International Journal of Modern Agriculture, Volume 10 No. 2, 2021
ISSN: 2305-7246

Ameliorative influences of Moringa oleifera leaves extract to protect the fertility from toxicity Cisplatin-induced in male rats

Amal M. Allbban

Nutrition and Food Science, (Applied Nutrition), Faculty of Education, Department of Family Education. University of Umm Al-Qura, Makka Al-Mukarama, Kingdom of Saudi Arabia
*Email:*amlbban@uqu.edu.sa

Abstract

Moringa oleifera leaves had contained high amounts of nutritive compounds like total protein, total lipids as well as minerals content (calcium, phosphorus, potassium, iron, and sodium). Moreover, it leaves extract is a good source for natural antioxidant as total phenolic acids, flavonoids compounds, chlorophyll and carotenoids and also antioxidant activity.
The biological experimental observed that the effect of *Moringa oleifera* leaves extract to protect the fertility from toxicity Cisplatin-induced in male rats. *Moringa oleifera* leaves extract was improvement oxidative stress and kidney functions. Furthermore, it was protective of the weight of the epididymis, vas deferens, and seminal vesicle as well as activity of serum testosterone (T), dehydroepiandrosterone (DHEA) levels and leptin. Moreover, the *Moringa oleifera* leaves extract could the improvement the fertility in rats from toxicity Cisplatin-induced when taken rats orally at 400ppm level from *Moringa oleifera* leaves extract. Histopathological investigation showed a betterment testicular tissue injury in *Moringa oleifera* therapy groups than the cisplatin group (control positive group).
From the obvious studies, it could be concluded that the *Moringa oleifera* leaves was influential in improving the toxic impacts of cisplatin on testicular tissue in rats.

Keywords: Histopathological, cisplatin, chlorophyll and carotonoids, testicular tissue, antioxidant activity

Introduction

Cisplatin (CP) or cis-diammine dichloroplatinum (II) with the molecular formula [*cis*-$PtCl_2(NH_3)_2$], is a platinum-based drug that is utilized against different of solid tumors and neoplasms. It is agreeable that CP is a DNA alkylating agent that kills cells by way of DNA injury, reactive oxygen species (ROS) production, and encourages apoptosis (Kart *et al.*, 2010).The side effects are referred to as oxidative and nitrosative damage generated by Cisplatin to both endocrine and exocrine compartments is influenced, resulting in weakened sperm, gonadal, and changing of Leydig cell functions. As well, it leads to decrease sperm motility and also chromosomal abnormalities in spermatozoa and temporary or permanent azoospermia are side effects of Cisplatin therapy (Afsar *et al.*, 2017).

Jahan *et al.* (2018) found that the Cisplatin therapy has happened significant reduction in daily spermatogonia production, lowering in head length and% DNA in the head. In addition, it was a reduction of epithelial cell height, tubular diameter, decreases of the number in sperm, increase in the thiobarbituric acid reactive substances (TBARS) and oxidative stress in testicular tissues, and alteration of the intra-testicular testosterone hormone concentrations.

At a recent time, the application of medicinal plants has attracted big attention, maybe due to the toxicity and side effects of especially chemically drugs but may be caused by low paying ability of the developing societies. As well, the evaluation of the medicinal plants as a base for new cure agents for being developed of therapeutic drugs (Verma and Singh, 2008).

The *Moringa oleifera* (MO) has high nutritional and medicinal influences (Almatrafi *et al.*, 2017). A new investigation has found the *Moringa oleifera* contains high amounts of antioxidants, anti-inflammatory, and anti-diabetic. (Zeng *et al.*, 2019).

Nayak *et al.* (2016) observed that the *Moringa oleifera* leaf which protective activities for spermatogonial cells and lowering the cell harm of mice injected with cyclophosphamide. In addition, testicular toxicity is a significant cause of male infertility that may happen caused by hormonal and nutritional dysfunction. The *Moringa oleifera* leaves have antioxidant, anti-inflammatory, anti-tumor properties, and lowering cardiovascular diseases. The results found that the *Moringa oleifera* leaves extract was an ameliorative effect on melamine-induced testicular toxicity in rats. Histopathological examination showed an improvement in testicular tissue harm which *Moringa oleifera* therapy groups than the melamine group. Therefore, *Moringa oleifera* was effective in becoming better the toxic impacts of melamine on testicular tissue in rats (Mansour *et al.*, 2020).

The goal of this investigation was to evaluate the influences of *Moringa oleifera* leaves extract in opposition to Cisplatin-induced testicular toxicity in male rats

Materials and Methods

Materials:

Cisplatin [cis-$PtCl_2$ $(NH_3)_2$], drug was purchased in vial contain 50 mg powder dissolved in 50 ml sodium chloride solution produced by Merck company / France. Also, Kits of different parameters were obtained from Bicon Diagnosemittel GmbH and Co. KG Hecke 8 made in Germany.

Moringa oleifera leaves were obtained from Al-Khaldiah Agricultural Farm situated in Riyadh, Kingdom of Saudi Arabia.

Methods:

Chemical composition and minerals content of *Moringa oleifera* leaves:

Chemical composition as moisture, crude protein, ether extract, ash, crude fiber and carbohydrates were determined by using the methods of the AOAC (2012). Minerals content (Na, Ca and K) were determined in the diluted solution of ash samples by using emission flam photometer (Model Corning 410). The other minerals (Zn, Fe and Mg) were determined by Atomic absorption spectrophotometer (PerKin – Elmer Instrument Model 2380) were determined by using the methods of the AOAC (2012).

Estimation of total phenolic acids and flavonoids compounds of *Moringa oleifera* leaves extract:

The total phenolic content in the mulberry extract was measured using the method of Qawasmeh *et al.* (2012) with Folin-Ciocalteu reagent.The UV reading was measured at 760 nm. Gallic acid was used as standard (1 mg/ml) and the results were expressed as gallic acid equivalent (GAE mg/100g of dry weight).

The total flavonoids content was determined by the method of Eghdami and Sadeghi (2010). The absorbance was measured against a blank solution at 510 nm and the total flavonoids content was expressed in terms of milligrams of quercetin equivalent (mg QE /100g DW).

