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THE POTENTIAL PROTECTIVE ACTIVITY OF DATE PALM (*PHOENIX DACTYLIFERA*) POLLEN AND *PANAX GINSENG* AGAINST CISPLATIN-INDUCED TESTICULAR TOXICITY IN RATS

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ABSTRACT

Cisplatin (CIS) is a potent drug used in clinical oncology but causes testicular damage. The present study was aimed to investigate the protective potential of date palmpollen (DPP) and *Panax ginseng* (*P. ginseng*) extracts against CIS-induced testicular toxicity in rats. The fertility experiment was done on six groups of male rats. The 1st group was kept as normal control, while the 2nd-6th groups were treated with single intraperitoneal injection of CIS at a dose of 3 mg/kg three times per week for 35 days on 1st, 3rd and 5thdays of the week. The 2nd group was left as CIS-control, while the 3rd and 4th groups were given orally DPP at doses of 200 and 400 mg/kg, respectively for 8 consecutive weeks. The 5th and 6th groups were given orally *P. ginseng* at doses of 200 and 400 mg/kg, respectively for 8 consecutive weeks. Male fertility was evaluated by estimating serum testosterone level, epididymal sperm characters and genital organs weight. Moreover, the concentration of lipid peroxidation product (MDA) and glutathione (GSH) contents was also estimated in the testicular homogenate. Mating success %, fertility success % and fertility index were also calculated. Fertility parameters were further confirmed by histopathological examination of testes.

The results of the present study have clearly shown that the impact of CIS on several reproductive parameters of male rats was improved by DPP and *P. ginseng* administration (400

mg/kg) in term of increased serum testosterone concentration, sperm count, sperm motility and sperm viability and genital organs weight. Moreover, the present study suggested that DPP and *P. ginseng* might have a protective effect against oxidative stress-induced impaired testicular functions in CIS-treated rats as they reduced MDA and elevated GSH levels in the testicular homogenate. Thus, the present results indicate the protective effect of DPP and *P. ginseng* against CIS-induced testicular toxicity.

Keywords: Date Palm Pollen, *Panax ginseng*, Testosterone, Fertility Index, Male Fertility

INTRODUCTION

Cancer is one of the most common fatal diseases in global society. In cancer, cells divide and grow uncontrollably, forming malignant tumors, and invading nearby parts of the body. CIS, a chemotherapeutic agent has been successfully used for treatment of different types of malignancies **[1]**. It is an efficient platinum-derived alkylating agent which acts against proliferating and resting cells **[2]**. CIS treatment is coupled with several toxic side effects including nephrotoxicity, oxidative stress injury and testicular damage **[3]**. Several studies suggested that CIS affects Sertoli cells and Leydig cells of testis, thereby causes anti-spermatogenic **[4]** and anti-steroidogenic **[5]** effects, respectively.

Herbal medicine is gaining importance day-by-day in the management of several disorders including male reproductive disorders. DPP and *P. ginseng* are well recognized to improve male fertility **[6]** and **[7]** respectively. The date palm (*Phoenix dactylifera*) belongs to the Aracaceae family and is indigenous to the Arabian Peninsula, the Mediterranean, and North Africa countries, parts of India and hotter parts of the USA. The date has always played an important role in the economy and social life of the people of arid and semiarid regions of the world **[8]**. Suspension of DPP is an herbal mixture that is widely used as a folk remedy for curing male infertility **[9]**. Many researchers have documented the antioxidant property of DPP **[10]**. It was reported that PDP extracts are rich in carotenoids, flavonoids, estrogenic materials, estrone, as gonad-stimulating compounds that improve male infertility and exhibit gonadotrophin activity in the rat **[11]**.

P. ginseng has been recognized as the most prized medicine among all herbal medicine. It has the most potent multiple pharmacologic actions for anticancer, antihypertension and anti-nociception effects and for improving weak body conditions **[12]**. It was also found

to possess anti-stress and anti-aging activities **[13]**. In addition, several researches give evidences that ginseng possesses anti diabetic properties through lowering blood glucose effect and stimulating sugar metabolism **[14]**. Kitts and Hu **[15]** mentioned that ginseng has powerful antioxidant properties that may explain its anti-inflammatory and antineoplastic effects. Ginseng contains many physiologically important constituents that include saponins, polyacetylenes, polyphenolic compounds and acidic polysaccharides **[16]**.

In the present investigation, we have investigated the potential beneficial effects of DPP and *P. ginseng* against CIS-induced testicular toxicity in male rats.

MATERIAL AND METHODS

Plant Material

DPP were collected from Giza province, Egypt during March 2010. The pollens were separated from the kernels with a fine gauze sieve. DPP (0.5 kg) were extracted by percolation in 2L of 80% aqueous ethanol with occasional shaking for 48 h. This process was repeated three times, and then the ethanolic extracts were combined and evaporated under reduced pressure to give 70 g of yellowish semisolid residue.

P. ginseng powder was purchased from a local herbal store in Al-Kharj, KSA. The powder of *P. ginseng* was soaked with water (1:25 W: V) in a glass flask for three hours. The container then was placed in boiling water bath for 40 min. The solid residue was subjected to the same process once again, and then the water fractions were combined and filtered through Whatman No. 1 filter paper.

Animals

Healthy adult male (185-200 g) and female (160-170 g) Wistar rats were used in the fertility study. Both sexes of adult albino mice (28–32 g b.wt) were used in the acute toxicity test. Animals were obtained from Lab Animal Care Unit, Pharmacy College, Salman bin Abdulaziz University, Al-Kharj, KSA. All animals were kept under uniform and controlled conditions of temperature and light/dark (12/12 h) cycles, fed with standard rodent diet and water *ad libitum*. The animals were allowed to acclimatize to the laboratory condition for one week before commencement of the experiment. The experimental tests on animals have been performed in accordance with the Institutional Ethical Committee approval.

