The Testicular Protection Effect of Thiamine Pyrophosphate Against Cisplatin-treated Male Rats

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Abstract:

Infertility is a worldwide problem affecting both genders, it can be defined as the inability of the adult males to make a fertile woman pregnant after one year of regular intercourse. Cisplatin considers one of the most potent antineoplastic drugs that is extensively used, alone or in combination with other antitumor agents, to manage solid and germ cell cancer. The major drawback in cisplatin treatment is its damaging consequence on various body tissue, including the testis, liver, renal and others. One of its pronounced adverse effects is testicular injury, which may proceed to end with infertility. Thiamine pyrophosphate is the active form of thiamine which has an important role in the oxidative phosphorylation pathway. It acts as a co-factor and energy source for many cellular enzymes, also it utilizes by pentose-phosphate shut that elevates NADPH and improves antioxidants level. This study aimed to evaluate the effect of thiamine pyrophosphate on sperm parameters and gonadotropic hormones (luteal and follicle-stimulating hormone) of male rats exposed to a single dose of cisplatin.

Twenty-eight albino male rats were randomly grouped into four groups. Control group: received normal saline, Cisplatin group: received normal saline and cisplatin, TPP50 group: received thiamine pyrophosphate (50mg/kg) with cisplatin, and TPP100 group: as third group (TPP50) but thiamine pyrophosphate dose was 100 mg/kg. Semen samples used to measure the sperms viability and morphology, while serum samples were gathered to measure the levels of gonadotropic hormones (FSH and LH).

This study revealed that rat's testicular function was notably deteriorated by cisplatin administration, represented by a reduction in sperm parameters (viability and normal morphology), and serum gonadotropic hormones (FSH and LH). In this work, thiamine pyrophosphate was act as a protective agent that ameliorates rat's testicular damage induced by cisplatin in a dose-dependent manner. The suggested mechanism may attribute to its antioxidant and anti-apoptotic action.

Key words: Cisplatin, Thiamine pyrophosphate, Male infertility.

دور بيروفوسفات الثيامين في حماية الخصية في ذكور الجرذان المعاملة بالسسبلاتين

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الخلاصة:

العقم مشكلة عالمية تؤثر على كلا الجنسين ، ويمكن تعريفه على أنه عدم قدرة الذكور البالغين على جعل المرأة الخصبة حاملا بعد عام واحد من الجماع المنتظم. يعتبر السسبلاتين أحد أقوى الأدوية المضادة للأورام التي يتم استخدامها على نطاق واسع ، بمفردها أو بالاشتراك مع ادوية أخرى مضادة للأورام ، لمعالجة سرطان الخلايا الصلبة والجرثومية. المأخذ