The content of chlorophylls a and b, and as well as total carotenoids, was determined using the method of Lichtenthaler (1987). Approximately one gram of *Moringa oleifera* leaves was extracted with 50 mL of 80% acetone (v/v) solution and filtration. Absorbance was read at 662 nm, 644 nm and 470 nm using spectrophotometer to measure the content of chlorophyll a, chlorophyll b and carotenoids, respectively. Total chlorophyll was calculated as the sum of chlorophylls a and b. Total chlorophyll and total carotenoid contents were expressed as mg/100g on a dry weight basis

Determination of antioxidant activity for *Moringa oleifera* leaves extract:

Antioxidant activity was determined according to the method described by Zhang and Hamauzu (2004) as follows: Five grams of *Moringa oleifera* different parts were extracted by 100 ml. 80 % methanol.

ABTS• (2, 2-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt)

The ABTS• assay was performed according to the method used by Miller and Rice-Evans (1997) and Ramesh Kumar and Sivasudha (2012). For the analysis, 1 mL ABTS• reaction solution was added to 100 µL sample extract, and the absorbance was measured at 734 nm immediately after 1 min of initial mixing.

DPPH (1,1-Diphenyl-2-picrylhydrazyl)

The DPPH assay was performed as described by Ravichandran *et al.* (2012) the absorbance of the mixture was measured at515 nm with the UV-Visible spectrophotometer. The following formula was used to determine the percentage of scavenging activity,

Percentage of inhibition (%) = $[(A_{control}-A_{sample}) /A_{control}] \times 100$

Where, $A_{control}$- absorbance of DPPH,

A_{sample}-absorbance reaction of mixture (DPPH with Sample**)**

Biological experiential

Male Wister albino weaning rats (36 rats) with weight ranging from 190-210g (n = 36) were purchased from Pharmacy Collage at King Saud University and delivered to the King Fahd Medical Research Center in Jeddah. Rats were housed in individual cages with screen bottoms and fed ad *libitum* on a basal diet for one-week for acclimatization, which containing casein (20 %), corn oil (8%), corn starch (31%), sucrose (32%), mgcellulose (4%), salt mixture (4%) and vitamin mixture (1%) according to the method Pell *et al*. (1992).

Experimental rats were fed on basal diet for 7 days and randomly divided into six groups six rats for each. The 1st main group was fed on basal diet for another 4 weeks and considered as control negative rats.

The six rat groups (30 rats) received intraperitoneal (i.p) injection of cisplatin (7 mg/kg) on day first and received saline for the next 13 days according to Jahan *et al.* (2018) and these groups were reclassified into control positive as a second group fed on basal diet. The third, fourth, fifth and sixth groups were taken orally 100, 200, 300 and 400 ppm from *Moringa oleifera* leaves extract are given daily for six weeks by gavage to rats and fed on basal diet for four weeks.

At the end of experimental period (four weeks), the blood samples were taken with drawn from the orbital plexus and centrifuged at 3000 rpm for 10 min to obtain the sera. After that, the sera were kept on a deep freezer at -20ºC until their biochemical measurements. Immediately after collecting blood, the two testes, epididymis, vas deferens and seminal vesicle from each rat were removed and weighed. The epididymis was homogenized in 5ml of 0.9% NaCl. Sperm counting was done using hemocytometer according to Adeeko and Dada (1998) and total number of sperm per gram of epididymis was then calculated.

The biochemical characteristics were serum testosterone (T) and dehydroepiandrosterone (DHEA) levels were evaluated, according to the methods of Tietz (1995) and Longcope (1996). Leptin was estimated according to the method of Considine and Siha (1996).

Kidney functions as serum creatinine, urea and uric acid concentrations were determined using the methods of Khozeimeh *et al.* (2017).

Oxidative stress as plasma Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GSH) were assayed by the method of Aebi (1995), Nishikimi *et al.* (1972), Paglia and Valentine (1967) and Factor *et al.* (1998), respectively. The lipid peroxidation was determined colorimetrically as malondialdehyde (MDA) by Yoshioka *et al.* (1979).

Histological examination

For histological studies, the testis was fixed overnight in Bouin's fluid, dehydrated in ethanol, and embedded in paraffin. Tissue sections (6 μm-thick) were cut on a microtome, mounted on glass slide. Staining of the section with hematoxylin and eosin (HE), the Seminiferous tubular diameter and germinal mass thickness were measured and the number of spermatogonia, primary spermatocyte, many spermatozoa and average interstitium showing Leydig cells were examined in each group under a light microscope (Rezvanfar *et al.*, 2013).

Statistical analysis:

The obtained data were exposed to analysis of variance. Duncan's multiple range tests at ($p \leq 0.05$) level was used to compare between means. The analysis was carried out using the PRO ANOVA procedure of Statistical Analysis System (SAS, 2004).

Results and Discussion

Chemical composition of the *Moringa oleifera* leaves

Chemical composition and minerals content were determined in the *Moringa oleifera* leaves and the results are reported in Table (1). Chemical composition as moisture, total protein, total lipids, crude fiber, ash content and total carbohydrates had found 8.73, 25.60, 15.72, 7.95, 10.62 and 40.11% on dry weight, respectively. These results are agree with Chatepa and Mbew (2018) who found that the *Moringa oleifera* leaves were estimated chemical composition as crude protein, ash, crude fat, crude fiber, and total carbohydrates had contained 22.60, 11.24, 13.40, 8.07, and 44.69% respectively.

Minerals content from the *Moringa oleifera* leaves in the same table like iron, potassium, calcium, sodium, zinc and phosphorus had contained 23.51, 27.86, 420.29, 25.49, 3.42 and 60.73 mg/100g, respectively. The *Moringa oleifera* had contained great significance minerals and it has protein, vitamins, B – carotene, amino acids, and different phenolic. The *Moringa oleifera* leaves had contained rich amounts from natural antioxidants and many other phytochemical for medicinal value like cardovascular diseases, posses' antitumor, anti-inflammatory, antihypertensive, hypolpidemic and hypodiabetic (Bukar *et al.*, 2010).

