ORIGINAL ARTICLE

Effects of cisplatin on oxidative stress parameters, hepatic biochemical and histological picture in male albino rats Role of propolis

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ABSTRACT

Aims: This study aimed to investigate the effects of cisplatin on oxidative stress parameters (malondelhyde MDA; Total antioxidant status TAS) , liver function tests with histological picture and any possible role for propolis in ameliorating toxic changes, in comparison to controls.

Materials and Methods: This experimental work takes 6 weeks and involved 60 male albino rats ,weighed 230±40g , divided into 6 groups, each of 10 members.

Group A, received 3 doses of cisplatin at 4 mg/kg intraperitoneally (I.P) at weekly intervals starting by the end of the 2nd week, and killed by the end of the 6th week.

Group B, received propolis at 120 mg /day orally for 6 weeks.

Group C, received propolis 120 mg/day initially then by the end of the 2nd week started cisplatin 4mg I.P weekly for 3 doses.

Group D, received propolis 60 mg/day for 6 weeks.

Group E, received propolis 60 mg daily for 6 weeks, starting cisplatin I.P 4 mg/kg 3 doses by the end of the 2nd week ,one week apart.

Group F, represent the control group, on normal saline orally for 6 weeks.

At the start the animals in all groups were weighed ,a blood sample was taken then, during the experiment , weighed , physical activity and food intake were recorded, by the end of the experimental duration weight were recorded, a blood sample were taken and the animals then were killed , livers were taken for their weight and histological section with H& E staining.

The blood samples taken before and after the experiment were analysed for ALT, AST, ALP, serum albumin, total serum bilirubin, direct and indirect, serum MDA and TAS levels.

Results: By observation: By comparison of before and 6 weeks after the experiment , there was a significant weight reduction in the group A, with loss of 2 members of this group after the 3rd dose of cisplatin. While group B showed a significant weight gain. Clear and significant changes were recorded in parameters of liver function tests, MDA and TAS levels in group A in comparison to control group, with a clear ameliorative effects of propolis especially noticed at 120 mg/d. Also there was a clear changes in the histological picture of the liver with obvious ameliorated effects of propolis especially noticed at 120 mg dose /day.

Conclusion: Propolis administration at a daily dose of 120 mg/day clearly ameliorated the toxic effects of cisplatin on the liver at both functional and histological pictures.

INTRODUCTION

Cisplatin (CP) is a commonly used chemotherapeutic agent in the management of a number of solid tumors, of them, testicular, ovarian, lung, brain , head and neck tumors (1). Its use carry a serious adverse effects of these especially concerned , nephrotoxicity (2),and hepatotoxicity (3), which could be explained by the fact that more than 90% of the drug in the blood is covalently bound to plasma proteins and after administration high levels of the drug can be recovered in tissues of the kidney, liver, intestine and testis (4). After only few days from initiation of therapy ,about one-third of the cisplatin treated patients exhibited reduced glomerular filtration rates (5). Cisplatin is metabolized primarily by the kidneys. Although renal cells exhibit a low rate of division, they are sensitive to toxic injury owing to their high blood flow and their ability to concentrate toxins in the medullary interstitium and tubule epithelium (6). Little information is known about the underlying mechanism of hepatotoxicity induced by CP , although reportedly CP may interfere with tissue antioxidant defense system and generates highly reactive oxygen species (ROS). Therefore CP can cause oxidative damage to the liver (7). Recently attention has been given to the possible protective roles of dietary antioxidant against cisplatin nephrotoxicity and hepatotoxicity, of these propolis, daidzein, grape pomace extract have been evaluated (8,9,10). Propolis is a glue material, collected by honeybees from buds and exudates of plants (11). It contains more than 300 component, among them flavonoids and phenolic acid and their esters (12). Many in vivo and in vitro researches showed that propolis has several biological actions, such as scavenging of free radicals , antioxidant , antitumor , and immunomodulatory effects (13,14). This study aimed to assess the possible antioxidant ameliorated effects of propolis at 2 dose levels opposing the hepatotoxicity of cisplatin at both biochemical and histological levels.

MATERIALS AND METHODS

A total of 60 adult male albino rats weighing 230±40 g ,obtained from the animal house in the College of

Veterinary Medicine-University of Mosul, randomly divided into 6 groups each of 10 rats. The animals were housed in metallic cages and subjected to an adaptation period of two weeks ,photoperiod of (12 h:12h light/dark), 25º C± 2 º C temperature and 45-50% humidity , receiving normal amount of water and food. Ethical approval reference number :UOM/COM/MREC/20-21(21).

Group A: received 4 mg/kg cisplatin I.P by the end of the 2nd week for 3 doses one week apart.

Group B: received propolis 120 mg daily orally for 6 weeks.

Group C: Received propolis 120 mg daily orally for 6 weeks and by the end of the 2nd week starting cisplatin I.P 4 mg/kg for 3 doses one week apart between a dose and the other.

