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A Physiological and Histopathological Study on the Protective Effect of Aqueous Leaves Extract of Moringaoleifera against Cisplatin Toxicity in Rats

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ABSTRACT

This study aims to determine the amount of protection of the aqueous extract of Moringaoleifera leaves grown in Iraq-Ramadi against the drug cisplatin used in the medicament of cancer. The test animals were split into six groups and given different doses with aqueous extract, and intraperitoneally injected with cisplatin. The results indicated that the aqueous extract reduced the hepatotoxicity and nephrotoxicity resulting from cisplatin injection by analyzing liver enzymes and kidneys' functions physiologically and histologically.

Keywords
Cisplatin, Moringaoliefera leaves, Hepatoxicity, Nephrotoxicity.

Introduction

Moringa tree is one of the most important medicinal- herbal plant, that has become commonplace in tropical and subtropical countries. Used in several terms Horse-radish tree, Mulangay, Mlonge, Benzolive, Drumstick tree, Sajna, Kelor, Saijihan ,Marango and AL-Rawag tree in arabic [1],[2] . Antioxidant compounds are contained in Moringa tree leaves because they contain large quantities of polyphenols [3]. As Moringa leaf extract, whether the leaves are ripe or immature, possesses strong Antioxidant action against free radicals, as it protects biological molecules from oxidative damage and provides great protection against oxidative stress [4]. Several researchers at the Asian Vegetable Research and Development Center (AVRDC) was referring that the leaves of four different moringa species were to be high in nutrients and antioxidants [5]. As is common practice, many vegetables lose their nutritional value when cooked; however, the leaves of M.oliefera have been found to retain their nutritional value when cooked or preserved as a dry powder for many months without refrigeration [6]. As the leaves that have been boiled lead to the provision of iron three times more than uncooked, where the crushed Moringa leaves produced the same findings. Moringa Oliveira has a great role in the medical fields, the most important of which is, Anti-fibrotic/ulcer, Moringa leaf extract has a protective effect against liver damage in mice caused by anticancer drugs such as Isoniazide, Rifampicin, and Payrazinamide [7], Also, the aqueous extract of Moringaolifera leaves has an anti-inflammatory effect of cytokines and aspirin-causing stomach ulcers [8]Anti-nephrotoxicity, M. olifera pod extract has anti-inflammatory activity induced by 7-12-Dimethylbenz [a] anthracene (DMBA) that causes leukemia in albino mice for 14 days. Biomarker values of oxidative stress in the kidney were observed as superoxide, lipid peroxidation, and the antagonist Renal oxidative catalase has reached normal values [9].

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Material and method

Collecting the leaves of the Moringaoliveira plant

The Moringa tree was grown in the home garden, taking into account the addition of fertilizers and daily watering, after which the leaves of the Moringa plant were collected and then washed and left to dry at room temperature in the shade, and after the leaves dried completely, the leaves were crushed and milled with an electric pulverizer and the powder was kept and placed in a clean, airtight container Cover with silica gel (moisture blocker).

Aqueous extract preparation

A 200 mL conical flask was filled with 20 grams of Moringa leaf powder and 100 mL of distilled water. The beaker nozzle was covered with aluminum foil and placed on the shaker for 24 hours for continuous mixing, stirring until the active substances dissolved well in the water. Then, the extract was filtered with a centrifuge followed by a filter paper after which the filtrate was placed in the dryer at a temperature of 50 ° C. The dry extract was collected and used in the experiment.

Collect experiment animals

60 adult white female rats were purchased from the Biotechnology Research Center at Nahrain University-Baghdad of Mus musculus at a weight rate of 150-160 g per rat, and appropriate conditions were created from a temperature of 24 ± 1 m and a standard light period (12 light and 12 dark), taking into account the provision of Food is made from a diet containing soy protein, cornstarch, a mixture of minerals and vitamins, with the provision of water.

Used drug

Cisplatin (an anti-cancer drug) was purchased from the Turkish company Kocakfarma from the pharmacy. It contains 50 mg of the active ingredient.