Table (1): Nutritive composition of Moringa oleifera leaves on dry weight

Chemical composition %	*Moringa oleifera* leaves	Minerals content mg/100g	*Moringa oleifera* leaves
Moisture	8.73±0.13	Iron	23.51±0.14
Total protein	25.60±0.24	Potassium	27.86±0.21
Total lipids	15.72±0.17	Calcium	420.29±10.38
Crude fiber	7.95±0.08	Sodium	25.49±0.31
Ash content	10.62±0.11	Zinc	3.42±0.02
Total carbohydrates	40.11±1.26	Phosphorus	60.73±1.25

Values are mean and SD (n = 3)

Antioxidants and their activities of the *Moringa Oleifera* leave extract

Flavonoids compounds, total phenolic, chlorophylls a and b, total carotenoids, antioxidant activity, ABTS• and DPPH were determined in the *Moringa oleifera* leaves extract and the resultant is tabulated in Table (2). The results indicated that the flavonoids compounds and phenolic content are major constituents of the *Moringa oleifera* leaves extract which had contained 70.28 mg QE/100g and 39.73 mg GAE/100g, respectively. Flavonoids have been to be of great significance as part of the human diet and are having been to be active principles in some medicinal plants. The antioxidant activity of flavonoids is an important efficient act to scavenging the free radical (Wang *et al.*, 2003).

More natural antioxidants in the *Moringa oleifera* leave extract like chlorophylls a and b, total carotenoids, and antioxidant activity was high contained amounts which 126.36, 42.15, 49.26, and 152.38%, respectively. In addition, the chlorophyll and carotenoid were found to give significantly to the antioxidant activity of plant species through their ability to scavenging free oxygen radicals, and peroxyl radicals (Bunea *et al.,* 2012).

From the results in the same table was found that the scavenging ability of DPPH and ABTS• free radical is extensively used to screen the antioxidant potential of natural plants. The results evaluation of the antioxidant capacity of the extract of the *Moringa oleifera* leaves showed good antioxidant capacity by 77.12 and 58.68%, respectively. The results observed that decreases the DPPH and ABTS• radical activity may be due to the scavenging ability of the *Moringa oleifera* leaves extract.

The *Moringa oleifera* extract had contained significant strong antioxidant activity for radical scavenging activities as DPPH and ABTS. In addition, the ABTS showed greater antioxidant activity than the DPPH radical, it could maybe this difference in the mechanism of action and reaction of DPPH and ABST radicals. As well as the ABTS was to be soluble in aqueous and organic solvents, to know both antioxidant capacities hydrophilic and/or lipophilic (Abegg *et al.,* 2012).

The *Moringa oleifera* extract showed that the potent antioxidant activity in free radical scavenging like DPPH and ABTS• has contained high amounts of phenolic compounds (Srikanth *et al.,* 2012).

Table (2): Antioxidant activities, Carotenoid and chlorophyll contents of *Moring oleifera*leaves extract

Antioxidant activity	*Moringa oleifera* leaves	Antioxidant activity	*Moringa oleifera* leaves
Total flavonoids mg QE/100g	70.28±2.57	Chlorophyll (a mg/100g)	126.36±8.69
Total phenolic mg GAE/100g	39.73±0.97	Chlorophyll (b mg/100g)	42.15±2.14
Antioxidant activity mg/100g	152.38±3.57	DPPH %	77.12±3.58
Carotonoids mg/100g	42.16±1.21	ABTS• %	85.68±5.16

Values are mean and SD (n = 3)

Effect of *Moringa oleifera* leaves extract on weight of sex organs and epididymis sperm count in male rats from Cisplatin-induced toxicity

Table (3) showed that the effect of *Moringa oleifera* leaves extract at levels 100,200, 300, and 400 ppm were separately taken orally daily on the weight and count of sex organs in male rats from Cisplatin-induced toxicity. The outcome of the weights of sex organs as testes, epididymis, seminal vesicle, and vas deferens in male rats from Cisplatin-induced toxicity taken orally 400 ppm observed that no considerable alterations compared with negative control healthy rats. Whilst, the epididymis sperm count was improved male rats from Cisplatin-induced toxicity, which took orally daily *Moringa oleifera* leave extract at level 400 ppm. These elevated results in sex organs and development for epididymis sperm count in male rats from Cisplatin-induced toxicity, which orally daily *Moringa oleifera* leaves extract at level 400 ppm may be caused by the *Moringa oleifera* leaves have contained riches amounts of polyphenolic compounds which have been advantageous to human health. The reproductive organs are dependent upon testosterone and other androgens. In addition, testosterone stimulates the growth and activity of the reproductive organs (Oberlander *et al.,* 1994).

Documented phytochemical screening of *Moringa oleifera* was found in high amounts of bioactive compounds like vitamins, minerals, and polyphenolic compounds. (Idris *et al.,* 2016). Some of these constituents – flavonoids and saponins, for example - have however been implicated in male reproductive toxicity. Flavonoid compounds have anti-mitotic, vasoprotective, and antihyperlipidemic

activity and it has been documented to cause alterations in the levels of testosterone and dihydrotestosterone (Becho *et al.*, 2015).

Table (3): Sex organs and epididymis sperm countin male rats from Cisplatin-induced toxicity treated with *Moringa oleifera* leaves extract.

Groups	Testis weight (g)	Epididymis weight (g)	Seminal vesicle weight (g)	Vas deferens weight (g)	Epididymis sperm count (10^4)/g
Control negative	1.45^{a}±0.13	0.56^{a} ±0.04	0.84^{a} ±0.04	0.27^{a} ±0.20	4.61^{a} ±0.12
Control positive	1.29^{d}±0.11	0.40^{d} ±0.01	0.68^{d} ±0.04	0.15^{d} ±0.01	1.72^{d} ±0.09
100 ppm	1.33^{c}±0.12	0.44^{c} ±0.01	0.72^{c} ±0.01	0.18^{c} ±0.01	2.44^{c} ±0.12
200 ppm	1.37^{b}±0.11	0.48^{b} ±0.01	0.76^{b} ±0.02	0.21^{b} ±0.1	3.16^{b} ±0.24
300 ppm	1.41^{ab}±0.14	0.52^{ab} ±0.02	0.80^{ab} ±0.03	0.24^{ab} ±0.2	3.88^{ab} ±0.22
400 ppm	1.44^{a} ±0.13	0.55^{a} ±0.02	0.84^{a} ±0.02	0.26^{a} ±0.03	4.60^{a} ±0.23

Each value represents the mean ±SD. Mean followed by different superscript letters in each column are significantly different ($p<0.05$).

