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Ameliorative Effect Of Thiamine Pyrophosphate Against Cisplatin-Induced Reproductive System Damage Within Male Rats

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ABSTRACT

Background: Cisplatin considers one of the most potent antineoplastic drugs that manage solid and germ cell cancer. The major drawback in cisplatin treatment is infertility. Thiamine pyrophosphate is the active form of thiamine which has an important role in the oxidative phosphorylation pathway.
Objective: This study aimed to evaluate the protective effect of thiamine pyrophosphate on the testes of male rats exposed to a single dose of cisplatin.
Methods: Twenty-eight albino rats were randomly grouped into four groups. Negative control group: received normal saline, Positive control group: received normal saline and cisplatin, Low-dose thiamine pyrophosphate group: received thiamine pyrophosphate (50 mg/kg) and cisplatin, High- dose thiamine pyrophosphate group: as above but thiamine pyrophosphate dose was 100 mg/kg. Semen samples used to measure the sperms motility, and concentration. Serum samples were gathered to measure the levels of Testosterone. Testicular samples were collected to determine testicular superoxide dismutase, glutathione peroxidase, and caspase-3 levels. The testes were harvested to achieve histopathological study and testicular prostaglandin F2α expression.
Results: Testicular gonadosomatic index, sperm motility and concentration, testosterone and antioxidant markers levels within testicular tissue were significantly decreased within positive control group (received just cisplatin) compared with the negative control, while co-treatment with thiamine pyrophosphate can significantly improve these parameters in a dose-dependent manner. Conversely, the testicular caspase-3 level was elevated markedly in the cisplatin alone group while reduced significantly in a dose-dependent manner when thiamine pyrophosphate was co-administered. In the cisplatin group, histopathological study demonstrated a marked alteration in the structure of testicular tissue while under immunohistochemical staining, the testicular prostaglandin F2α expression was significantly overexpressed, whereas co-treatment with thiamine pyrophosphate can significantly reduce this up-regulation and reverse histopathological findings.
Conclusion: Thiamine pyrophosphate may act as a protective agent that ameliorates rat's testicular damage induced by cisplatin treatment in a dose-dependent manner. The suggested mechanism may attribute to its antioxidant and anti-apoptotic action.

Keywords: Cisplatin, Thiamine pyrophosphate, Male infertility

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INTRODUCTION

Infertility can be defined as the inability of the adult male to make fertile women be pregnant after one year of regular intercourse [(1)]. There are several mechanisms for medications that could damage spermatogenesis and alter semen parameters, like impaired spermatogenesis e.g. colchicine, methotrexate and other chemotherapies like cisplatin [(2)]. Treatment of male infertility depends on the underlying causes. It required several months to years of treatment for fertility to be reached. The major goal of male infertility treatment is to reduce the damage, improve or even normalize the fertility state [(3)].

Cisplatin, besides its beneficial effect in various cancers treatment, can causes either permanent or transient infertility [(4,5,6)]. Patients with cancer, especially testicular cancer, have defects in the spermatogenic process. Besides, those patients who take cisplatin as treatment will suffer from further impairment in spermatogenesis; the majority of the patients show azoospermic or oligozoospermic infertility for a long time [(7,8)]. Cisplatin treatment can lead to infertility in males due to the apoptotic effect on germ cells of the testis [(9)]. Cisplatin can induce apoptosis and activate caspases family (cysteine proteases) which include: caspases 8 (has a role in the stimulation of death receptors in the plasma membrane), caspases 9, caspases 3, 6 and 7 (executioner caspases that control execution phase of apoptosis by managing DNA fragmentation) [(4)].

Thiamine pyrophosphate (TPP) is the active form of thiamine which is important in the oxidative phosphorylation pathway, it acts as a co-factor that responsible for generating energy for many enzymatic reactions like alfa-ketoglutarate dehydrogenase, alfa-ketoacid dehydrogenase, branch-chain amino acid dehydrogenase, pyruvate dehydrogenase and transketolase [(10)].