Acute Toxicity Test (LD50)

Acute toxicity study was performed according to Ghosh **[17]**. Two groups of mice (n=6) were fasted overnight then treated orally with the extracts of DPP and *P. ginseng*, respectively at a dose of 2000 mg/kg using

intragastric tube. Another control group was given an equal volume of the vehicle (3% v/v Tween 80 in distilled water) and kept under the same conditions without any treatment. General symptoms of toxicity and mortality were observed for 24 hours for any sign of delayed toxicity. Since, there was no mortality at this level; the dose of both extracts was increased to 4000 mg/kg and animals were observed for another 48 h.

Justification for Dose Selection

Extracts of DPP and *P. ginseng* were nontoxic at the dose of 4000 mg/ kg so, 1/20th and 1/10th of this dose (200 and 400 mg/kg, respectively) were selected for the study.

Experimental Design

Thirty-six sexually mature male Wistar rats (185-200 g b. wt) were randomly divided into six groups (n=6). Rats of the 1st group received the vehicle in a dose of 5 mL/kg and kept as normal control. The 2nd-6th groups were treated with single intraperitoneal injection of CIS at a dose of 3 mg/kg three times per week for 35 days on 1st, 3rd and 5th days of the week **[18]**. In addition, the 2nd group was left as CIS-control, while the 3rd and 4th groups were given orally DPP at doses of 200 and 400 mg/kg, respectively for 8 consecutive weeks. The 5th and 6th groups were given orally *P. ginseng* at doses of 200 and 400 mg/kg, respectively for 8 consecutive weeks.

Sacrification Schedule

Twenty-four hours after last dose of the tested extracts, the rats were weighed and sacrificed under light ether anesthesia.

Parameters

Estimation of Testosterone and Oxidative Stress Markers

Blood samples were collected from rats for estimations of serum levels of testosterone. Sera were separated into clean bottles, stored frozen and used within 12 h of preparation for the estimation of testosterone using commercial diagnostic kits and ELISA kits **[19]**. The concentration of lipid peroxidation product, MDA, was determined in homogenates of liver using the thiobarbituric acid according to Ohkawa *et al*., **[20]**. The same homogenates of liver were used in determination of the concentration of endogenous non enzymatic antioxidant, GSH **[21]**.

Assessment of Sperm Motility and Count

Progressive motility was tested immediately. The right cauda epididymidis was incised and semen was squeezed on a pre-warmed slide. Two drops of warm 2.9% sodium citrate was added to semen and mixed by a cover-slip. The percentage of progressive sperm motility was evaluated visually at 400× magnification

[22]. Motility estimates were performed from three different fields in each sample. The mean of the three successive estimations was used as the final motility score. For sperm count, the left cauda epididymidis was incised and semen that oozed was quickly sucked into a red blood pipette to the 0.5 mark, and then diluted with warm normal saline up to the 101 mark. A drop of the semen mixture was placed on the Neubauer counting chamber and viewed under the magnification of ×40 **[22]**. The total numbers of sperm cells were counted and expressed as 10^6/mL.

Sperm Viability Assay (Percentage of Live Spermatozoa)

A drop of semen was squeezed onto a clean microscope slide and mixed with 2 drops of eosin/nigrosin stain. Slides were prepared and incubated for 2 min at room temperature before being evaluated using a light microscope at ×400 magnification. Two hundred sperm were counted for each sample and viability percentages were calculated **[23]**. Viable sperms were not stained while dead sperms stained pink.

Sperm Morphological Study

To determine the percentage of morphologically abnormal spermatozoa, the slides stained with eosin–nigrosin viewed under a light microscope at 400× magnifications. Two hundred sperm cells were examined per animal **[23]**. Any disorders in the morphology and structure of either head or tail or both were considered as abnormal.

Mating Trial Test

Mating trial test of male rats was done, 2 weeks before the termination of the experiment. Each male rat was cohabitated overnight with 2 estrous females and housed in a single cage. Positive mating was confirmed by presence of sperm and vaginal plug in the vaginal smear the following morning **[24]**. Each sperm positive female was kept under observation and the resultant pregnancies were noted, when dam gave birth. The following reproductive parameters were then computed: mating success % = ([number mated/number paired] × 100); fertility success % = ([number pregnant/number paired] × 100); Fertility index = ([number pregnant/number mated] × 100).

Body and Sex Organs Weights

The initial and final body weights of the animals were recorded. The testes, seminal vesicle and ventral prostate were dissected out, freed from adherent tissues and blood, and weighed to the nearest milligram. Organ weights were reported as relative weights (organ weight/body weight ×100).

Histological Analysis

Testes were carefully dissected out following abdominal incision and fixed in 10% formol-saline and processed routinely for paraffin embedding. Sections of 5 μm were obtained with rotary microtome, stained with Hematoxylin and Eosin Stalin (H/E) and observed under a light microscope.

Statistical Analysis

The values are expressed as mean ± SEM of six observations in each group. All groups were subjected to one-way analysis of variance (ANOVA), which was followed by Dunnett's post hoc test to determine the intergroup variability by using SPSS ver. 14.0. A comparison was made with the experimental control group. Differences were regarded statistically significant at the $P \leq 0.05$ level.

RESULTS

Acute Toxicity Test

Extracts of DPP and *P. ginseng* were well tolerated by mice at doses up to 4g/ kg. No mortalities observed with oral administration of both extracts during 48h of observation even at the highest dose. DPP and *P. ginseng* did not produce any symptom of acute toxicity and none of the mice exhibit hyperactivity, convulsions, sedation, hypothermia, and respiratory distress. Accordingly; it suggested that oral LD50 of both extracts are higher than 4g/ kg b.wt in mice.

