Studies on *Curcuma longa, Chicorium intybus* and *Petroselinum sativum* Water Extracts against Cisplatin-Induced Toxicity in Rats

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ABSTRAC: This study aimed to investigate the prophylactic effect of three water extracts; *Curcuma longa* (CL), *Chicorium intybus* (CI), and *Petroselinum sativum* (PS) alone or in combination with silymarin (SN) as standard antioxidant compound on cisplatin induced acute toxicity. Group 3, 4 and 5 received a daily dose of each extract (250mg/kg b.w.) by oral administration. Groups 7, 8 and 9 were received each extract by the same dose and SN by the dose of (70mg/kg b.w.). All groups except control (treated with tween 80, p.o.) received a single dose of cisplatin (CPN) by dose of (7.5 mg/kg b.w., i.p.) on the fifth day. After 7 days, all animals were decapitated; blood was collected and analyzed for the biochemical parameters; GSH content, total antioxidant capacity (TAC), total protein, ALT, AST and MDA levels. SOD, CAT, MDA, TP and DNA concentrations were determined in liver and kidney. Also, histophathological examinations were done in liver. The results revealed that the body and organs weight affected by CPN and plant extracts. The level of MDA, AST and ALT were increased by CPN and it appeared to be improved by the plant extract either alone or in combination with SN. Also, total protein level, the total antioxidant capacity and GSH contents appeared to be improved by the plant extracts against CPN-induced toxicity. CPN treatment caused a significant decrease in the level of CAT & SOD in rat liver and kidney as compared to control, while the level was increased after plant extract treatments in both liver and kidney by different values. DNA damage was occurred as compared to control after CPN treatment, it was ameliorated after each plant extract treatments alone or in mixture with SN at the present of cisplatin by different percentage values. Histopathological examination exerted a severe damage in the liver under the effect of CPN. The liver morphology was characterized by severe activation of Kupffer cells, degenerated hepatocytes and moderate enlargement of sinusoids. Plant extract administrations exerted an ameliorative effect by decreasing pathological lesions as inflammatory cell aggregates and fibrosis areas. The results support significant antioxidant effect of each plant extract by different values and it may involve prevention of lipid peroxidation and tissue damage in liver and kidney. [Journal of American Science 2010; 6(9):545-558]. (ISSN: 1545-1003).

Keywords: *Curcuma longa, Chicorium intybus*, *Petroselinum sativum,* silymarin, cisplatin, MDA, SOD, CAT, DNA and GSH

INTRODUCTION

Epidemiological studies have shown that consumption of vegetables and fruits protect against a variety of diseases including cancer **(Tiwari, 2001).** Many fruits, vegetables and foods have been reported to have potential anti-mutagenic or anti-carcinogenic effects **(Vijayalaxmi and Venu, 1999)**. Flavonoids are a family of phenolic compounds that have many biological properties, including hepatoprotective, antithrombotic, antibacterial, antiviral and anticancer activity. These physiological benefits thought to be due to their antioxidant and free radical scavenging properties **(Tiwari, 2001 and Hosseinimehr and Karami, 2004).**

The methanolic fraction of *Chicorium intybus* was found to possess a potent anti-hepatotoxic activity comparable to the standard drug silymarin **(Ahmed et al., 2002).** Curcumin is a naturally occurring phenolic compound isolated as a yellow pigment from *Curcuma longa*, it has been reported to possess a variety of biological and pharmacologyical activities including antioxidative and anti-inflammatory effects **(Rukumani et al., 2003; Chainani-Wu, 2003; Ramachandran et al., 2005, and Shi et al., 2006).** *Petroselinum sativum* contains large amounts of flavonoids (apigenin, kaempferol, quercetin, hesperetin, luteolin), polyphenols and tannins, classified as flavones and flavanol class of flavonoids. It is believed to be an excellent source of natural antioxidants, anti-inflammatory stimulant and may be particularly important in protecting cells against free radicals and chronic diseases **(Lugasi and Hovari, 2000**).

