Effect of Dietary Supplementation with Doum on Liver and Kidney Injury Induced By Cisplatin in Experimental Rats.

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Abstract

This study was carried out to investigate the effect of dietary supplementation with doum on liver and kidney injury induced by cisplatin in experimental rats. Forty two adult male albino rats weighing 130-150 g were randomly distributed after adaptation period (7 days) into 6 main groups (7 of each). The first main group (n=7) was as a negative control group (-ve) and fed on basel diet. Group 2 rats injected cisplatin (10 mg/kg i.p., once) as a positive control group (+ve). Group 3 and 4 were injected by cisplatin as the same of group 2 then rats fed on basel diet supplemented with 5, 10 % doum powder for 5 weeks, respectively. Group 5, 6 rats fed on basel diet supplemented with doum powder 5, 10 %, respectively among three weeks then rats injected by cisplatin (10 mg/kg i.p., once) then rats fed on basel diet supplemented with 5, 10 % doum powder for 2 weeks, respectively. At the end of the experimental period (5 weeks) blood and urine samples were taken for biochemical analyses such as liver and kidneys functions were determined. Also, antioxidants enzymes as well as MDA were analyzed. Histobathological examination liver and kidneys were done. The results indicated that liver enzymes alkaline phosphatase (ALP), aspartate aminotransferase and alanine aminotransferase (ALT) were decreased significantly in the groups 3,4,5, and 6, respectively compared with the positive control group, however serum albumin was increased in this groups when compared with the positive control group. Uric acid, urea nitrogen and creatinine in addition MDA were decreased significantly in the groups 3,4,5, and 6, respectively compared with the positive control group. However, antioxidant enzymes (SOD, CAT, and GSH) were significantly increased in groups 3,4,5, and 6, respectively compared with the positive control group. The useful effect of doum could be due to the antioxidants effect. Finally, this study suggests that doum may be useful for patients who suffer from renal and liver diseases.

Key words: Liver injury, Kidney injury, Doum, Cisplatin, Rats, Antioxidant Enzymes, Histopathology.

Introduction

The liver is a vital organ has many functions for the body as converting nutrients derived from food into essential blood components , regulating blood clotting, storing vitamins and minerals, maintaining hormone balances, producing proteins and enzymes, and metabolizing and detoxifying substances that would otherwise be harmful to the body. The liver also makes factors that help the human immune system fight infection, removes bacteria from the blood, and makes bile, which is essential for digestion. Liver disease is any condition that causes liver inflammation or tissue damage and affects liver function ***(Ward and Daly, 1999).***

The kidneys are a major elimination pathway for many antineoplastic drugs and their metabolites, and renal impairment can result in metabolism of chemotherapeutic, delayed drug excretion and agents, and increased systemic toxicity. Many drugs require dose adjustment when administered in the setting of renal insufficiency. Minimizing non renal systemic toxicity may be a particular problem in patients on chronic hemodialysis, especially when the details of drug elimination and metabolism are not fully known. Nephrotoxicity induced by several synthetic drugs represents a major problem of modern age population.

Doum (*Hyphaene thebaica*) is an African palm tree, common in Upper Egypt, originally native to the Nile valley, bearing an edible fruit is a desert palm native to Egypt, sub-Saharan Africa and West India. It is listed as one of the useful plants of the world. Research on the fruit pulp showed that it contains nutritional trace minerals, proteins and fatty acids, in particular the nutritionally essential linoleic acid ***(Cook et al., 2000).*** Identification of compounds, by thin-layer chromatography, showed that the fruit contains significant amounts of saponins, coumarins, hydroxycinnamates, essential oils and flavonoids ***(Hsub et al., 2006).*** It was found that the administration of flavonoid extracts to diabetic rats significantly increased adiponectin levels that stimulate the hypoglycemic action of insulin without altering the concentration of insulin in blood and decrease the weight and volume of contents of granuloma in inflammation.

Doum, a well-known plant for its antioxidant, anticancer and anti-Inflammatory potential because of its phenolic and flavonoid content was explored for its antimicrobial potential against various Gram-negative and Gram- positive bacterial and fungal pathogens. The total

flavonoids content in different extracts of doum fruit extracts varied widely ranging from 24.04 to 47.17 mg rutin/g DW ***(Mohamed et al., 2010)***. HPLC analysis of aqueous doum fruit extracts showed flavonoid compounds. The highest concentrations were quercetin, hesperetin, naringin and rutin compounds ***(Aamer, 2016).***

The anti-inflammatory activity of doum was possibly due to its saponin content which acts against the oxidative damage and suppresses the serum transforming growth factor- (TGF) expression. So, doum administration declines the oxidative damage and the renal interstitial fibrosis in rats ***(Xie et al., 2009).*** Doum fruits as hematinic potentials, hypolipidemic, improve the hepatorenal functions.