Effect of *Moringa oleifera* leave extract on hormonal profile in male rats from Cisplatin-induced toxicity

The results in Table (4) showed that the influence of *Moringa oleifera l*eaves extract at level 100, 200, 300, and 400ppm on serum testosterone (T), dehydroepiandrosterone (DHEA) levels and leptin, in male rats from Cisplatin-induced toxicity. The results from *Moringa oleifera* leave extract at level 400 had significant prevention on hormonal profile male rats from Cisplatin-induced toxicity and no significant changes compared with negative control.

Leptin is a protein hormone and its physiological role is to adjust appetite and body weight, but in obese subjects levels of leptin are frequently increased leading to harmful impacts, especially on male fertility (Phillips and Tanphaichitr, 2010).The greatest significance of these influences is that elevated levels of leptin may be doing as an inhibitory signal for testosterone synthesis out of membrane receptors on testicular cells (Khullar *et al*., 2012). Jope *et al.* (2003) suggested that elevates of leptin may immediately influence sperm production independent of variations in testosterone production. Plants and their derivatives have played a major role in global health and have long been known to have biological activity. As well as, plants have long in helping fertility, and aphrodisiacal qualities (D'Cruz *et al.*, 2010).

Table (4): influence of *Moringa oleifera* leaves on hormonal profile in male rats from Cisplatin-induced toxicity

Groups	Serum Testosterone (ng/ml)	Serum DHEA (ng/ml)	Serum Leptin (ng/ml)
Control negative	5.21^{a} ±0.71	6.51^{a} ±0.7.6	15.94^{d} ±0.94
Control positive	3.11^{d}±0.22	2.15^{e}±0.15	35.24^{a} ±1.45
100 ppm	3.63^{c}±0.24	3.24^{d}±0.22	31.01^{a} ±1.26
200 ppm	4.15^{b}±0.35	4.33^{c} ±0.37	26.18^{b}±1.28
300 ppm	4.67^{ab}±0.31	5.42^{b}±0.54	21.36^{c} ±2.23
400 ppm	5.19^{a}±0.42	6.50^{a} ±0.56	16.45^{d}±0.98

Each value represents the mean ±SD.Mean followed by different superscript letters in each column are significantly different ($p<0.05$).

Effect of *Moringa oleifera*leaves extract on kidney functions in male rats from Cisplatin-induced toxicity

Kidney functions like urea, creatinine, and uric acid were determined in rats' fertility Cisplatin-induced toxicity and the results in Table (5) indicates the control positive had the highest in urea, creatinine, and uric acid were 49.34, 2.01, and 6.69 mg/dl, respectively. These results increased may be caused by the Cisplatin (CP)-induced nephrotoxicity is related to its accumulation in the proximal tubule cells (Kim *et al*., 2015). Sen *et al.* (2018) and Singh *et al.* (2018) indicated that increasing the levels of blood urea nitrogen and serum creatinine in the cisplatin group caused by increases in oxidative stress in kidney tissues. Injection of cisplatin increased serum urea, uric acid, and creatinine as indicators of nephrotoxicity.

Meanwhile, the rats' groups were taken orally/day with 100, 200, 300, and 400 ppm with *Moringa oleifera* leave extract, the results observed a decrease in kidney function from 43.40 at 100 ppm level to 33.12 for 400 ppm level mg/dl in urea, whilst in creatinine was decreased from 1.65 to 0.52 mg/dl and finely in uric acid was lowering from 5.91 to 3.51 mg/dl, respectively. The results observed that the control negative rats group was 33.45, 0.55, and 3.48mg/dl, respectively. These results were equal to the results from the rats' group from Cisplatin-induced toxicity which treated using *Moringa oleifera* leaves extract at 400ppm.

Kou *et al.* (2018) stated that *Moringa oleifera* has a renoprotective effect and lowering effect on kidney functions parameters due to bioactive compounds and also, it had contained high amounts of natural antioxidants which can protect human health.

Effect of *Moringa oleifera*leaves extract on serum antioxidant enzymes in rats from Cisplatin-induced toxicity

Table (6) estimated that the antioxidant enzymes as Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GSH) and malondialdehyde (MDA) in *Moringa oleifera* leaves extract at level 100, 200, 300, and 400ppm and compared with control rat health group. The results indicated that the control positive treated with cisplatin and fed on basal diet were lowering in CAT, SOD, GPx, and GSH by 3.57, 4.97, 20.39 and 15.37 U/L, respectively, whilst, MDA were elevated to 390.28 nmol/ml. Oxidative stress plays a great significant role in the development and evolution of the inflammatory response to exposure to different pollutants; thus, the interrelationship between oxidative harm and inflammatory processes was considered (Khalil *et al.*, 2019). Oxidants stress act all phases of an inflammatory process, like production of pro-inflammatory cytokines, activation of signaling pathways, and tan adaptive cellular response (Lugrin *et al.*, 2014).

Table (5): Effect of *Moringa oleifera*leaves extract on kidney functions in rats from Cisplatin-induced toxicity

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control negative	33.45^{e} ±2.4	0.55^{e} ±0.11	3.48^{e} ±0.25
Control positive	49.34^{a} ±3.0	2.01^{a} ±0.24	6.69^{a} ±0.61
100 ppm	43.40^{b}±1.83	1.65^{b} ±0.32	5.91^{b} ±0.31
200 ppm	37.45^{c}±1.72	1.02^{c} ±0.21	5.11^{c} ±0.24
300 ppm	30.52^{d}±1.94	0.81^{d} ±0.22	4.32^{d} ±0.45
400 ppm	33.12^{e}±1.68	0.52^{e} ±0.16	3.51^{e} ±0.75

Each value represents the mean ±SD.Mean followed by different superscript letters in each column are significantly different ($p<0.05$).

Moreover, the results from CAT, SOD, GPx, and GSH found that the rats groups were taken orally *Moringa oleifera* leaves extract at level 100, 200, 300 and 400ppm increasing from 4.47 to 7.17 U/L in CAT, 7.02 to 13.14 U/L in SOD, 27.60 to 49.23 U/L in GPX and GSH was elevated from 17.55 to 24.16 U/L, respectively. Our results corresponding to Karthivashan *et al*., (2016) who demonstrated that *Moringa oleifera* leaves extract increases the capability of the antioxidant system and found that a modulator influence on inflammatory cytokines in renal tissues that evidence by elevated SOD,

CAT and GPx activities and decreased the levels of MDA in the groups treated with *Moringa oleifera* leaf extract. These results illustrated that Moringa oleifera leaves extract productivity to regulate and restore the antioxidant status of cisplatin-intoxicated rat renal.

