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Original Article

Effect of Paracetamol on Sperm Biological Parameters and Testosterone Level in Albino Male Mice

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ABSTRACT

Backgrounds and objectives. The wide use of paracetamol at high doses was found to alter sperm parameters especially sperm morphology, and thus its fertilizing capability. Therefore, the present study was designed to use different doses of paracetamol to identify its effect on sperm parameters and testosterone levels in adult male mice. **Methods.** Forty adult male albino mice were divided into four equal groups, the first group injected with distilled water, the three treated groups injected with different doses of paracetamol (20, 40, 80 mg/kg body weight /day) over a period of 42 days. All doses were given once daily via intraperitoneal injection. **Results**. The results showed that paracetamol causes a significant decrease in body weight, non-significance effect on sperm parameters at doses of 20 and 40 mg/kg, while it led to a significant effect on sperm parameters at a dose of 80 mg/kg. Also, there was no difference in testosterone level between control and the treated groups (20 and 40mg/kg). But it showed a significant decrease in testosterone level at dose 80 mg/kg treated groups. **Conclusion**. It is considered safe to use paracetamol at doses 20 and 40 mg/kg but the dose 80 mg/kg has adverse effects on sperm parameters and testosterone level.

Keywords: Paracetamol, Sperm Parameters, Mice.

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INTRODUCTION

Infertility, defined as the inability to achieve pregnancy after 12 months of regular intercourse, affects 15% of reproductive-aged couples [1]. A quarter of these cases can be explained by male-only factors, and up to 50% can be explained by combined male and female factors. Many infertile men have abnormal semen analyses, of which the etiology is often poorly explained. These etiologies can include environmental, dietary, medical, genetic, and physiologic factors [2]. Male infertility was increased during the last years, in recent years, there has been

an increasing awareness and realization of the genotoxic potentials of a wide variety of drugs such as paracetamol.

Paracetamol is a nonsteroidal anti-inflammatory drug used widely as a painkiller for various diseases and as the symptomatic flu cure in several countries worldwide habitual ingestion of paracetamol which is capable of inducing structural chromosomal aberrations in somatic cells in vivo [3]. This awareness follows the recent development of appropriate, sensitive and practical methods for detecting and estimating the toxic effects of these substances and their impact on environmental health. DNA synthesis in the testis, spleen, thymus, stomach, small intestine and bone marrow was reported to have been inhibited by 70-90% at 1hour following an oral dose of 1g/kg of paracetamol in rats [4]. Increases incidence of abnormal sperm has been reported in albino rats treated with formaldehyde, [5] and also high temperatures, extreme nutritional deficiencies and some diseases in a wide range of species including mice and man [6]. paracetamol has also been shown to inhibit replicative DNA synthesis in V79 cells, without causing cell injury, at concentrations over about 0.5 mM [7]. Inhibition of DNA synthesis by paracetamol is due to a specific inhibition of the enzyme nucleotide reductase which catalyzes the reduction of ribonucleotides to deoxyribonucleotides. paracetamol destroys the tyrosyl free radical of the active site located on the small subunit of the enzyme [8].

METHODS

Preparation of the drug

About 200mg of paracetamol tablets have been dissolved in 20 ml of distilled water to prepare a stock solution 10 mg/ml, then 2, 4 and 8 ml of this stock solution were taken and added to 8-, 6- and 2-ml distilled water respectively, to prepare the concentration of 2, 4 and 8 mg/ml, that used to dose T1, T2, T3 animal's groups at a dose 0.1 ml/10g body weight, which is equivalent to a concentration of 20, 40, and 80 mg/kg body weight /day respectively.

Ethical approval

All experimental procedures and animal maintenance were performed according to the bioethical research guide established by the Libyan National Committee for Biosafety and Bioethics.

Laboratory animals and sample collection

Male albino mice were bred in the animal house of the Zoology department, Faculty of Science, University of Tripoli, Libya. Mice were housed in plastic cages containing wooden flakes in an air-conditioned room. They were kept under standard laboratory conditions (24 to 26^0C, and 55 to 60% humidity) with a 12-hourlight/dark cycle, and fed standard commercial laboratory chow. Water and standard pellet diet were available ad libitum throughout the experimental period.

