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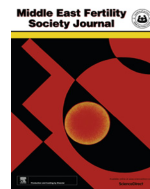

Original Article

Short and long term effects of different doses of paracetamol on sperm parameters and DNA integrity in mice

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ABSTRACT

The aim was to survey the impact of normal and high doses of paracetamol consumption on sperm parameters and DNA integrity in mice. A total of 36 adult male mice were divided into three groups: mice of group A served as control fed on basal diet, group B received normal dosage of Paracetamol (66 mg kg/day) and basal diet, group C received high dosage of Paracetamol (100 mg kg/day) and basal diet for 35, 70 and 105 days. The cauda epididymitis of each mouse was dissected and placed in 1 ml of pre-warm Ham's F10 culture medium for 20 min. The swim-out spermatozoa were analyzed for count, motility, morphology and viability. Sperm chromatin quality was evaluated by chromomycin A3 staining (CMA3), aniline blue staining and sperm chromatin dispersion test (SCD). The results showed that almost all of the sperm parameters significantly decrease following consumption of normal and high dosage of Paracetamol in three periods of experiments in mice ($p < 0.05$). Regarding to SCD test, we found a highly significant difference only in dose effect, but in CMA3 test and aniline blue staining there was a significant difference ($p < 0.05$) in both dose and time effects. According to our results, paracetamol as an analgesic and antipyretic may have detrimental effects on sperm parameters and DNA integrity in mice.

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1. Introduction

Infertility is a major problem in up to 15% of the sexually active population and male factor is responsible in 50% of these cases [1]. As far as, lifestyle has some directly impresses on male infertility, sperm DNA quality and sperm parameters [2], we study paracetamol as a common pain killer. Acetaminophen (paracetamol) is widely used as an analgesic and antipyretic without prescription. The most commonly used over-the-counter (OTC) pain medication is acetaminophen along with aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) [3]. Paracetamol has a range of action like that of NSAIDs and be similar to particularly the COX-2 selective inhibitors. It is usually accepted that it inhibits COX-1 and COX-2 through metabolism by the peroxidase function of these isoenzymes. This results in inhibition of phenoxyl radical formation from a critical tyrosine remains needed for the cyclooxygenase activity of COX-1 and COX-2 and prostaglandin (PG) synthesis. Paracetamol is, on average, a weaker analgesic than NSAIDs or COX-2 selective inhibitors but is often chosen because of its better tolerance [4].

It is shown that overdosing with acetaminophen can cause hepatic necrosis in both humans and laboratory animals [5], and prolonged human use has been implicated in chronic renal disease [6] necrotic changes in lung [7], testis, lymphoid tissue of mice [8] and asthma in children [9]. Moreover, genotoxic effects of acetaminophen have been observed [10]. In vivo, acetaminophen has been shown to cause chromosome aberrations in bone marrow cells from exposed mice [3]. High doses of acetaminophen have also been reported to lead to testicular atrophy and decrease of testosterone hormone in vitro in rat and human [11–13].

There is a clear negative relationship between sperm chromatin/DNA damage and reproductive outcomes. Furthermore, it is generally accepted that the sperm chromatin condensation has a key role in male fertility, early embryonic growth and pregnancy outcomes [14]. The inter and intra molecular disulphide bonds of protamine molecules are essential for sperm nuclear compaction and stabilization. It is believed that this kind of nuclear compaction protects sperm genome from external damages including oxidative stress; temperature height and acid-induced DNA denaturation [15]. There are some kinds of tests for sperm chromatin/DNA evaluation which show different forms of damages. Chromatin struc-

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tural probes by nuclear dyes with cytochemical bases are sensitive, easy and economical which do not need exclusive device like flow cytometry [16]. There are some studies that showed effects of paracetamol on sperm parameters in mice and Rat [3,12,17]. Hence, we designed this study for the first time to evaluate Short and long term effects of different doses of paracetamol on sperm parameters and chromatin condensation in three period of spermatogenesis in mice by cytocemical tests.

2. Materials and methods

2.1. Animal

In this experimental study, totally 36 adult male NMRI mice with average weight 37 g and 11 ± 0 weeks old which were obtained from the animal house of Research & Clinical Center for Infertility of Shahid Sadoghi University of Medical Sciences. These mouse were divided into 3 groups; control (group I, n = 12), normal dosage (group II, n = 12) and high dosage (group III, n = 12) and each group was treated during three different periods with considering of mice spermatogenesis duration. Hence each group was divided into three subgroups (each n = 4) by 35, 70 and 105 days with or without treatments. Group II received normal dosage of acetaminophen which was calculated by the formula

$$D = MTR \tag{1-1}$$

where M is maximum recommended daily human dosage in tablets, R is ratio of weight of rat to average adult human weight of 60 kg and Tis weight of each tablet [18]. So, 66 mg/kg body weight acetaminophen was dissolved in daily water. The last group was given 100 mg/kg acetaminophen [19], but, in control group, we did not receive any medication. As we know acetaminophen is very bitter is the reason why we dissolved 0.002% and 0.003% saccharine in the daily water of group II and III. During experiments, animals were kept in standard condition with a temperature range of 25 ± 3 °C and mean relative humidity of 50 ± 5% in the animal house. This experimental study was approved by ethical committee of clinical center for infertility Shahid Sadoghi University.