Distribution of animals used

The rats were split into six groups, each with ten female rats (Mus musculus) for each group, Control Group. second group, which were treated with aqueous extract of Moringa leaves orally using Gavage(150 mg / kg), third group, were injected with cisplatin only once at a concentration of 5 mg / kg, after which they were dosed orally with the aqueous extract of Moringa leaves for 8 days with the same concentration studied. Fourth group, they were injected with Cisplatin once only by Intraperitoneal at a concentration of 5 mg / kg and left without treatment for 8 days. Fifth group, are injected with cisplatin at a concentration of 5 mg / kg only once, left without treatment for 16 days Sixth group, are injected with cisplatin only once (5 mg / kg), after which they were dosed orally with aqueous extract for 16 days with the same concentration studied.

Animal anatomy and serum collection

After the experimenyal treatments ended,the animals were collected and anesthetized with chloroform, after which the blood was drawn from the animals by heart stabbing [10] and the blood was separated using a centrifuge for 10 minutes for the purpose of obtaining serum, and it was stored in the freezer until the studied biochemical tests. After that, the liver and kidneys of each species were removed after they were dissected. The excised samples were preserved in a plastic cup containing 10% formalin for the purpose of preparing them for the histological sections.

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Biochemical measurement

The activity of liver enzyme (aspartate aminotransferase AST, alanine amino transferase ALT) was estimated using standard protocols of MIND-RAY commercial kits [11]. and alkaline phosphatase (ALP) in the different treatment groups were assayed using standard protocols BioMerieux commercial kits [12].Kidney function tests(Creatiine and Blood urea) were estimated according to the laboratory kit Linear chemicals [13],Also, lipid peroxidation (MDA) [14],Superoxide dismutase(SOD) [15],Catalase(CAT) [16] and Glutathion peroxidase(GPx) [17], was estimated using a diagnostic kit from ElabScience .

Histological examination

The organs that were removed were preserved with a 10% formalin solution [18]. The tissues were cut after being removed from the stabilizing solution and placed in special capsules and then placed in an Automatic processor device that contained eight vats of which five contained ethyl alcohol at different concentrations (50, 70,90,100,100), a sixth basin containing xylene, and two other basins containing paraffin wax, the melting point of which is (60,58). The samples remain in each basin for a specified time. Then the samples were placed in special molds and the wax was poured with a melting point of 80 ° C, and the mold was left to harden. Then the mold was cut with a microtome to get rid of the excess wax, then the molds were cut with a thickness of 4 micrometers and then the sections were loaded onto glass slides after coating them with glycerol with egg white in order to stick Sections on slides, Then the samples were placed in special molds and the wax was poured with a melting point of 80 ° C, and the mold was left to harden. Hematoxylin and eosin dye is used to stain sections on slides.

Statistical analysis

The study of the hepatic and renal parameters was completed using a large gap one-way analysis of variance (ANOVA) (p. 0.01).

Results and Discussion

The current study found that there was a substantial rise inactivity of ALT and AST enzymes in the group (3,4, 5) at the probability level of s (P <0.001) a large rise in behavior as opposed to the control group of the enzyme ALP in the group (3,4, 5) in comparison to a reference sample, as shown in Table (1).

Table (1) Estimation of activity of liver enzymes (ALT,AST,ALP) In the serum of rats, treated with aqueous extract of Moringaolifera leaves and cisplatin.

Parameters / Groups	ALT U/L (mean±SD	AST U/L (mean±SD	ALP U/L (mean±SD
Control (G1)	cd 24.900±1.791	d 26.300±2.110	C 75.698±3.653
Moringa/8 days (G2)	d 24.000±1.763	d 25.100±2.330	c 74.467±3.311
Moringa+Cisplatin/8 days (G3)	b 34.500±6.620	b 40.000±4.521	b 85.195±9.438
Cisplatin/ 8 days (G4)	a 48.700±9.043	a 48.100±48.100	a 96.290±10.476
Cisplatin/16 days(G5)	b 35.900±5.279	c 33.900±7.062	b 83.929±5.527

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Moringa+Cisplatin/16 days(G6)	C 29.300±5.334	d 28.700±6.236	bc 70.528±26.009