Cisplatin (dichlorodiamino platinum) is an inorganic platinum-based chemotherapeutic agent that is widely used in the treatment of a variety of solid malignant tumors such as neck and head, lung, ovarian, and bladder cancers. The use of cisplatin is frequently limited by various significant side effects such as bone marrow suppression, peripheral neuropathy, ototoxicity, and nephrotoxicity ***(Kodama et al., 2014)***. The mechanism involved in cisplatin-induced nephropathy includes inhibition of protein synthesis, DNA damage, mitochondrial injury and apoptotic cell death in renal tubules.

The platinum compounds (Cisplatin) generally are not considered to be hepatotoxic, but cisplatin has been associated with a low rate of serum enzyme elevations during therapy. These elevations are usually mild, self-limited and asymptomatic, rarely requiring dose modification. In one instance, steatosis and necrosis (steatohepatitis) was found by liver biopsy in a patient who developed liver enzyme elevations 4 weeks after starting a regimen of cisplatin. In addition, hepatocellular liver injury was described. The number of cases of liver injury attributed to cisplatin has been too few to characterize the liver injury clinically. Recently, oxaliplatin when given in multiple courses has been linked to development of nodular regenerative hyperplasia and non-cirrhotic portal hypertension ***(Pollera et al., 1987)***.

Cisplatin is a rare cause of hepatic toxicity (steatosis and cholestasis) at standard doses, but minor AST elevations are not uncommon. At high doses, it has been reported to produce abnormal liver tests, especially AST and ALT ***(Hruban et al., 1991)***. The authors suggested that cisplatin-induced acute hepatic injury is dose-related. A case of cisplatin induced liver failure has been reported. A case of

autopsy-documented hepatic veno-occlusive disease has been reported in a patient who received high-dose cisplatin ***(Christian, 1989.***

The pathophysiology of cisplatin-induced kidney diseases involves 4 major mechanism such as proximal tubular injury, oxidative stress, inflammation, and vascular injury in the kidney. Proximal tubular injury involves several different mechanisms including apoptosis ***(Wei et al., 2007)***, autophagy ***(Yang et al., 2008)***, dysregulation of cell-cycle proteins ***(Megyesi J et al., 1998)***, activation of the mitogen-activated protein kinase (MAPK) signaling pathways ***(Jo S-K et al., 2005)***, direct toxicity to renal tubular epithelial cells, DNA damage ***(Leibbrandt et al., 1995)***, and mitochondrial dysfunction ***(Sugiyama et al., 1989).*** The present study was therefore carried out to investigate the effect of dietery supplementation of *doum* on kidney and liver injury induced by cisplatin in experimental rats.

Materials and Methods

Materials:

Cisplatin, casein, vitamins, minerals, cellulose and formalin were obtained from Elgomhoria Pharmaceutical Company, Cairo, Egypt. Doum as well as starch were obtained from the local market. Doum was finely grinded into fine powders and kept for further use. Chemicals for histopathological examinations were obtained from Elgomhoria Pharmaceutical Company.

Methods:

Experimental Animals and Design:

Forty two adult male rats of Sprague Dawley strain weighing 130-150 g body weight and 12-14 weeks old were used in this study. The rats were purchased from the Laboratory Animal Colony, Helwan, Egypt. The animals were housed under hygienic conditions at a room temperature of 25 ± 2 °C with relative humidity of 50–60% and on 12 hrs light/12 hrs dark cycles in the Animal House of Agricultural Research Center, Giza, Egypt. Basal diet and water were allowed *ad libitum.*

Preparation of Basal Diet:

Basal diet was prepared according to ***Reeves et al. (1993)***. It consists of 20 % protein, 10 % sucrose, 4.7% corn oil, 2% choline chloride, 1% vitamin mixture, 3.5 % salt mixture and 5% fibers. The remainder was corn starch up to 100 %.

Animals were divided into six main groups (n=7, once). The first main group (n=7) were fed on the basal diet during the experimental

period as a negative control group (-ve). The second main group rats were intraperitoneally (IP) injected with a single dose of cisplatin at 10 mg/kg body weight (BW) as a positive control group (+ve). This dosage was proved to be effective on kidney and liver functions from the earlier report ***(Osman et al., 2000, Saad et al., 2007 and Sarawoot and Chuchard, 2013).*** Group 3 and 4 were injected by cisplatin as the same of group 2 then rats fed on basel diet supplemented with 5, 10 % doum powder for 5 weeks, respectively. Group 5, 6 rats fed on basel diet supplemented with doum powder 5, 10 %, respectively among three weeks then rats injected by cisplatin (10 mg/kg i.p., once) then rats fed on basel diet supplemented with 5, 10 % doum powder for 2 weeks, respectively.

At the end of the feeding trail (5weeks), animals were fasted over–night, lightly anesthetized under ether. Blood was withdrawn into clean dry centrifuge plastic tubes. Blood samples were centrifuged and serum were obtained then stored at -20º C in a clean well stopped vial until analysis.

