Evaluation the Protective Effect of *Capparis spinosa* Fruits Hydroalcoholic Extract Against Hepatotoxicity Induced by Cisplatin in Rats

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Abstract

The present study was carried out to evaluate the liver abnormality change, hepatotoxicity after using the cisplatine and investigate the protective effect of capparis spinosa fruit extract against these effects and evaluate the liver function (AST, ALT, ALP), serum lipid profile (HDL, LDL, VLDL, Triglyceride and cholesterol) and histopathological changes of liver. Twenty-one male rats were randomly divided into three groups (7rats/ each). First group was received distilled water orally for 10 days and served as negative control group. Second group was received cisplatin only (10 mg/kg, i.p.) as a single dose at day 8 and served as a cisplatin treated group. Third group was received capparis spinosa fruit extract (100 mg/kg B.W) for 10 days and 10 mg/kg cisplatin as a single dose at day 8. The results showed that the animals that treated with cisplatine alone were suffered from liver damage by significant increasing in the liver enzymes, induced histopathological changes, significant increasing in lipid profile (except HDL, which significantly decreased). While the third group (that treated with hydroalcoholic extract of capparis spiosa fruit showed significant decreasing in the liver enzymes as compared with second group and decreasing in the histopathplogical changes in the liver, while there were significant decreasing in lipid profile (except HDL, which significantly increased). Conclusion: In this study cisplatine induced histopathplogical changes, alteration in the liver function and produced increasing in the lipid profile, while the (hydroalcoholic extract of capparis spiosa fruit) was have ability to maintain liver function and induce the decreasing in the lipid profile.

Keywords: Capparis spinosa, Hepatotoxicity, Cisplatin.

Introduction

Hepatotoxicity is the term used to describe liver damage or injury caused on by exposure to medications or other nonpharmacological substances (1) , (2). Acute and chronic hepatitis, cholestasis, granulomatous hepatitis, fulminant hepatitis, ductopenia, and steatosis steatohepatitis, micro vesicular steatosis or macro vesicular are some of the clinical and pathological symptoms of hepatotoxicity (3) , (4). Excluding illicit drugs and natural ingredients, it is estimated that 1100 medications cause hepatotoxicity responses (5). Cisplatin, also known as (SP-4-2)-diamminedichloridoplatinum, is one of the most common and successful drugs for the treatment of numerous solid malignancies (II) (6). Hepatocyte oxidative damage and mitochondrial dysfunction are the causes of cisplatin-induced hepatotoxicity (7). Additionally, cisplatin activated kinases of MAP (Mitogen Activated Protein) like kinases of p38 and kinases of c-Jun N-terminal. These kinases are involved in liver damage by phosphorylating Jun N-terminal Kinases JNKs, which then causes the transcription genes of pro-inflammatory like Inducible nitric oxide synthase I. (AP-1), Cyclooxygenase (COX-2) (8). Numerous factors, such as an increased oxidative state, an inflammatory response, and apoptosis, contribute to the toxicity caused by cisplatine (9) and (10).

The caper, *Capparis spinosa* L., belongs to the family Capparidaceae and is widely cultivated around the world (11). *Capparis spinosa* Extract shows a noteworthy protective effect contra oxidative stress and breaks the ROS signal loop in systemic sclerosis (12). Due to it is content of phytochemical and nine compounds, including luteolin, catechin, coumarin, rutin, kaempferol, vanillic acid, epicatechin, resveratrol and gallic acid, the fruit of *Capparis spinosa* has been used for liver disease treatment for centuries. These compounds have hepatoprotective, and antioxidant properties (13). The molybdate assay's determination of the total capacity of antioxidant in *Capparis spinosa* pollen ranged from 99.54 mg to 0.9 mg of AAE (Ascorbic Acid Equivalent) per g (14).

Caper fruits were distinguished by having a green exocarp throughout all phases of growth, a decline in protein content as the fruit grew, and high levels of flavonoids, total phenols, and flavanols (15). Previous studies using ethanol extracts of the *Capparis spinosa* fruit shown hepatoprotective properties, and histological research revealed that the extracts may prevent tissue fibrosis (16). All biochemical indicators were brought back to normal with a regular intake of Capparis spinosa leaf or buds corrected liver damage and offered varied percent of organ protection because of the herb's antidiabetic and antihyperlipidemic properties (17). Hence, this study aimed to evaluating the protective effect of *capparis spniosa* fruits extract by reduce hepatoxicity induced by cisplatin in rats.