This study was conducted to evaluate the effect of thiamine pyrophosphate as protective against reproductive system damage induced by cisplatin in male rats.

MATERIALS AND METHODS

Drugs and chemicals

All chemicals and reagents used were of highest available purity, their origin was as follow: Cisplatin (Koçak-Farma-Turkey), Thiamine pyrophosphate (Sigma-

Aldrich-Germany), Testosterone and Caspase-3 (Mybiosource-USA), Superoxide dismutase (SOD) and Glutathione peroxidase (GPX) (Elabscience-USA), Anti-prostaglandin f2 Alfa receptor (PTGFR) primary antibody and Goat anti-rabbit IgG H&L secondary antibody (Abcam-USA). All measurements were depend on enzyme-linked immunosorbent assay (ELISA) technique.

Animal's selection

Twenty eight, non-previously treated male albino rats, weighing 210-320gm, were gained from the National Center for Drug Control and Research/Ministry of Health. Before the study began, the animals were housed in well ventilated condition (controlled temperature and humidity) and freely access to food and water in experimental cage (20x25x35 cm) at 22°C ± 3° with normal light/dark cycle in animal house at the college of pharmacy/ Al- Mustansiriyah University, where the study was begin after taking approval from the scientific and animal ethics committee within the department of pharmacology and toxicology.

Experimental design

Those twenty-eight male albino rats were randomly grouped into four groups, seven rats in each. The study protocol and drugs-dose selection were conducted according to the results obtained from previous literature [11,12] and our preliminary study (figure 1).

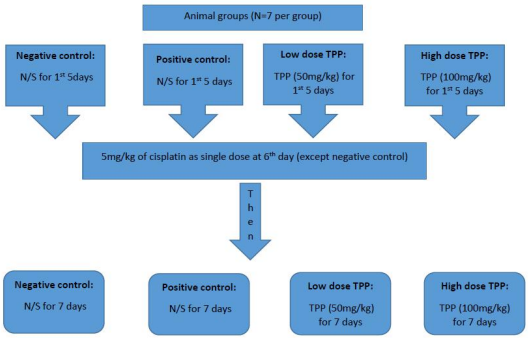


Figure 1: Study design and animal groups.
N/S= normal saline, TPP= thiamine pyrophosphate, all treatments was given by IP route.

Samples collection

Tissue sample of rat's testes were rapidly excised after laparotomy, testes were cleared from adhered connective tissue then weighted for estimating gonadosomatic index (GSI) depending on the following equation [13]:

$$\text{Gonadosomatic index (GSI)} = \frac{\text{gonadal weight (g)}}{\text{total body weight (g)}} \text{x } 100$$

The blood samples were collected via cardiac puncture by 10ml syringe, gauge 23 and kept in the gel/serum separating tubes and left for 30 minutes to clot, then centrifuged for 15 minutes at 1000 RPM and froze in Eppendorf tubes (1.8ml) at -20 C° [14]for measurement of testosterone level.

The right testis was placed in formalin: phosphate buffer saline (PBS) solution in a ratio of (1:9) for histopathological and immunohistochemical (IHC) analysis.

Testicular tissue homogenization was adapted from Hamzeh (2019). The homogenization was achieved using tissue homogenizer and mortar in a cold environment. The homogenate then centrifuged at 4000 round per minute for 15 minutes at 4C° [15] and used to estimate the levels of glutathione peroxidase (GPx), superoxide dismutase (SOD), and caspase-3.

Semen samples were collected to determined sperms concentration and motility. The left epididymis was carefully dissected into three parts by scissor then placed in a petri dish contain 1 ml of pre-wormed (37C°) PBS solution (PH: 7.4) and incubate at 37C° for 5 minutes. Approximately, 10 µl of epididymal fluid was loaded on the cover slide within a hemocytometer for 5 minutes before counting the sperms by a light microscope at magnification (X100) to evaluate different fields. Sperm motility was estimated by calculating the mean of the three fields in each sample that were observed under a light microscope at magnification (X400), it was expressed as a percentage of total visible sperms (total 100 sperms)[16].