Group D: received propolis 60 mg daily orally for 6 weeks.

Group E: received propolis 60 mg daily orally for 6 weeks and by the end of the 2nd week starting cisplatin 4 mg I.P for 3 doses one week apart between a dose and the other.

Group F: Control group receiving normal saline orally.

At the start of the experiment and by the end of the 6th week , a blood samples were taken from all the animals under study, for assessing

a. Liver function tests including ALT, AST, ALP, serum albumin, total serum bilirubin, direct and indirect.

b. Oxidative stress parameters (MDA, TAS).

By the end of the end of the experiment duration animals were weighed, anesthetized and scarified, livers were weighed and preserved and later examined microscopically with H&E stain.

Statistical Analysis of the data: The results were expressed as mean± SD; Mann-Whitney test was used to compare of pre and post administration results within the group. Improvement (change) rate was calculated as

Improvement rate = result of pre-administration – result of after administration/ result of pre-administration x 100.

One-way ANOVA test with Tukey's Pair wise was used to compare results between different groups. The Statistical Minitab version 18 software program was used to perform statistical analysis of the data.

RESULTS

A/ Biochemical results

1. Liver function tests

a. Group A. By comparison of pre and post administration of cisplatin, there was a significant increase in the serum levels of SGOT,SGPT, ALP and serum albumin, with insignificant differences in total, direct and indirect bilirubin levels. Table1.
b. Group B. By comparison of pre and post administration of 120 mg daily propolis, there was a significant reduction in SGOT, and ALP, with insignificant effects on the other parameters of liver function tests. Table 2.
c. Group C. By comparison of pre and post administration , there was a significant increase in the serum levels of SGOT, SGPT, with a significant reduction in ALP and serum albumin, with insignificant effects on the other parameters of liver function. Table 3.
d. Group D. On comparison between results of pre and post administration, there was insignificant effects on all the parameters of liver function tests . Table 4.
e. Group E. There was a significant increase in SGOT and a significant decrease in serum albumin, with insignificant effects, on the other parameters of liver function tests, on comparison between pre and post administration results. Table 5.
f. Group F. represent the control group, there was a non-significant differences on comparing results of pre and post-administration of placebo. Table 6.

Table 7 shows a comparison between the 6 groups with regard parameters of liver function tests. There was a significant differences with regard serum levels of SGOT, SGPT and serum albumin levels with insignificant differences between values of total serum bilirubin , direct and indirect.

2. Oxidative stress parameters (MDA, TAS)

a. Group A. There was a significant increase in serum MDA levels , with a significant decrease in TAS levels on comparison of pre- and post-administration results. Table 8.
b. Group B. There was a significant reduction in serum MDA levels with a significant increase in TAS levels on comparison of pre- and post-administration results. Table 9.
c. Group C. There was a significant increase in serum MDA levels with a non-significant effects on TAS levels on comparison of pre- and post-administration results. Table 10.
d. Group D. Group C. There was a significant increase in serum MDA levels with a non-significant increase in TAS levels on comparison of pre- and post-administration results. Table 11.
e. Group E. There was a significant increase in serum MDA levels , with a significant decrease in TAS levels on comparison of pre- and post-administration results. Table 12.
f. Group F. No significant differences between results of pre- and post- administration of placebo. Table 13.

Table 14 shows, comparison between all the 6 groups with regard MDA and TAS levels.

special notice in that there was a significant different between group A and Group F with regard and MDA levels.

B/ Observational

1. Observational before dissection

a. Group A. Two of the members of this group died 3 days after the 3rd I.P dose of cisplatin both showed yellowish disclouration of the whole body(jaundice) , the other members showed clear weight loss ,with decrease in physical activity and food intake.
b. Group B. No death was reported in this group, members of this group looks fully active, with good food intake and obvious weight gain.
c. Group C. No death was reported in this group, with mild decrease in physical activity and food intake and mild weight loss.
d. Group D. No death was reported in this group, physically active with good food intake.
e. Group E. one death was recorded in this group ,with mild reduction in physical activity and food intake, with obvious weight loss.
f. Group F. No losses was reported in this group, all members active physically with good food intake.

Table 15 (a) shows comparison between the 6 groups with regard weight changes before and after the 6 weeks intervention.

2. Observational after dissection

Table 15 (b) showed insignificant differences with regard liver/body weight ratio.

