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Protective effect of pomegranate *(Punica Granatunm)* on the sperm DNA integration induced by cadmium chloride in adult mice

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Abstract
This study was designed to investigate the potential protective effects of pomegranate (*punica granatunm*) on sperm DNA integration induced by cadmium chloride in adult male mice. Plant (*Punica granatum*) was collected from the local market and prepared from its water extract, Thirty Adult male mice were used in this study. The animals were eight weeks old and weighing 26±30g each. The mice were split into control (10) and treated group (20) treated group, split into two group one received cadmium chloride dissolved in distilled water administered orally at a dose 50 mg/kg/BW and group two received cadmium chloride plus *punica granatunm* water extract administered orally at a dose 50 mg/kg/BW duration of experiment 21 days.
The current study is showed that the average body weight was statistically significant ($p<0.05$) decreased in the experimental group treated with Cadmium chloride and increased in the group treated with Cadmium chloride plus *punica granatunm* compared with the control group. The average testis weight decreased in all treated groups. Besides, the results state that, the sperm concentration was statistically significant decreased at ($p<0.05$) in group treated with cadmium chloride groups and cadmium chloride plus *punica granatunm* in respect to the control group. However, there were highly significant decreases at ($p<0.05$) in motility and viability of sperm in cadmium chloride treated groups at ($p<0.05$) compared with the control group. Moreover, sperm motility and viability in cadmium chloride plus *punica granatunm* treated groups as well. Total antioxidant capacity (TAC) and malondialdehyde (MDA) in cadmium chloride treated groups and Cadmium chloride plus *punica granatunm* were highly significant decreases at ($p<0.05$). The study found that sperm with normal morphology was 86% in control, 49% in cadmium chloride treated groups and 63% in cadmium chloride plus *punica granatunm*, and immature sperms were 6% in control, 10% in Cadmium chloride treated groups and 8% in cadmium chloride plus *punica granatunm*. Where the DNA damage was 8% in control, 41% in cadmium chloride treated group and 25% in cadmium chloride *plus punica granatunm*. The microscopic examination of sperms in control and cadmium chloride plus *punica granatunm* treated group was shown a normal structure of sperms in contrast in cadmium chloride treated group showed abnormal sperms (head and tail).

Keywords: protective, *Punica granatunm*, sperm DNA, cadmium chloride, mice

Introduction
The recent fertility evaluation in animals and human depends on microscopic examination of sperms and sperm DNA integrity. Routine semen investigation, which includes sperm parameters that give an idea about fertility, but does not always reflect the quality of sperm especially in DNA integration. Men with normal spermograms (semen analysis) may still be infertile; the cause could be related to abnormal sperm DNA (Nasrin Sheikh *et al* 2008) [16]. Sperm DNA integrity has an important role not only for fertilization but also for a normal embryo. Generally, some studies concluded that the exogenous DNA interaction with, and/or internalization in, spermatozoa triggers a metabolic activation of these cells, otherwise regarded as being inert in DNA modification and metabolism (Maione B, *et al*, 1997) [14]. Cadmium chloride is considered as a highly polluting material and its toxic effects in liver, kidney, vascular system and reproduction in humans and animals. (Eybl and Kotyzová, 2010) [5] reported that cadmium chloride causes damage to tissues and potentially leads to carcinogenesis. Cadmium chloride reduces electron transportation in mitochondria by stimulates the production of reactive oxygen species (ROS). As a result, lipids are oxidized resulting in damage to membranes. Long-term exposure to cadmium chloride-induced testicular toxicity and apoptosis in testicular germ cells of rats.
The effects of cadmium chloride on sperm functional parameters were evaluated on male mice and reported that, short-term effects of cadmium chloride resulted in an increased fraction of sperm with abnormal morphology, and reduced motility. Late term effects included a drastic reduction of sperm cell numbers and sperm motility. An increase in DNA fragmentation (Helena O., 2009) [10]. Other study by (Saber A. *et al* 2013) [19] found that, mice treated with cadmium chloride showed decreased testosterone level, increased lipid peroxidation, and caused degeneration of testicular germ cells. In the normal physiological status, the seminal plasma contains antioxidant enzyme that is capable of reducing these ROS as well as protecting the spermatozoa against any likely damage. Principally, an antioxidant such as vitamin E and C, carotenoids and carnitine have been found valuable in restoring a balance between ROS generation and scavenging activities (Quintanilha A *et al*., 1982). There is further evidence that herbal products can also improvement male reproductive functions. (Adewoyin, M *et al*, 2017) [1]. Recent studies suggested that pomegranate (*Punica granatum*) or its active ingredients employ pharmacological actions such as antioxidant, anti-