Biochemical Analysis:-

Determination of Serum Liver Enzymes:

The enzyme alanine amino transeferase (ALT) was determined in serum according to ***Sherwin (1984).*** The enzyme aspartate amino transeferase (AST) was determined according to ***Young (1990).*** Serum alkaline phosphatase (ALP) was determined according to the method described by ***Roy (1970).***

Determination of Serum Albumin Concentration:

Serum albumin level was determined as described by ***Young (1990)*** using spectrophotometer at 630 nm.

Determination of Serum Kidney Functions:

Serum urea nitrogen and serum uric acid concentration were determined according to the method described by ***Fossati et al., (1980)*** using Spectro-photometer (DU 4700) adjusted at 580 nm. Creatinine was determined according to the method described by ***Henry (1974)*** using spectrophotometer (DU 7400) adjusted at 580 nm.

Determination of Serum Malondialdehyde:

Serum malondialdehyde (MDA) content was determined by the method of ***Draper and Hadly (1990).***

Determination of Superoxide Dismutase (SOD):

The kidney SOD activity was measured according to ***Nishikimi et al. (1972)***. This assay relies on the ability of the enzyme to inhibit the

phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. The activity of SOD was expressed as Unit of activity/mg protein.

Determination of Catalase (CAT):

Renal CAT activity was measured in tissue homogenate according to ***Aebi (1984).*** The assay is based on that catalase reacts with a known quantity of hydrogen peroxide. This reaction is stopped after exactly one minute with catalase inhibitor. In the presence of peroxidase, the remaining hydrogen peroxide reacts with 3, 5-Dichloro-2-hydroxybenzenesulfonic acid and 4-aminophenazone to form a chromophore with a colour intensity inversely proportional to the amount of catalase. The activity of CAT was expressed as nmol of H_2O_2 utilized/min/mg protein.

Determination of Reduced Glutathione (GSH):

GSH content of kidney tissue was determined according to ***Ellman (1959).*** The assay is based on the reduction of 5, 5´dithiobis (2-nitrobenzoic acid) with glutathione producing a yellow compound. The reduced chromogen was directly proportional to GSH concentration and its absorbance was measured at wave length 412 nm.

Statistical Analysis:

Data were statistically analyzed using computerized program at scientific computer center, Faculty of Agriculture, Ain Shams University, Cairo, Egypt using SPSS, PC statistical software (version 11.0; SPSS INC, chincago, USA). Results were expressed as mean ± SE. Differences among groups were analyzed by analysis of variance (ANOVA) using Duncan's test. A p value of < 0.05 was considered statistically significant according to ***Armitage and Berry, (1987)***.

Results and Discussion

The Effect of Dietary Supplementation with Doum on Serum Concentr-ations of AST, ALT, ALP, and Albumin on Experimental Rats.

The effect of doum on liver functions such as ALT, AST, ALP and serum albumin are showed on table (1). Tabulated results showed significantly increased of serum ALT, AST and ALP in positive control group when compared with the normal control group. However, serum albumin was increased significantly in negative control group with a mean value of 4.23±0.14 U/L when compared with the positive control group with a mean value 2.53±0.21 U/L. When rats fed on basel diet supplemented with doum 5 % and intraperitoneal injection of a single

dose of cisplatin at 10 mg/kg body weight in group 3 serum ALT, AST and ALP decreased significantly ($P < 0.05$) with a mean value of 47.63±1.48 U/L, 62.93±2.03 U/L and 767.16±8.24 U/L, respectively when compared with the positive control group as shown in table (1). In addition when rats fed on basel diet supplemented with doum 10 % and intraperitoneal injection of a single dose of cisplatin at 10 mg/kg body weight in group 4 serum ALT, AST and ALP decreased significantly ($P < 0.05$) with a mean value of 41.90±1.10 U/L, 55.53U/L and 645.40±9.07 U/L, respectively when compared with the positive control group. However, serum albumin was increased significantly in group 3 and 4 with a mean value of 3.20±0.15 U/L and 3.56±0.20 U/L respectively when compared with the positive control group with a mean value 2.53±0.21 U/L.

Data in table (1) manifest that when rats fed on basel diet supplemented with doum 5 % among three weeks then rats were injected intraperitoneally with a single dose of cisplatin at 10 mg/kg then rats fed on basel diet supplemented with 5% doum powder for 2 weeks others serum ALT, AST and ALP decreased significantly ($P < 0.05$) with a mean value of 35.46±1.29 U/L, 45.33±2.40 U/L 573.53±5.94 U/L, respectively when compared with the positive control group. Also, serum ALT, AST and ALP of rats in group 6 decreased significantly ($P < 0.05$) with a mean value of 30.00±1.32U/L, 43.83±1.48 U/L and 542.93±6.17 U/L, respectively when compared with the positive control group. However, serum albumin was increased significantly in group 5 and 6 with a mean value of 3.68±0.17 g/dl and 4.10±0.08 g/dl, respectively when compared with the positive control group with a mean value 2.53±0.21 g/dl.