Histopathological and immunohistochemical study

Histopathological changes were microscopically assessed by the blind method using an arbitrary scale by a qualified pathologist. Testicular damage was graded by Jonson's scoring system which involve ten grades, where 10 indicates maximum spermatogenesis activity, whereas 1 indicates complete absence of germ cells[17].

For immunohistochemical (IHC) staining assessment, a digital microscope with a Leica DM4000 B LED system was used to capture five random areas of the slide at X400 magnification. The IHC staining score system was used for detecting antiprostaglandin F2-receptors expression adapted from Van Diest (2007) [18] by measuring the staining intensity within tissues in random fields, where the intensity of stain was reflected by using a criteria from 3 scales, 0: negative stain, 1: weak stain signal, 2: moderate stain signal, and 3: strong stain signal. Scores were calculated according to the following equation [19]:

(Number of positive stained cells / total number of cells) x 100

Statistical analysis

The collected data were expressed as mean ± standard error of mean (M±SEM). The results were analyzed by Statistical Packages for Social Sciences (SPSS-18). The significance of different means was analyzed by one-way analysis of variance (ANOVA) test, then the least significant difference (LSD) was used for comparison between different groups. The results were considered as statistically significant difference when *P*-value ≤0.05 [20].

RESULTS

Effect of TPP on GSI of rats exposed to cisplatin

Regarding the effect of cisplatin on relative testis weight, the gonadosomatic index (GSI) was significantly decreased in the cisplatin group compared with the control group (*p*-value ≤ 0.05). Conversely, thiamine pyrophosphate (TPP) treated rats (50 and 100 mg/kg) showed significant elevation in GSI compared with rats given just cisplatin in a dose-dependent manner, as shown in figure 2 and 3A.

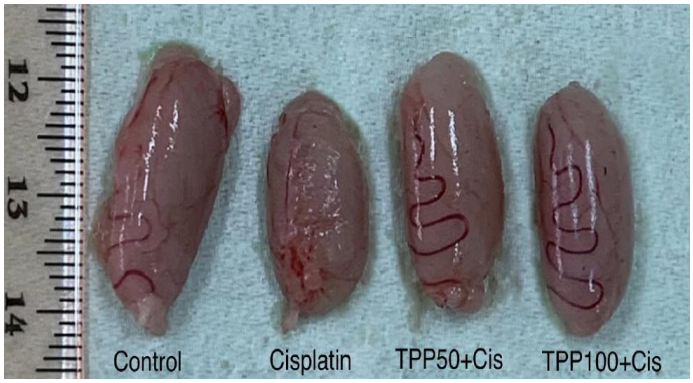


Figure 2: Effect of TPP on testicular size of rats exposed to cisplatin.

Effect of TPP on sperm parameters of rats exposed to cisplatin

Semen analysis showed a significant improvement in the sperm parameters (motility and concentration) when rats exposed to cisplatin received thiamine pyrophosphate (*p*-value ≤ 0.05) in a dose dependent manner, as shown in figure 3B and 3C.