inflammatory, and neuroprotective properties. *Punica granatum* pretreatment resulted in a significant decrease in DNA damage. Suggesting that pomegranate has the potential to be used as a new therapeutic strategy for neurodegenerative disorders. (Forouzanfar, F *et al*, 2013) [9]. Pomegranate *(Punica granatum*), is a shrub mostly available in the Mediterranean Sea region. The fruits of pomegranate have many medicinal properties due to possessing secondary metabolites such as phenolic compounds. (Muhammad Saeed, *et al* 2018) [15].

Materials and Methods

1. Plant Samples Collection

Plant pomegranate *(Punica granatum*) was collected from the local market then authenticated in Botany department on the basis of taxonomic characters, and prepared from its water extract and keep it in -4c until used.

2. Animals

Thirty Adult male mice were used in this study. The animals were eight weeks old and weighing 25-30g each. Animals were housed and treated under standard Laboratory Animal Care (Imam Mohammed Bin Saud Islamic University). The animals were randomly split into control (10) and treated (20) groups. The control group was given distilled water only and treated groups divided into two groups each were include ten mice. Group one treated with cadmium chloride in distilled water administered orally at a dose 50 mg/kg/BW and group two received cadmium chloride plus *Punica granatunm* water extract administered orally at a dose 50 mg/kg/BW for 21 consequence days.

3. Sperm parameters measurement

Sperms collected from cauda epididymis of male mice after sacrificed. Sperm concentration determined using the standard hemocytometric method and sperm viability using eosin stain and sperm motility according to (WHO, 2013) [23]. Damage of sperm DNA was used method reported by (Wong *et al*., 2008) [22]. Seminal MDA levels were analyzed according to (Feng and Ochi, 2001 and Rao et al. 1989) [8, 18]. MDA was assessed by using thiobarbituric acid at 534 nm. TAC was measuring according to (Niehaus W *et al*., 1968, Lowry O *et al*., 1995) [17] based on the ferric reduction antioxidant power. The protein level was estimated by the method of Lowry et al.

Statistical analysis

Statistical analysis was performed by SPSS to compare data in the control group and the treated groups. The results expressed as mean ± S.E.M (standard error of means). A significant difference was written in parentheses.

Results and Discussion

Results

As shown in Table.1., the average of body weight decreased in experimental group treated with Cadmium chloride and increased in the group treated with Cadmium chloride and *Punica granatunm* compared to control; however, this decrease was statistically significant in body weight ($p<0.05$). The average weight of testis decreased in both experimental groups treated with Cadmium chloride and Cadmium chloride plus *Punica granatunm* when compared to control; however, this reduction was not significant. It was also showed that, the sperm concentration decreased in group treated with Cadmium chloride groups and Cadmium chloride plus *Punica granatunm* relative to the control, although this decrease was significantly at level ($p<0.05$). Nevertheless, there were highly significant decreases ($p<0.05$) in sperm motility and viability in groups treated with Cadmium chloride when compared the control group. In the same way, the sperm motility and viability in groups treated with Cadmium chloride plus *Punica granatunm* were decreased significant at level ($p>0.05$). Total antioxidant capacity (TAC) and Malondialdehyde are measured and the data are shown in (Fig1 and Fig2). (TAC) in groups treated with Cadmium chloride 0.700±0.21µmol/l were highly decreases at ($p<0.05$) when compared to control 1.30±0.42 µmol/l, on the other hand, the groups treated with Cadmium chloride plus *Punica granatunm* 1.12±0.21µmol/l were decreases significant at level ($p>0.05$). (MDA) in groups treated with Cadmium chloride 1.59±0.06µmol/mg were highly significant decreases at ($p<0.05$). On the other hand, the groups treated with Cadmium chloride plus *Punica granatunm* 2.31 ±0.05µmol/mg were decreases significant at level ($p>0.05$) when compared to control 3.55±0.01µmol/mg.

