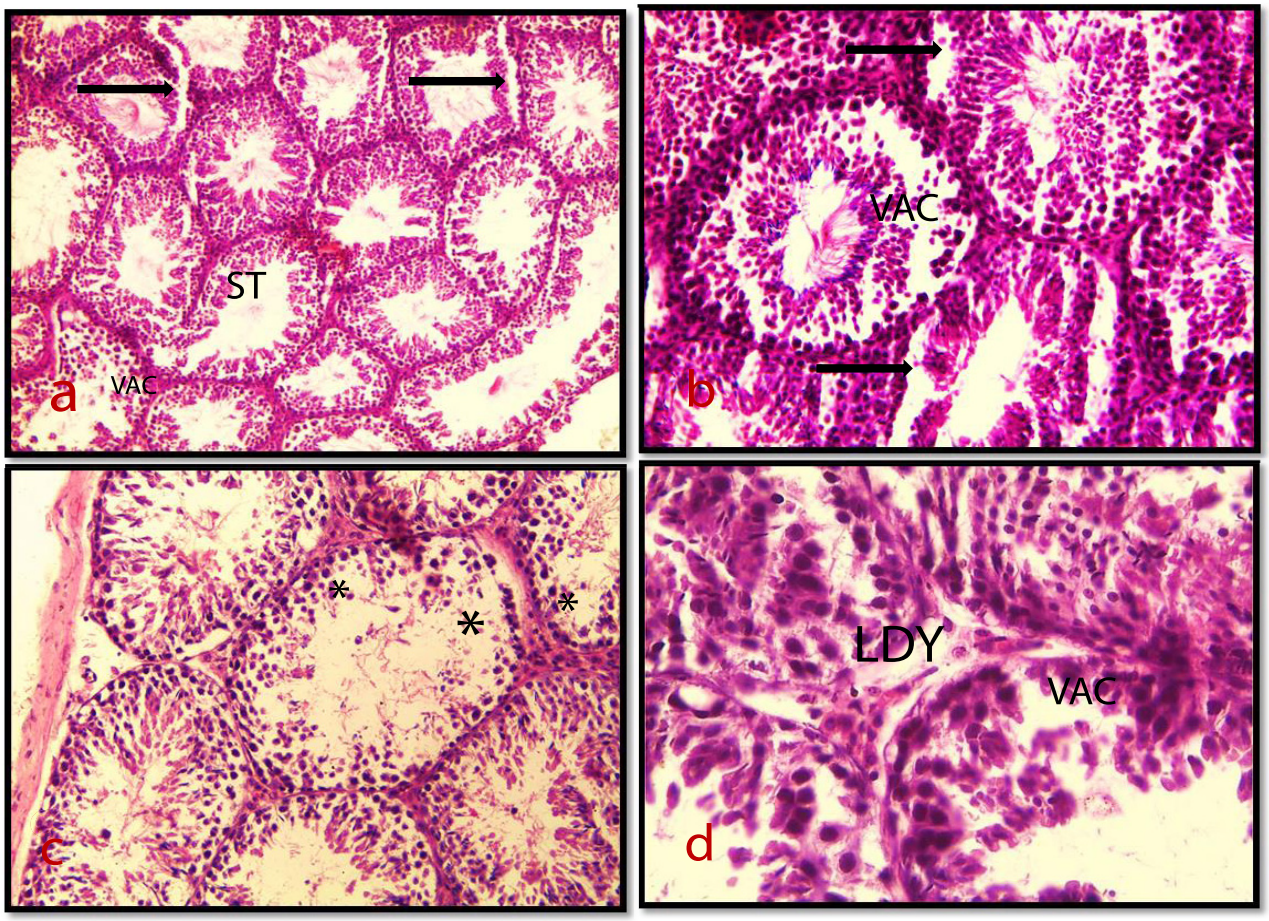

Figure 3 : A photomicrograph of a Testis section of group III showed;
a) Affected seminiferous tubules (ST) with detachment of spermatogonia from the basement membrane (⟶), other tubules showed intercellular vacuolations (VAC) **, (H&E,10x) .**
b) Affected seminiferous tubules (ST) with detachment of spermatogonia from the basement membrane (⟶), other tubules showed intercellular vacuolations (VAC) , **(H&E,20x)** .
c) The seminiferous tubules showed completely loss of spermatogenic cells (*), **(H&E,20x).**
d) Some leydig cell were completely destroyed (LYD) **, (H&E,40x).**