الرئيسي في علاج سيسبلاتين هو تأثيره الضار على أنسجة الجسم المختلفة ، بما في ذلك الجهاز التناسلي والكبد والكلى وغيرها. أحد آثاره السلبية الواضحة هو إصابة انسجة الخصية ، والتي قد تنتهي بالعقم. بيروفوسفات الثيامين (TPP)هو الشكل النشط للثيامين الذي يلعب دورًا مهمًا في مسار الفسفرة المؤكسدة, لكونه يعمل كعامل مساعد ومصدر للطاقة للعديد من الإنزيمات الخلوية ، كما أنه يستخدم عن طريق إغلاق فوسفات البنتوز الذي يرفع ال NADPH ويحسن مستوى مضادات الأكسدة. هدفت هذه الدراسة لتقييم تأثير بيروفوسفات الثيامين على المؤشرات المنوية ومستوى الهرمونات المنشطة للجهاز التناسلي في ذكور الجرذان(LH ,FSH) الذين تعرضوا لجرعة واحدة من دواء السيسبلاتين.
تم استخدام ثمانية وعشرون من ذكور الجرذان البيضاء غير المعالجة وقسمت بشكل عشوائي الى أربع مجموعات. تلقت المجموعة الاولى محلول ملحي متساوي التوتر (9.0 ٪) فقط بواسطة الحقن داخل الصفاق, تلقت المجموعة الثانية المحلول الملحي متساوي التوتر(9.0 ٪) لمدة خمسة أيام متبوعة بجرعة واحدة من السيسبلاتين داخل الصفاق (5 ملغم / كغم) مع الاستمرار باعطاء المحلول الملحي لغاية اليوم الثاني عشرمن التجربة, المجموعة الثالثة تمت معالجتها باعطاء بيروفوسفات الثيامين (50 ملغم / كغم) لمدة خمسة أيام ثم حقنت بجرعة واحدة من السيسبلاتين (5 ملغم / كغم) عبر الصفاق في اليوم السادس تلاها اعطاء بيروفوسفات الثيامين (50 ملغم / كغم) لمدة سبعة أيام , وبالمثل فإن البروتوكول العلاجي للمجموعة الرابعة كان مشابها للمجموعة الثالثة لكن جرعة بيروفوسفات الثيامين كانت (100 ملغم / كغم).
جمعت عينات السائل المنوي واستخدمت لقياس حيوية الحيوانات المنوية ، وشكلها و تم جمع عينات المصل لقياس مستويات الهرمونات المنشطة للجهاز التناسلي.(LH ,FSH)
كشفت هذه الدراسة أن وظيفة الخصى يمكن ان تتدهور بشكل ملحوظ بعد إعطاء عقار السيسبلاتين ؛ والذي تمثل في انخفاض مؤشرات الحيوانات المنوية (نسبة الحيوية والتشكل الطبيعي) ومستويات الهرمونات المنشطة للجهاز التناسلي (LH ,FSH) . من خلال هذه الدراسة, قد يعمل بيروفوسفات الثيامين كعامل وقائي لمنع تلف الخصى الناجم عن علاج السيسبلاتين بطريقة تعتمد على الجرعة المعطاة. ممكن ان تعزى آلية عمل بيروفوسفات الثيامين في كونه مضاد فعال للأكسدة ، وبالتالي يعمل على تحسين وظائف الجهاز التناسلي الذكري.
الكلمات المفتاحية: السسبلاتين, بيروفوسفات الثيامين, العقم في الذكور.

Introduction

Infertility is well-defined as the incapability of the adult male to make fertile women 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, it causes either permanent or transient infertility [4] 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 [5,6]. Cisplatin treatment can lead to infertility in males due to the apoptotic effect on germ cells of the testis [7]. Cisplatin can induce apoptosis and activate caspases family (cysteine proteases) which include: caspases 8, caspases 9, caspases 3, 6 and 7 (executioner caspases that can activate and cleave poly polymerase to control execution phase of apoptosis by managing DNA fragmentation) [5].

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 dehydro-genase, branch-chain amino acid dehydrogenase, pyruvate dehydrogenase and transketolase [8].

This study aimed to evaluate the effect of thiamine pyrophosphate on sperm parameters and gonadotropic hormones (FSH and LH) of male rats exposed to a single dose of cisplatin.

Materials and Methods

Chemicals

All chemicals and kits used in this work were of highest available purity, their origin was as follow: Cisplatin (Koçak-Farma-Turkey), Thiamine pyrophosphate (Sigma-Aldrich-Germany), FSH and LH (Mybiosource-USA). All measurements depended on enzyme-linked immunosorbent assay (ELISA) technique.

Animals

Twenty-eight, non-previously treated male albino rats, weighing approximately 200-300gm, 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 group. The doses of TPP and cisplatin were selected according to the results obtained from our preliminary study and previous literature [9,10], as below:

a) Group I (control): received normal saline (0.9%) by intraperitoneal route for 12 days.

b) Group II (Cisplatin): received normal saline (0.9%) for five days followed by a single dose of cisplatin (5mg/kg) then normal saline again for seven days, all by intraperitoneal route.

c) Group III (TPP50+Cis): received thiamine pyrophosphate (50 mg/kg) for 5 consecutive days (day1-5) followed by a single dose of cisplatin (5 mg/kg) in the sixth day, then thiamine pyrophosphate again (50 mg/kg) for seven days (day 6-12), all by intraperitoneal route.

d) Group IV (TPP100+Cis): similar to group 3 but TPP dose was 100mg/kg.

Samples collection

For measurement FSH and LH levels, blood samples 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^o [11]. The kits utilize a competitive ELISA technique. The primary antibody and sample incubate to form a specific antigen-antibody complex, then added to the plate. Each plate was pre-covered with a specific antigen, followed by a washing- out process which is done to remove the unbounded antibodies then incubate with enzyme conjugated antibody then wash. Finally, the substrate is added to produce a calorimetric signal that can be read in the plate reader [12].