Effect of TPP on testosterone levels of rats exposed to cisplatin

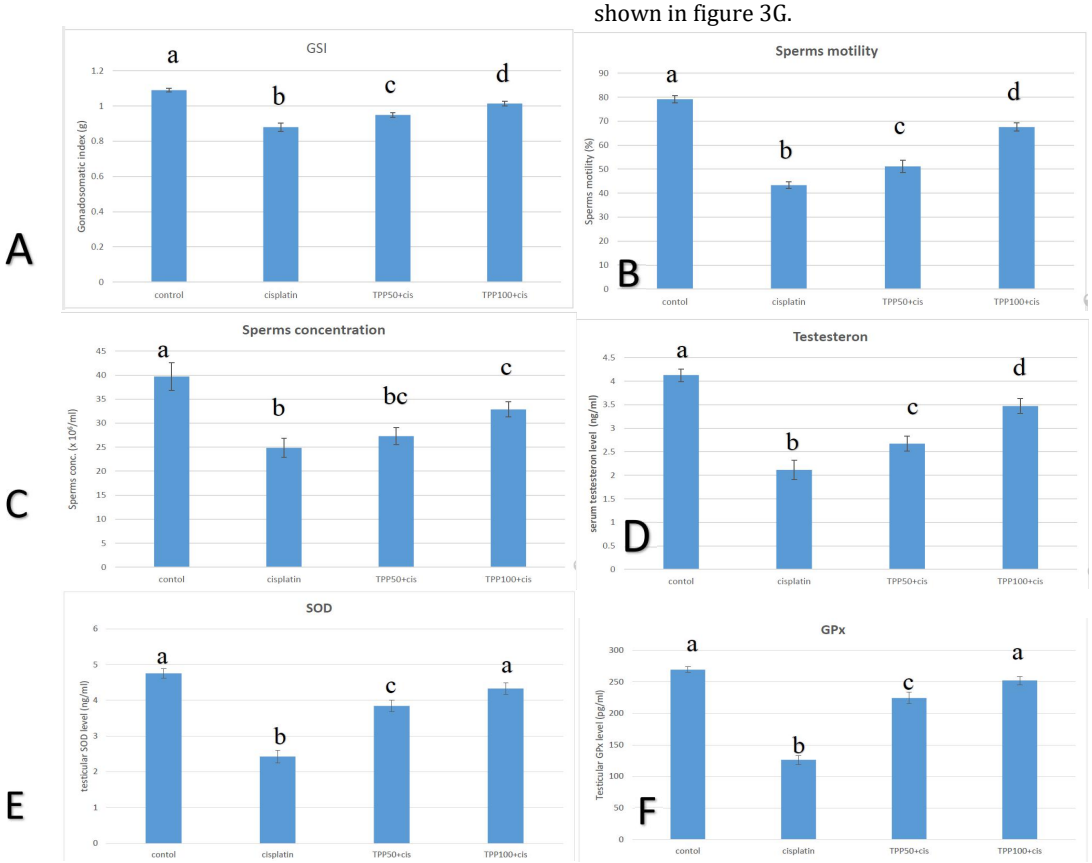
The mean of testosterone level was significantly decreased when rats treated with cisplatin, compared with the control group (*P*-value ≤ 0.05), while rats within TPP50 and TPP100 -treated group reported a significant increment of testosterone mean levels in a dose-dependent manner, as shown in figure 3D.

Effect of TPP on oxidative stress status of rats exposed to cisplatin

Cisplatin significantly diminished the level of testicular SOD and GPx when compared with that of control group (*P*-value ≤ 0.05). Rats within TPP50+cis and TPP100+cis groups significantly showed an elevation of testicular SOD and GPx levels compared to just cisplatin-treated rats, in a dose-dependent manner, as shown in figure 3E and 3F.

Effect of TPP on testicular caspase-3 level of rats exposed to cisplatin

Cisplatin caused a significant elevation of testicular caspase-3 level compared with the control group (*P*-value ≤ 0.05). Meanwhile, the administration of TPP within TPP50+cis and TPP100+cis groups significantly reduced the cisplatin effect on the caspase-3 level in a dose-dependent manner, as shown in figure 3G.