Blood Testosterone Level

The results presented in **Table 1** showed that CIS significantly decreased the serum testosterone level (2.42±0.16 ng/mL) as compared to normal control group (3.18±0.24 ng/mL). Administration of CIS + DPP and CIS + *P. ginseng* at 400 mg/kg to rats for 8 weeks improved the level of testosterone in their blood (5.65±0.38 and 5.15±0.47 ng/mL, respectively) when compared with that of the CIS-controls.

Testicular MDA and GSH

MDA level in the testicular tissue was found to be significantly higher in rats treated with CIS alone than those in the normal control group. GSH level in testicular tissue of rats treated with CIS alone was significantly lower than those in normal control group. The testicular MDA was significantly declined while the testicular GSH activity was significantly elevated following CIS + DPP and CIS + *P. ginseng*- medication of rats at a dose of 400 mg/kg for 8 weeks as compared with those of CIS-control rats.

Epididymal Sperm Characters

The average sperm count, motile sperm and viable sperm in the normal control rats were found to be 82.2±3.53 millions/mL, 86.4±3.31% and 87.4±3.53%, respectively. A

significant decrease in epididymal sperm count (24.7±1.84%), motility (37.8±2.46%) and viability (42.7±2.14%) was observed in CIS-control rats as compared to normal controls (Table 2). Moreover, the percentage of abnormal sperms in CIS-control rats was also increased significantly (31.65±2.51%) as compared to normal controls (8.53±0.33%). The most common abnormalities seen in the examined seminal smears of CIS-control rats were bent and coiled tail sperms **(Figure 1)**. Administration of DPP and *P. ginseng* at a dose of 400 mg/ kg to CIS-treated rats significantly increased the sperm count (63.7±3.35 and 60.4±3.34%, respectively), motility (67.5±3.53 and 66.7±4.52%, respectively) and viability (67.5±3.75 and 64.7±2.49%, respectively) and decreased the percentage of abnormal sperms (20.58±1.42 and 23.64±1.75%, respectively) as compared to CIS-controls. However, no significant changes were observed in sperm count, motile sperm, viable sperm and abnormal sperms in CIS + DPP and CIS + *P. ginseng*-treated rats at a dose of 200 mg/ kg as compared to CIS-control rats **(Table 2)**.

Body and Genital Organs Weight

Total average body weights of the rats were measured at baseline and at the completion of the experiment (8 weeks). At the end of the experiment, CIS-control rats showed high significant decrease in their body weight gain (192.3±6.50g) compared to normal control group (221.5±7.35g). The body weights of rats in CIS +DPP (400 mg/kg) group and in CIS+*P. ginseng* (400 mg/kg) group were significantly improved compared with those in CIS-control group. While the difference between the body weights at completion of the experiment were not significant in CIS +DPP (200 mg/kg) and CIS+*P. ginseng* extracts (200 mg/kg) groups, compared with CIS-control group.

The relative weights of testes, seminal vesicles and ventral prostate of CIS-control rats were significantly lower than those of normal controls. Relative weights of testes, seminal vesicles and ventral prostate increased significantly in CIS+DPP (400 mg/kg) and in CIS+*P. ginseng* (400 mg/kg) groups in comparison with CIS-control group. On the other hand, administration of DPP or *P. Ginseng* at a dose of 200 mg/kg to CIS-treated rats had no effect on the weights of testis, seminal vesicles and ventral prostate as compared to CIS-control rats.

Mating Trial

Mating trial between normal control males and normal females resulted in normal pregnancy outcome. The fertility success and fertility index of CIS-control male rats were significantly declined as compared to normal

controls **(Table 3)**. The fertility of male rats was improved following CIS + DPP and CIS + *P. ginseng* treatment (400 mg/kg) as evidenced by the number of mated females (8/12 & 7/12, respectively), fertility success (50.0 & 33.33%, respectively) and fertility index (75.0 & 57.14%, respectively).

Histopathological Evaluation

Histological observations of the testes of normal control rats revealed normal architecture of the seminiferous tubule **(Figure 2-A)**. The seminiferous tubules were rounded and are separated by a thin intertubular connective tissue. The germinal epithelia are formed of normal spermatogenic layers represented by spermatogonia, primary and secondary spermatocytes, spermatids and sperms. CIS-control rats showed testicular atrophy, and degenerative changes in spermatogonia cells lining the seminiferous tubules, associated with incomplete spermatogenesis **(Figure 2-B)**. The intercellular spacing became wider and the seminiferous tubules were shrunken and greatly depleted of germ cells. Improvement in the histopathological picture was noticed in examined sections from rats treated with CIS + DPP (400 mg/kg) as well as rats treated with CIS + *P. ginseng*(400 mg/kg) as the examined sections revealed apparent normal seminiferous tubules and interstitial spaces occupied by the Leydig cells. Most of their seminiferous tubules were close together with regular outlines and narrow interstitium **(Figure 2-C & D)**.

DISCUSSION

In the current study, oral administration of DPP and *P. ginseng* extracts at doses up to 4g/kg did not produce any symptom of acute toxicity and none of mice died during 48 h of observation. Accordingly, it suggested that oral LD50 of the tested extracts was higher than 4g/kg. Therefore, DPP and *P. ginseng* can be categorized as highly safe since substances possessing LD50 higher than 50 mg/kg are non-toxic **[25]**.

Numerous studies have shown that CIS therapy induces oxidative stress in testicular tissues **[18]**. Moreover, CIS causes lipid peroxidation and decreases the activity of enzymes that protect against oxidative damage in testicular tissue of CIS-treated rats. The present study showed that CIS lead to an increased oxidative stress in testes, which was manifested by an increase in MDA level and decrease in antioxidant GSH. The level of tissue MDA is reported to be a reliable marker of lipid peroxidation **[26]**. The present observationis in agreement with previous findings showing an increase in renal MDA level and decrease in the testicular antioxidant activity of GSH in CIS-medicated rats **[27]**.