Cisplatin (CNP) is used as anti-neoplastic agent for the treatment of metastatic tumors and many other solid tumors (**Sweetman, 2002**). Although higher doses of cisplatin are more efficacious for the suppression of cancer, high dose

therapy manifests irreversible renal dysfunction and other toxicities yet (**Halliwell and Cross, 1994**). There is a suggestion that the drug accumulates in significant amounts in hepatic tissue particularly when injected in high doses **(Liu et al., 1998)**.

This study was undertaken to assess the prophylactic effect of different plant extract as *Curcuma longa* (CL), *Chicorium intybus* (CI), and *Petroselinum sativum* (PS) with or without silmarin on Cisplatin-induced toxicity. Also, we studied the protective effect of these plant extracts on different biochemical parameters as well as the histological study was performed.

MATERIALS & METHODS

Plant materials and Preparation of plant extract

The plant materials of *Curcuma longa*, *Chicorium intybus* and *Petroselinum sativum* were obtained from local market (Giza, Egypt). The plants were identified by Faculty of Pharmacy, Cairo University, Egypt. Dried and ground leaves of PC and CI (about 50g) were extracted by boiled water overnight and the extraction of each plant was filtered, concentrated and freeze dried. The dried rhizome of CL was powdered mechanically and extracted by boiled water for overnight. The process was reported three times, pooled, concentrated under reduced pressure and freeze dried **(Lee et al., 2002, and Feresin et al., 2002).**

Animals

Experiments were performed using male albino rats (200-250 g) supported from the animal house of NODCAR, Egypt. Animal were kept in a room at a constant temperature 22±1°C with 12h light-dark cycles and had free access to diet and tap water.

Chemicals and Drugs

All chemicals were obtained from Sigma Company; Santa Lewis, USA, they obtained in analytical and purified grade, SN, TBARS, GSH were purchased from Merck India Ltd., and cisplatin (CPN) supplied from raw material laboratory, NODCAR, EGYPT.

Experimental designs

The rats were divided into nine groups (n= 8) as the following:

-Group 1 received tween 80 as vehicle as normal control.

-Group 2 received a single dose (7.5mg/kg b. w., i.p) of CPN at the fifth day as positive control **(Dickey et al., 2007)**.

-Group 3, 4 and 5 received the aqueous extract of CL, CI and PS (250mg/kg b.w.) by oral administration for seven days **(Ajith et al., 2007)**.

-Group 6 received a daily dose of SN by 70mg/kg b.w. **(Vengerovskii et al., 2007)** for 7days.

-In Group 7, 8 and 9 received each extract by 250mg/kg b.w. plus SN (70mg/kg b.w.) orally for 7 days.

-Group 3, 4, 5, 7, 8 and 9 were given a single dose of CPN at the fifth day as group 2

Blood were collecting at the initial time (zero), then after 7 days, all animals were sacrificed and decapitated after blood collecting for determination of ALT, AST in serum, TP and MDA in plasma, GSH in whole blood and total antioxidant capacity (TAC). The liver and kidney were kept at -80°C until use for measurement of SOD, CAT, MDA, TP and genomic DNA concentrations. A part of liver was fixed in 10% formaldehyde for histopathological examinations.

Determination of serum transaminases

Two transaminases; Alanine aminotrans-aminase (ALT) and aspartate aminotransaminase (AST) were determined according to **Young (2001).**

Determination of total protein (TP)

Total protein level was determined in plasma, by a reagent kit based on the method of **Gornal et al. (1949),** and in liver and kidney homogenates according to **Lowry et al. (1951).**

Determination of lipid peroxidation concentration (MDA)

Lipid peroxidation formation was determined in plasma and in liver and kidney homogenate as TBARS substances. It was determined according to the method of **Buege and Aust (1978)** with slight modification in the incubation period according to the method of **Deniz et al. (1997)** giving pink color, which has an absorption on 535 nm.

Total antioxidant capacity (TAC) assay in serum

The determination of the antioxidative capacity is performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H_2O_2). The antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual H_2O_2 is determined colorimetrically by an enzymatic reaction which evolves the conversion of 3, 5 dichloro-2-hydroxy benzensulphonate to a colored product **Koracevic and Koracevic (2001)**.