These findings are consistent with previous researches [19],[20], since, despite the excellent anti-cancer activity of cisplatin Its usage has been related to significant organ injury in which it accumulates such as the liver and kidneys [21]. Therefore, numerous studies have been conducted to reduce the nephrotoxic and hepatic toxicity by means of antioxidants [22]. As repeated high doses of cisplatin cause tremendous hepatotoxicity, including hepatic cord degeneration and focal inflammatory and necrosis lesions [23], and low doses cause hepatotoxicity [24]. The damage caused by cisplatin to the liver results from an increase As a consequence of the hydrolysis of cisplatin, the amount of Reactive Oxygen Species has increased. An increase in the level of INF-interferon gamma, which causes damage to the liver cells and thus an increase in the level of liver enzymes, as interferon kama stimulates inflammation. Liver [25]. A large decrease in the percentage of enzyme activity was seen in this group (6) treatment with cisplatin once and aqueous extract of Moringaoleifera leaves for 16 days compared to groups (3, 4, 5), implying that the activity of liver enzymes in group A was not significantly different from group (6) of liver enzymes was found to be significantly reduced in the community No. (3) Compared to group (4,5). The aqueous extract of Moringaolifera leaves has also minimized the function of liver enzymes in the group (2), treatment only with Moringa extract in comparison to a reference sample.

Previous results using the aqueous and alcoholic extract of Moringaoleifera leaves as a protective agent against the hepatotoxicity of cisplatin showed that there was a decrease in the effectiveness of liver enzymes treated with cisplatin and extracts of Moringaoleifera compared to the group treated with cisplatin only [26].

The current research found that the levels of creatinine and blood urea serum in the groups increased (3, 4, 5) in comparison to a reference sample, as shown in the table (2).

Table (2) Estimation of kidney function tests (creatinine,Blood urea) In the serum of rats, treated with aqueous extract of Moringaolifera leaves and cisplatin.

parameters / Groups	B.Urea mg/dl (mean±SD	Createnin mg/dl (mean±SD
Control	cd 27.00±2.357	d .455±.455
Moringa/8 days	d 23.600±1.837	d .455±.071
Moringa+Cisplatin/ 8 DAYS	bc 34.500±6.620	b 1.204±.462
Cisplatin/ 8 days	a 51.000±9.018	a 2.760±.728
Cisplatin/16 days	b 37.200±3.614	bc 1.034±.261
Moringa+Cisplatin/16 days	bcd 30.300±7.746	cd .756±.385

The treatment of rats with cisplatin significantly increased blood urea and creatinine levels [27], Treatment of rats resulted in elevated creatinine level and TNF-α [28].Nephrotoxicity results from the release of DAMPs danger-associated molecular patterns [29]. DAMPs activates the TLR4 Toll-like receptor 4 protein that causes the

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activation of TNF-α cytokines, the tumor necrosis factor that causes renal inflammation, as cisplatin increases the renal expression of TNF, which causes damage to the epithelial cells that extend to the renal tubules and thus severe renal failure [30]. The current study's findings revealed that lipid peroxidation levels have increased MDA in the groups (3,4,5) There was a reduction in the effectiveness of the tested antioxidant enzymes in the cisplatin-treated relative to the control group, SOD enzyme in the group (4,5), CAT enzyme in group (3,4,5) and GPx enzyme in group (4) as shown in Table No.(4). The aqueous extract of M. olifera leaves (150 mg / kg) led to a decrease in the levels of oxidation in group 5, treated with the studied extract for 16 days, as the MDA values reached normal values compared to the group that did not treat with the aqueous extract (4,5) and the group (3) That was treated with aqueous extract for 8 days, as shown in the table 3. Also, a significant increase in the effectiveness level of CAT was observed in group (2) treatment with aqueous extract of moringa plant only for 8 days compared to the control group in addition to group (6) compared to the control group and group (4,5), and the aqueous extract of M. olifera leaves also caused a decrease in The level of MDA oxidation in group (2,3,6) compared to the control group (1) and group (4,5) treated with cisplatin only. The results also showed that the aqueous extract of Moringa leaves caused an insignificant increase in the level of GPx enzyme activity in group (2,6) compared to the control group, while the aqueous extract of leaves caused an increase in the level of GPx in group (3) compared to group (4), the enzyme level reached normal values. The ability of MoringaOlivera leaves to reduce hepatotoxicity and decrease the effectiveness of liver enzymes is due to the leaves containing high concentrations of phenols, especially flavonoids, high concentrations of metabolites, which have the ability to scavenge and reduce free radicals [31] .These compounds have different biological activities, including antioxidants and anticancers. In addition, such as amino acids, fatty acids, carbohydrates and organic acids [32]. Moringaoleifera controls lipid oxidation and reactive oxygen species causing nephrotoxicity [33]. The aqueous extract of Moringaoleifera leaves reduced nephrotoxicity by reducing the expression of KIM-1, NF-κB, TNF-α, and HSP-70 [34].

