Cytological and Histological Effect of Paracetamol on the Testis and Liver in Albino Mice *Mus musculus*

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Abstract

The purpose of this study is to evaluate the acute toxicity effects of pure paracetamol tablets on laboratory mice. In this study *Mus musculus* mice (6-8) weeks old were used. The toxicity effects were evaluated on fifteen mice, in which they were divided equally into three groups, each one consists of five individual mice. The first group was labeled as a vehicle group and received 5mL/kg DW. The second and third groups were labeled as experimental groups which received 2 g/kg and 5 g/kg of paracetamol respectively. After fifteen days the animals were examined for toxicity signs and then the liver and testis were processed for histological examinations. Furthermore, the sperm abnormalities were recorded. The results of this study demonstrated that the low dose did not show any morphological changes while some changes such as shivering, nausea and mortality were manifested after administration of high dose of paracetamol. Many histological changes have been observed after high dose treatment of paracetamol, the frequencies of abnormal sperm were increased as well. Therefore, Paracetamol, which is believed to be a strong pain killer for the hangover headache may damage liver and testis when consumes beyond the recommended dose.

Keywords: Paracetamol; Histology; Hepatotoxicity; Testicular toxicity and Sperm abnormalities

1. Introduction

Nowadays, so many drugs are used as medicines. Oral drug delivery is the easiest way of administering dosage form. People are consuming such medicines unknowing their nature, properties, mechanism of action, toxicity and their side effects on the body [1].

Paracetamol (PAR) is one of the widely used drugs to get relief from fever, headache and certain pains such as muscle aches, arthritis, backache, toothache and cold. As a therapeutic agent, it has been estimated that 10 to 20 thousand tons of paracetamol are consumed annually in the United States. The easy accessibility to this drug masks the potential danger of its misuse, for example when taken in lethal overdoses by children. It has also been reported that Paracetamol shows some life threatening effects like Liver damage which in turn leads to liver failure and death [2].

The pharmacological effects of PAR are generally, act based on inhibition of prostaglandin synthesis [3].

A study in 2008 on long term side effects of PAR tablets in children found that administering PAR for fever in the first year of life was linked with an increase in the incidence of asthmatic symptoms at age of 6-7 year. It was stated that use of PAR (both in the first year of life and in children aged 6-7 years) was associated with an increased incidence of rhino conjunctivitis and eczema [4]. It has also

been reported that high dose-usage (greater than 2,000 mg per day) of PAR increases the risk of upper gastrointestinal complications like stomach bleeding. Furthermore, heavy use of PAR (300 grams a year or 1 g per day on average) has been linked to a condition known as 'Small Indented and Calcified Kidneys (SICK) [5].

It has also been reported that PAR have genotoxicity [6] hepatotoxicity [7-9], interferes with DNA synthesis and carcinogenicity effects [10]. Exposure to chemicals that could produce pituitary-hypothalamic or sex hormonal effects which in turn could affect spermatogenesis and exposure of the seminal fluid to chemicals, resulting in functional or structural impairment of sperm cells [11].

PAR is metabolised by the liver and excreted in the urine mainly as glucuronide and sulphate conjugates; less than 5% is excreted as unmodified PAR. Binding to the plasma proteins is minimal at therapeutic concentrations [1]. Further explanation has been demonstrated about PAR metabolism, in which reported that PAR is metabolized in the liver via three pathways: glucuronidation, sulfation or via the hepatic cytochrome P450 enzyme system, which is responsible for the toxic effects of Paracetamol due to alkylating metabolite N-acetyl-P-benzo-quinone imine (NAPQI) [12]. In this pathway, PAR is converted to a metabolite which is toxic to liver cells. Glutathione (a tripeptide) 4 then binds to this toxic metabolite resulting in a non-toxic compound. Hepatotoxicity occurs when glutathione stores are depleted faster than they can be regenerated and the toxic metabolite is left to accumulate. The metabolism of PAR is an excellent example of intoxication.

This study aimed to highlight the acute toxicity effect of PAR on histological changes of liver and testis. Furthermore, the effect of PAR on spermatogenesis and sperm structure of albino mice *Mus musculus* had been studied.

2. Materials and Methods

2-1 Animals

In this experiment, healthy adult mice *Mus musculus* aged 6-8 weeks old and weighing approximately 25gm were used. The animals were obtained from the Department of Biology/College of Education/University of Salahaddin. They were maintained at a controlled temperature and 12-h intervals of light and dark, with supplying of water and food.

2-2 Paracetamol

The pure PAR tablet, made by S. D. I. -IRAQ, was administered orally in the doses of 2 gm/kg and 5gm/kg.