Serum ALT and AST are the most sensitive markers employed in the diagnosis of liver injury. When the liver cell plasma membrane is damaged, varieties of enzyme normally located on the cytosol are released into the bloodstream. Their estimation in serum is a useful quantitative marker of the extent and type of hepatocellular damage. Serum alkaline phosphate (ALP) activity is another common hepatotoxic biomarker, its activity increases in most types of liver injury ***(Friedman et al., 1996).***

These results agreed with the results of ***Liao et al., (2008)*** who showed that all cisplatin-treated groups had continuously increased ALT and AST concentrations when compared with the control group. Consequently, cisplatin is capable of causing liver function alterations,

as indicated by markedly increased ALT and AST levels, especially 48 and 72 h after treatment.

Evidence of cisplatin-induced liver injury has been demonstrated by ***Kim et al., 2004, Işeri et al., 2007,*** **and** ***Liao et al., (2008)*** whose reported that cisplatin-induced hepatotoxicity involved membrane rigidification, lipid peroxidation, and oxidative damage of cardiolipin. ***İşeri et al., (2007)*** found that a single intravenous injection of cisplatin at a dose of 2.5 mg/kg BW in Sprague-Dawley rats impaired both kidney and liver functions, characterized by significant increases in serum ALT, AST and ALP levels with compared with a control group. ***Liao et al., (2008)*** demonstrated that cisplatin altered liver function of male albino mice and that this was accompanied by significantly increased ALT and AST concentrations through the underlying mechanism of cisplatin-induced inflammationin and a mechanism of oxidative stress caused by increased MDA and reduced GSH levels.

Huda and Nabila (2018) demonstrated that doum fruit pulp contains 4.91% proteins, 5.26% fat, 4.5% ash and 85.33% total carbohydrate with fatty acids and also provided the proximate composition and the content of the minerals such as phosphorus, calcium and iron. Doum was reported to contain important substances including tannins, saponins and flavonoids; hence the use of doum, which is rich in flavonoids and saponins, in folk medicine is not surprising ***(Dosumu et al., 2006)***. Aqueous ethanolic extract of doum leaves appeared to be a potent scavenger of reactive oxygen species.

Table (1): The Effect of Dietary Supplementation with Doum on Serum Concentrations of AST, ALT, ALP, and Albumin on Experimental Rats.

Parameters / **Groups**		ALT **(U/L)**	AST **(U/L)**	ALP **(U/L)**	Albumin (g/dl)
G 1 (- Ve control)		24.80±1.53 ^{f}	32.90±1.64^{e}	351.73±3.79^{f}	4.23±0.14 ^{a}
G 2 (+Ve control)		63.66±2.60 ^{a}	90.66±2.40 ^{a}	867.60±4.25 ^{a}	2.53±0.21 ^{d}
G 3 (Doum 5%)	Treated groups	47.63±1.48 ^{b}	62.93±2.03 ^{b}	767.16±8.24 ^{b}	3.20±0.15 ^{c}
G4 (Doum 10%)		41.90±1.10 ^{c}	55.53±1.44 ^{c}	645.40±9.07 ^{c}	3.56±0.20 ^{bc}
G 5 (Doum 5%)	Prevented groups	35.46±1.29 ^{d}	45.33±2.40 ^{d}	573.53±5.94 ^{d}	3.68±0.17 ^{bc}
G6 (Doum 10%)		30.00±1.32 ^{e}	43.83±1.48 ^{d}	542.93±6.17 ^{e}	4.10±0.08 ^{ab}

Mean values in the same column sharing the same superscript letterers are not statistically significant, $P \leq 0.05$. Parameters (Mean ±SE)
ALT: Alanine Aminotransferase. AST: Aspartate Aminotransferase. ALP: Alkaline phosphatase.

Effect of Dietary Supplementation with Doum Powder on Serum Concent-rations of Uric Acid, Creatinine, and Urea Nitrogen on Experimental Rats.

The effect of doum on kidney functions such as uric acid, creatinine and urea nitrogen are showed on table (2). Results showed significantly increased of serum uric acid, creatinine and urea nitrogen in positive control group when compared with the negative control group. When rats fed on basel diet supplemented with doum 5 % and intraperitoneal injection of a single dose of cisplatin at 10 mg/kg body weight in group 3 serum uric acid, creatinine and urea nitrogen decreased significantly ($P < 0.05$) with a mean value of 4.60±0.35 U/L, 62.93±2.03 U/L, 1.10±0.05 U/L and 39.16±1.42 U/L, respectively when compared with the positive control group as shown in table (2). Also when rats fed on basel diet supplemented with doum 10 % and intraperitoneal injection of a single dose of cisplatin at 10 mg/kg body weight in group 4 serum concentration of uric acid, creatinine and urea nitrogen were decreased significantly ($P < 0.05$) with a mean value of 4.51±0.10 U/L, 1.01±0.08 U/L and 35.13±1.48 U/L, respectively when compared with the positive control group.