Exogenous protective agents with antioxidant properties were reported to show some protective effects against CIS-induced toxicity. DPP and *P. ginseng* are promising agents against various toxicities associated with oxidative stress. In the current study, DPP and *P. ginseng* (400 mg/kg) ameliorated CIS toxicity, indicated by significant reduction in the elevated MDA and increase testicular GSH level. These results were confirmed by the histopathological examinations. Previous studies indicated that DPP **[28]** and *P. ginseng* **[29]** had potent antioxidant activity. In this respect, Conklin **[30]** mentioned that using of antioxidants during cancer chemotherapy may enhance therapy by reducing the generation of oxidative-stress induced aldehydes **[30]**.The antioxidant activity of DPP is attributed to the wide range of active compounds including sterols, flavonoids and triterpenoidal **[31]**. Accordingly, DPP could possibly have a testicular protective effect in CIS-treated rats by exerting its beneficial effect via modulating the antioxidant system. This finding was supported by Mansouri *et al*., **[32]** who indicated that the aqueous extracts of dates have potent antioxidant activity. Protection against oxidative damage of chemicals by ginseng was also demonstrated by Shukla and Kumar **[33]**. It has long been known that *P. ginseng* has protective properties against free radical attack **[34]**. In a different study, ginseng has the ability to protect liver cells against oxidative damage by diminishing ROS and hepatic lipid peroxidation **[35].**

The study of Maines *et al*., **[36]** demonstrated that exposure to CIS, lowered serum testosterone levels in rats. One of the molecular mechanisms of anti-gonadal effects induced by CIS is by mediating dysfunction of biosynthesis of testosterone **[37]**. In the current study, serum testosterone levels were significantly reduced in CIS-treated rats compared to normal controls. Cao *et al*., **[38]** indicated that excessive oxidative stress reduced levels of key enzymatic and non-enzymatic antioxidants in Leydig cells, and resulted in decline in testosterone secretion. Accordingly, the reduced serum testosterone level in CIS-treated rats in our study could be attributed to the impairment of Leydig cells. The administration of DPP and *P. ginseng*at a dose of 400 mg/ kg caused a significant increase in testosterone level in serum of male rats. These results agree with Kostyuk **[39]** who indicated that DPP increases the plasma levels of testosterone in rats. Moreover, rats that received 5% ginseng experienced a significant increase in blood testosterone level **[40]**.

Gonadal dysfunction and decrease in testosterone production are the consequence of cancer chemotherapeutic agents **[41]**. Moreover, sperm motility and count are considered as the important factors that affect male fertility **[42]**. In this investigation, the reduction in epididymal sperm motility and count of CIS-control rats could be connected to the reduction in the serum testosterone level. We concurred in this matter with Kishore *et al*., **[43]** and Ciftci *et al*., **[44]** who reported significant decrease in sperm count, sperm viability and reduced testosterone in rats exposed to CIS. Gong and Han **[45]** confirmed this explanation as they stated that lowering of epididymal sperm motility and count suggested an undersupply of testosterone to the epididymis. Besides hormonal alteration, the spermatogenic inhibition may also be due to the generation of ROS by CIS in the testicular tissue and the consequential elimination of sperm cells at different stages of development **[46]**.

Our results showed that all the sperm parameters were recovered after treatment with CIS+DPP (400 mg/kg) and CIS+*P. ginseng* (400 mg/kg). The improvement that observed in spermatogenesis among CIS+DPP and CIS+*P. ginseng*-delivered rats may be associated with the antioxidant properties of their extracts. The deleterious impact of CIS on sperm viability and abnormalities in the present study is in agreement with previous studies **[27]**. The increase in sperm abnormalities indicates that CIS induced DNA damage in germ cells leading to altered sperm morphology. The percentages of sperm viability were significantly improved while those of sperm abnormalities were significantly reduced in rats medicated with CIS+DPP (400 mg/kg) and CIS+*P. ginseng* (400 mg/kg) compared to CIS-control rats. This result may suggest that DPP and *P. ginseng* could have an effect on spermiogenesis process and they could favor normal sperm production. Experimentally, date extracts have been shown to increase sperm count in guinea pigs and to enhance spermatogenesis in rats **[47]**. Additionally, testosterone deficiency (like that observed in our CIS-control rats) produces immature sperm by early sloughing of spermatids from the Sertoli cells **[48]**. However, in the present study, the most notable abnormalities of the epididymal sperm of CIS-control rats were bent and coiled tails. The improvement in percentages of sperm abnormality and viability, in the present study, coincided with the increased testosterone level in the serum of DPP and *P. ginseng*-treated rats.

It is well known that low sexual desire and penile erection are related to low serum

testosterone level **[49]**. In the current study, the fertilizing ability of CIS-control males was significantly reduced. The reduction in mating and fertility success of CIS-control rats could be due to decreased testosterone level that reduces androgen-dependent parameters like mating behavior, libido and penile erection **[50]**. Pregnancy rates of the untreated female rats were reduced following mating with CIS-control males. The decrease in the pregnancy rate might be due to the effects of CIS on the progressive epididymal sperm motility as sperm motility is positively correlated with fertilization of oocytes and pregnancy rates **[51]**. The percentages of mating success, fertility success and male fertility index were improved in rats medicated with CIS+DPP (400 mg/kg) and CIS+*P. ginseng* (400 mg/kg) compared to CIS-control rats. The decrease in sperm count is an important factor leading to male infertility **[52]**. Moreover, motility is critical in enabling the sperm to ascend the female reproductive tract to the site of fertilization and also is necessary to achieve fertilization **[53]**. Thus, the observed increase in fertility success and index in CIS+DPP and CIS+*P. ginseng* compared to CIS-control rats could be attributed in part to the concomitant increase in sperm count and motility.