2-3 experimental Design

The experiment was conducted in order to determine the toxicity dose of PAR. Acute toxicity study was used to calculate a lethal dose of any compound that may kill 50% of the animals. Two different doses such as 2 gm/kg and 5gm/kg have been used to evaluate the acute toxicity test following the standard methods described by a previous study [13] fifteen mice were divided equally into three groups of five each. The first group was labeled as a vehicle group and received 5mL/kg DW. The second and third groups were labeled as experimental groups which received 2 g/kg and 5 g/kg of PAR respectively. Mice were fasted over-night (food but not water) the day before experiments. Food was withheld for a further 3-4 hrs after dosage. All animals were monitored for 30 mints, 2, 4, 8, 24 and 48 hrs intervals after the administration for the onset of clinical or toxicological symptoms. Mortality of mice was observed over a period of 2 weeks. On day 15, all the animals were

killed ethically. The livers and testis were fixed in 10% formalin for histological examination.

2-4 Histological Examination

For histological examination the liver and testis were fixed in 10% of buffered formalin solution. The tissues were processed (dehydration, cleaning and infiltration). Then, they were embedded in paraffin wax and sectioned with microtome to produce 5 μm paraffin wax tissue sections. After that, the sections were stained with Haematoxylin & Eosin followed by mounting with DPX mounting media. Next, the sections were examined using light microscope (Carl Zeiss, Japan).

2-5 Sperm abnormalities

Following the method described by Saleem [14], the sperm film from epididymis and vas deferens were prepared with slight modification. The epididymis and vas deference were put in a small petri-dish containing 90% of normal saline. Then the sperm were extracted, smeared and stained using 1% Eosin for 10 min. Sperms was counted and the data were analyzed by completely randomized design (CRD). Then, all the sperms have been examined with microscope to determine the abnormalities.

3. Results and Discussions

3.1 Paracetamol and Toxic signs

The results revealed that using of PAR in low dose (2 gm/kg) did not show any morphological changes. Whereas after using high dose (5gm/kg) of PAR caused different toxic signs such as nausea, salivary, shivering, and mortality. After 24 hrs of treatments the behavior of mice increasingly changed. This result supported by Rumack and Matthew [15] in which reported that overdose of paracetamol causes nausea, vomiting, pallor, and shivering. However, patients often have no specific symptoms or only mild symptoms in the first 24 hours of poisoning. Rarely, after massive overdoses, patients may develop symptoms of metabolic acidosis and coma early in the course of poisoning [16].

3-2 Paracetamol and Hepatotoxicity

The toxicity test of PAR showed that PAR significantly caused liver injury and so many undesirable changes occurred in the liver tissue particularly after administration of high dose (5 g/kg).

The effects of using high dose (5g/kg) are shown as lymphatic infiltration (Figure 2), hemorrhage as in Figure 3, dead cell as in Figure 4, fatty cell accumulation as in Figure 5 and acute inflammatory cells and kupfer cell hyperplasia (Figure 6). These results are in agreement with those reported by Abreu and Moraes [10] who found that drug accumulates in significant amounts in hepatic tissue particularly when injected in high doses causes liver toxicity. Events that produce hepatocellular death following the formation of acetaminophen protein adducts are poorly understood [1]. Primary cellular targets have been postulated to be mitochondrial proteins, with resulting loss of energy production, as well proteins that are involved in cellular ion control [17].

On the other hand, it has been recorded by other researcher that PAR is metabolized in the liver and converted to a metabolite which is toxic to liver cells [18].