Assessment of effect of chloride and cadmium chloride plus *Punica granatunm* on DNA integrity, immature and morphology were assessed. The study found that, sperm with normal morphology were 86±0.21% for control and 49±0.05%, 63±0.07% for Cadmium chloride and cadmium chloride plus *Punica granatunm* respectively, moreover, immature sperms were 6±0.042% in control, 10±0.02% in Cadmium chloride groups and 8±0.037% in cadmium chloride plus *Punica granatunm*. The DNA integrity was 8.0±0.13% for control and 41±0.24% 25±0.07% for Cadmium chloride group and cadmium chloride *plus Punica granatunm* respectively as shown in Fig.3 and 4). The microscopic examination of sperms is illustrated in plate.1,2 and,), abnormal structure (head and tail) was seen in Cadmium chloride group only, while the control and cadmium chloride plus *Punica granatunm* don't show any abnormality.

Table 1: Effects of pomegranate *Punica granatunm* on the average of body, testis weight (g), sperm count (10^6/ml), sperm motility (%) and sperm viability (%) on male mice treated with Cadmium chloride

Parameter	Control (n=10)	G1. Cadmium chloride treated (n=10)	G2. Cadmium chloride+ *Punica granatunm* treated (n=10)
Body (gr)	29.20±0.47	27.5±0.66*	30.5±0.85
Testis (gr)	0.22±0.055	0.16±0.012	0.19±0.098
Sperm concentration (total count) (No of sperm/rat (10^6)	69.30±3.06	44.31±3.73**	55.70±2.59*
Motility (%)	82.19±4.30	42.13±1. 86**	75.00±2.14*
Viability %	88.55±7.26	48.17±5.80**	79.22±4.36*

LS, level of sig., NS: not significant P<0.05 *significant at P>0.05. **significant at P>0.01.

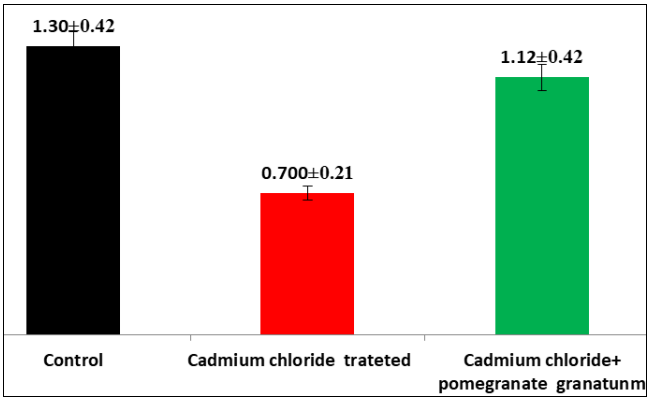

Fig 1: Total Antioxidant capacity (TAC) on male mice treated with Cadmium chloride and cadmium chloride plus *Punica granatunm*

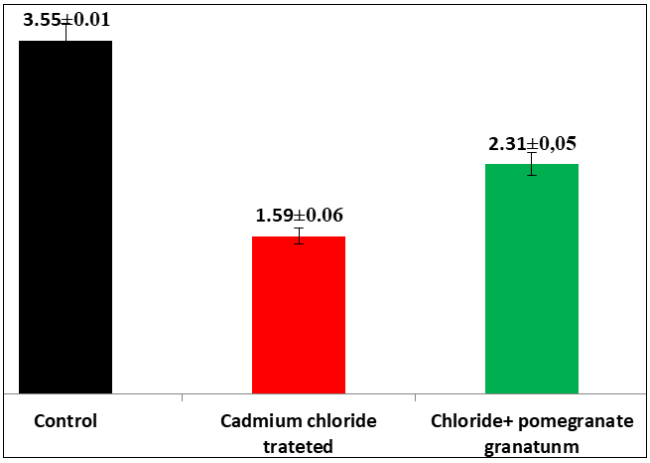

Fig 2: Malondialdehyde (MDA on male mice targeted with Cadmium chloride and cadmium chloride plus *Punica granatunm*