2.2. Epididymal sperm preparation

We studied the spermatozoa after 1, 2 and 3 durations of spermatogenesis following drug treatments [21]. So, the mice were killed after 35, 70 and 105 days by cervical dislocated and the cauda epididymis of each animal was cut and placed in 1 ml Ham'sF10 medium. The dishes were incubated at 37 °C and 5% CO2 for 10 min to make spermatozoa swim out [21].

2.3. Sperm analysis

The sperm count, motility, normal morphology and viability (%) were evaluated for at least 200 spermatozoa from each animal. Sperm count and motility were evaluated by Meckler Chamber (Sefi Medical Co., Haifa) and light microscopy (Olympus Co., Tokyo, Japan). Motility indices were expressed as the percentages of progressive motility (rapid and slow), non-progressive and immotile spermatozoa. The morphologically normal spermatozoa and the percentage of viable sperm cells were assessed by Papanicolau staining and Eosin test respectively [22].

2.4. Sperm chromatin and DNA study

Sperm DNA integrity and chromatin condensation were assessed using Sperm Chromatin Dispersion (SCD) test and chromomycin A3 (CMA3) staining respectively.

2.4.1. Chromomycin A3 staining

CMA3 is a fluorochrome antibiotic which competes with the protamines for binding to the minor groove of DNA and show protamine deficiency. Briefly, the smears were dried and fixed in Carnoy's solution at 4 °C for 10 min. The slides were treated with 150 µl of CMA3 (0.25 mg/ml) in McIlvain buffer for 20 min. After staining in darkroom, they were washed in buffer and mounted with buffered glycerol. In each sample, at least 200 spermatozoa were counted under fluorescent microscope with a 460-nm filter and 100X eyepiece magnification and the percentage of $CMA3^+$ spermatozoa was reported. Bright yellow-stained chromomycin-reacted spermatozoa ($CMA3^+$) were considered as abnormal and yellowish green-stained or no reacted spermatozoa ($CMA3^-$) were considered as mature sperm with normal protamination [23].

2.4.2. Sperm chromatin dispersion (SCD) test

We used the sperm chromatin dispersion test for the assessment of sperm DNA fragmentation. The SCD test was performed via the Halosperm® Kit (INDAS laboratories, Madrid, Spain). Briefly, after adding 50 µl of semen to 100 µl of low melting agarose, 8 µl of this suspension was decanted on coated slide of the kit. A small lamella was put on it and kept on 4 °C for 5 min. The slide was immersed in Denaturant Agent (for 7 min) and Lysis Solution (for 20 min). After dehydrating, the slides were stained with Staining Solution A & B for 7 min [24].

2.4.3. Aniline blue

Aniline blue staining was indicated the surplus histone in chromatin structure. After preparing sperm and spread onto glass side, wait to air-dry. The smears slides were fixed in 3% buffered glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 30 min at room temperature. Afterwards, the smears slides were stained by aniline blue solution for 15 min.

3. Calculation

Statistical analysis was performed by SPSS version 20 for Windows (SPSS Inc., Chicago, IL, USA). Repeated measures analysis of variance (ANOVA) was applied to evaluate the significant differences between dose and time effects. The term 'statistically significant' was used to signify a two- sided P-value <0.05 for sperm parameters and cytochemical tests**.** All data were expressed in mean ± SD.

4. Results

It is significant to be mentioned that our study was performed in three different periods of spermatogenesis and the p-value of Mauchly's Test of Sphericity were more than 0.05 in all obtained results. Hence, we could use repeated measures of variance (ANOVA) with three levels (three different periods) as the most suitable test. As far as we were willing to figure out the effect of two different subjects effect (time and dose) on sperm quality, two-way repeated measures of variance analysis was performed. Summary of this analysis results was expressed separately in two different tables.

Table 4.1 demonstrates means, standard deviation and pairwise comparisons of sperm parameters. The part of pairwise comparisons in Table 4.1 has two subparts. One of them illustrates the comparison among three levels of time (one, two, and three periods of spermatogenesis). The results of this part will be able to answer a question that acetaminophen consumption for three periods of spermatogenesis can affect male fertility more than using it for two or less periods of spermatogenesis. A significant change between using paracetamol for one and three period of spermato-

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