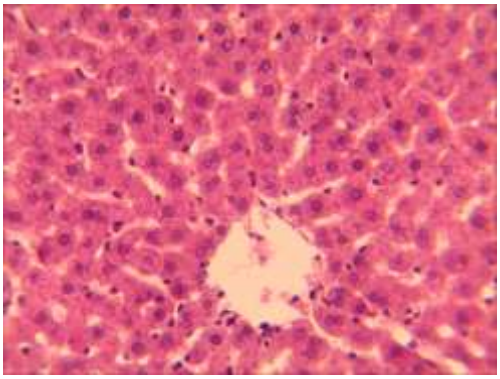

Fig. 1. Shows normal liver using DW (5mL/kg) 40X

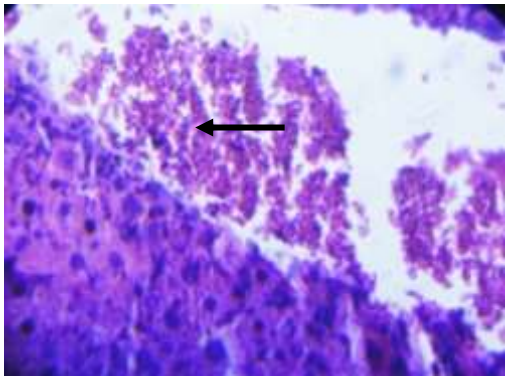

Fig. 2. Shows lymphatic infiltration after using high dose (5g/kg) of PAR 40X

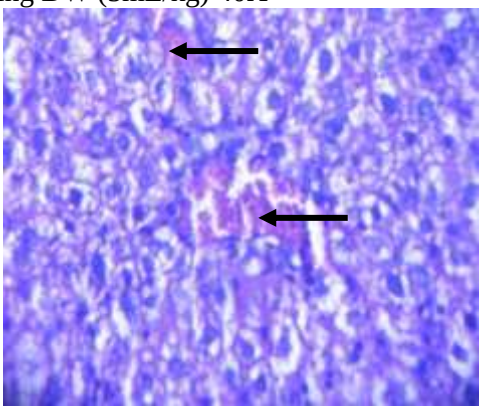

Fig. 3 Shows hemorrhage after using high dose (5g/kg) of PAR 40X

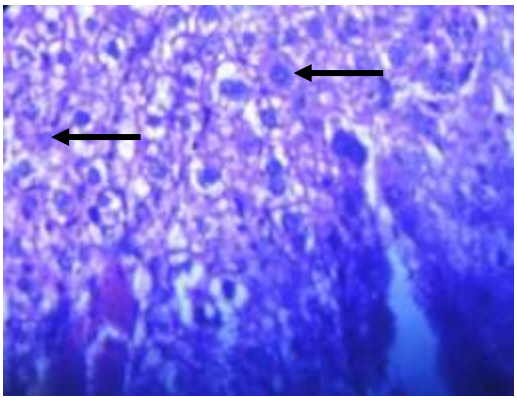

Fig. 4. Shows dead cell (the glassy eosinophilic cytoplasm) using high dose (5g/kg) of PAR 40X

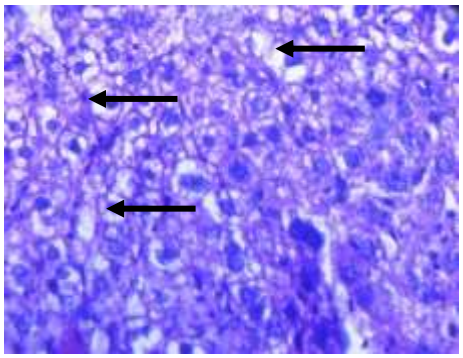

Fig. 5. Fatty cell accumulation using high dose (5g/kg) of PAR 40X

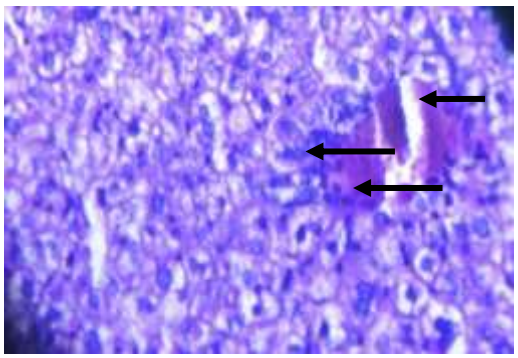

Fig. 6. Acute inflammatory cells and kupfer cell hyperplasia using high dose (5g/kg) of PAR 40X.

3-3 Paracetamol and Testicular Toxicity

In the present study, PAR caused testicular atrophy of several seminiferous tubules, some of which were virtually devoid of germinal epithelium and contained mostly sertoli cells with small amount of sperm cell as in Figure (7). Moreover, significant alterations have been seen in testes on high dose treatment, such as the loss of contact between basal cells is apparently due to sertoli cell fragmentation as in Figure (8) at all levels of the seminal epithelium and a decrease in the diameters of some seminiferous tubules as in Figure (9). Generally, defects of seminal epithelium cells that provide support and nutrition for the spermatic cells result in loss of spermatic cells and may lead to the destruction of this tissue and infertility [19]. Many similar observations are also revealed that PAR-induced testicular toxicity in mice [20] and in rats [21] previously. The acceptable explanation of testicular toxicity could be related to a greater loss of cells to the degeneration of tubules, leaving open lacunae, as found in this study. The frequent observation of poorly condensed chromatin in late spermatids could be the result of the covalent binding of some PAR metabolites to DNA [19].