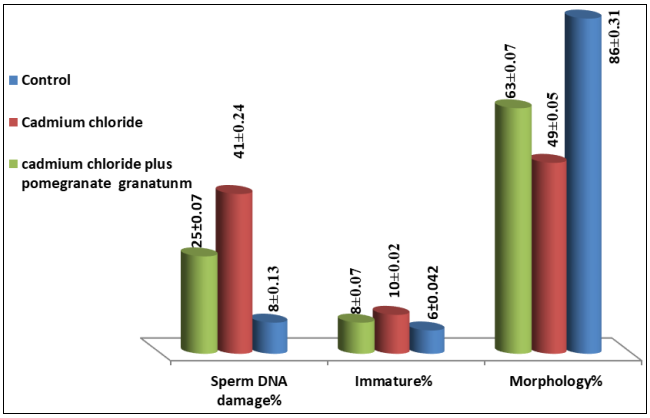

Fig 3: Comparison of DNA integrity%, immature % and Morpholoy % on male mice treated with Cadmium chloride and cadmium chloride plus *Punica granatunm*

Plate 1: normal structure of sperm in control group

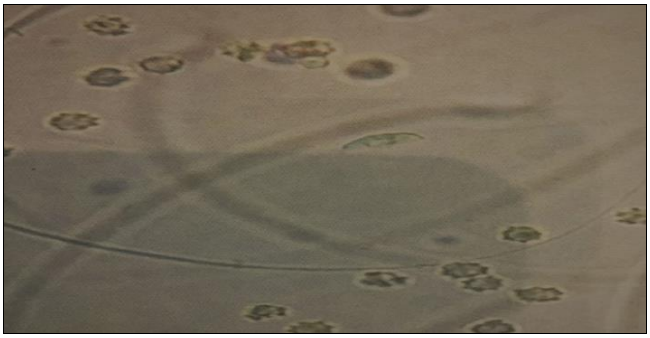

Plate 2: The cadmium chloride treatment group shows abnormal structure of sperms

Plate 3: The cadmium chloride treatment group shows abnormal structure of sperms head

Discussion

Pomegranate (*Punica granatum*) belonging to the family Punicaceae, is one of the oldest known edible fruit and distributed in middle east countries (Fadavi *et al*., 2016) [6]. The plant has much active ingredients material. Seed oil contains 80% of punicic acid, sex steroid, estrone, at a concentration of 17 mg/kg dried seed. *Punica granatum* aqueous juice has a protective role against oxidative damage (Al-Olayan., *et al*, 2014) [2] and (Kim *et al*., 2002) [11]. The antioxidants play important role in cellular response against oxidative stress (Ergul Belge Kurutas., 2016) [4].

In the present study, the results were supported by (Leiva,. *et al* 2011) [12], reported that, ethanolic extract of pomegranate (*punica granatum*) can be useful for treatment deleterious effect of heavy metals such as lead acetate in sperm parameters. Also, he supposes the antioxidant activities have a major role in recovered damage produced by lead acetate on spermatogenesis. Other researcher found that, pomegranate fruit showed a positive effect on seaman quality such as increased motility and concentration and morphology (Fedder, *et al*, 2014) [7]. Our study was consistently with result obtained by (Avdatek F *et al*,. 2018**)** [3] on the the effect of pomegranate for reproductive parameters in male rabbits and antioxidant status, the current study was found increased significantly at ($p \leq 0.05$) when he reported decreased significantly ($p \leq 0.05$) this variation between studies due to the type of animals used and treatment inducer. (Türk., *et al*, 2016) [21] used pomegranate juice to protective damage induced by carbon tetrachloride (CCl4) in sperm and testicular apoptosis, the finding of this study similar to results obtained by current study and state that, *pomegranate* consumption improves sperm quality and antioxidant activity of rats. Pomegranate extract also significant elevation the concentration of sexual hormones such as testosterone, luteinizing hormone and follicle stimulating hormone (Al-Olayan E., *et al*., 2014) [2]. Our result for morphology, concentration of (MDA) and (TAC) and androgen are comparable to results were

reported by (Türk G *et al*., 2008) [20] and by (Helena O *et al*., 2009) [10] their result indicate that, pomegranate consumption provided an increase in epididymal sperm concentration, sperm motility, spermatogenic cell density And diameter of seminiferous tubules and germinal cell layer thickness, and it decreased abnormal sperm rate when compared to the control group.

The present experiment concluded, that, the *pomegranate granatunm* treatment group showed a protective effect due to possess antioxidant and androgenic activity therefore a useful effect on spermatogenesis and positive effect on reproductive parameter of mice.

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