To determined sperms morphology and viability, semen samples were collected. The left epididymis was carefully dissected into three parts by scissor then placed in a petri dish contain 1 ml of pre-wormed (37C^o) PBS solution (PH: 7.4) and incubate at 37C^o 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 [13-14], while sperm viability was determined within 3-4 minutes after adding eosin stain (1%) under magnification (X400). Sperms with redhead consider dead while unstained considered viable for 100 sperm/sample [15].

Statistical analysis

The collected data were expressed as mean ± standard error of mean (M±SEM). The percentage difference was calculated as the

following equation: % of change = (new No.-original No.) ÷ Original No. × 100. 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.

Results

Effect of thiamine pyrophosphate on sperms parameters of rats exposed to cisplatin

The percent of morphological changes in sperms displayed in figure (1) shows a significant difference between control and cisplatin group (*P*-value ≤ 0.05). In cisplatin group, sperms normal morphology was about 42.4% lower than that of control. Both TPP50 and TPP100-treated groups showed significant improvement in sperms shape, compared with cisplatin alone group (*P*-value ≤ 0.05). This improvement was about 32.8% and 45.9%, respectively in TPP50 and TPP100- treated groups, where the percent with higher dose nearly approached to that of control, as shown in figure (1).

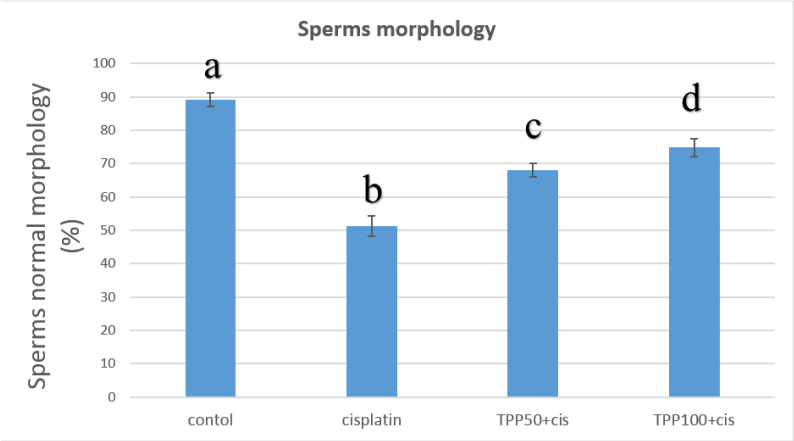

Figure (1): Effect of thiamine pyrophosphate on the sperms morphology of rats exposed to cisplatin.

Data were expressed as Mean ± SEM. Different small letters indicate statistically significant difference among groups. *P*-value ≤ 0.05 considered statistically significant difference. TPP50= Thiamine pyrophosphate50mg/kg, TPP100= Thiamine pyrophosphate 100mg/kg Cis= cisplatin.

Regarding the percent of sperms viability in rats given just cisplatin, it was significantly lower (21.8%) than that observed with control rats (*P*-value ≤ 0.05). This effect was reversed when rats exposed to cisplatin were co-administered with TPP50 and TPP100, where the viability of sperms was increased by about 7.4% and 15% respectively, compared with the cisplatin group. Even though, no statistically significant difference was found between the two doses of TPP (50 and 100 mg/kg) when compared to each other (*P*-value = 0.122), but the viability percent within higher dose was approached to that of control, as presented in figure (2).

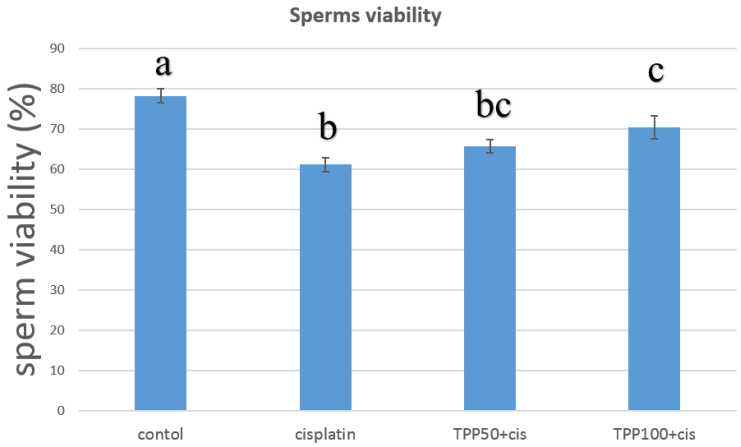

Figure (2): Effect of thiamine pyrophosphate on the sperms viability of rats exposed to cisplatin.