Catalase (CAT), and superoxide dismutase (SOD) as antioxidants enzyme are that repairs cells and reduces the damage done to them by superoxides (Dinkova-Kotsova, 2002). The primary role of catalase is to scavenge hydrogen peroxide that has been produced by free radicals. The two play key roles in the protection against the injurious effects of lipid peroxidation. Where SOD stops its function, catalase exerts its function (Petrulea *et al*., 2012).

The results from Lipid peroxidation as Malondialdehyde (MDA) in the same table was decreased from 339.14 to 185.72 nmol/ml. This was probably due to *Moringa oleifera* leaves extract direct antioxidant effects or the enhanced biosynthesis of GSH and the other antioxidant enzymes (Ghanbarzadeh *et al.,* 2014). In addition, *Moringa oleifera* leaves extract reduces lipids' availability for peroxidation by transporting fatty acids into the mitochondria for β-oxidation and consequently mitigates the production and accumulation of lipid peroxidation products (Dokmeci *et al.,* 2005 and Derin *et al.,* 2006). Moreover, the Moringa oleifera leaves extract oxidative stress by attenuating MDA production and improving the antioxidant status in testicular tissues via augmentation of SOD, CAT, GPx, and GSH levels. Our results were in harmony with earlier reports showing that pomegranate *Moringa oleifera* leaves extract attenuated lipid peroxidation and enhanced the antioxidant balance in rat testicular tissues (Yuncu *et al.,* 2015).

The results from rats group fed on basal diet as control group was the highest in all parameters except the MDA were the lowest and these results were equal the results from rats group taken at 400ppm *M. oleifera* leaves extract.

Table (6):Effect of *Moringa oleifera*leaves extract on oxidative stress in rats from Cisplatin-induced toxicity

Groups	CAT (U/L)	SOD (U/L)	GPX (U/L)	GSH (U/L)	MDA (nmol/ml)
Control negative	7.21 ^{a} ±0.14	13.15 ^{a} ±0.27	49.25 ^{a} ±2.48	24.18 ^{a} ±2.11	185.69 ^{d} ±6.38
Control positive	3.57^{d} ±0.08	4.97 ^{d} ±0.06	20.39 ^{d} ±2.39	15.34 ^{d} ±1.72	390.28 ^{a} ±9.84
100 ppm	4.47^{c} ±0.05	7.02 ^{c} ±0.04	27.60 ^{c} ±2.57	17.55 ^{c} ±0.98	339.14 ^{ab} ±7.56
200 ppm	5.37^{b} ±0.04	9.07 ^{b} ±0.11	34.81 ^{b} ±3.19	19.76 ^{b} ±1.28	288.00 ^{b} ±4.39
300 ppm	6.27^{ab} ±0.06	11.12 ^{ab} ±0.14	42.02 ^{ab} ±3.94	21.97 ^{ab} ±1.64	236.86 ^{c} ±4.15
400 ppm	7.17a ^{a} ±0.03	13.14 ^{a} ±0.16	49.23 ^{a} ±4.15	24.16 ^{a} ±2.13	185.72 ^{d} ±5.73

Each value represents the mean ±SD. Mean followed by different superscript letters in each column are significantly different ($p<0.05$).

Histopathological testis:

Figure (1and 2) negative control group (1): testis showed average tunica albuginea, average sub-capsular blood vessels, average sized tubules with average germinal lining up to complete spermatogenesis, and average interstitium with average leydig cells.

Figure (3 and 4) positive control group (2) control cisplatin: testis showed thick tunica albuginea, widely-spaced small-sized distorted tubules with markedly thickened basement membrane, marked reduction of germinal lining and no spermatids and spermatozoa in some tubules , and average interstitium showing Leydig cells. These results confirmed with Kata (2013) found that the

results of the histological examination indicated that CP administration caused a significant decrease in seminiferous tubular diameter, seminiferous tubules epithelial height, tunica albuginea height, increase in the tubular lumen, interstitial space, and reduction in germ cell number and deceleration in spermatogenesis.

Figure (5 and 6) group (3) taken orally at 100 ppm *Moringa oleifera* leaves extract: testis showed average tunica albuginea, average sub-capsular blood vessels, average sized tubules with average germinal lining with full spermatogenesis, scattered tubules with mildly thickened basement membrane, and mildly edematous interstitium with average Leydig cells.

Figure (7 and 8) group (4) taken orally at 200 ppm *Moringa oleifera*leaves extract: testis showed average tunica albuginea, average sub-capsular blood vessels, average sized tubules with average germinal lining with full spermatogenesis, few scattered tubules with mildly thickened basement membrane, and average interstitium with average Leydig cells.

Figure (9 and 10) group (5) taken orally at 300 ppm *Moringa oleifera* leaves extract: testis showed average tunica albuginea, average sub-capsular blood vessels, average sized tubules with thin detached germinal lining with full spermatogenesis, scattered tubules with mildly thickened basement membrane and reduction of spermatogenesis, and markedly edematous interstitium with dilated thrombosed interstitial blood vessels.

Figure (11and 12) group (6) taken orally at 400 ppm *Moringa oleifera* leaves extract: testis showed average tunica albuginea, average sub-capsular blood vessels, average sized tubules with average germinal lining with full spermatogenesis, few scattered distorted tubules with mildly thickened basement membrane, and average interstitium with average Leydig cells.

These results agree with Abarikwu *et al.* (2012) who found that the Rutin may be caused by lowering in Cyclophosphamide induced oxidative stress and testicular damage by sustaining antioxidant levels in epididymis and testis.

From the results, it could be noticed that the *Moringa oleifera* treatment reversed the toxic effects of cisplatin on spermatogenesis.