In this study, the final body weight of CIS-control rats was significantly reduced compared to normal controls. The decline in body weight was attenuated by treatment with the tested extracts at 400 mg/kg suggesting that DPP and *P. ginseng* could possess some protective constituents that prevented the loss of weight in rats exposed to CIS. Creasy **[54]** has reported that weights of testes and accessory sex organs are sensitive end points that can be used in evaluation of negative effect on male reproduction. In the present study, there was a significant decrease in the weights of testes and the accessory reproductive glands in CIS-control rats compared to the normal controls. This suggests that testis and accessory sex organs are vulnerable targets to CIS in rats. Reductions of testicular and accessory sex organs weights in CIS-control animals, shown in this study, have already been described by Malarvizhi and Mathur **[55]** and can be related to the reduced level of testosterone. Accordingly, the significant increase in the reproductive organ weights of rats that medicated with CIS +DPP (400 mg/kg) and CIS +*P. ginseng* (400 mg/kg) may be attributed to the improvement in testosterone levels. This effect can be explained also by the anti-oxidant property of DPP and *P. ginseng* that prevents cellular damage

occurring as a result of oxidative stress in spermatogenic cells of seminiferous tubules and Leydig cells of the testes.

IN CONCLUSION

Present study showed that CIS treatment has a deleterious impact on blood testosterone level and semen quality of rats. Oral administration of DPP and *P. ginseng* at 400 mg/kg for 8 weeks has a protective effect against testicular damage induced by CIS. Thus, this study revealed that pretreatment with DPP and *P. ginseng* protected against CIS-induced testicular toxicity.

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REFERENCES

[1] Khynriam D and Prasad SB, Changes in endogenous tissue glutathione level I relation to murine ascites tumour growth and the anticancer activity of cisplatin, Braz. J. Med. Res., 36, 2003, 53-63.

[2] Lirdi LC, Stupp T, Sasso-Cerri E, and Miraglia SM, Amifostine protective effect on cisplatin-treated rat testis, The Anatomical Record, 291, 2008, 797-808.

[3] Yao X, Panichpisal K, Kurtzman N, and Nugent K, Cisplatin nephrotoxicity: A review, Am. J. Med. Sci., 334, 2007, 115-124.

[4] Zhang X, Yamamoto N, Soramoto S and Takenaka I, Cisplatin-induced germ cell apoptosis in mouse testes, Arch. Androl., 46, 2001, 43-9.

[5] Malarvizhi D, and Mathur PP, Effect of cisplatin on testicular function in rats, Indian J. Exp. Biol., 34, 1996, 995-8.

[6] El-Neweshy M, El-Maddawy Z and El-Sayed Y, Therapeutic effects of date palm (Phoenix dactylifera L.) pollen extract on cadmium-induced testicular toxicity, Andrologia, 45 (6), 2013, 369-378.

[7] Salvati G, Genovesi G, Marcellini L, Paolini P, De nuccio I, Pepe M, and Re M, Effects of Panax ginseng C.A. Meyer saponins on male fertility, Panminerva Med., 38 (4), 1996, 249-254.

[8] Besbes S, Blecker C, Deroanne C, Drira NE and Attia H, Date seeds: Chemical composition and characteristic profiles of the lipid fraction, Food Chem., 84, 2004, 577-584

[9] Bahmanpour S, Talaei T, Vojdani Z, Panjehshahin MR, Poostpasand A, Zareei S and Ghaeminia M, Effect of Phoenix Dactylifera Pollen on Sperm Parameters and Reproductive system of Adult Male Rats, Iran J. Med. Sci., 31, 2006, 208-212.

[10] Assi MA, Study the A thesis of master, college of veterinary medicine, University of Baghdad, 2009.

[11] Dostal LA, Faber CK, and Zandee J, Sperm motion parameters in vas deferens and cauda epididymal rat sperm, Reprod. Toxicol., 10, 1996, 231-235

[12] Kaneko H and Nakanishi K, Proof of the mysterious efficacy of ginseng: basic and clinical trials: clinical effects of medical ginseng, korean red ginseng: specifically, its anti-stress action for prevention of disease, J. Pharmacol. Sci., 95, 2004, 158-62.

[13] Kim CS, Park JB, Kim KJ, Chang SJ, Ryoo SW, and Jeon BH, Effect of Korea red ginseng on cerebral blood flow and superoxide production, Acta Pharmacol. Sin., 23, 2002, 1152-6.

[14] Xie JT, Wang CZ, Kim S and Yuan CS, The anti-hyperglycemic property of different ginseng partitions, Oriental Pharm. & Experimen. Med., 5, 2005, 1-15.

[15] Kitts DD, Wijewickreme AN, and Hu C (2000). Antioxidant properties of a North American ginseng extract. Mol Cell Biochem.; 203: 1-10.

[16] Kim MK, Lee JW, Lee KY and Yang D, Microbial conversion of major ginsenoside Rb1 to pharmaceutically active minor ginsenoside Rd, J. Microbiol., 43 (5), 2005, 456-462.

[17] Ghosh M, Fundamentals of Experimental Pharmacology, 2nd Ed., Scientific Book Agency, Kolkatta, 1994, 153-158.

[18] Ganga U, Kishori B, and Reddy P, Cisplatin and/or etoposide induces oxidative stress in testicular, hepatic and kidney tissues in male albino mice, J. Biol. Earth Sci., 3 (2), 2013, B249-B254.

[19] Ismail AA, The role of testosterone measurement in the investigation of androgen disorders, Ann. Clin. Biochem., 23, 1986, 113-134.