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Materials and methods:

Plant Materials and Extraction

From an Iraqi local market, *Capparis spinosa* fruits were collected . The plant was recognized and authorized in the Ministry of Agriculture /State Board for Seed Certification and Testing in (Abu Graib-Baghdad) at the certification number (2859) in 11/11/2021 . Fruits were washed thoroughly in water, dried in shade at 25°C cut into small pieces and grind until it becomes a powder. 90 grams of the capers fruit powder was then poured into a one-liter flask using a Soxhlet extractor, and hydro alcoholic extract (ethanol 70 percent) was added to keep the powder level covered. The solution was filtered after 72 hours, and the ethanolic extract was then concentrated using a rotary evaporator apparatus at 50 ° C with a turn speed of 70 rpm to **(19).**

Animals

Twenty one (21) male rats that were 200–250 g in weight and were around three months old . Were employed to carry out the study's experiment. Rats were kept in plastic cages and kept in the College of Veterinary Medicine's special housing area for two weeks so they could become acclimated to it. Tap water and common rodent food (commercial feed pellets) were also freely available. Housing conditions were kept at 20-25 Co in air-conditioned rooms, where the air was regularly changed using ventilation vacuums and the light/dark cycle was 14/10. The cages' litter was replaced once a week.

Experimental Design

Twenty one (21) three groups of 7 male rats each were created at random from the total. First group was received distilled water orally for 10 days and served as negative control group. Second group was received cisplatin only (10 mg/kg, i.p.) as a single dosage at day 8 (19) and worked as a cisplatin treated group (positive control). Third group was received *capparis spinosa* fruit extract (100 mg/kg B.W) for 10 days (20) and 10 mg/kg cisplatin as a single dosage at day 8.

In every group 7 of animals were sacrificed in day 11 and the sample of blood was collected directly from the heart while the liver served for histopathology.

The Alanine Aminotransferase (ALT or GPT) converts alanine to the 2-oxoglutarate by transferring the amino group, which results in the synthesis of pyruvate and glutamate. The average of NADH reduction, evaluated at 340 nm, is used to compute the catalytic concentration using the lactate dehydrogenase (LDH) linked reaction (21). Oxalacetate and glutamate are produced when The amino group is transferred from aspartate to 2-oxoglutarate by Aspartate Aminotransferase (AST or GOT). Using the malate dehydrogenase (MDH) linked reaction, The rate of NADH reduction, which is measured at 340 nm, is used to estimate the catalytic concentration (22). Alkaline Phosphatase (ALP): By catalyzing the transfer of the phosphate group from 4-nitrophenylphosphate to diethanolamine (DEA) in an alkaline environment, alkaline phosphatase (ALP) creates 4-nitrophenol. The ratio of 4-nitrophenol synthesis is monitored at 405 nm, and the catalytic concentration is calculated from this data (23).

Estimation of Lipid Profile

Cholesterol: Through the coupled processes outlined below, Using spectrophotometry, it is possible to identify the colored combination that is produced by the sample's free and esterified cholesterol (24). Triglycerides in the sample product a colored combined that may be detection by spectrophotometry through the linked processes outlined below (25).

The sample's low density lipoproteins (LDL) and very low density lipoproteins (VLDL) precipitate with magnesium ions and phosphotungstate. High density lipoproteins are present in the supernatant (HDL). Then, using the coupled processes, the HDL cholesterol is determined spectrophotometrically (26). According to Friedewald method

Triglyceride = VLDL. (27).

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Low density lipoproteins (LDL); according to Friedewald method: LDLc=TC-HDLc-TG/5(VLDL) (28).

Histopathology

Rat livers from all groups were utilized as samples for a histopathological analysis at 11 days of the experiment. After the animal was given a chloroform anesthesia (by inhalation), the liver were removed and placed in plastic containers with formalin solution (10%) and tissue produced in accordance with (29).

Results and discussion

The Effects of Cisplatine, and Hydro Alcoholic Extract of Capparis Spinosa Fruits on Serum Liver Enzymes (AST, ALT, ALP).