C. **Histological picture excessive +++; moderate ++;mild +**

a. Group A. There was excessive changes in hepatic portal pattern,with moderate hepatic sinusoidal dilatation, vacuolar degeneration with cell swelling, pyknosis of nuclei, cell necrosis, apoptosis with infiltration of inflammatory cells, excessive congestion, hyperplasia of bile duct, with mild degree of hemorrhage, hypertrophy of hepatocytes and fibrosis. Figure 1
b. Group B. There was a mild degree of changes in hepatic portal pattern, apoptosis, inflammatory cell infiltration with congestion Figure 2.
c. Group C. There was a moderate changes in hepatic portal pattern, with a mild degree hepatic sinusoidal dilatation, vacuolar degeneration with cell swelling, pyknosis of nuclei, cell necrosis, apoptosis with infiltration of inflammatory cells, congestion, hyperplasia of bile duct, with mild degree of hemorrhage, and hyperplasia of bile ducts. Figure 3.
d. Group D. There was a mild degree of changes in hepatic portal pattern, with pyknosis of nuclei, necrosis and apoptosis of cells, with inflammatory cell infiltration with mild congestion and hypertrophy of hepatocytes Figure 4.
e. Group E. There was a moderate changes in hepatic portal pattern, infiltration of inflammatory cells, congestion, hyperplasia of bile duct, with mild degree of dilatation of hepatic sinusoidal dilatation, pyknosis of nuclei and apoptosis of cells with hypertrophy of hepatocytes and fibrosis. Figure 5.
f. Group F. Normal histological picture of the liver Figure 6.

Table 16 shows shows the significantly of kupffer cells infiltration and the diameter of sinusoids (Mml).there was a significant different between Group A group C in comparison with the other groups under the study.

Regarding sinusoidal diameter changes, also there is a significant difference between group A and group C in comparison with other group.

DISCUSSION

1. **Effects on body weight:** The reduction in body weight observed in this study in the cisplatin group , could be correlated with the reduced food intake noticed during the period of the experiment. Weight gain have been noticed in group C by adding propolis at 120 mg/d, but not at 60 mg/d, this is in agreement with the study conducted by El-Naggar et al., (8). They reported that propolis treatment after cyclophosphamide injection could protect partially the body from weight loss.

This is in agreement with study conducted by Denli *et al*, (15), whom reported that the addition of propolis in the diet significantly increase the growth parameter of quail chicks such as body weight gain and feed consumption and improvement feed efficacy compared with controls and they suggested that it could be due to antimicrobial activity of the propolis extract that resulted in improvement of intestinal hygiene that lead to improved digestion and absorption, beside that it has been suggested that bee propolis contain protein, amino acids, vitamins, and flavonoids, for this resinous it has been used by some people as a nutritional supplement (16).

2. **Effects on liver:** Hepatotoxicity induced by cisplatin is recognized by alteration of biochemical and histological picture (17, 18). The biochemical changes can be recognized by certain liver enzymes and since the enzyme activities of the liver is about 1000 times more than that of the sera, so if only 1% of hepatocytes damaged, the enzyme activities in the sera will be doubled . As SGPT mainly exists in liver cell cytoplasm and mitochondria , so it is regarded as one of the most sensitive parameters of liver function tests recommended by the WHO (19). In this study , there was a significant increase in serum levels of SGOT, SGPT and ALP with a significant reduction in serum albumin. The enzyme SGOT has 2 isoenzymes (ASTs; ASTm). In normal condition, AST exists mainly as ASTs and when necrosis occur, ASTm is released from hepatic mitochondria and its level in the sera increase , so ALT and AST can be regarded as the marker key indexes measuring the liver cell injury (20). Our results was in agreement with many research wokers (17,18,19). All reported that the activities of ALT; AST; and ALP increased by cisplatin as compared to controls.

In this study , by adding propolis at 120 mg/d , in group C, the levels of SGOT and SGPT although remain still high but not to the same level reached on giving cisplatin alone. Propolis as an antioxidant ,was evaluated therapeutically by Badr, (21) , as a water extract on methotrexate induced liver toxicity in mice. He concluded that the administration of propolis was associated with a hepatoprotective effects against degenerative effects of methotrexate. Santos and Cruz, (22), showed that the antioxidant properties of propolis could decrease the adverse effects caused by chemotherapeutic agents without adversely affecting the therapeutic effects.

The present study showed many histological evidence of hepatotoxicity, since the liver is known to accumulate significant amount of cisplatin , second only to the kidney (23), thus hepatotoxicity could be predicted during cisplatin therapy (24). Apoptosis is a common feature of hepatotoxicity induced by many drugs and chemicals, it may precede necrosis or occur at the same time with necrosis . Cisplatin is thought to kill cells mainly by forming DNA adducts causing G2 arrest in the cell cycle, trigging apoptosis (25). It might induce apoptosis by oxidative stress, by reactive oxygen species or depletion of intracellular antioxidant (26). Our histological finding were in agreement with the reports of many research workers (24,27,28). In this study, giving propolis especially at a dose of 120 mg/d ameliorated the effects of cisplatin on the liver, this goes with the findings of Kaya et al., (11), as they reported that propolis protect against the furan induced hepatoxicity and oxidative stress in rats.Also in lines with the study conducted by Tanvir et al (29) .They reported that the phenolic compounds identified in propolis were effective against tetracycline-induced hepatic and renal toxicity .Many reseach workers used antioxidant as vitamin