Experimental design

A total of 40 white albino male mice aged between 8-10 weeks and their weights between 26-28 grams were used in this study. Mice were divided into four groups of ten mice each. The first group (control) injected with distilled water intraperitoneally (i.p.), the second group (T1) injected with 20 mg/kg body weight/day, third group (T2) injected with 40 mg/kg body weight and fourth group (T3) injected with 80 mg/kg body weight/day body weight of paracetamol respectively for 42 days. Mice weighed at the beginning of the experiment, before injection, and before sacrifice. At the end of the experiment, mice were killed by decapitation. Mice were processed and evaluated for sperm count, sperm motility and sperm morphology (abnormalities). Blood samples from the control and treated mice were collected for estimation of testosterone level in (different groups).

Seminal Fluid collection

Groups of treated and untreated mice were killed by cervical dislocation. Sperm of each mouse were obtained by squeeze the vasa differentia 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 [9].

Determination of sperm count

Sperm count was made using previous method [10]. The sperm were counted by charging both chambers of improved Neubauer hemocytometer (American optical Co., Buffalo. N.Y) 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 magnifications for morphological abnormalities. The criteria for abnormal sperm morphology included tail and head abnormalities. The result was expressed as percentage of abnormal sperm [11], Also mutation factor and mutation indices were calculated by the following equations [12]:

$$\text{Mutation factor (MT)} = \frac{frequency\ of\ abnormal\ sperm\ heads(treated)}{\text{frequency of abnormal sperm heads(control)}}$$

$$\text{Mutation Index } (MI) = \frac{\text{frequency of abnormal of sperm heads(treated}-\text{control)}}{\text{frequency of abnormal sperm heads(control)}}$$

Determination of sperm motility

The percentage of sperm motility was microscopically evaluated according to previous method [13]. A drop of diluted sperm suspension was loaded onto the improved Neubauer hemocytometer and the number of motile and non-motile sperms was counted under a magnification 400X. The number of motile and nonmotile spermatozoa was expressed as percentage from the total number of counted spermatozoa.

Determination of serum testosterone levels

Testosterone levels were determined by enzyme-linked immunosorbent assay commercial kit, following the procedures outlined by the manufacturer (BioChek). Clotted blood samples of control and treated groups were centrifuged for 15 minutes at 3,000 rpm to separate the serum and were stored at −20°C until measurement of testosterone hormone level.

Statistical analysis.

The data were expressed as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's test, to compare the significance of the difference between the control and treated groups for body weight and sperm parameters. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Body weights

The results of this study showed that administration of different doses of paracetamol for 42 days caused a change in body weight of albino male mice when compared with control group (table 1). Body weights of the control, T1, T2 and T3 were 28.8±0.8g, 26.8±1.8g, 24.4±2.5g and 26.8±1.6g before injection respectively, while at the end of experiment the body weight of control and treated groups were respectively, 30 ±1, 25.8 ± 0.8, 25.4 ± 1.8 and 25.8 ± 0.8. Apparent decrease of body weight was observed in T1 and T3.

Table 1. Effect of paracetamol treatment on mean value of mice body weight (g).

Groups	Weight before treatment	Weight after treatment
Control	28.8±0.8[a]	30±1.0a
T1 (20 mg/kg)	26.8±1.8[a]	25.8±0.8[b]
T2 (40 mg/kg)	24.4±2.5[b]	25.4±1.8[c]
T3 (80 mg/kg)	26.8±1.6[a]	25.8±0.8b[c]

Values are presented as means ± SD (n=10). The mean difference is significant at the P ≤ 0.05 level. a, b,c, show the significant difference at (P ≤ 0.05).