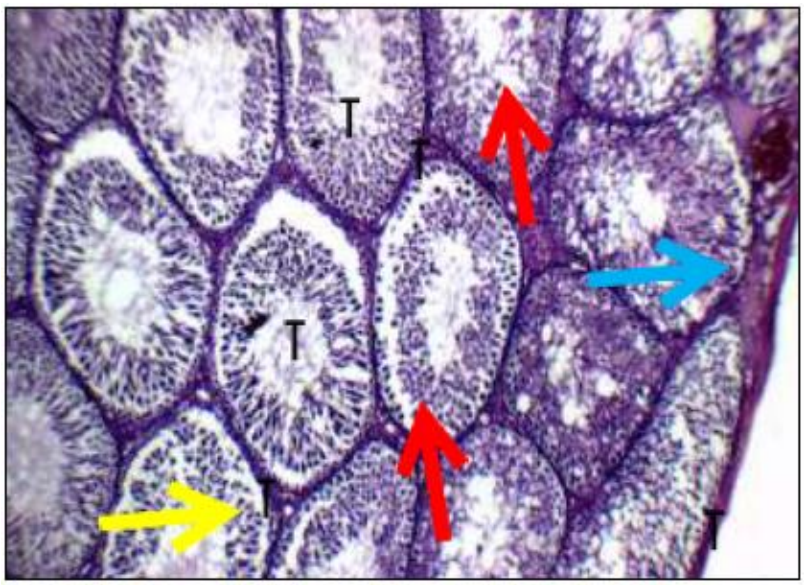

Fig 1: Control: slide G1: high power view showingaverage tunica albuginea (black arrow), average sub-capsular blood vessels (blue arrow), average sized tubules (T), average germinal lining with full spermatogenesis (yellow arrow), and average interstitium (red arrow) (H&E X 200)

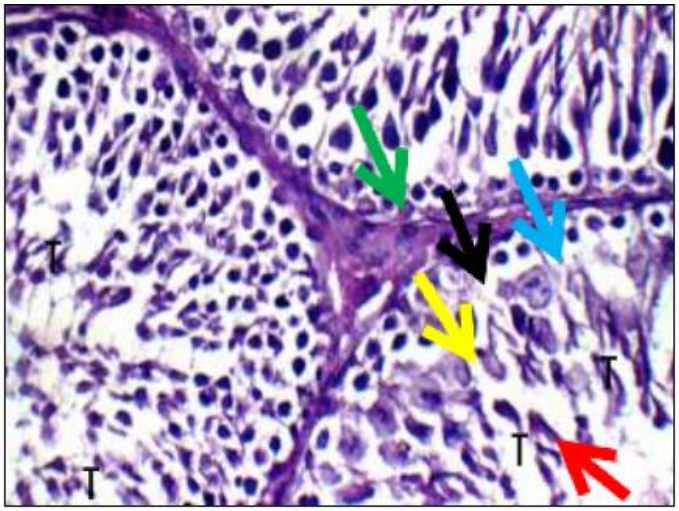

Fig 2: Control: slide G1: higher power view showingtubules with average basement membrane (black arrow), spermatogonia (blue arrow), primary spermatocyte (yellow arrow), many spermatozoa (red arrow) and average interstitium showing Leydig cells (green arrow) (H&E X 400)

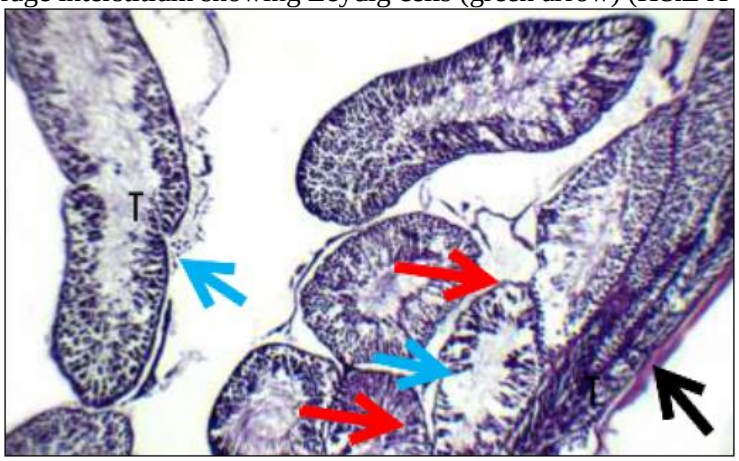

Fig 3: Cisplatin: slide G2: high power view showingthick tunica albuginea (black arrow), widely-spaced small-sized distorted tubules with destructed basement membrane (blue arrow) and marked reduction of germinal lining (red arrow) (H&E X 200)

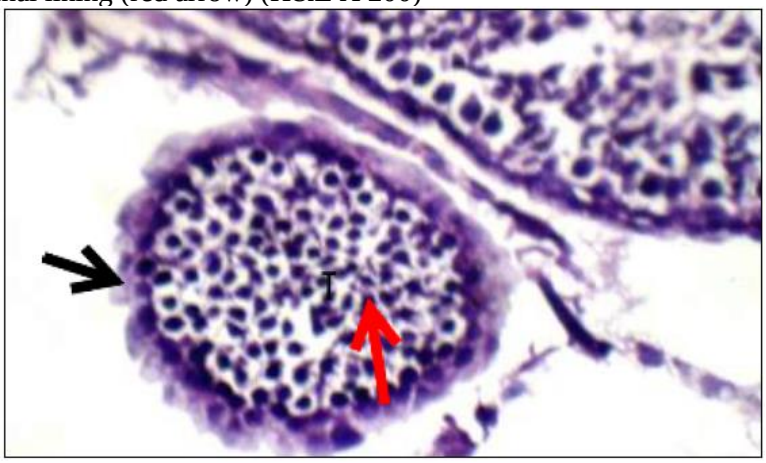

Fig 4: Cisplatin: slide G2: another view showingmarkedly distortedtubules with markedly thickened basement membrane (black arrow), and no spermatids or sperms (red arrow) (H&E X 400)

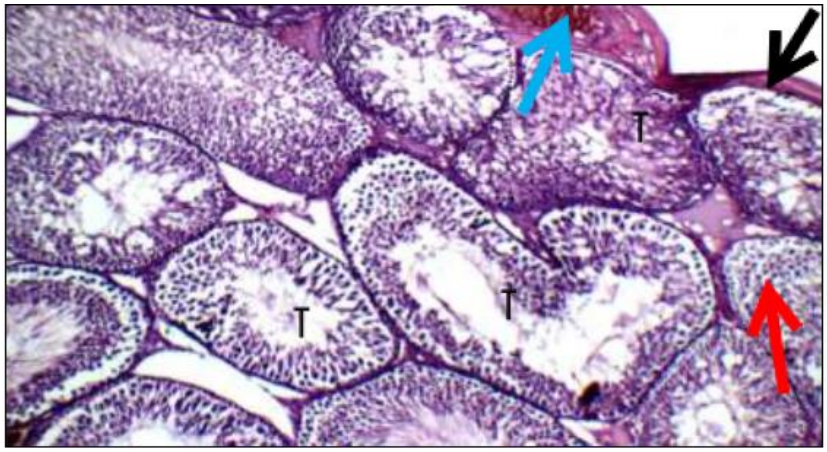

Fig 5: Group 3: slide G3: high power view showingaverage tunica albuginea (black arrow), average sub-capsular blood vessels (blue arrow), average sized tubules (T), and mildly edematous interstitium (red arrow) (H&E X 200)