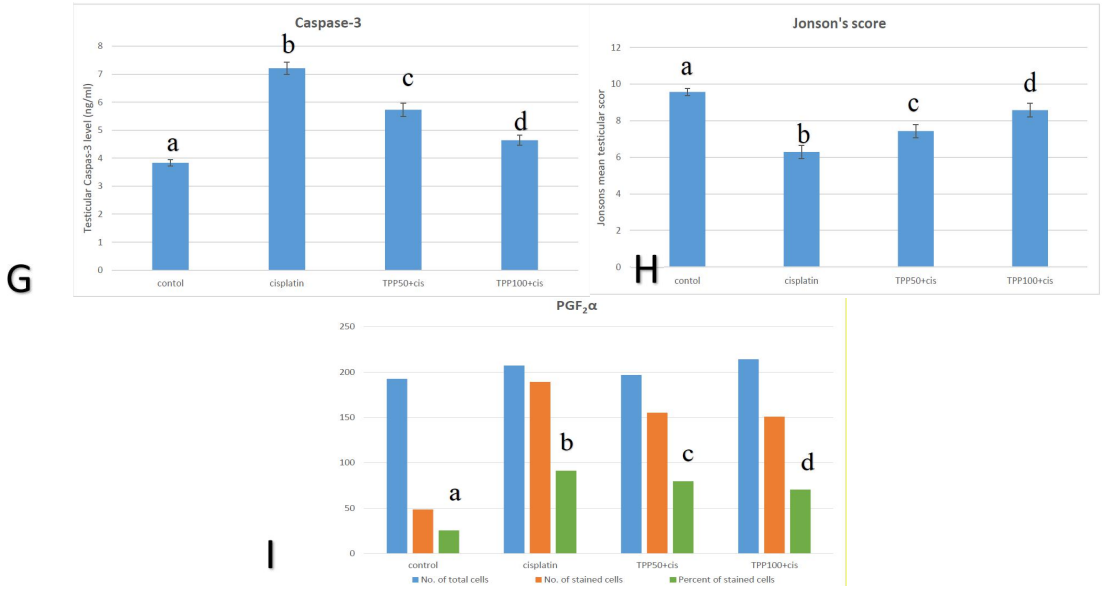


Figure 3: Efffect of TPP on the studied parameters in male rats exposed to cisplatin.

A= effect on GSI, B = effect on sperms motility, C = effect on sperms concentration, D = effect on serum testosterone level, E= effect on testicular SOD, F= effect on testicular GPx, G= effect on testicular caspase-3, H= effect on Jonson's score, and I= effect on PGF_2-α. Data were expressed as Mean ± SEM. Different small letters indicate statistically significant difference among groups (*P*-value ≤ 0.05). GSI= gonadosomatic index, SOD=superoxide dismutase, GPx= glutathione peroxidase, PGF_2-α= prostaglandine F_2-α.

Histopathological evaluation

Rats with control group showed a normal appearance of testicular tissues, represented by intact spermatogenic cells, epithelial layers, and seminiferous tube aspects (figure 4A and B). On the other side, cisplatin group developed severe destruction of Leydig cells, ending with a defect in the formation of spermatids and mature sperms, as shown in figure 4C and D. Meanwhile, rats that were treated with a low dose of TPP (50mg/kg) exhibited protection against testicular tissue injury induced by cisplatin, where the seminiferous tube gradually back to normal shape (figure 4E and F). Also, a high dose of TPP (100mg/kg) significantly reduced cisplatin damaging effect on spermatogenic cells (Leydig and Sertoli cells) and tubular architecture approximately returned to its normal appearance (figure 4G and H).