Preparation of liver samples

The remaining part of liver was divided into two portions. The first one was homogenized in 0.1 M Tris-HCl buffer, pH 7.4 using an electric homogenizer to prepare 20 % homogenate samples (1.0g / 5ml) the homogenate was centrifuged at 5000 rpm/10 min. The supernatant was collected **(Orafidiya et al., 2004).** The second portion was

homogenized in lyses buffer and was used for the preparation of DNA concentrations.

Extraction and purification of DNA from rat liver

Isolation of DNA was performed according to the method described by **Sambrook et al. (1989).** Determination of DNA concentrations and purity were applied according to the method of **Wilfinger et al. (1997).**

Determination Superoxide dismutase (SOD) activity

Liver and kidney SOD were estimated according to the method of **Marklund and Marklund (1974)** depends on spontaneous auto-oxidation of pyrogallol in alkaline media, produces superoxide anion radical, which in turn enhances more oxidation. The presence of SOD in the reaction medium inhibits the pyrogallol auto-oxidation by scavenging the superoxide radical.

Determination Catalase (CAT) activity

It was assayed by the method of **Aebi (1984)** based on the decomposition of H_2O_2 followed directly by the decrease in absorbance at 240nm. The difference in absorbance/unit time is a measure of the catalase activity.

Histological examination

After the experimental period animals were decapitated, livers removed immediately, sliced and washed in saline. Liver pieces were preserved in 10% formalin for histophathological studies. The pieces of liver were processed and embedded in paraffin wax. Sections were taken and stained with hematoxylin and eosin and photographed **(Coskun et al., 2000).**

Statistical analysis of the results

All values are mean ± SE obtained from eight animals. For statistical analysis, one way ANOVA with Duncan's variance (SPSS 10) was used to compare groups. In all the cases a difference was considered significant when P was < 0.05.

RESULTS

Effect of water extract of CL, CI, PS and SN on body and liver weights

Treatment of cisplatin (7.5mg/kg b.w., i.p.) for acute toxicity (7 days) caused a significant decrease in the body and liver weight of rats (6% and 18%, respectively). While this effect was attenuated by the treatment with plant extracts **(Table 1)**. Also, PS extract exerted a highly percentage value more than other plant extract by 15.2% and 25.4% for the body and the liver weights, respectively.

Effect of water extract of CL, CI, PS and SN on GSH contents in whole blood

Glutathione reduced levels after cisplatin (CPN) treatment showed a statistically significant decrease by 60.6% as compared to normal rat **Table (2)**. This effect was ameliorated by the plant extracts, the highly percentage values of the GSH level appeared to be for PS by 225.0% and 319.5% either PS alone or PS in the presence of SN, respectively. The lowest effect was to CL then CI extracts.

Effect of water extract of CL, CI, PS and SN on total antioxidant capacity (TAC)

The total antioxidant capacity (TAC) exerted a significant decrease after treatment with CPN. This effect was ameliorated with the plant extracts. As revealed in **Table (2)** the percentage values increased for PS extract and it is appeared to be higher than CI and CL either with CPN or SN by 71.8% and 117.6% for PC 67.4% and 108.8% for CI and 62.1% and 98.2% for CL.

Effect of water extract of CL, CI, PS and SN on lipid peroxidation (MDA) concentration in plasma

As showed in **Table 2** cisplatin caused a marked increase in MDA of blood plasma level as compared to control by 89%, treatment with the different extracts, the effect was reduced by 38.7%; 40.6%, and 42.6% for CL, CI and PS in the presence of CPN and by 45.2%; 46.5%; 45.8%; 44.5% for these extracts with SN in presence of CPN. The effect on MDA level was approved to be closely together around 38-45% inhibitory effect.

Effect of water extract of CL, CI, PS and SN on liver function (AST and ALT) in serum

As indicated from results that cisplatin caused a significant increase in serum ALT and AST (23% and 82.7%) when compared to control group (**Table 3).** Treatment with each plant extract for 7 days markedly improved enzyme activities by decreasing the level of ALT and AST activity. The percentage of reduction for AST and ALT enzyme activity was highly at PS in mixture with SN by 45.1% and 53.7%, then CI plus SN by 36.7% and 49.9%, then CL plus SN by 33.2% and 47.5%, respectively as compared to control group.