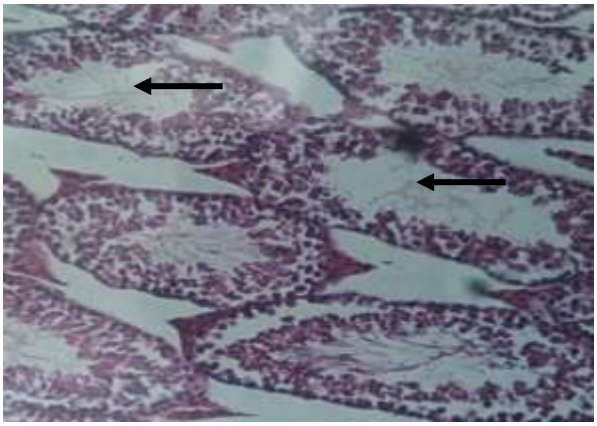

Fig. 7. shows atrophy of some seminiferous tubules with small amount of sperm cell. Using high dose (5g/kg) of PAR 20X .

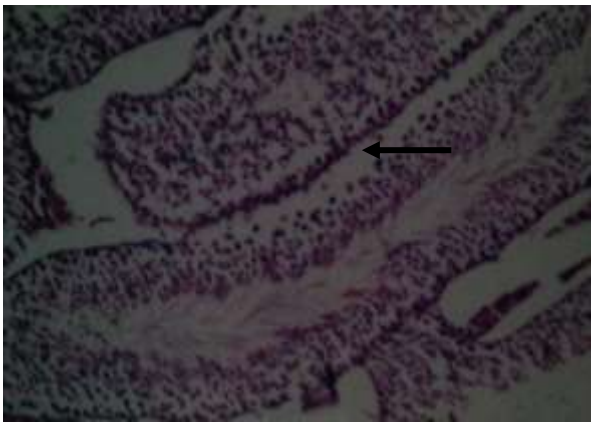

Fig. 8. shows loss of contact between basal cells due to sertoli cell fragmentation. Using high dose (5g/kg) of PAR 20X

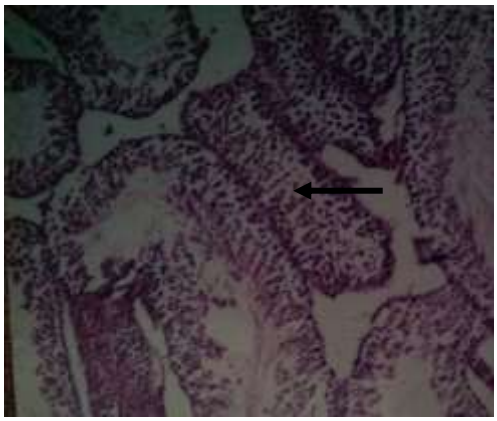

Fig. 9. shows disruption of some seminiferous which contained few cells, had considerably smaller diameters than normal tubules.using high dose (5g/kg) of PAR 40X.

3-4 Paracetamol and Sperm Abnormalities

In this study, the sperm abnormalities were observed in the spermatozoa of treated mice. Different abnormalities has been monitored such as Blunt hook sperm (BHS); Sperm without tail (SWT); Coiled tail sperm (CTS); Defective head sperm (DHS) and Swollen head sperm (SHS) as in Table (1) and Fig (10). Increases in the incidence of abnormal sperm have been reported after treatment of male albino rats with formaldehyde and analgesics. It has been reported that high temperatures, extreme nutritional deficiencies, some drugs and some diseases can cause sperm abnormalities in a wide range of species including mice and man [11]. Although, the precise mechanism by which paracetamol cause sperm abnormalities has not been fully established, generally, damage to the sperm cell by substances may occur by one of three mechanisms: physiological, cytotoxic and genetic. The morphological abnormalities might have been caused by alterations (deletions, point mutation or a combination of both) in testicular DNA that in turn disrupts the process of differentiation of spermatozoa, exposure to chemicals that could produce pituitary-hypothalamic or sex hormonal effects which in turn could affect spermatogenesis and exposure of the seminal fluid to chemicals, resulting in functional or structural impairment of sperm cells [11].

Table (1): Mean ± S.E of the effect of different concentrations of paracetamol on sperm abnormalities in male mice.