Data in table (2) revealed that when rats fed on basel diet supplemented with doum 5 % among three weeks then rats were injected intraperitoneally with a single dose of cisplatin at 10 mg/kg then rats fed on basel diet supplemented with 5% doum powder for 2 weeks others serum concentration of uric acid, creatinine and urea nitrogen decreased significantly ($P < 0.05$) with a mean value of 3.75±0.13 U/L, 0.80±0.03 U/L 30.26±0.37 U/L, respectively when compared with the positive control group. In addition, serum concentration of uric acid, creatinine and urea nitrogen in group 6 decreased significantly ($P < 0.05$) with a mean value of 3.66±0.06 U/L, 0.72±0.03 U/L and 30.13±1.04 U/L, respectively when compared with the positive control group.

These results agreed with the results of ***Shalpy et al., (2012)*** who found that addition of doum to the diet of the experimental animals produced a significant improvement in kidney functions and inflammatory status. The anti-inflammatory activity of doum was possibly due to its saponin content which acts against the oxidative damage and suppresses the TGF-beta1 expression. So, doum administration declines the oxidative damage and the renal interstitial fibrosis in rats. Our results also confirmed by ***Aida E. (2016)*** who

reported that doum fruit administration at 0.5 g/kg b.wt. or 2 g/kg b. wt. improved lipid profile (serum triglycerides, total lipids, cholesterol), hepato-renal functions such as serum AST, ALT, ALP, urea and creatinine, protein profile.

Cisplatin induced injury in renal vasculature and result in decreased blood flow and ischemic injury of the kidneys, contributing to a decline in glomerular filtration rate. These events, together, culminate in the loss of renal function during cisplatin nephrotoxicity, triggering acute renal failure. Cisplatin enters renal cells by passive and/or facilitated mechanisms. Cisplatin may also induce injury in renal vasculature, leading to ischemic tubular cell death and decreased glomerular filtration rate (GFR). Together, these pathological events culminate in acute renal failure (***Pabla, 2008)***.

Doum decreased hepatic and renal function parameters, indicating hepatic and renal impairments. This was attributed to the rich amount of flavonoids such as luteolin, which inhibits lipid oxidation by scavenging free radicals or by other mechanisms, such as singlet oxygen and lipoxygenase inhibition ***(Saravanan and Leelavinothan 2012)***.

Liao et al., (2008) suggests that cisplatin can cause kidney function impairment via elevation of kidney function biomarker levels including BUN and creatinine, which is associated with kidney pathologies depending on the dose and time of treatment. The toxic effects of cisplatin by intravenous cisplatin administration (7 mg/kg BW) caused abnormal kidney function in male Wistar albino rats, as evidenced by markedly increased levels of serum BUN and creatinine compared with a control group. They suggested the underlying mechanism was cisplatin-induced oxidative stress through elevation of ROS and reactive nitrogen species (RNS), and reduction of the antioxidant defense. Cisplatin administered at 12 mg/kg BW in male Swiss albino mice for four days increased the levels of MDA, indicating lipid peroxidation and reduced levels of GSH, catalase, SOD, and glutathione peroxidase (GPx) in the kidneys.

Table (2): The Effect of Dietary Supplementation with Doum Powder on Serum Concentrations of Uric Acid, Creatinine, and Urea Nitrogen on Experimental Rats.

Parameters / Groups		Uric Acid *(mg/dl)*	Creatinine *(mg/dl)*	Urea Nitrogen *(mg/dl)*
G 1 (- Ve control)		2.90±0.15 [d]	0.58±0.03 [d]	25.96±0.93[e]
G 2 (+Ve control)		5.50±0.26 [a]	1.43±0.08 [a]	43.40±1.97 [a]
G 3 (Doum 5%)	Treated groups	4.60±0.35 [b]	1.10±0.05 [b]	39.16±1.42 [b]
G4 (Doum 10%)		4.51±0.10 [b]	1.01±0.08 [b]	35.13±1.48 [c]
G 5 (Doum 5%)	Prevented groups	3.75±0.13 [c]	0.80±0.03 [c]	30.26±0.37 [d]
G 6(Doum 10%)		3.66±0.06 [c]	0.72±0.03 [cd]	30.13±1.04 [d]

Mean values in the same column sharing the same superscript letterers are not statistically significant, $P \leq 0.05$. Parameters (Mean ±SE).

Effect of Dietary Supplementation with Doum Powder on Serum Concentra-tions of Malondialdehyide and Antioxidant Activity on Experimental Rats.