This explains the protective role of Moringaoleifera leaves, as it contains antioxidant compounds such as flavonoids and vitamin C, and nutrients such as amino acids [35].

Table (3) Estimation of lipid peroxidation (MDA), Antioxidant enzymes (SOD,CAT,GPx) In the serum of rats, treated with aqueous extract of Moringaolifera leaves and cisplatin.

Parameters / Groups	MDA U/m (mean±SD	SOD U/m (mean±SD	CAT U/m (mean±SD	GPxU/m (mean±SD
control	c 1.198±.256	bc 1.164 ± .110	c 1.451±.359	ab 1.253±.297
Moringa/8 days	c 1.074±.367	ab 1.514 ± .419	a 2.790±.335	a 1.581±.361
Moringa+Cisplatin/ 8 dats	b 1.769±.469	ab 1.354 ± .483	b 1.938±.367	ab 1.070±.3044
Cisplatin/ 8 days	a 3.073±.725	d .539 ± .35288	d .967±.383	b .6950±.2843
Cisplatin/16 days	a 2.763±561	cd .850 ± .217	cd 1.149± .122	b 1.152±.449

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Moringa+Cisplatin/16 days	c 1.156±.335	a 1.543 ± .492	b 2.059±.636	a 1.561±.380

The results of the histological diagnosis of the liver showed damage to the liver through the presence of blood congestion in the central and portal veins in the fourth and fifth groups and dilated of the sinuses compared to other groups.