[20] Ohkawa H, Ohishi N, and Yagi K, "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction," Analytical Biochem., 95 (2), 1979, 351-358.

[21] Ellman G, Tissue sulfhydryl groups, Arch. of Biochem. and Biophy., 82 (1), 1959, 70-77.

[22] Sonmez M, Turk G and Yuce A, The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats, Theriogenol., 63, 2005, 2063-2072.

[23] Rezvanfar M, Sadrkhanlou R, Ahmadi A, Shojaei-Sadee H, Rezvanfar M and Mohammadirad A, Protection of cyclophosphamide-induced toxicity in reproductive tract histology, sperm characteristics, and DNA damage by an herbal source; evidence for role of free-radical toxic stress, Hum. Exp. Toxicol., 27 (12), 2008, 901-910.

[24] Dehghan MH, Martin T and Dehghanan R, Antifertility effect of Iranian neem seed alcoholic extract on epididymal sperm of mice, Iran. J. Reprod. Med., 3, 2005, 83-89.

[25] Buck W, Osweiter G, and Van Gelder A, Clinical and Diagnostic Veterinary Toxicol., 2nd Ed., Kendall/Hunt Publishing Co., Iowa, USA., ISBN-13: 9780840307200, 1976, Pp: 380.

[26] Patel N, Joseph C, Corcoran GB and Ray SD, Silymarin modulates doxorubicin-induced oxidative stress, Bcl-xL and p53 expression while preventing apoptotic and necrotic cell death in the liver, Toxicol. and Appl. Pharmacol., 245, 2010, 143-152.

[27] Ilbey YO, Ozbek E, Cekmen M, Simsek A, Otunctemur A and Somay A, Protective effect of curcumin in cisplatin-induced oxidative injury in rat testis: mitogen-activated protein kinase and nuclear factor-kappa B signaling pathway, Human Reproduction, 24 (7), 2009, 1717-1725.

[28] Abbas F and Ateya A, Estradiol, Esteriol, Estrone and Novel Flavonoids from Date Palm Pollen, Aust. J. Basic and Appl. Sci., 5 (8), 2011, 606-614.

[29] Liu ZQ, Wang ZC, and Sun YX, In vitro study of the relationship between the structure of ginsenoside and its antioxidative or prooxidative activity in free radical induced hemolysis of human erythrocytes, J. Agric. Food Chem., 51, 2003, 2555-2558.

[30] Conklin KA, Chemotherapy-associated oxidative stress: impact on chemotherapeutic effectiveness,

Integrative Cancer Therapies, 3, 2004, 294-300.

[31] Bennet RD, Ko ST and Heftman E, Isolation of estrone and cholesterol from the date palm Phoenix Dactylifera. Phytochem., 5, 1966, 231-235.

[32] Mansouri A, Embarek G, Kokkalou E and Kefalas P, Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (Phoenix dactylifera), Food Chem., 89, 2005, 411-420.

[33] Shukla R, and Kumar M, Role of Panax ginseng as an antioxidant after cadmium-induced hepatic injuries, Food Chem. Toxicol., 47, 2009, 769-773.

[34] Zhu D, Wu L, Li CR, Wang XW, Ma YJ, and Zhong ZY, Ginsenoside Rg1 protects rat cardiomyocyte from hypoxia/reoxygenation oxidative injury via antioxidant and intracellular calcium homeostasis, J. Cell Biochem., 108, 2009, 117-24.

[35] Bak MJ, Jun M and Jeong WS, Antioxidant and hepatoprotective effects of the red ginseng essential oil in H_2O_2-treated hepG2 cells and CCl_4-treated mice, Int. J. Mol. Sci., 13, 2012, 2314-2330.

[36] Maines MD, Sluss PM, and Iscan M, cis-Platinum-mediated decrease in serum testosterone is associated with depression of luteinizing hormone receptors and cytochrome P-450scc in rat testis, Endocrinol., 126, 1990, 2398-2406.

[37] Vawda AI, Effect of testosterone on cisplatin-induced testicular damage, Arch. Androl., 32, 1994, 53-7.

[38] Cao L, Leers-Sucheta S and Azhar S, Aging alter the functional expression of enzymatic and non-enzymatic anti-oxidant defense systems in testicular rat Leydig cells, J. Steroid Biochem. Mol. Biol., 88, 2004, 61-7.

[39] Kostyuk VA, Potapovich AI and Strigunova EN, Experimental evidence that flavonoid metal complexes may act as mimics of superoxide dismutase, Arch. Biochem. Biophys., 428, 2004, 204-8.

[40] Fahim MS, Fahim Z, Harman JM, Clevenger TE, Mullins W and Hafez ESE, Effect of Panax Ginseng on Testosterone Level and Prostate in Male Rats, Systems Biol. in Reproductive Med., 8 (4), 1982, 261-263.

[41] Dohle GR, Male infertility in cancer patients: Review of the literature. Int. J. Urol. 17, 2010, 327-331

[42] Joshi SC, Mathur R and Gulati N, Testicular toxicity of chlorpyrifos (an organophosphate pesticide) in albino rats, Toxicol. and Industrial Health, 2007, 23 (7), 439-444.

[43] Kishore R, Sreenivasula R, and Shivalingam M, Protective Effects of Testosterone on Cisplatin Induced Impairment of Spermatogenesis and Steroidogenesis in Rats, Res. J. Pharm. and Technol., 3 (2), 2010, 535-539.

[44] Ciftci O, Beytu A, Cakir O, Gurbuz N and Vardi N, Comparison of Reproductive Toxicity Caused by Cisplatin and Novel Platinum-N-Heterocyclic Carbene Complex in Male Rats, Basic & Clinical Pharmacol. & Toxicol., 109, 2011, 328-333.