C , daidzein as an attempt to ameliorate the hepatotoxic effects of cisplatin(30,31). Eman.,, concluded that aqueous extract of propolis could reduce the damage and toxicity effects on liver cells induced by octylphenol owing to its antioxidant properties. (32)

El-Menyiy et al (33) evaluated the hepato-renal protective activities of propolis in paracetamol toxicity in rats and concluded that propolis significantly prevents, renal, hepatic toxicity caused by paracetamol overdose and that the mode of action could be related to its anti-inflammatory and antioxidant effect . In the same line propolis significantly ameliorated the histological picture induced by cyclophosphamide on the liver and kidney(8). The hepatoprotective effect of propolis could be derived from its inhibitory effects on lipid peroxidation mechanism, its inhibition of free radicals or its scavenging effect on free radicals. All this could be related to caffeic acid phenethyl ester (CAPE) as an active component of propolis. Many studies reported that CAPE is a potent agent in preventing oxidative stress in the liver for various reasons (34,35). Other possible mechanisms for the hepatoprotective effects of propolis might include inhibitory effects on the synthesis of reactive metabolites by cytochrome p450 pathway and enhanced activities of conjugation enzymes associated with detoxification (36,37).

3- Effect on oxidative stress, our study revealed that the use of cisplatin was associated with oxidative stress as reflected by a significant increase in MDA and a significant reduction in TAS, while the use of propolis was associated with a significant reduction in MDA with a significant increase in TAS levels and that preceding cisplatin by propolis , although still causing a significant increase in MDA but does not reach the high levels reached with cisplatin alone and TAS levels increased at both dose levels of propolis ,60 and 120 mg/d.This is in agreement with the study conducted by Singla et al (33),whom concluded that propolis has strong antioxidant and protective effects against doxorubicin-induced toxicity in rats as reflected by improved serum biochemical parameters and restoration of antioxidant oxidative status.

CONCLUSION

Giving propolis with cisplatin ameliorating the hepatotoxicity induced by cisplatin alone both at functional and histological picture levels.

Table (1): Effect of cisplatin 4 mg /kg[group A] on the LFT of sampled rats.

Parameters	Before Mean ± SD	After Mean ± SD	% Improvement rate	P-value*
SGPT (U/L)	32.88 ± 10.34	53.38 ± 10.61	- 62.4 %	0.002
SGOT (U/L)	140.25 ± 20.15	187.63 ± 16.99	- 33.8 %	0.003
ALP (U/L)	194.9 ± 78.7	477.4 ± 129.1	- 144.9 %	0.000
S. Albumin (g/dl)	4.073 ± 0.158	2.23 ± 0.397	45.3 %	0.001
TSB (mg/dl)	0.687 ± 0.083	0.702 ± 0.076	- 2.2 %	0.554
Direct	0.442 ± 0.095	0.437 ± 0.052	1.1 %	0.978
Indirect	0.245 ±0.071	0.240 ± 0.091	2.0 %	0.674

* Mann-Whitney test was used. % Improvement rate = [(before – after) / Before] × 100.

Table (2): Effect of propolis 120 mg [group B] on the LFT of sampled rats.

Parameters	Before Mean ± SD	After Mean ± SD	% Improvement rate	P-value*
SGPT (U/L)	47.69 ± 5.97	40.19 ± 4.47	15.7 %	0.031
SGOT (U/L)	174.63 ± 24.68	160.13 ± 22.68	8.3 %	0.206
ALP (U/L)	398.0 ± 72.2	324.5 ± 76.5	18.5 %	0.003
S. Albumin (g/dl)	4.075 ± 0.128	4.119 ± 0.189	- 1.1 %	0.406
TSB (mg/dl)	0.811 ± 0.215	0.727 ± 0.103	10.4 %	0.386
Direct	0.487 ± 0.188	0.430 ± 0.073	11.7 %	0.413
Indirect	0.324 ± 0.172	0.297 ± 0.080	8.3 %	0.7564

* Mann-Whitney test was used. % Improvement rate = [(before – after) / Before] × 100.

Table (3): Effect of propolis 120 mg with cisplatin 4 mg [group C] on the LFT of sampled rats.

Parameters	Before Mean ± SD	After Mean ± SD	% Improvement rate	P-value*
SGPT (U/L)	27.75 ± 5.80	35.50 ± 5.50	- 27.9 %	0.031
SGOT (U/L)	101.50 ± 14.57	129.38 ± 13.51	- 27.5 %	0.003
ALP (U/L)	200.1 ± 36.9	122.8± 35.8	38.6 %	0.004
S. Albumin (g/dl)	4.225 ± 0.2659	3.887 ± 0.113	8.0 %	0.014
TSB (mg/dl)	0.787 ± 0.236	0.812 ± 0.155	- 3.2 %	0.789
Direct	0.487 ± 0.146	0.462 ± 0.168	5.1 %	0.667
Indirect	0.300 ± 0.107	0.350 ± 0.093	- 16.7 %	0.318

* Mann-Whitney test was used. % Improvement rate = [(before – after) / Before] × 100.