Table (3) showed the effect of dietary supplementation with doum on serum concentrations of malondialdehyide (MDA) on Experimental Rats. Results showed significantly increased of malondialdehyide in positive control group when compared with the negative control group. Serum MDA in groups 3, 4, 5 and 6 groups were decreased significantly ($P < 0.05$) with a mean value of 104.26±3.90, 93.86±1.18, 80.33±4.33 and 70.00±2.51 nmol/min/mg protein , respectively when compared with the positive control group with a mean value of 160.26±2.36 μmol/dL.

The effect of dietary supplementation with doum on antioxidant activity such as SOD, CAT and GSH are showed on table (3). Tabulated results showed significantly decreased of serum SOD, CAT and GSH in positive control group when compared with the normal control group. However, serum SOD, CAT and GSH were increased significantly in groups 3, 4, 5, and 6 when compared with the positive control group.

Antioxidants are compounds that can delay and/or inhibit oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reaction. In the present study which agreed with ***Safia M. and Eman A. (2017)*** who's demonstrated that doum increased GSH concentration, GSH-Px, GST and CAT activities as antioxidant potential and reducing the MDA concentration as a

marker of lipid peroxidation. Doum possess strong antioxidant activity which provokes free radical scavenging enzyme system. Additionally, the elevated level of GSH-Px, GST and CAT by doum may have facilities the conjugation reaction of xenobiotics metabolism and may have increased the availability of non-critical nucleophile or inactivation of electrophiles and therefore might be playing a major role in metallo-protection.

Safia M. and Eman A. (2017) illustrated that the pretreatment with *Doum* increased total protein and albumin levels in serum resulted mainly from elevation concentration of GSH which protects cellular protein against oxidation through glutathione redox cycle and directly detoxifies reactive species. The protective effect of *doum* is probably due to its high contents of powerful antioxidants, particularly: saponins, coumarins, hydroxycinnamates, essential oils, flavonoids, alkaloids, reducing sugars, glycosides, and water-soluble phenolic contents, polyphenols and polyunsaturated/unsaturated fatty acid which are known as powerful antioxidants ***(Heta et al., 2006).*** These compounds make doum an important source of antioxidant, which certainly play an important role in *vivo*. Pro-inflammatory cytokines are potent inducers of free radicals and inflammatory mediators.

Both doses of doum extract (especial high dose) were able to significantly prevent oxidative damage and cellular toxicity through scavenging the free radicals and terminating the membrane lipid peroxidation (MDA) by improving cellular antioxidants and pro-inflammatory cytokines (TNF-α and IL-1β). Finally, the antioxidant property of *doum* is claimed to be one of the mechanisms of hepato-/ nephro-protective against oxidative damage. On the other hand, *doum* significantly increased and decreased (P<0.05) the concentration of hepatic GSH and MDA concentrations, respectively, in rats **(*Safia M. and Eman A. 2017)*.**

Hossam et al., (2018) indicated that doum is one of the commonly consumed traditional beverages in Egypt and is rich in polyphenolic compounds. Several studies have recorded that doum fruit extracts contain high amount of flavonoids, phenols and used as antioxidant and antibacterial activities which can alleviate the adverse efects of oxidative stress and prevent diseases caused by pathogenic bacteria. It is well-known that plant phenolic compounds are highly effective free radical scavengers. The antioxidant activity increased with the increase in concentration and the consumption of doum plant

which would exert several beneficial effects by the value of its antioxidant and antimicrobial activities.

For over a decade, oxidative stress has been recognized as an important factor that contributes to cisplatin nephrotoxicity. Increases of various reactive oxygen species (ROS) occur during cisplatin treatment of cultured renal tubular cells, kidney slices, and in vivo in whole animals. Three mechanisms have been proposed to account for ROS generation under these pathological conditions. First, once in a cell, cisplatin is aquated into a highly reactive form, which can rapidly react with thiol-containing molecules including glutathinone, a well-recognized cellular antioxidant. Depletion or inactivation of glutathione and related antioxidants by cisplatin is expected to shift the cellular redox status, leading to the accumulation of endogenous ROS and oxidative stress within the cells (***Liao et al., 2008)***.

Table (3): Effect of Dietary Supplementation with Doum Powder on Serum Concentrations of Malondialdehyide and Antioxidant Activity on Experim-ental Rats.