[45] Gong Y and Han XD, Effect of nonylphenol on steroidogenesis of rat leydig cells, J. Environ. Sci. Health B, 41, 2006, 705-715.

[46] Yeni D, Gundogan M, Cigerci IH, Avdatek F and Fidan AF, Seasonal variation of oxidative stress parameters in ram seminal plasma, J. Anim. Vet. Adv., 9, 2010, 49-55.

[47] El-Mougy SA, Abdel-Aziz SA, Al-Shanawany M and Omar A, The gonadotropic activity of Palmae in mature male rats, Alexandrina, J. Pharm. Sci., 5, 1991, 156-159.

[48] O'Donnell L, Pratis K, Stanton PG, Robertson DM and McLachlan RI, Testosterone-dependent restoration of spermatogenesis in adult rats is impaired by a 5α-reductase inhibitor, J. Androl., 20, 1999, 109-117.

[49] Jannini EA, Screponi E, Carosa E, Pepe M, Lo Giudice F, Trimarchi F and Benvenga S, Lack of sexual activity from erectile dysfunction is associated with a reversible reduction in serum testosterone, Int. J. Androl., 22, 1999, 385-392.

[50] Gauthaman K, Adaikan PG and Prasad RNV, Aphrodisiac properties of Tribulus terrestris extract (Protodioscin) in normal and castrated rats, Life Sci., 71, 2002, 1385-1396.

[51] Donnelly ET, Lewis SEM, McNally JA and Thompson W, In vitro fertilization and pregnancy rates: The influence of sperm motility and

morphology on IVF outcome, Fertil. Steril., 70, 1998, 305-314.

[52] Meistrich ML and Brown CC, Estimation of the increased risk of human infertility from alterations in semen characteristics, Fertil. Steril., 40 (2), 1983, 220-230.

[53] Aitken RJ, Free radicals, lipid peroxidation and sperm function, Reprod. Fertil. Dev., 7, 1995, 659-668.

[54] Creasy DM Evaluation of testicular toxicology: A synopsis and discussion of the recommendations proposed by the Society of Toxicologic Pathology, Birth Defects Res., Part B: Dev. Reprod. Toxicol., 68, 2003, 408-415.

[55] Malarvizhi D and Mathur PP, Effect of cisplatin on physiological status of normal rat testis, Indian J. Experimen. Biol., 33 (4), 1995, 281-283.

Table 1: Effect of Oral Administration of DPP and *P. ginseng* Extracts for 8 Weeks on Blood Levels of Testosterone and Testicular Levels of MDA and GSH of CIS-Medicated Male Rats

Groups	Doses (mg/kg)	Testosterone (ng/mL)	MDA (nmol /min/g tissue)	GSH (µmoles/ g tissue)
Normal control	0.0	9.32±0.52	1.63±0.12	0.20±0.02
CIS- control	3.0	3.18±0.24[a]	6.85±0.39[a]	0.06±0.01[a]
CIS+ DPP	3.0+200	3.84±0.25	5.94±0.32	0.09±0.01
	3.0+400	5.65±0.38[b]	3.55±0.19[b]	0.13±0.01[b]
CIS+ *P. ginseng*	3.0+200	3.74±0.28	5.76±0.41	0.08±0.01
	3.0+400	5.15±0.47[b]	3.84±0.20[b]	0.12±0.01[b]

NOTE: The Values are Presented as Means ± SEM, (n = 6); [a] Significant Differences as Compared With Normal Control Group at P < 0.05; [b] Significant Differences as Compared with - Control Group at P < 0.05

Table 2: Effect of Oral Administration of DPP and *P. ginseng* Extracts for 8 Weeks on the Epididymal Sperm Characters of CIS-Medicated Rats

Groups	Doses (mg/kg)	Sperm count (X 10^6/mL)	Sperm motility (%)	Sperm viability (%)	Sperm abnormalities (%)
Normal control	0.0	82.2±3.53	86.4±3.31	87.4±3.53	8.53±0.33
CIS- control	3.0	24.7±1.84[a]	37.8±2.46[a]	42.7±2.14[a]	31.65±2.51[a]
CIS+ DPP	3.0+200	28.5±1.96	41.5±2.12	46.6±2.74	27.52±1.90
	3.0+400	63.7±3.35[b]	67.5±3.53[b]	67.5±3.75 [b]	20.58±1.42[b]
CIS+ *P. ginseng*	3.0+200	27.7±1.79	40.6±2.55	44.8±2.43	29.66±1.83
	3.0+400	60.4±3.34[b]	66.7±4.52[b]	64.7±2.49 [b]	23.64±1.75[b]

NOTE: The Data are Presented as means ± SEM, n = 6; [a] Significant Difference as Compared with Normal Control Group (P < 0.05); [b] Significant Difference as Compared with Control Group (P < 0.05)

Table 3: Effect of Oral Administration of DPP and *P. ginseng* Extracts for 8 Weeks on the Mating Trial of CIS-Medicated Male Rats With Normal Untreated Females (Mating Ratio = 1 Male: 2 Females)

Groups	Doses (mg/kg)	No. of females mated[a]	Mating success (%)[b]	No. of females pregnant	Fertility success (%)[c]	Male fertility index (%)[d]
Normal control	0.0	12/12	100.0	12/12	100.0	100.0
CIS- control	3.0	0/12	0.0	0/12	0.0	0.0
CIS+ DPP	3.0+200	2/12	16.66	0/12	0.0	0.0
	3.0+400	8/12	66.66	6/12	50.00	75.0
CIS+ *P. ginseng*	3.0+200	1/12	8.33	0/12	0.0	0.0
	3.0+400	7/12	58.33	4/12	33.33	57.14