All rats that treat with cisplatine (10 mg/kg b.w IP) alone showed significant increasing (P≤0.05) the serum of the liver enzymes include (AST , ALT and ALP) compared to negative control group, showed the group that treated with cisplatine only (10 mg/kg b.w IP) were showed significant increasing in the level of liver enzyme in second period, these result agreed with those obtained by (30) The CP-induced hepatotoxicity in this investigation was demonstrated by significance variations in the serum liver enzyme (AST, ALT, and ALP) as in table (1). The liver absorbs cisplatine, which builds up in hepatocytes and causes cellular damage that eventually results in an increase in the levels of circulating liver enzymes (31). Hepatocyte membranes become damaged and necrose as a result of the free radicals' attachment to them. This causes structural breakdowns in the membranes, which caused intracellular cytosol (AST, ALT, ALP,

and LDH) to be released into the blood (32).
In other hand rats treated with ethanolic extract of *capparis spinosa* fruit (100 mg/kg b.w orally) with cisplatine administration showed significant decreasing in level of liver enzyme (AST, ALT, ALP) as compared with administration of cisplatine alone, However there was a significant decreasing (P≤0.05) in level of liver enzyme become near to control levels . These result is in agreement with those obtained by (33) The flavonoids quercetin and rutin, which have antioxidant qualities and aid in reducing oxidative damage to the liver, are thought to be responsible for the actions of *Capparis spinosa* on the liver. It has been noted that administering *Capparis spinosa* caused ALT and AST levels to drop in a dose-dependent manner.

Table (1): The Effects of Cisplatine and Ethanolic Extract of *Capparis Spinosa* Fruits on Serum Liver Enzyme Enzymes (AST,ALT,ALP).

Groups	ALT mu/ml	AST mu/ml	ALP mu/ml
Negative control	87.29 ±0.90 B	29.13 ±0.87 C	29.42 ±0.46 B
Cisplatine 10 mg/k ip	180.16 ±6.09 A	58.74 ±1.24 A	54.87 ±1.63 A
Capparis spinosa fruit extract 100 mg /k orally and Cisplatine 10 mg/k ip	94.14 ±2.53 B	32.04 ±2.62 B	31.38 ±1.88 B
LSD value	13.45	4.87	4.29
Means having with the different letters in same column differed significantly. * (P≤0.05),			

The Effects of Cisplatine and Hydro Alcoholic Extract of Capparis Spinosa Fruit on Serum lipid profile .

Rats that treated with cisplatine (10 mg/kg b.w IP) alone showed decreasing in the HDL as compared with negative control group, while (LDL, VLDL, Triglyceride and cholesterol) were significantly elevated (P≤0.05) contrasted with the negative control group, as in table (2). These results are in agreed with those obtained by (34). Circulating the cholesterol, triglycerides, and LDL cholesterol all elevated noticeably after receiving cisplatine, whereas HDL cholesterol fell. Plasma cholesterol levels are significantly controlled and regulated by the liver. So, when medication therapy results in hepatic dysfunction, serum total cholesterol (TC) and LDL-cholesterol levels rise. According to the results of the study, the deleterious effects of cisplatin, which result in hepatocellular dysfunction and altered lipid metabolism, are likely responsible for the significant increase in level of the serum triglycerides, total cholesterol, and LDL-cholesterol after cisplatin exposure in rats (35).

Table (2) :The Effects of Cisplatine and Ethanolic Extract of *Capparis Spinosa* Fruits on Serum lipid profile

Group	Mean ± SE (mg/dl)				
	Cholesterol	Triglyceride	HDL	LDL	VLDL
Negative control	128.79 ±1.83 B	125.06 ±9.67 B	30.06 ±3.95 A	73.71 ±5.96 B	25.01 ±1.93 B
Cisplatine 10 mg/k ip	232.74 ±5.42 A	189.63 ±6.86 A	12.67 ±2.32 B	182.14 4.69± A	37.92 ±1.37 A
Capparis spinosa fruit extract 100 mg /k orally and Cisplatine 10 mg/k ip	132.70 ±10.02 B	130.40 ±4.45 B	31.66 ±1.49 A	74.61 ±11.20 B	26.08 ±0.89 B
LSD value	20.0	24.7	10.2	21.2	4.05
Means having with the different letters in same column differed significantly. * (P≤0.05),					