	Sperm abnormalities				
Treatments	**Blunt hook sperm**	**Sperm without tail**	**Coiled tail sperm**	**Defective sperm head**	**Swollen sperm head**
Control	1.030±0.13	1. 40 ± 0.119	0.200±0.030	0.50 ± 0.023	0.020±0.010
Low Dose	4.330±0.413	6.000±1.303	8.500±1.392	5.000±0.303	2.000±0.003
High Dose	7.40±0.907	12.30±0.900	10.00±1.157	9.800±1.077	8.600±1.157

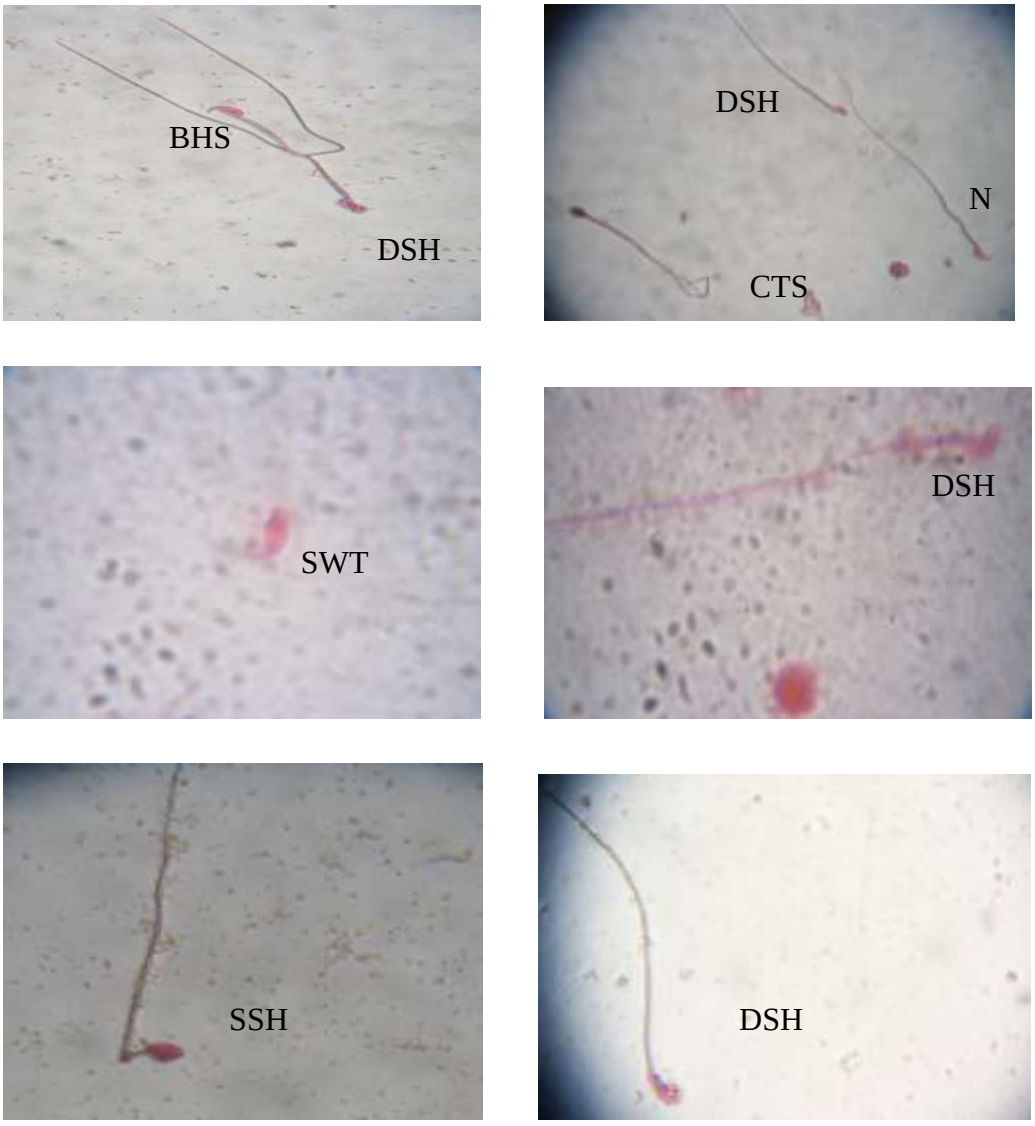

Fig. 10. shows effect of PAR on sperm abnormalities after using high dose (5g/kg) of PAR 40X. (N): Normal sperm; (BHS): Blunt hook sperm; (SWT): Sperm without tail; (CTS): Coiled tail sperm; (DSH): Defective sperm head and (SSH): Swollen sperm head.

Conclusion

Our data demonstrated that after 15 days of PAR administration in laboratory mice is damaging liver and testis as well as affecting on the spermatocytes and increased the frequency of sperm by changes in chromatin structure. Though PAR gives positive results in relieving pain, it is found to be too toxic when it consumed beyond the recommended dose.

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