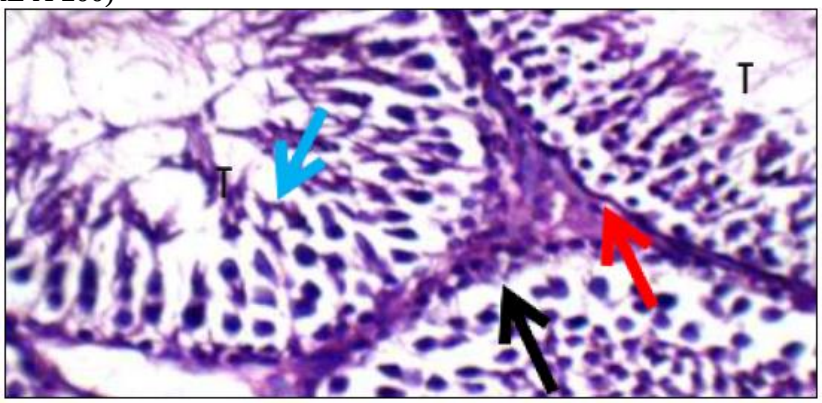

Fig 6: Group 3: slide G3: another view showingtubules with mildly thickened basement membrane (black arrow), average germinal lining with full spermatogenesis (blue arrow), and average interstitium with average Leydig cells (red arrow) (H&E X 400)

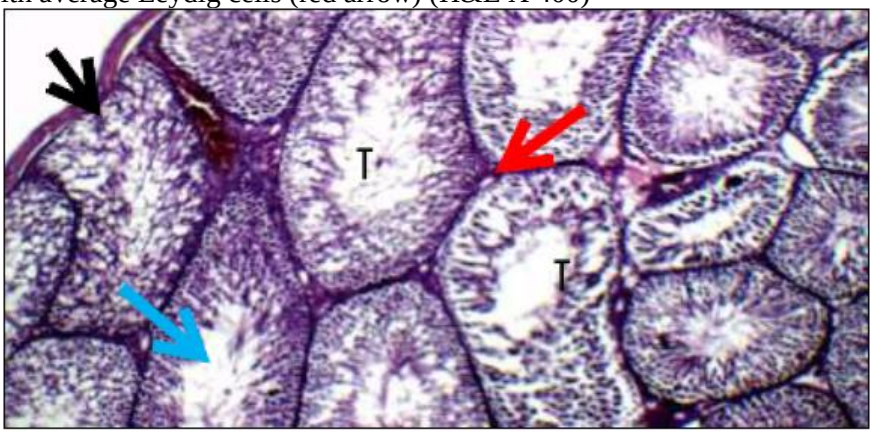

Fig 7: Group 4: slide G4: high power view showingaverage tunica albuginea (black arrow), average sized tubules (T) with average lining (blue arrow), and average interstitium (red arrow) (H&E X 200)

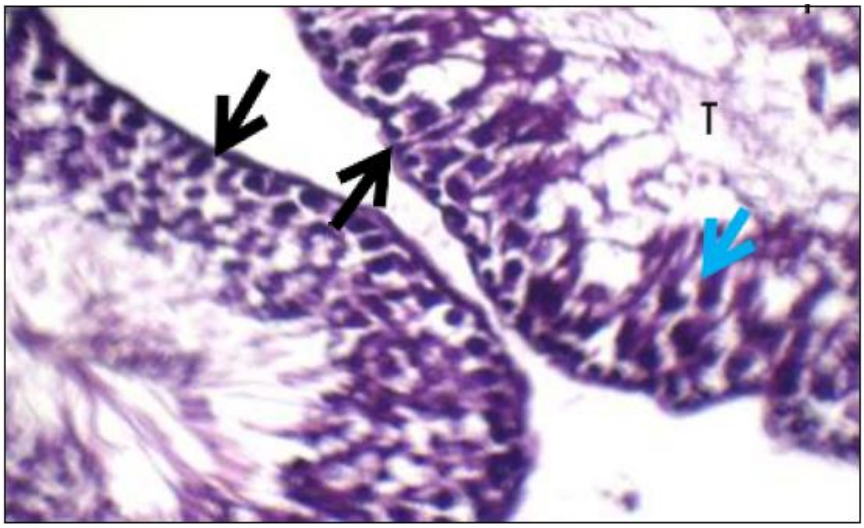

Fig 8: Group 4: slide G4: another view showingtubules with average basement membrane (black arrow), average germinal lining with full spermatogenesis (blue arrow) (H&E X 400)

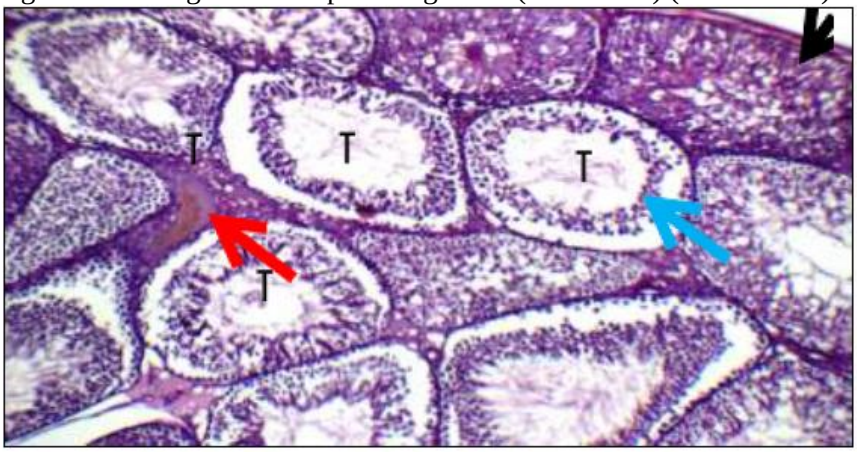

Fig 9: Group 5: slide G5: high power view showingaverage tunica albuginea (black arrow), average sized tubules (T) with thin detached lining (blue arrow), and markedly edematous interstitium (red arrow) (H&E X 200)