The rats that treated with ethanolic extract of capparis spinosa (100 mg/kg b.w orally) before cisplatine administration showed increase in the HDL level and became nearly to negative control group, while (LDL, VLDL, Triglyceride and cholesterol) were significantly decreased (P≤0.05) as combined to the positive control group, these result agreed with those obtained by (36) , (37) found that type 1 diabetic wistar rats' plasma TG and cholesterol levels were decreased by the capparis spinosa fruit extract (200 mg/kg) (38) also showed that two weeks of treatment with an liquid extract of capparis spiosa fruits (20 mg/kg) reduction in plasma concretion triglycerides significantly and levels of cholesterol in type 1 diabetics Rats. The potential of Capparis spinosa to lowering cholesterol may be due to improved uptake of LDL and LDL receptors, decreased intestinal cholesterol absorption, and other factors, lowering cholesterol production, and increased lecithin cholesterol acyltransferase (LCAT) activity. The effects of hypotriglyceridemia may be caused by a reduction in fatty acid synthesis, an increase in LDL catabolism, an increase in tissue lipases and lecithin cholesterol acyltransferase (LCAT) activity, and an inhibition of acetyl-CoA carboxylase (39). Another study used capparis spiosa fruit ethanol extract to treat diabetic rats given streptozotocin to lower their glucose-6-phosphate

activity of enzymes, hepatic triglyceride and cholesterol concentrations, in addition to their mRNA expressions (40) . High concentrations of phytosterols found in capparis spiosa have been shown to mediate the reduce in cholesterol levels (41). It has been hypothesized that it has antilipidemic effects via reducing bile acid action on intestinal absorption of cholesterol and by diminishing 3-hydroxy-3-methylglutaryl coenzyme activity (42). By boosting the activity of lecithin cholesterol acyl transferase (LCAT), which may aid in the management of blood lipids, it may also improve the uptake of low density lipoproteins. The increase in HDL levels observed with capparis spiosa bud and leaves may be caused by LCAT, which facilitates the incorporation of free cholesterol into HDL (43).

The Effects of Cisplatine and Hydro Alcoholic Extract of Capparis Spinosa Fruit on Histopathology of Liver Tissue.

Histopathological sections of liver in the first group (negative control) showed normal architecture the center of lobule is C.V and the hepatocyte H arranged as cords of cells with kupffer cell K ,. Sinusoids as in (figure 1).

While in the second group (cisplatine only), hepatic manifestation showed slight per portal MNCS infiltration mixed with few neutrophils accompanied with nu clear anisonucleosis of adjacent hepatocytes and showed focal MNCS aggregation with anisonucleosis as in(figure 2). The liver section moderate destruction of hepatic tissue accompanied with focal and diffuse necrosis with sever congestion and hemorrhagic areas as in together with evidence of sinusoids dilation and congestion accompanied with nuclear pyknosis of adjacent hepatocytes and focal MNCS infiltration as in (figure 3), mild sinusoids congestion among swelling hepatic cord ,these results have been previously mentioned by (44). Cisplatine is a powerful cytotoxic drug that is frequently used in the treatment of many cancer types. However, when the liver suffers oxidative stress, such as those related to cisplatin's hepatotoxic effects, liver cells come into contact with massive levels of reactive oxygen species that exceed their ability for detoxification (45). Then, reactive oxygen species harm the hepatocyte through direct effect on signal pathways, lipid peroxidation, protein oxidation, growth factor inhibition, and DNA damages (increased cell death, decreased proliferation) (transcription factor activation, reduced angiogenesis) (46).

Histopathological sections of liver in the third group (hydro alcoholic extract of *capparis spinosa* fruit with cisplatine) showed moderate MNCS infiltration in liver tissue mainly in central region of hepatice lobules with peripheral hepatocyte swelling and sinusoids congestion as in (figure 4), (figure 5) . This scavenging ability of phenolic compounds is probably due to their antiradical scavenging action (47).

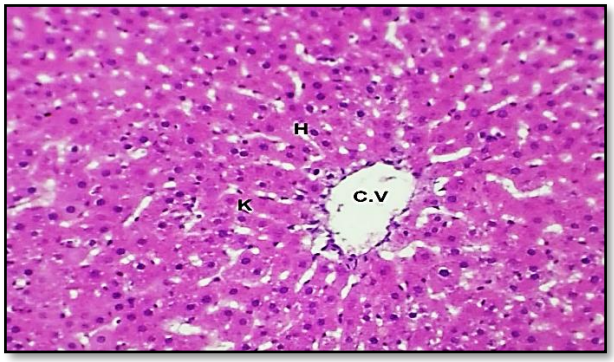

Figure 1: Histological section in the liver of rats (negative control group), with normal limit structure aspect (C.V, H, Kupffer cells) (H&E stain, X10).