Data were expressed as Mean ± SEM. Different small letters indicate statistically significant difference among groups. *P*-value ≤ 0.05 considered statistically significant difference. TPP= Thiamine pyrophosphate, Cis= cisplatin.

Effect of thiamine pyrophosphate on gonadotropic hormones of rats exposed to cisplatin

Figure (3) presents a statistically significant decline of LH mean levels by 48.6% for rats received just cisplatin, compared with the control group (*P*-value ≤ 0.05). Meanwhile, the effect of cisplatin on LH level was significantly revered within TPP50 and TPP100 -treated groups (*P*-value ≤ 0.05), presented by elevation of LH mean levels by 43% and 61% respectively, approaching to control group, especially with the higher dose of TPP, where there was a significant difference in the extent of elevation of LH level between the two doses of TPP, in a dose-dependent manner (*P*-value ≤ 0.05).

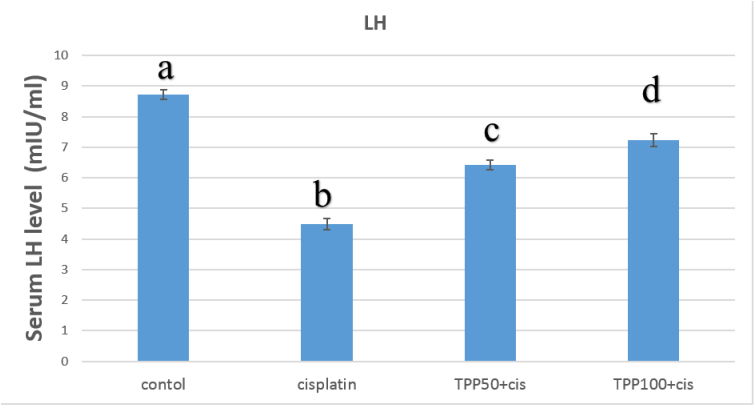

Figure (3): Effect of thiamine pyrophosphate on serum LH levels of rats exposed to cisplatin.

Data were expressed as Mean ± SEM. Different small letters indicate statistically significant difference among groups. *P*-value ≤ 0.05 considered statistically significant difference. TPP= Thiamine pyrophosphate, Cis= cisplatin, LH= luteal hormone.

In this study, the mean serum level of FSH within the cisplatin group was significantly lower than that of control by about 40% (*P*-value ≤ 0.05). On the other side, levels

of this hormone were significantly increased in TPP50 and TPP100 - treated groups (by 30.5% and 39.5%, respectively) when compared to the cisplatin alone group (*P*-value ≤ 0.05), and even though the higher TPP dose group approached more to control, no statistically significant difference was observed (*P*-value = 0.065) between the two studied groups of TPP, as shown in figure (4).

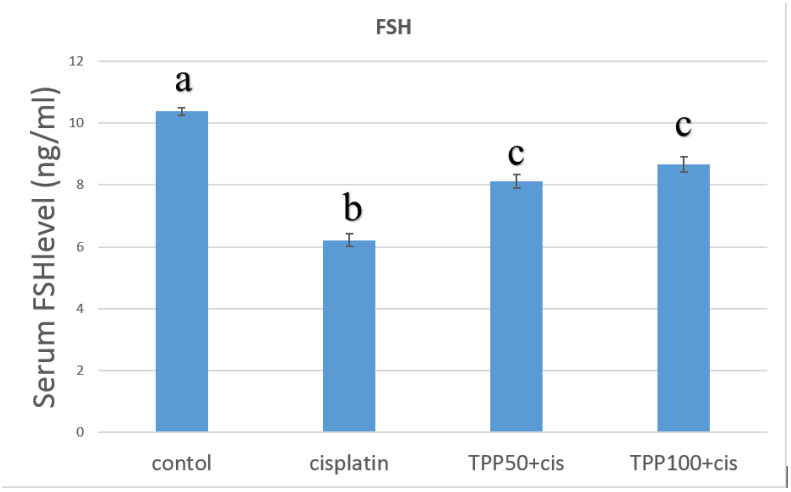

Figure (4): Effect of thiamine pyrophosphate on serum FSH levels of rats exposed to cisplatin.