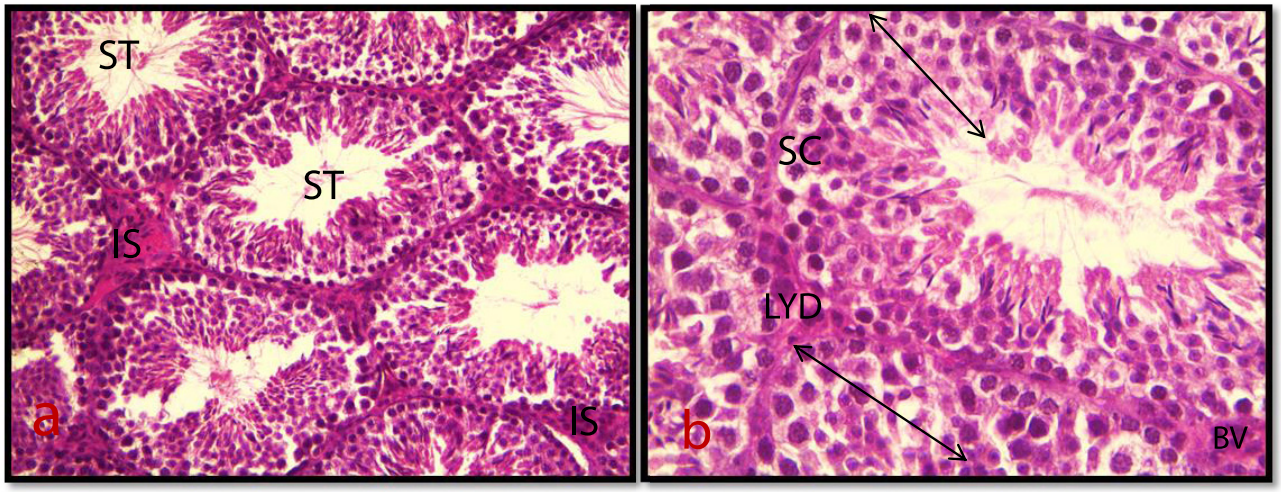

Figure 4 : A photomicrograph of a testis section of group IV showed;
a) Close to normal (ST) , and intersteium (IS) , **(H&E,10x) .**
b) The interstitial spaces containing interstitial Leydig cells (LYD) around blood Capillaries (BV). The seminiferous tubules were lined by normal spermatogenic cells (⟷) and Sertoli cells (Sc) ,many spermatozoa (SP) present and in the seminiferous tubules, **(H&E,40x) .**

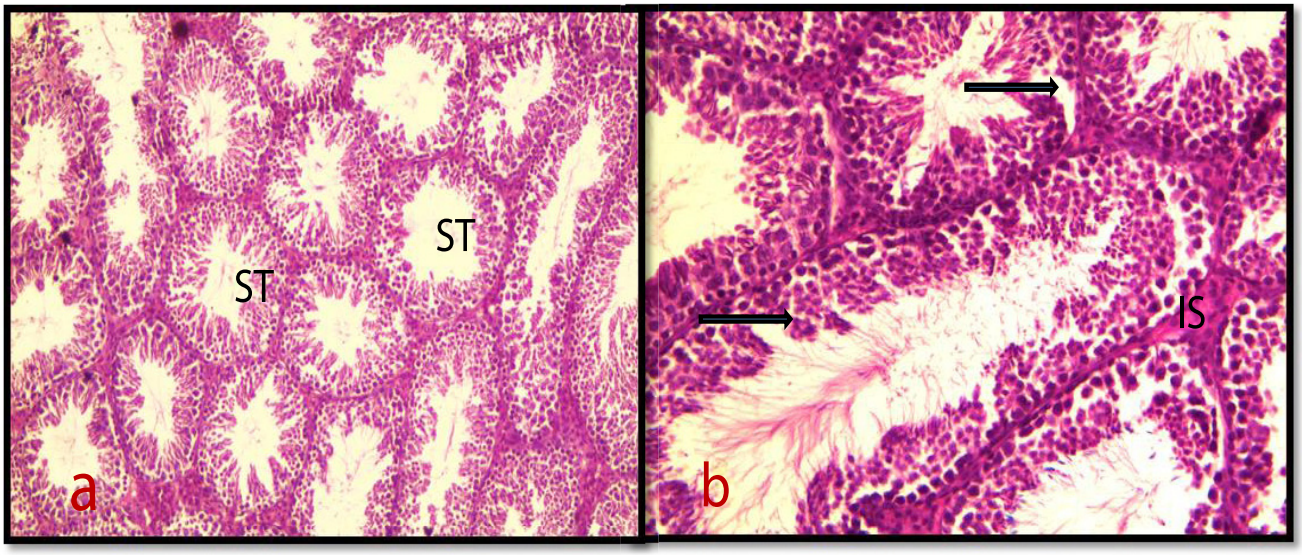

Figure 5: A photomicrograph of a Testis section of V group showed;
a) The testicular tissue showing some seminiferous tubules (ST) with more or less normal cellularity **(H&E,10x) .**
b) other tubules (ST) still show intercellular vacuolation (⟶)with relatively normal Spermatogenic cells and normal interstetium (IS) **, (H&E,20x) .**

DISCUSSION

Formaldehyde toxicity on sperm count and percentage of motile sperm were not changed. Sperm abnormalities are increased, while healthy sperms are decreased. Formaldehyde produces histological degeneration/damage to the male mice testis.