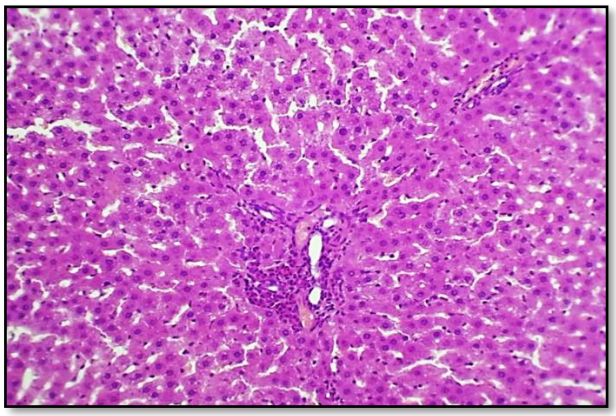

Figure 2: Histological section in the liver of rats showed periprotal MNCS infiltration with few neutrophils accompanied with anisonuclosis (positive control group) (H&E stain, X10).

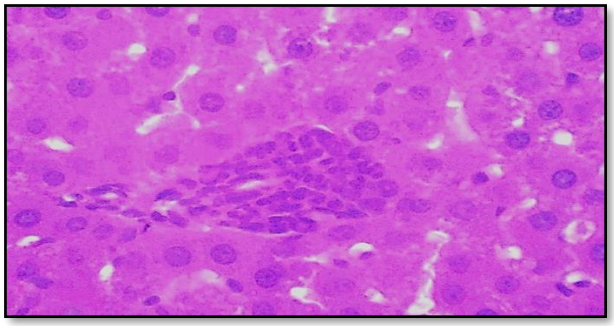

Figure 3: Histological section in the liver of rats (positive control group) showed focal MNCS aggregation with anisonucleosis (H&E stain, X40).

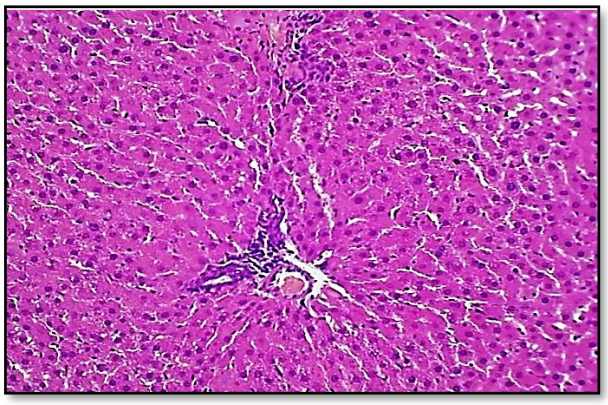

Figure 4: Histological section in the liver of rats (ethanol extract of capparis spinosa fruit), show perivascular MNCS aggregation with kupffer cell hyperplasia (H&E stain, X10).

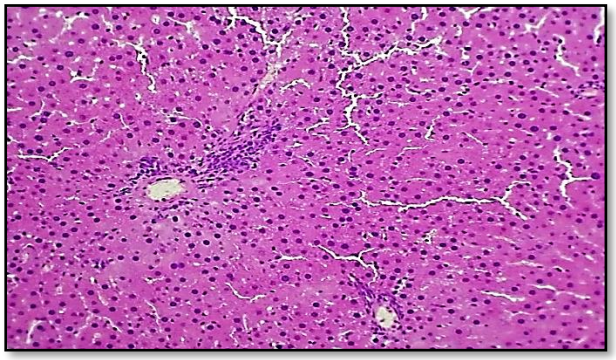

Figure 5: Histological section in the liver of rats (ethanol extract of capparis spinosa fruit), show MNCS infiltration in central region of hepatic lobules with peripheral hepatocyte swelling (H&E stain, X10).

Conclusion

In this study cisplatine induced histopathplogical changes, alteration in the liver function and produced increasing in the lipid profile, while the (hydroalcoholic extract of *capparis spiosa* fruit) was have ability to maintain liver function and induce the decreasing in the lipid profile.

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