Sperm parameters

The effect of paracetamol injection on sperm motility, count and abnormality is shown in (Table 2). The results of this study showed that there was non-significance increase (P > 0.5) in percentage of abnormal sperm morphology and non-significance decrease (P > 0.5) in both sperm counts and sperm motility at the doses of 20 and 40 mg/kg paracetamol compared to the control group. However, administration of paracetamol at a dose of 80 mg/kg caused a significant (P < 0.05) decrease in sperm counts and sperm motility with associated significant (P < 0.05) increase in the percentage of abnormal sperms morphology as compared with the control group. Sperm morphology test of treated mice with different doses of paracetamol showed different sperm phenotype abnormalities such as normal sperm, distal bent tail, bent middle piece, medial protoplasmic sperm, folded mid piece, amorphous head, fused head, proximal bent (fig.1). In addition to sperm morphology test, a sperm morphology assay tail showed a significant increase in mutation factor and mutation index at the highest dose of paracetamol (80 mg/kg) when compared with the other groups T1, T2 and control (Table 3).

Table 2. Effects of different doses of paracetamol on sperm parameter of male mice.

Treatment	Sperm count (10^6/ml)	Motile sperm (%)	Abnormal sperm morphology (%)
Control	26.2±1.3[a]	94.9±2.6[a]	14.1±2.5[b]
T1(20 mg/kg)	26.2±0.8[a]	93.4±2.0[a]	16.3±2.3[b]
T2(40 mg?kg)	25.8±0.8[a]	92.0±2.8[a]	17.0±1.8[b]
T3(80mg/kg)	22.8±1.3[b]	36.5±30.1[b]	32.2±10.0[a]

Values are presented as means ±S D (n=10). The mean difference is significant at the P ≤ 0.05 level. a, b shows the significant difference at (P ≤ 0.05).

Table 3. Effect of different doses of Paracetamol on the frequency and mutagenicity of abnormal sperm of male mice.

Analgesic Paracetamol	Frequency of Abnormal sperm (%)	Increase in Frequency of (%) abnormal sperm	Mutation factor	Mutation index
Control	12.32	.000	1.00	00.0
T1	14.44	12.54	1.17	0.17
T2	14.52	8.58	1.18	0.18
T3	22.76*	29.38*	1.84*	0.84*

*Values are presented as means ±S D (n=10). The mean difference is significant at the P ≤ 0.05 level. * Significant difference (P < 0.05) as compared with control group*

Table 4. Effect of different doses of paracetamol on the level of testosterone (ng/ml) of male mice.

Testosterone levels (ng/ml)	Control	T1	T2	T3
	4.0±1.4[a]	3.4±2.4[a]	2.5±0.8[a]	2.4±0.8[b]

Values are presented as means ±S D (n=10). The mean difference is significant at the P ≤ 0.05 level, a, b show the significant difference at (P ≤ 0.05).

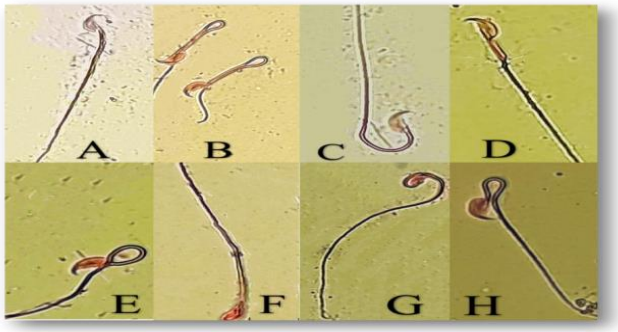

Figure 1. Different shapes of sperm morphology: normal sperm (A), distal bent tail(B), bent middle piece(C), medial protoplasmic sperm(D), folded mid piece(E), amorphous head(F), fused head(G), proximal bent tail(H).

Testosterone level in the blood

The mean value of testosterone levels in control and treated groups were 4.0±1.4, 3.4±2.4, 2.5±0.8 and 2.4±0.8 ng/ml, respectively. Testosterone level was slightly reduced in T1 and T2 group (20 and 40 mg/kg), while it deceased significantly in T3 (P≤ 0.05) in comparison with the control group indicates harmful changes in the Leydig interstitial cells of testes, which are responsible for testosterone biosynthesis and secretion.