Data were expressed as Mean ± SEM. Different small letters indicate statistically significant difference among groups. *P*-value ≤ 0.05 considered statistically significant difference. TPP= Thiamine pyrophosphate, Cis= cisplatin.

Discussion

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 viability and morphology [16]. In this study, cisplatin significantly reduces sperm viability, also it increases abnormal sperms. A similar observation was described by Amir *et al.* study (2019), who demonstrated that cisplatin reduces sperms viability and DNA integrity [17]. The testicular damage caused by cisplatin treatment is mainly attributed to increased ROS production. The testicular damage caused by cisplatin treatment is mainly attributed to the increased reactive oxygen species (ROS) production through 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 viability and morphology [18].

Thiamine pyrophosphate, in the current study, significantly reduced the damage caused by cisplatin in a dose-dependent manner. These results are in line with Shan et al. (2009) confirmed that co-administration of vitamin C and thiamine together can regularize testicular damage produced by the heavy metal "lead" administration, which is characterized by low sperms concentration, motility and abnormal germ cells morphology [19]. 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,

viability, morphology and concentration [20-21].

The Hypothalamus-Pituitary-Gonadal (HPG) axis and Hypothalamus-Pituitary-Adrenal (HPA) axis are neuroendocrine systems that controlled by the circadian cycle and stress. Hypothalamic neurons after stimulation can release adrenocorticotrophic hormone (ACTH) and gonadotropin-releasing hormone (GnRH) from the anterior pituitary gland, where ACTH is responsible for glucocorticoid release. The sensitivity of the HPG axis is controlled by a negative feedback mechanism through the stimulation of cortisol receptors within the pituitary gland and hypothalamus. At a physiological level, ROS has an essential role in controlling the HPA axis hemostasis [22]. Treating with a low or therapeutic dose of cisplatin may increase cortisol level [23]. Moreover, oxidative damage and elevation of ROS level may cause alteration to the HPA and Hypothalamus-Pituitary-Adrenal (HPA) axis and/or interrupt crosstalk between hormones that may elevate cortisol levels, leading to inhibit LH and FSH secretion by inhibiting GnRH from the hypothalamus and diminish the sensitivity of pituitary gland to GnRH [24-25].

Regarding the findings of the present study, the cisplatin-treated group showed a remarkable and significant diminish in serum LH and FSH level when compared with the control group. These results were consisted with Shakibaie *et al.* (2020) [26], who found that a single injection of cisplatin can reduce gonadotropic hormone levels (LH and FSH) within lab animals. Anterior pituitary gland secret LH in response to GnRH which in turn stimulates Leydig cells to produce testosterone. Therefore, the expected 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, or indirectly by downregulating the sensitivity hypothalamic-pituitary axis to the negative feedback mechanism, i.e., cisplatin stimulate inhibitory neuron circuit that controls feedback mechanism, including arginine vasopressin (AVP) in the CNS [27]. This, in turn, stimulates Corticotrophin-Releasing Hormone (CRH) and ACTH from the medullary region of the adrenal gland in the PKC-dependent pathway [28]. This can inhibit the HPG axis and end with a decrease in GnRH secretion from the hypothalamus [29]. Furthermore, it may cause hypopituitarism which may be established by the decline in serum FSH level.

In this study, the cisplatin effect on gonadotropic hormones was significantly reversed by thiamine pyrophosphate via raising serum LH and FSH level in a dose-dependent manner. These results were in line Shati *et al*. (2019) [30] who co-administered resveratrol with cisplatin and elevate serum level of LH and FSH by inhibiting ROS produced by cisplatin.

Conclusion:

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 sperms parameters and gonadotropic hormones. The proposed protective mechanism of thiamine pyrophosphate against cisplatin testicular toxicity may attribute to its antioxidant activity.

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