Table (4): Effect of propolis 60 mg [group D] on the LFT of sampled rats.

Parameters	Before Mean ± SD	After Mean ± SD	% Improvement rate	P-value*
SGPT (U/L)	33.25 ± 6.34	33.00 ± 5.76	0.8 %	0.988
SGOT (U/L)	146.1 ± 33.6	116.88 ± 14.17	20.0 %	0.052
ALP (U/L)	263.0 ± 71.4	221.9 ± 75.6	15.6 %	0.318
S. Albumin (g/dl)	4.112 ± 0.155	4.111 ± 0.114	0.0 %	0.996
TSB (mg/dl)	0.862 ± 0.177	0.900 ± 0.169	- 4.4 %	0.753
Direct	0.500 ± 0.151	0.550 ± 0.141	- 10.0 %	0.483
Indirect	0.363 ± 0.092	0.375 ± 0.089	- 3.3 %	0.956

* Mann-Whitney test was used. % Improvement rate = [(before – after) / Before] × 100

Table (5): Effect of propolis 60 mg with cisplatin 4 mg [group E] on the LFT of sampled rats.

Parameters	Before Mean ± SD	After Mean ± SD	% Improvement rate	P-value*
SGPT (U/L)	28.13 ± 7.10	30.63 ± 8.23	- 8.9 %	0.527
SGOT (U/L)	104.00 ± 11.56	139.88 ± 19.84	- 34.5 %	0.002
ALP (U/L)	234.9 ± 85.0	175.1 ± 64.5	25.5 %	0.189
S. Albumin (g/dl)	4.037 ± 0.092	3.712± 0.210	8.1 %	0.004
TSB (mg/dl)	0.812 ± 0.173	0.812 ± 0.203	0.0 %	0.914
Direct	0.512 ± 0.196	0.500 ± 0.160	2.3 %	0.989
Indirect	0.300 ± 0.093	0.312 ± 0.113	- 4.0 %	0.912

* Mann-Whitney test was used. % Improvement rate = [(before – after) / Before] × 100.

Table (6): Comparison in LFT after 6 weeks in control group.

Parameters	Beginning Mean ± SD	After 4 weeks Mean ± SD	% Improvement rate	P-value*
SGPT (U/L)	22.25 ± 2.816	25.38 ± 4.21	- 14.1 %	0.128
SGOT (U/L)	129.38 ±15.01	137.88 ± 9.99	- 6.6 %	0.115
ALP (U/L)	192.9 ±35.9	209.3 ± 47.8	- 8.5 %	0.343
S. Albumin (g/dl)	4.162 ± 0.141	4.112 ± 0.164	1.2 %	0.529
TSB (mg/dl)	0.800 ± 0.177	0.800 ± 0.233	0.0 %	0.998
Direct	0.512 ± 0.113	0.412 ± 0.146	19.5 %	0.093
Indirect	0.287 ± 0.099	0.287 ± 0.125	0.0 %	0.953

* Mann-Whitney test was used. % Improvement rate = [(before – after) / Before] × 100.

Table (7): Comparison in LFT among the six groups after 6 weeks of intervention.

LFT	Groups						P-value*
	A Mean ± SD	B Mean ± SD	C Mean ± SD	D Mean ± SD	E Mean ± SD	Control Mean ± SD	
SGPT (U/L)	53.38 ± 10.61 ^{A}	40.19 ± 4.47 ^{B}	35.50 ± 5.50 ^{BC}	33.00 ± 5.76 ^{BC}	30.63 ± 8.23 ^{BC}	25.38 ± 4.21 ^{C}	0.000
SGOT (U/L)	187.63 ± 16.99 ^{A}	160.13 ± 22.68 ^{B}	129.38 ± 13.51 ^{C}	116.88 ± 14.17 ^{C}	139.88 ± 19.84 ^{BC}	137.88 ± 9.99 ^{BC}	0.000
ALP (U/L)	477.4 ± 129.1 ^{A}	324.5 ± 76.5 ^{B}	122.8 ± 35.8 ^{C}	221.9 ± 75.6 ^{BC}	175.1 ± 64.5 ^{C}	209.3 ± 47.8 ^{BC}	0.000
S. Albumin (g/dl)	2.23 ± 0.397 ^{C}	4.119 ± 0.189 ^{A}	3.887 ± 0.113 ^{AB}	4.111 ± 0.114 ^{A}	3.712 ± 0.210 ^{B}	4.112 ± 0.164 ^{A}	0.000
TSB (mg/dl)	0.702 ± 0.076 ^{A}	0.727 ± 0.103 ^{A}	0.812 ± 0.155 ^{A}	0.900 ± 0.169 ^{A}	0.812 ± 0.203 ^{A}	0.800 ± 0.233 ^{A}	0.229
Direct	0.437 ± 0.052 ^{A}	0.430 ± 0.073 ^{A}	0.462 ± 0.168 ^{A}	0.550 ± 0.141 ^{A}	0.500 ± 0.160 ^{A}	0.412 ± 0.146 ^{A}	0.315
Indirect	0.240 ± 0.091 ^{A}	0.297 ± 0.080 ^{A}	0.350 ± 0.093 ^{A}	0.375 ± 0.089 ^{A}	0.312 ± 0.113 ^{A}	0.287 ± 0.125 ^{A}	0.123

* One-way ANOVA-test with Tukey's Pair wise comparisons. Means that do not share a letter are significantly different.