DISSCUSION

Paracetamol is used widely as a painkiller in various diseases in several countries. Although, it is accepted to be safe when taken in doses recommended by physicians. Regular usage of the drug is a risk factor for drug toxicity, which appear in certain types of tissues including the liver, kidneys and testes.[14]

The evaluation of male reproductive status depends on the measurements of sperm parameters, which include sperm count, sperm motility, sperm viability, and sperm morphology [15].

The present study showed a significance reduction in the body weight of the paracetamol treated groups (T1 and T3) when compared to control group. The decline in body weight may be due to decrease in food intake or over activity, these results were similar to the study of Ali [16] who elucidate that injection of male mice with paracetamol caused a considerable decline in body weight when compared with control group, while this study was disagree with other studies [17,18] who showed that paracetamol injection did not decrease the body weight.

Treatment of mice with paracetamol caused a non-significant decrease in sperm count and percentage of sperm motility together with a non-significant increase in abnormal sperm morphology at doses of 20 and 40 mg/kg compared with the control group. On the other hand, there was a significant decrease in both sperm count and sperm motility at a dose of 80 mg/kg of paracetamol, this agrees with the study of [19] who found similar effects following treatment of rats with 500 mg/kg paracetamol for 5 days. On the contrary, 80 mg/kg of paracetamol caused a considerable increase in abnormal sperm morphology as compared with the control group. These results are consistent with previous studies [20, 21] who illustrated that administration of 500 mg/kg of paracetamol to rats for 30 days [20] and to mice for 4 days [21] led to a significant increase in abnormal sperm morphology.

In addition, paracetamol injection showed gradual increase of mutational effects on the sperm morphology in different doses, which was in agreement with a study by Ekaluo et al., [22].

The reduction in sperm count that noticed in this study at 80 mg/kg could be attributed to the decline in the concentration of testosterone through induction of Leydig cell damage that disrupts testosterone synthesis. Adequate levels of testosterone are needed to maintain normal spermatogenesis. The toxicological effects of high doses of paracetamol are probably related to increased reactive oxygen species (ROS) which induced apoptosis and DNA damage [23,24].

Moreover, the results of this study showed that there was no significance difference in the serum testosterone levels at doses 20 and 40 mg/kg when compared with the control, a similar result was also reported by Hassan [25] who found that a low dose of paracetamol has no effect on the testosterone level of male rabbit. However, the highest dose of paracetamol (80 mg/kg) caused a significant decrease in testosterone level, it might be due to harmful changes in the Leydig interstitial cells of testes, which are responsible for testosterone biosynthesis [26]. However, the current result was consistent with other studies [19, 27] which showed a significant difference in testosterone level following administration the high dose of paracetamol (500 mg/kg) [19] and 350 mg/kg [27]. This indicates that only high doses of paracetamol cause significance reduction of serum testosterone levels. This evidence indicates that paracetamol has an antigonadotrophic

effect in males, which may alter semen quality and sperm parameters.

Several studies have been conducted to explain the increase in the frequency of occurrence of sperm anomalies in organism exposed to some environmental chemicals. In general, damage to the sperm cell might be happen by either physiological, cytotoxic or genetic mechanism. According to Agarwal et al., Nowicka-Bauer, and Nixon [28,29] oxidative stress is linked with the overproduction of free radical, such as reactive oxygen species (ROS) which have been considered as pathophysiology of male infertility. Physiological levels of ROS are essential for natural sperm function and in the development of sperm hyper activation and capacitation [28, 30]. On the other hand, overproduction of ROS induced lipid peroxidation in the sperm membrane leading to loss of motility [28], damage to the acrosomal membranes, and DNA oxidation and thus loss the ability of fertilization [31]. Occurrence of sperm abnormalities have been attributed to chromosomal aberrations during the packaging of genetic material or occurrence of point mutation in testicular DNA or of natural occurring level of mistakes in sperm differentiating process [32 ,33].

CONCLUSION

The results of the present study showed that the continues injection of high dose of paracetamol drug caused significant testicular damage, decline in sperm count motility and testosterone levels, and an increase in morphological sperm abnormalities. Therefore, prolonged injection or exposure may induce reproductive failure and could be a cause of male infertility.

Disclaimer

The article has not been previously presented or published, and is not part of a thesis project.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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