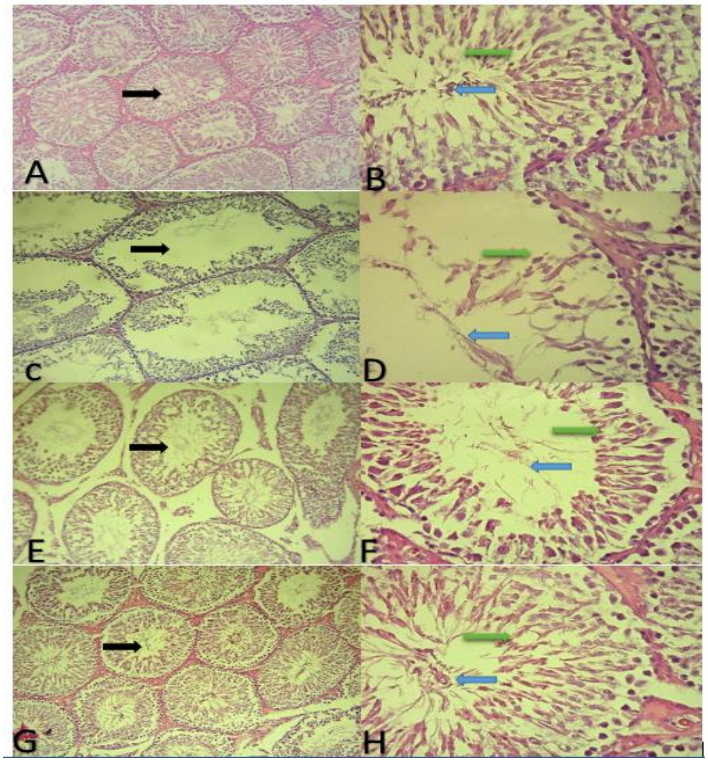


Figure 4: Cross section of testicular rat's tissue under H&E stain. Black arrow = tubular lumen, green arrow= lumen epithelial layers, blue arrow= sperm presence. A,B=normal group, C,D=cisplatin group, E,F=TPP50+cis group, G,H=TPP100+cis group. A, C, E and G= X100 magnification, B, D, F and H= X400 magnification.

Jonson's score evaluation

Cisplatin group showed a significant reduction in the mean of Jonson's score compared to the control group (*P*-value ≤ 0.05). On the other hand, rats within TPP50+cis and TPP100+cis groups showed a significant elevation of score means compared to those within cisplatin group, in a dose-dependent manner, as shown in figure 3H.

Effect of TPP on prostaglandin F2α-receptors of rats exposed to cisplatin (immunohistochemical staining):

The distribution of testicular stained cells within cisplatin group was significantly higher than control (*P*-value ≤ 0.05). Meanwhile, the percent of staining intensity for TPP50+cis and TPP100+cis groups were reduced significantly (*P*-value ≤ 0.05) compared to that of cisplatin alone group (+2 score and +1 score respectively), where the staining intensity score of TPP-high dose was equal to that of control (figure 2I and 5).

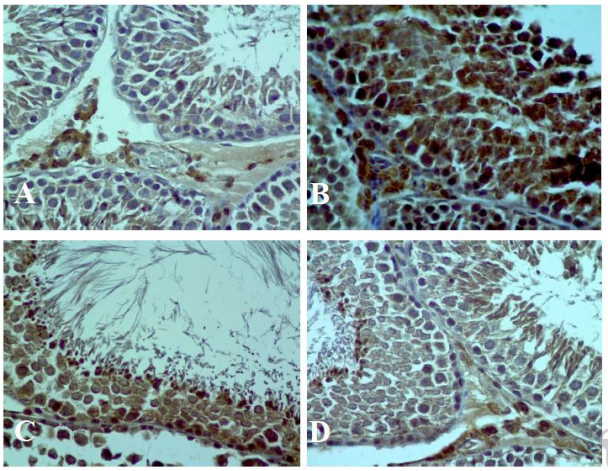


Figure 5: Photomicrographs of rat's testicular cross sections showing the effect of TPP on intensity of PGF2α-receptors during exposure to cisplatin under light microscope (immunohistochemical staining, magnification: X400). A: control group, stain intensity (+1). B: cisplatin group, stain intensity (+3). C: TPP50+ cisplatin group, stain intensity (+2). D: TPP100+cisplatin group, stain intensity (+1).