Table (8): Effect of cisplatin 4 mg [group A] on the oxidative stress parameters TAS and MDA of sampled rats.

Parameters	Before Mean ± SD	After Mean ± SD	% Improvement rate	P-value
Total antioxidant (mM)	0.155 ± 0.033	0.094 ± 0.052	39.4 %	0.024
S. MDA (µmol\L)	14.39 ± 2.11	27.46 ± 1.43	- 90.8 %	0.000

* Mann-Whitney test was used. % Improvement rate = [(before – after) / Before] × 100

Table (9): Effect of propolis 120 mg [group B] on the oxidative stress parameters TAS and MDA of sampled rats.

Parameters	Before Mean ± SD	After Mean ± SD	% Improvement rate	P-value*
Total antioxidant (mM)	0.154 ± 0.051	0.248 ± 0.044	- 61.0 %	0.001
S. MDA (µmol\L)	13.01 ± 1.58	19.97 ± 1.36	- 53.5 %	0.000

* Mann-Whitney test was used. % Improvement rate = [(before – after) / Before] × 100

Table (10): Effect of propolis 120 mg with cisplatin 4 mg/kg [group C] on the oxidative stress parameters TAS and MDA of sampled rats.

Parameters	Before Mean ± SD	After Mean ± SD	% Improvement rate	P-value*
Total antioxidant (mM)	0.199 ± 0.026	0.206 ± 0.019	- 3.5 %	0.597
S. MDA (µmol\L)	14.36 ± 1.74	23.44 ± 2.91	- 63.2 %	0.000

* Mann-Whitney test was used. % Improvement rate = [(before – after) / Before] × 100

Table (11): Effect of propolis 60 mg [group D] on the oxidative stress parameters TAS and MDA of sampled rats.

Parameters	Before Mean ± SD	After Mean ± SD	% Improvement rate	P-value*
Total antioxidant (mM)	0.132 ± 0.036	0.160 ± 0.020	- 21.2 %	0.092
S. MDA (µmol\L)	13.51 ± 1.44	22.23 ± 2.54	- 64.5 %	0.000

* Mann-Whitney test was used. % Improvement rate = [(before – after) / Before] × 100

Table (12): Effect of propolis 60 mg with cisplatin 4 mg/kg [group E] on the oxidative stress parameters TAS and MDA of sampled rats.

Parameters	Before Mean ± SD	After Mean ± SD	% Improvement rate	P-value*
Total antioxidant (mM)	0.172 ± 0.041	0.130 ± 0.026	24.4 %	0.018
S. MDA (µmol\L)	14.20 ± 1.69	25.76 ± 1.90	- 81.4 %	0.000

* Mann-Whitney test was used. % Improvement rate = [(before – after) / Before] × 100

Table (13): Comparison in oxidative stress parameters TAS and MDA after 6 weeks in control group.

Parameters	Beginning Mean ± SD	After 4 weeks Mean ± SD	% Improvement rate	P-value*
Total antioxidant (mM)	0.139 ± 0.029	0.131 ± 0.026	5.8 %	0.562
S. MDA (µmol\L)	13.83 ± 1.20	13.72 ± 1.21	0.8 %	0.658

* Mann-Whitney test was used. % Improvement rate = [(before – after) / Before] × 100

Table (14): Comparison in oxidative stress parameters TAS and MDA among the six groups after 6 weeks of intervention.

Items	Groups						P-value*
	A Mean ± SD	B Mean ± SD	C Mean ± SD	D Mean ± SD	E Mean ± SD	Control Mean ± SD	
TAO (mM)	0.094 ± 0.052 ^{D}	0.248 ± 0.044 ^{A}	0.206 ± 0.019 ^{AB}	0.160 ± 0.020 ^{BC}	0.130 ± 0.026 ^{CD}	0.131 ± 0.026 ^{CD}	0.000
S. MDA (µmol\L)	27.46 ± 1.43 ^{A}	19.97 ± 1.36 ^{D}	23.44 ± 2.91 ^{BC}	22.23 ± 2.54 ^{CD}	25.76 ± 1.90 ^{AB}	13.72 ± 1.21 ^{E}	0.000

Table (15)a: Comparison in rats' weights before and after 6 weeks of intervention among the six groups.