NOTE: Data are expressed as numbers and % of 6 males and 12 females; [a] Evidenced by vaginal plug and sperm in a vaginal smear; [b] Mating success % = ([number mated/number paired] × 100); [c]Fertility success % = ([number pregnant/number paired] × 100); [d]Male fertility index % = ([number pregnant/number mated] × 100)

Table 4: Effect of Oral Administration of DPP and *P. ginseng* Extracts for 8 Weeks on the Body Weight and Relative Weight of Reproductive Organs of CIS-Medicated Male Rats

Groups	Doses (mg/kg)	Initial body weight(g)	Final body weight (g)	Relative weight of reproductive organs (g/100 g b.wt)		
				Testes (Pair)	Seminal vesicles	Ventral prostate
Normal control	0.0	188.2±6.43	221.5±7.35	1.83±0.13	0.62±0.04	0.50±0.03
CIS- control	3.0	189.8±6.25	192.3±6.50[a]	0.85±0.06[a]	0.21±0.02[a]	0.16±0.02[a]
CIS+ DPP	3.0+200	192.5±7.27	196.5±6.22	0.93±0.14 [a]	0.26±0.02 [a]	0.20±0.02[a]
	3.0+400	184.2±6.22	217.4±6.36[b]	1.30±0.11 [b]	0.51±0.03 [b]	0.39±0.03[b]
CIS+ *P. ginseng*	3.0+200	193.6±7.17	194.3±6.25	0.95±0.09	0.25±0.02	0.19±0.02
	3.0+400	186.1±6.50	214.9±6.14[b]	1.17±0.10 [b]	0.50±0.03 [b]	0.36±0.02 [b]

NOTE: The Values are Presented as Means ± SEM, (n = 6); [a] Significant Differences as Compared with Normal Control Group at P < 0.05; [b] Significant Differences as Compared with Control Group at P < 0.05

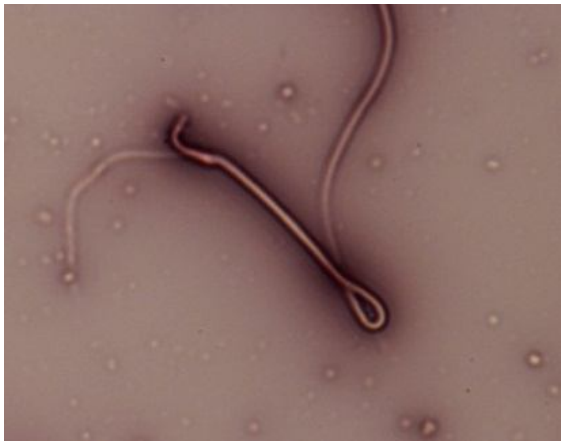

Figure 1: Showing Sperm With Bent Tail Obtained From CIS-Control Rats (Eosin-Nigrosin, X 400)

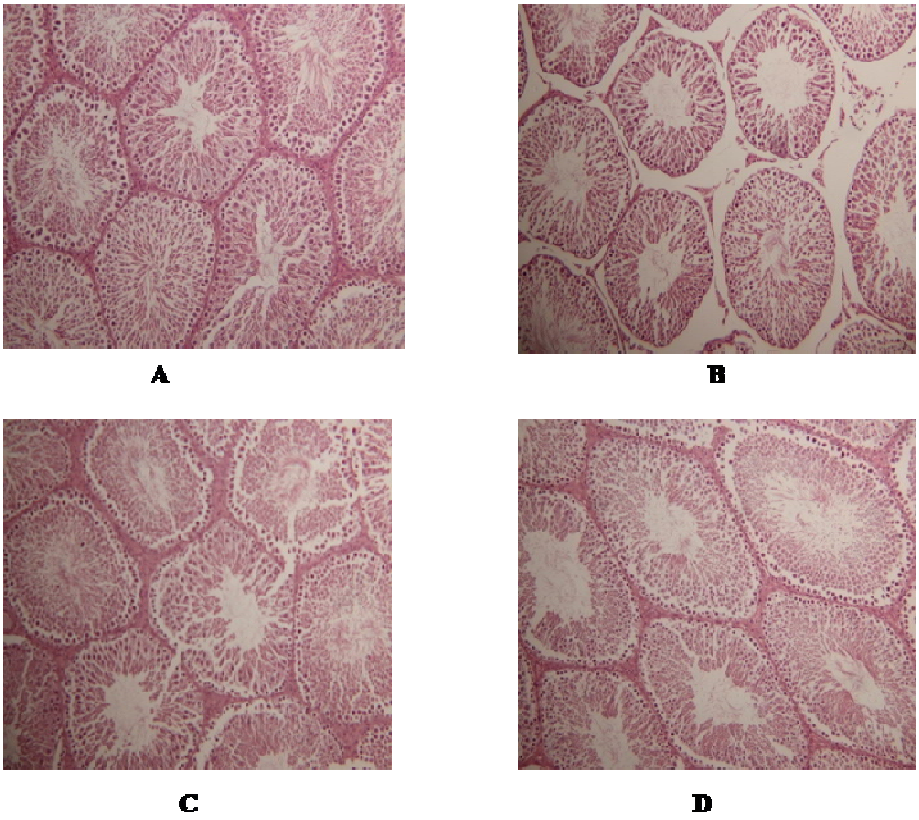

Figure 2: Photomicrograph of Testicular Histology of the (A) Control Rats Showing Normal Seminiferous Tubules and normal Structure of Germinal Epithelium. (B) CIS-Control Rats, Showing Atrophic and Degenerated Seminiferous Tubules Associated with Incomplete Spermatogenesis and Sloughing of Degenerated Germ Cells. (C) CIS + DPP at a Dose of 400 mg/kg. (D) CIS + *P. ginseng* at a Dose of 400 mg/kg Restored the Degenerative Changes in the Seminiferous Tubules Towards Normality. (H & E, X 200)