Formaldehyde is a human carcinogen, its reproductive toxicity showed more in male than female exposure studies; this skewing towards male studies may be because the effects on male reproduction are more readily observable and require fewer invasive procedures.[28]

Formaldehyde significantly decreased the activities of superoxide dismutase and glutathione peroxidase, also the activations of necrosis factor-κB (NF-κB) and activator protein 1 were induced by the formaldehyde treatment.[29] Formaldehyde is genotoxic, inducing chromosomal aberrations[30], DNA breakage[31] and DNA-protein crosslink.[22,32]

Excessive oxygen species production can cause developmental toxicity through oxidative damage to key cellular components such as DNA, proteins and lipids.[28] Reactive oxygen species-mediated oxidative damage resulting from formaldehyde exposure has been detected in distal cells and tissues, including the reproductive tissues.[33]

Rodents exposed to formaldehyde by inhalation exhibited lipid peroxidation[33]. Lipid peroxidation product commonly used as a biomarker of oxidative damage[34], was significantly increased in the testicular tissues of male mice treated with formaldehyde.[10] Formaldehyde impaired antioxidant cellular defenses and enhanced lipid peroxidation.[10,29]

Antioxidant enzymes (GSH-Px, SOD, CAT and GSH) have been demonstrated in male reproductive tissue; GSH-Px levels and GSH levels were lowered in formaldehyde exposed testicular tissue in mice; while superoxide dismutase (SOD) and catalase (CAT) levels were significantly elevated.[35]

Lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH) are involved in the maturation of spermatogenic cells, testis and spermatozoa and with the energy metabolism of spermatozoa. In mice, SDH activity was decreased in testicular tissue after exposure to formaldehyde.[36] Succinate dehydrogenase activity was positively correlated with sperm cell counts, and negatively correlated with the abnormal rate of sperm heads.[10]

Apoptosis rate was increased in the testicular tissue of rats exposed to formaldehyde; morphological abnormalities of the testicles and an increased number of abnormal sperm were also observed in the exposed rats.[37]

Formaldehyde-induced male reproductive toxicity mediated through aberrant DNA methylation. Abnormal DNA methylation has been associated with male gametogenic defects.[38]

Serum testosterone levels were decreased in male mice and rats subjected to formaldehyde exposure leading to disruption of male reproductive function.[37,39,40] Formaldehyde may exert adverse effects on the reproductive system without reaching it, through a stress-induced mechanism; it was found that severe stress leads to decrease in sperm count, motility and morphology in men.[41] Stress-induced reproductive toxicity could be mediated by effects on the endocrine or other regulatory systems.[28]

In formaldehyde toxicity sperm count and the percentage of motile sperm were not changed, this may be due to the short period of formaldehyde administration.

In this study, selenium has prophylactic and curative effects against formaldehyde-induce testis and sperm parameters toxicity.

There is significant positive correlations with selenium concentration and different reproductive organs; it was found that the testes have the highest concentrations of selenium.[42] In selenium-deficient rats, flagellar abnormalities during

spermatogenesis and post-testicular sperm development were observed, also the selenium-deficient animals exhibited extensive flagellar disorganization. Loss of male fertility in selenium deficiency results from the sequential development of sperm defects expressed during both spermatogenesis and maturation in the epididymis.[43]

Pretreatment with selenium counteracted the formaldehyde-induced oxidative stress, ameliorated DNA–protein cross-links and attenuated the activation of necrosis factor-κB. Selenium attenuated the formaldehyde-induced genotoxicity through its reactive oxidative species scavenging and anti-DNA–protein cross-link effects[28]; it may protect sperm DNA against oxidative stress damage.[14]

CONCLUSION

Formaldehyde toxicity on sperm count and percentage of motile sperm were not changed. Sperm abnormalities had increased, while healthy sperms had decreased. Formaldehyde produced histological degeneration/damage to the male mice testis.

Selenium alone produced an increase in sperm count, the percentage of motile sperms had increased, the volume of seminal fluid was larger, the normal sperms shape had increased, while abnormal sperms had decreased. There were no changes in the seminiferous tubules cells, it was found that the testicular architecture is closed to normal morphology.

Selenium and formaldehyde combination (Prophylaxis) produced no change in sperm count, the percentage of sperms motility had increased, the normal sperms morphology had increased, while abnormal sperms shape had decreased. The testicular tissue showed some seminiferous tubules changes with more or less normal cell structure.

Selenium and formaldehyde combination (Curative) produced a slight increase in sperm count, the percentage of motile sperms increased; the normal sperms shape increased, while abnormal sperms decreased. The seminiferous tubules showed relative structural changes with some normal cell structure.

From this study, we found that selenium has prophylactic and curative effects against formaldehyde-induce testis and sperm parameters toxicity. Our recommendation for future work is to find out if selenium protective effect is through antioxidant or other mechanisms.

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