Rats weight	Groups					
	A Mean ± SD	B Mean ± SD	C Mean ± SD	D Mean ± SD	E Mean ± SD	Control Mean ± SD
Before	210.8 ± 7.73	190.6 ± 7.25	205.0 ± 11.9	233.4 ± 20.46	244.4 ± 23.30	204.6 ± 17.29
After	157.9 ± 17.47	241.2 ± 13.32	218.2 ± 20.40	250.9 ± 27.3	205.3 ± 28.88	217.0 ± 16.73
% Improvement rate	25.1 %	- 26.6 %	- 6.5 %	- 7.5 %	16.0 %	- 6.1 %
P-value*	0.000	0.000	0.200	0.122	0.008	0.132

* Mann-Whitney test was used. % Improvement rate = [(before – after) / Before] × 100

Table (15)b: Comparison in rate weight and rat`s liver weight among the six groups after 6 weeks of intervention.

Weights	Groups						P-value*
	A Mean ± SD	B Mean ± SD	C Mean ± SD	D Mean ± SD	E Mean ± SD	Control Mean ± SD	
Rate (g)	157.9 ± 17.47 ^{D}	241.2 ± 13.32 ^{AB}	218.2 ± 20.40 ^{BC}	250.9 ± 27.3 ^{A}	205.3 ± 28.88 ^{C}	217.0 ± 16.73 ^{BC}	0.000
Liver (g)	5.17 ± 1.13 ^{C}	8.21 ± 0.62 ^{A}	6.29 ± 0.71 ^{BC}	8.50 ± 0.66 ^{A}	6.11 ± 1.17 ^{C}	7.43 ± 0.61 ^{AB}	0.000
Liver\body weight ratio	32.90 ± 4.92 ^{A}	33.71 ± 2.95 ^{A}	33.50 ± 2.66 ^{A}	34.06 ± 2.80 ^{A}	29.49 ± 5.45 ^{A}	34.50 ± 1.85 ^{A}	0.070

Table 16: shows shows the significantly of kupffer cells infiltration and the diameter of sinusoids (Mml) in the 6 different groups.

group / parameter	Control Group F	Group A Cisplatin 4mg	Group B Propolis 120mg	Group C Propolis 120+ cisplatin 4mg	Group D Propolis 60mg	Group E Propolis 60mg+ cisplatin 4mg
No. of kupffer cells /46000 µm² / FIELD 40X	17.43±0.7 a	13.28±1.3 b	20.57±0.76 a	16.57±0.5 b	20±1.4 a	17.57±1.1 a
Diameter of sinusoids /µm	5.47±0.54 a	7.54±0.77 b	5.6±0.60 c	5.92±0.9 c	5.9±0.41 c	6.68±0.58 c

The similar letters in rows means with no significant at ($p \leq 0.05$).
The different letters in rows means with significant difference at ($p \leq <0.05$).

GROUP (A) Cisplatin 4mg

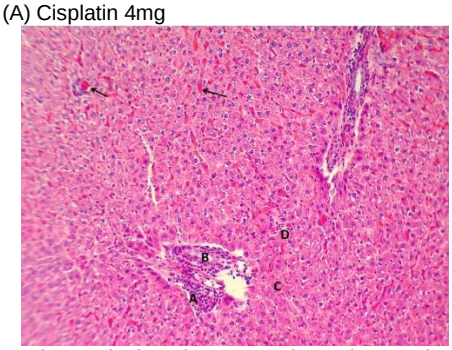

Fig. 1: photomicrograph Liver shows Sever hemorrhage and congestion of sinusoids (↘), hepatic portal pattern represented by infiltration of inflammatory cells in portal area (A), hyperplasia of bile ductule (B), necrosis of hepatocytes (C) and apoptosis of hepatocytes (D). H&E stain. 100X. H&E stain. 100X.

GROUP B/ Propolis 120mg

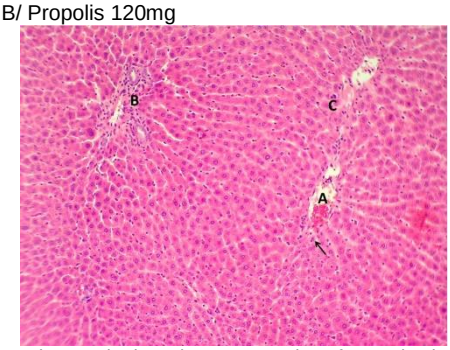

Fig. 2: photomicrograph Liver shows congestion of central vein (A), hepatic portal pattern represented by infiltration of inflammatory cells (B), increase number of kuppfer cells (↘) and apoptosis(C). H&E stain. 100X.