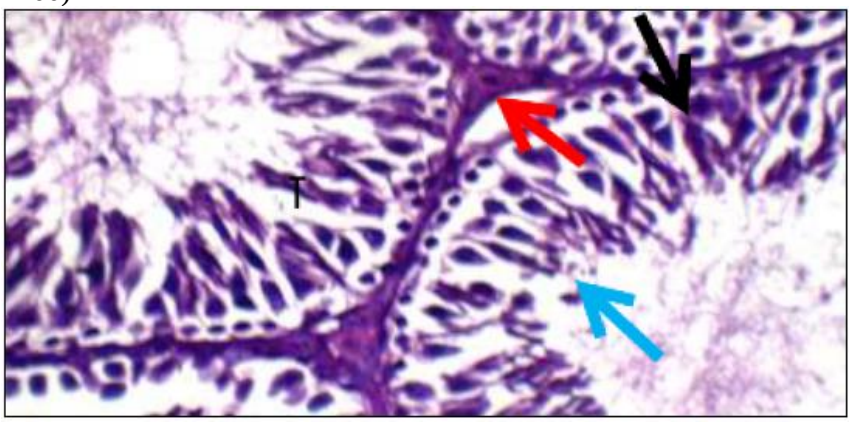

Fig 10: Group 5: slide G5: another view showingtubules with average basement membrane (black arrow), average germinal lining with full spermatogenesis (blue arrow), and average interstitium with average Leydig cells (red arrow) (H&E X 400)

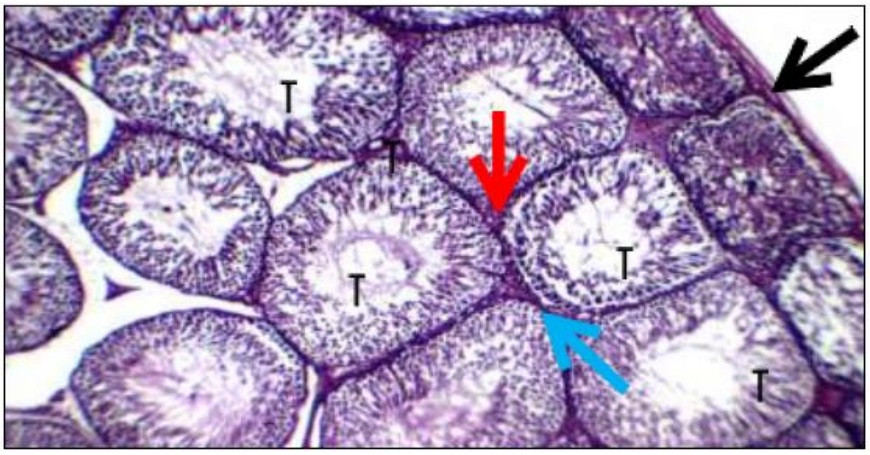

Fig 11: Group 6: slide G6: high power view showingaverage tunica albuginea (black arrow), average sized tubules (T) with average germinal lining (blue arrow), and average interstitium (red arrow) (H&E X 200)

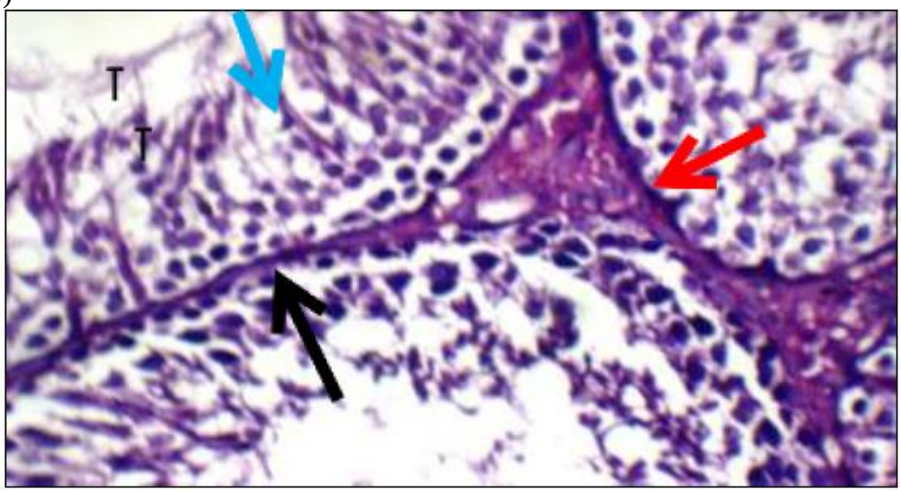

Fig 12: Group 6: slide G6: another view showingtubules with average basement membrane (black arrow), average germinal lining with full spermatogenesis (blue arrow), and average interstitium with average Leydig cells (red arrow) (H&E X 400)

Conclusion

From the results, it could be concluded that the *Moringa oleifera* leaves extract had the highest amounts of protein, total lipid, minerals content, and total phenolic, flavonoids compounds, and antioxidants.

The effect of *Moringa oleifera* leaves extract to protect the fertility from toxicity Cisplatin-induced in male rats, the results illustrated that improver the weighed of epididymis, vas deferens, and seminal vesicle as well as serum testosterone (T) dehydroepiandrosterone (DHEA) levels and leptin. Moreover, the *Moringa oleifera* leaves extract could the improvement the fertility in rats from toxicity Cisplatin-induced and also Kidney functions and Oxidative stress were improvements. These results were confirmed by histological experimental in the testes. Thus, it could be pointed out that Moringa oleifera may be utilized as a therapeutic agent combined with CP to lowering its side effects for infertility in rats.

Ethical Clearance

This study is cleared by the ethical committee of Nutrition and Food Science, (Applied Nutrition), Faculty of Education, Department of Family Education. University of Umm Al-Qura, Makka Al-Mukarama, Kingdom of Saudi Arabia

Conflict Of Interest

The researcher advertises no discrepancy of interest with any organization related to the materials researched in this paper.

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