DISSCUSION

In this study, GSI was significantly reduced in cisplatin-treated group compared with control, these results were agreed with previous studies, where Azouz *et al.* (2020)[21] and sheriff *et al*. (2019) [22] confirmed that the administration of a single dose of cisplatin to rats can reduce body and testis weight when compared to the untreated group. The testicular mass reduction may occur due to suppression of spermatogenic function caused by cisplatin[21,22]. Body weight reduction induced by cisplatin may result from anorexia which is attributed to the reduction of ghrelin production triggered by serotonin release (5-HT2B and 5-HT2C)[23], also it may cause emetogenic effect, nausea, diarrhea and malabsorption[24]. Co-treatment of TPP with cisplatin significantly improves GSI when compared with the cisplatin group, These results were similar to that of Azarbarz *et al*. study (2020), who used hydrogen sulfide (H_2S), which has potent antioxidant and anti-apoptotic activity, in the management of weight reduction induced by cisplatin treatment [25]. This improvement in testis and body weight is supposed to be related to the effect of TPP in decreasing the concentration of leptin hormone "the satiety hormone" which is an essential hormone that controls food consumption and energy balance, leading to an increase in appetite and food intake[26].

According to world health organization (WHO) guidelines, male infertility can predict from the seminal fluid analysis by assessment of sperms parameters that relies on sperms motility and concentration[27]. In this study, cisplatin significantly hindered sperms motility and reduces sperm concentration. A similar observation was described by Amir *et al.* study (2019), who demonstrated that cisplatin reduces sperms count and motility[28]. This mainly attributed to increased ROS production. This elevated level of ROS can cause injury to the plasma membrane of sperms and cause lipid peroxidation, DNA fragmentation and mitochondrial ATP depletion, these changes in sperms membrane will damage membrane fluidity and affect motility and count[29]. Additionally, sperms mitochondrial impairment that is produced from cisplatin can damage Na+ /K + -ATPase as inhibiting ATP production, and ultimately damage flagellar motility of sperms and inhibit fertilizing potential[30]. Thiamine pyrophosphate, in the current study, significantly reduced the damage caused by cisplatin in a dose-dependent manner. These results are in line with Zhang *et al.* study (2020), who demonstrate that vitamin C can recover sperms count, viability and normal morphology that deteriorated by cisplatin injection[31]. This effect may be related to the ability of TPP for suppressing sperms membrane lipid peroxidation by acting as co-factor with transketolase enzyme that mediates pentose- phosphate shunt, which is responsible for the production of NADPH, where NADPH is required for scavenging and neutralizing ROS that produced sperms damage. Also, it encompasses the bioenergetics pathway which leads to the creation of ATP as an essential molecule for improving sperms linear motility and concentration[32,33].

The determination of serum testosterone levels can use as predictive biomarker for testicular function and diagnosis of male infertility[34]. Regarding the findings of the present study, the cisplatin-treated group showed a remarkable and significant diminish in serum testosterone, this result was consisted with Shakibaie *et al.* (2020)[35]. Low level of testosterone may be due to the direct chemical influence of cisplatin on Leydig cells and the effect of epithelial layers of germ cells on LH- Leydig cell- axis. The cisplatin effect on sex hormones was significantly reversed by thiamine pyrophosphate via raising testosterone level in a dose-dependent manner. This finding was in line with Hernadez *et al*. (2014) study, who showed that the administration of TPP can decrease age-related testicular alteration by raising the rat's serum testosterone level[36]. The current study disagreed with that of Adelakun *et al.* (2018)[37], who observed no

significant difference in using ascorbic acid (50 mg/kg) in reversing cisplatin-induced suppression to sex hormones.