GROUP (C) Prplopolis 120 mg & cisplatin 4 mg

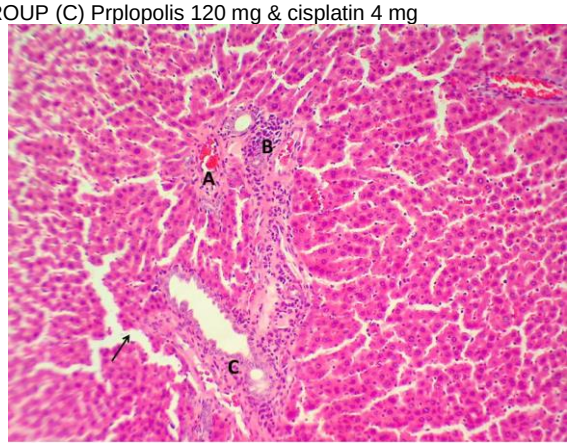

Fig. 3: photomicrograph liver shows hepatic portal pattern represented by congested B.V. (A), infiltration of inflammatory cells around portal area (B), hyperplasia of bile ductule (C) and dilation of hepatic sinusoids (↘). H&E stain. 100X. H&E stain. 100X.

GROUP (D) propolis 60

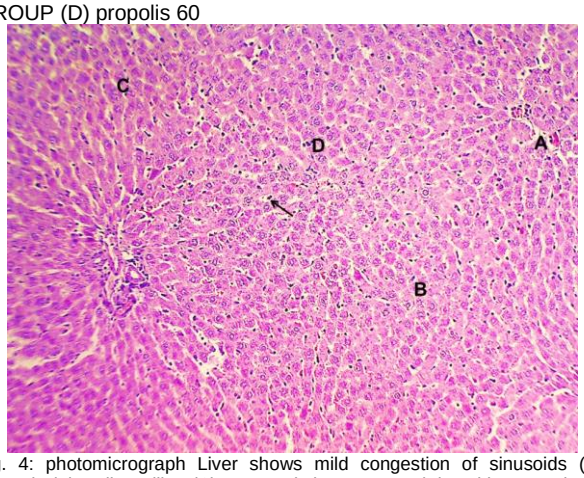

Fig. 4: photomicrograph Liver shows mild congestion of sinusoids (A), necrosis (B), cell swelling (C), apoptosis hepatocytes (D) and increase in the NO. of kuppfer cells(↘). H&E stain. 100X. H&E stain. 100X.

GROUP (E) Propolis 60 mg + cisplatin 4 mg

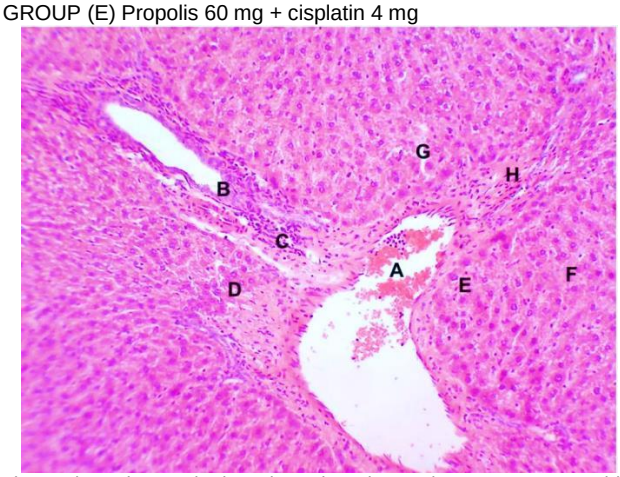

Fig. 5: photomicrograph Liver shows hepatic portal pattern represented by congestion of portal venule (A), bile ductule hyperplasia (B) and infiltration of inflammatory cells (C), also there are heprtrophy of hepatocytes (D), degeneration (cell swelling) (E), necrosis of hepatocytes (F), hepatic sinusoids dilation (G) and mild fibrosis (H). H&E stain. 100X. H&E stain. 100X.

CONTROL - Group (F)

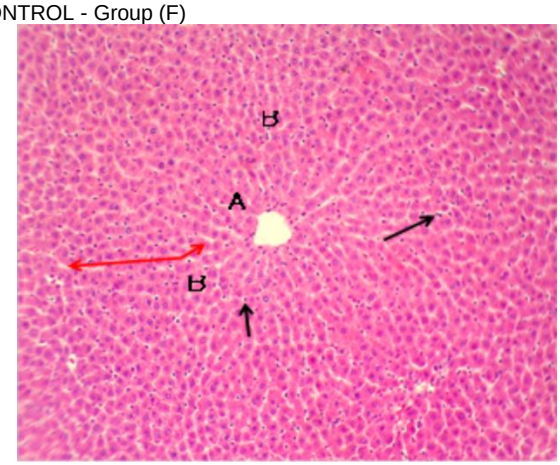

Fig.6: Histological section of liver of control group showing the normal architecture of liver, central vein(A), hepatocytes (B), sinusoids(↙) (red arrow) and kupffer cells (↘)(black arrow). H &E stain, 100X.

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