Parameters / Groups		SOD (U/mg protein)	CAT (nmol/min/mg protein)	GSH (nmol/min/mg protein)	MDA (nmol/min/mg protein)
G 1 (- Ve control)		1027.20±16.30 a	115.56±3.26 a	5.15±0.08 a	55.36±2.69 f
G 2 (+Ve control)		625.96±9.77 e	57.13±2.74 e	2.77±0.06 d	160.26±2.36 a
G3 (Doum 5%)	Treated groups	787.60±5.43 d	74.83±2.12 d	3.47±0.20 c	104.26±3.90 b
G4(Doum 10%)		900.00±5.77 c	87.83±1.30 c	3.72±0.10 c	93.86±1.18 c
G5 (Doum 5%)	Prevented groups	914.43±13.20 c	96.73±1.82 bc	4.77±0.06 b	80.33±4.33d
G6 (Doum 10%)		958.33±11.31b	99.00±5.68 b	4.91±0.04 ab	70.00±2.51 e

Histopathological Examination: -

Liver and kidney tissue specimens were fixed in 10% neutral buffered formalin. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin, sectioned at 4-6U thickness and stained by hematoxylin and eosin were used according to ***Bancroft et al., (2013).***

Histological Grading of liver injury according to ***Plaa*** **and** ***Charbonneau (1994):***

Grade	Grade Description
G 1 (- Ve control)	No apparent injury by light microscopy
G 2 (+Ve control)	Swelling of hepatocytes
II	Ballooning of hepatocytes
III	Lipid droplets in hepatocytes
IV	Necrosis of hepatocytes

Grading System for Renal Lesions

Score	Lesion
G 1 (- Ve control)	Normal histology
G 2 (+Ve control)	Tubular epithelial cell degeneration, without significant necrosis or apoptosis
G3	Tubular epithelial cell necrosis and apoptosis <25%
G4	Tubular epithelial cell necrosis and apoptosis <50%
G5	Tubular epithelial cell necrosis and apoptosis <75%
G6	Tubular epithelial cell necrosis and apoptosis≥ 75%

Photomicrograph of Liver Section:

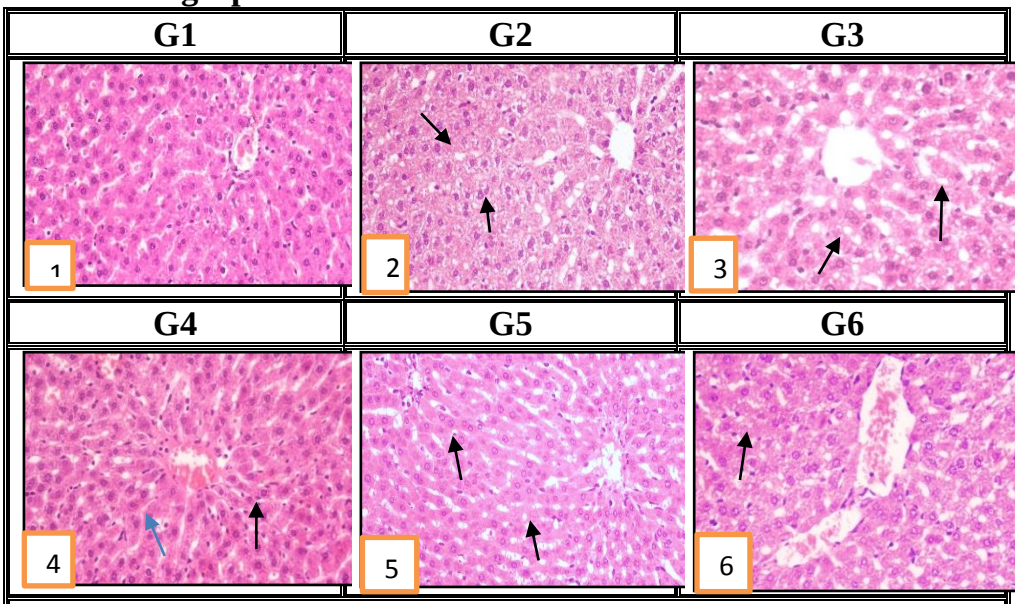

Fig. 1showing normal histological structure of hepatic lobules. **Fig. 2** showing swelling of hepatic cells and narrowing of hepatic sinusoids. **Fig.3** showing mild swelling of hepatocytes and granularity of its cytoplasm. **fig.4** showing swelling of hepatic cell sarrow with marked hyperplasia of Kuppfer cells. **Fig. 5,6** showing mild swelling of hepatocytes (H&E X200).

Photomicrograph of Kidney Section:

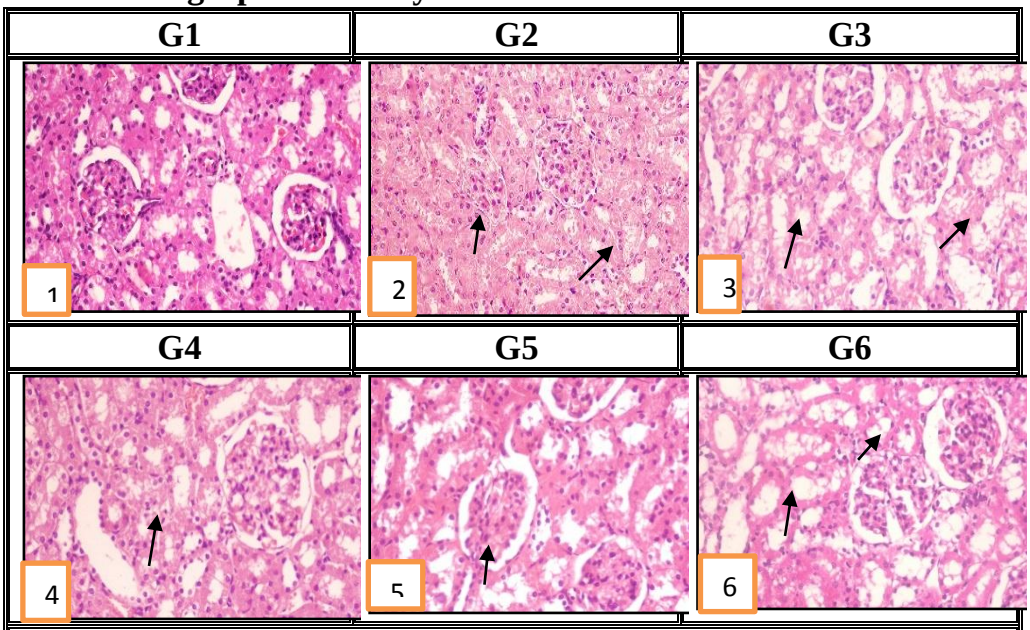

Fig.1 showing normal histological structure of both renal glomerulus and tubules scoring. **Fig.2** showing shrinkage of capillary tufts and tubular epithelial cell degeneration and necrosis scoring**. Fig. (3,4,5 and 6)** showing tubular epithelial cell degeneration scoring.

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تأثير التدعيم الغذائى بالدوم على اصابة الكبد والكلى المسبب بالسيسبلاتين على فئران التجارب

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تهدف هذه الدراسة الى معرفة التدعيم الغذائى بالدوم على اصابة الكبد والكلى المحدثة بالسيسبلاتين على فئران التجارب. تم استخدام اثنان واربعون فأرا يزنوا من ١٣٠-١٥٠ جرام وتم توزيعهم بعد فترة التكيف (٧ أيام) الى ٦ مجموعات رئيسية (٧ فأر للمجموعه) . المجموعة الاولى (العدد = ٧ فئران) وتعتبر المجموعة الضابطة السالبه وتم تغذيتها الغذاء الاساسى. المجموعه التانيه تم اعطاؤهم السيسبلاتين (٧ ملجم /كيلوجرام/ مرة) وتعتبر المجموعه الضابطة الموجبة. المجموعه الثالثة والرابعه تم استخدام السيسبلاتين كما فى المجموعه التانيه ثم تناولوا الغذاء الرئيسى مدعم ب ٥ و ١٠% دوم بودرلمدة خمس أسابيع على التوالى. تم تغذية المجموعه الخامسه والسادسه على الغذاء الرئيسى المدعم ب ٥ و ١٠% بالدوم على التوالى لمدة ٣ أسابيع ثم تم اعطاء الفئران السيسبلاتين (٧ ملجم / كجم/ مرة) لمدة اسبوعين . وفى نهاية فترة التجربة (٥ أسابيع) تم ذبح الفئران والحصول على السيرم للاجراء التحاليل المعملية مثل وظائف الكبد والكلى . أيضا ، تم تقدير مضادات الاكسدة بالاضافة الى مستوى المونالدهيد (MDA) . تم اجراء الفحوص التشريحية للكبد والكلى. أشارت النتائج الى ان هناك انخفاض معنوى فى انزيمات الكبد مثل ALP و ALT و AST للمجموعات ٥,٤,٣ و ٦ على التوالى بالمقارنة بالمجموعه الضابطة الموجبة ولكن مستوى الالبيومين ارتفع فى هذه المجموات بالمقارنة مع المجموعه الضابطة الموجبه. هناك انخفاض معنوى فى حمض اليوريك والكرياتينين ونيتروجين اليوريا بالاضافة الى مستوى المونالدهيد (MDA) فى المجموعات ٥,٤,٣ و ٦ على التوالى بالمقارنة بالمجموعه الضابطة الموجبة. ولكن هناك ارتفاع معنوى فى الانزيمات المضادة للأكسدة مثل مستوى الكتاليز فى السيرم (CAT) ، مستوى جلوتاثيون (GSH) و SOD فى المجموعات ٥,٤,٣ و ٦ على التوالى بالمقارنة بالمجموعه الضابطة الموجبة. التأثير المفيد للدوم قد يكون من خلال تأثيره كمضاد للأكسده. فى النهاية ، هذه الدراسه تقترح ان الدوم مفيد للمرضى الذين يعانوا من أمراض الكلى والكبد.

الكلمات المفتاحية:

اصابة الكبد، اصابة الكلى، الدوم، السيسبلاتين، الفئران، مضادات الاكسده و الفحوص التشريحية.