Cisplatin administration in the present study was reflected in a significant reduction of testicular SOD and GPx. These results were in line with Ekinci *et al.* (2019)[38] study. Cisplatin can increase the production of ROS, including superoxide radical, that will consume the enzymes responsible for ROS removal, including SOD and GPx. This inhibition in the antioxidant system will increase H_2O_2 levels which further proceed to Fenton's reaction to produce hydroxyl radical that may responsible for protein disruption, lipid peroxidation, and DNA damage, ending with a testicular tissue injury[36]. The co-administration of TPP to cisplatin in this study showed significant improvement in testicular SOD and GPx levels in a dose-dependent manner, these findings were similar to Irfan *et al*. (2020) data, who showed that TPP can inhibit oxidative stress markers, including SOD, after methotrexate treatment[39]. Thiamine pyrophosphate has a role in the pentose-phosphate pathway and in NADPH production, which is required by glutathione peroxidase for reduced glutathione formation, where it considers as an essential defensive antioxidant in spermatozoa, since they have a small cytoplasmic size[33,40].

In the current study, cisplatin has a significant role in the up-regulation of testicular caspase-3 activities, this outcome was comparable with that of Saad *et al.* (2020)[41]. Oxidative stress can increase calcium influx that triggers both dependent and independent-apoptotic pathway [42]. Meanwhile, TPP was significantly minimized the caspase-3 elevation induced by cisplatin in a dose-dependent manner. This result was in line with other study that showed the inhibitory effect of resveratrol on cisplatin-induced testicular caspase-3 elevation, as reported by Aly *et al.* (2020)[43]. This finding suggest that thiamine pyrophosphate may down-regulate apoptotic-related genes and altering signaling pathway that causes spermatogenic cells apoptosis which may further interfere with maturation and proliferation of these cells[44].

This study also established that cisplatin markedly induced damage to seminiferous tubules and spermatozoa, which is characterized by a decrease in tubular layers thickness, developed severe destruction of Leydig cells, ending with a defect in the formation of spermatids and mature sperms, these findings were concur with that of Kohsaka *et al*. (2020)[45]. The co-treatment of TPP with cisplatin significantly recovered the histo-morphologic integrity of the testis and Jonson's score in a dose-dependent manner, these data were in line with previous study that used resveratrol in amelioration of histopathological damage induced by cisplatin, as reported by Özyılmaz *et al*. (2019)[46].

Prostaglandin F2α- receptors (PGF2α-R) are found mainly in Leydig cells, and to less extent in Sertoli cells and seminiferous tubule walls[47]. A previous study showed the effect of a low physiological level of PGF2α in the enhancement of reproductive function via stimulating sperms motility that assists in raising the number of sperms that bind to zona pellucida, the glycoprotein layer that surrounds the plasma membrane of mammalian oocytes[48]. Also, it may act as a negative regulator for steroidogenesis[49]. In the current study, cisplatin markedly increased PGF2α-R expression within testicular tissue. This result was similar to Yamaguchi *et al*. study (2007), who demonstrated that cisplatin can increase testicular cyclooxygenase-2 (COX-2) and PGF2α levels[50]. At physiological concentration, PGF2α is important for normal spermatogenesis, whereas altering in PGF2α level can cause variation in testosterone levels, also it can affect sperms concentration [51]. This study reveals that TPP significantly inhibits testicular PGF2α-R over-expression in a dose-dependent manner, where the staining intensity score of TPP-high dose declined and was equal to that of control. This outcome is parallel to Gunnarsson *et al*. (2004) who demonstrated that zinc can inhibit PGF2α over-expression and thus ameliorates testicular damage and testosterone suppression[52].

CONCLUSIONS

From this study, one can concludes that rat's testicular damage induced by cisplatin can be ameliorated with thiamine pyrophosphate in a dose-dependent manner, represented by the improvement of gonadosomatic index, sperms parameters, testosterone, oxidative stress status, histopathological morphology, and over expression of rats testicular PGF2α-R. The proposed protective mechanism of thiamine pyrophosphate against cisplatin testicular toxicity may attribute to its antioxidant activity and anti-apoptotic effect, represented by the reduction of testicular caspase-3 levels.

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