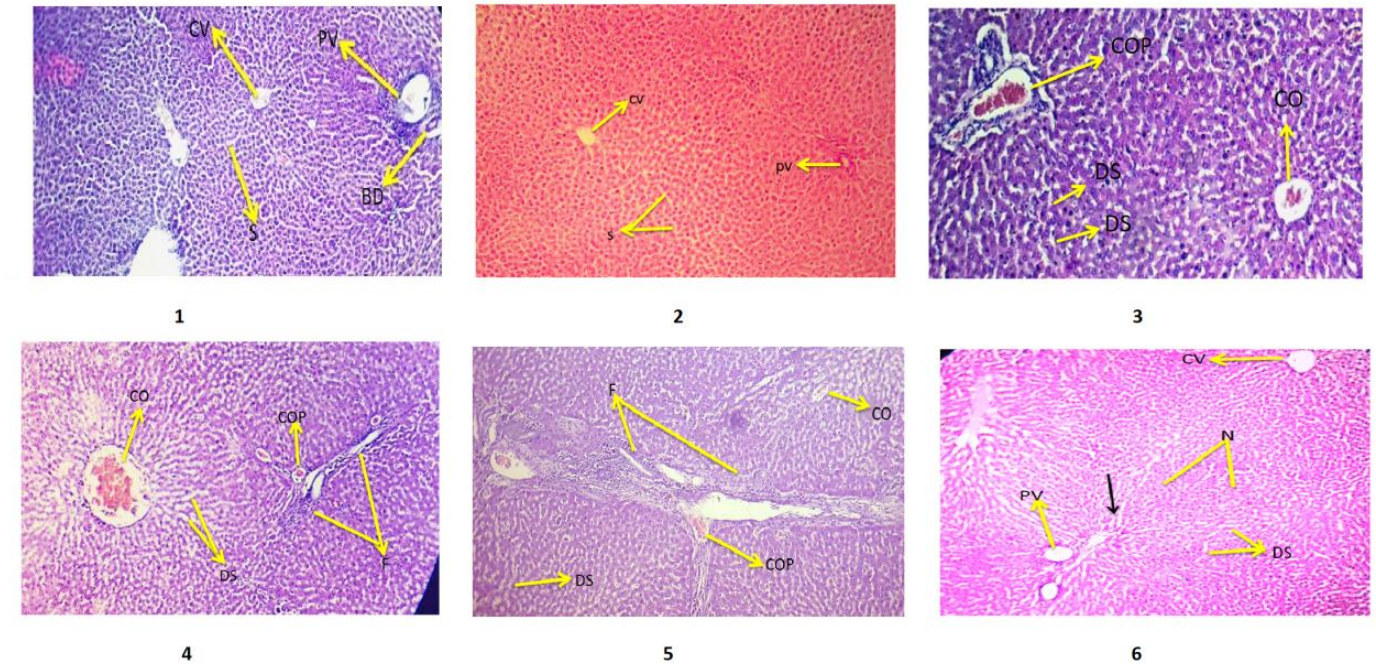

Figure (1) : section of liver showed the protective effect of aqueous leaves extract of M.oliefera against cisplatin (anti cancer drug) in rats.1: control group , 2: treated with aqueous leaves extract(8 days), 3: is M.oliefera extract + cisplatin(8 days) ,4:is cisplatin (8days), 5:is cisplatin (16 days), 6:is M.oliefera extract +cisplatin 16 days.S(sinusoid),CV(central vein), DS(Dilated sinusoid), F (Fibrosis) ,CO(Congested central vein,PV (Portal vien),CPV(Congested portal vein)BD(Bile duct).

The results of the histological diagnosis also showed that there was glomerular and tubular necrosis in the group treated with cisplatin only, in addition to the presence of inflammation of leukocytes(Indicated by a black arrow) and fibrosis .The histological diagnosis of the liver and the kidney showed the protective effect of the aqueous leaves extract of Moringaoliefera, especially in group 6 treatment with cisplatin and aqueous extract for 16 days ,figure (2).

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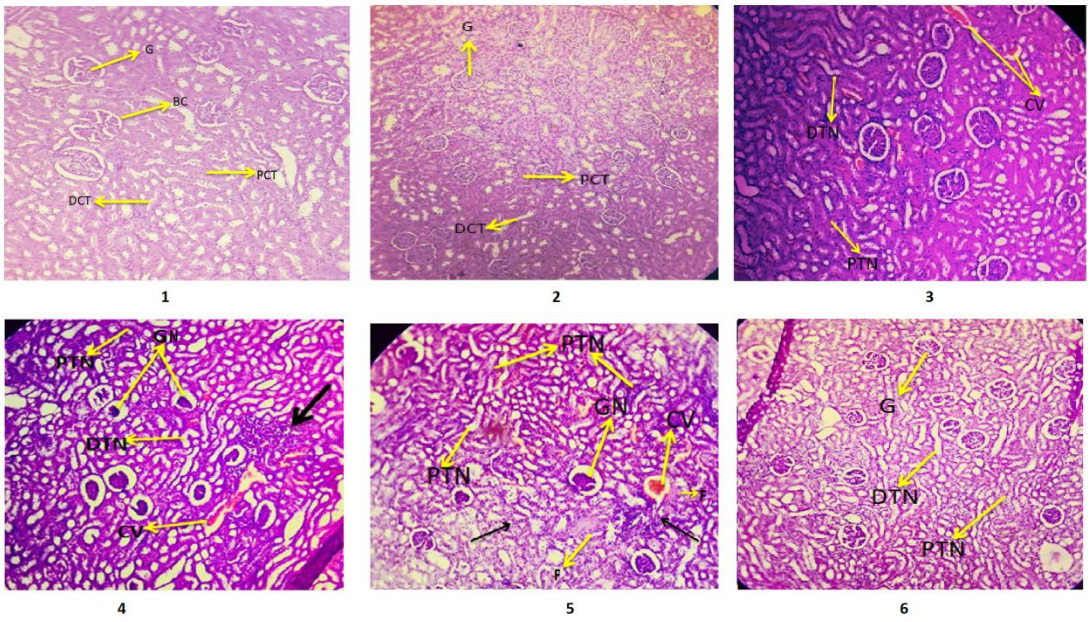

Figure 2: Cross section showed the protective effect of aqueous leaves extract of M.oliefera against cisplatin (anti cancer drug) in rats kidney.**1**: control group , **2**: treated with aqueous leaves extract(8 days), **3**: is M.oliefera extract + cisplatin(8 days) ,**4**:is cisplatin (8days), **5**:is cisplatin (16 days), **6**:is M.oliefera extract +cisplatin 16 days.**G(**G**l**umerulus),**BC**(Bowman's capsule),**PCT**(Proximal convoluted tubule),**DCT**(Distal Convoluted tubule),**CV**(Congested vessels),**GN**(Glumerular necrosis), F(fibrosis),DTN(Distal tubule necrosis and PTN (Proximal tubule necrosis).

Conclusion

Moringaolifera has a protective role against cisplatin because it contains biologically active component that have a great role in reducing hepatotoxicity and nephrotoxicity by scavenging free radicals, and this was evident in groups 3 and 6 where enzyme levels in the liver, creatinine and blood urea reached normal values, and the histological diagnosis was clearly prove it.

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