Effect of water extract of CL, CI, PS and SN on protein concentration in plasma

A significant decrease in total protein levels (45.7%) was recorded after cisplatin treatment **(Table 3)**, while the plant extract treatments ameliorated this effect. CI extract revealed a highly percentage increase of the total protein by 104.5% and 118.2% either alone or in combination with SN, while the lowest one was for PS extract by 77.3% and 79.5%, respectively.

Effect of water extract of CL, CI, PS and SN on MDA and TP in liver and kidney tissues

As revealed in **Table 4 and 5** that MDA was increased after CPN treatment while total protein was decreased. This effect was improved after administration of each plant extract. The effect of each plant extracts in the presence of SN plus each extract exerted more decrease on MDA and increase on total protein concentrations.

Effect of water extract of CL, CI, PS and SN on SOD and CAT in liver and Kidney tissues

The activities of both SOD and CAT in liver and kidney under the effect of cisplatin alone, CPN plus each plant extract, and CPN plus SN and each plant extract were performed as revealed in **Table 4 and 5**. Liver and kidney SOD and CAT activity were decreased significantly ($P<0.05$) in the CPN group as compared to the control group, while the CAT and SOD activities were increased by treatment with plant extracts as compared to control group. In addition the activity of these enzymes in the mixture of each plant extract with SN exerted more active than the plant extract alone.

Effect of water extract of CL, CI, PS and SN on genomic DNA

Table 6 showed DNA concentrations and purity of rat liver either in CPN induced toxicity or water plant extract in the presence of SN. The purity of DNA should be between (1.8-2.0) at absorbance (A_{260}/A_{280}), this indicated that the nucleic acid was relatively free of protein contamination **(Vidal and Garcia, 2000).** As revealed from the data DNA concentrations were increased in CPN treatment group, while DNA concentrations were decreased after plant extract administrations.

Effect of water extract of CL, CI, PS and SN on histological examination

The microscopically examination of cross section through the liver of untreated rats negative control (G1) revealed a normal appearance in hepatic lobular architecture, with normal vasculature. These changes were cleared in peripheral zone (Fig G1a). On the other hand, blood sinusoid showed obliterated and normal hepatocytes with vesicular nuclei were observed in Fig. G1b. While, the microscopical picture of liver treated by CPN (G2) revealed loss of lobular architecture, portal area, fibrosis, and vacuolated hepatocytes and focal areas of hepatic necrosis and pyknotic nuclei were cleared some areas (Fig. G2a). Also, dilated congested sinusoid, Kupffer's cell were prominent, mild scattered congested, portal and central veins accompanied by thickened, hyalinized in their wall and few fibrous tissues. These vessels showed obliterated by clotted blood (Fig. G2b) accompanied by mild to moderate inflammatory cells aggregated in portal areas and proliferated bile ducts, however, small patch of esinophilic materials were seen in some portal areas (Fig. G2c).

The histopathological changes in treated groups (3, 4 and 5) revealed mild improved as compared to group (G 1and 2), this improvement was more appeared in group (3).

Animals treated by SN plus CPN (G6), revealed moderate changes in form of hepatic cell necrosis (autolysis) with extravagated R.B.C.s and loss in their cell bounders in focal areas (Fig. 6), prominent kupffer cells accompanied by swollen, vacuolated hepatocytes in some portal pyknotic nuclei areas with moderate inflammatory cells aggregates were observed (Fig. 6b). However, animals treated with CL+CPN+SN (G7); CI (G8), and PS (G9), showed mild to moderate improved companied to (G2), the pathological changes in form of moderate to marked dilatation central, portal venous channels and some thickening, hyalinized in their wall with congested sinusoid (G7, 8 and 9). In addition to, proliferated bile ducts together with mild swollen vacuolated hepatocytes were also seen in (G7 fig7); beside to dilate congested blood sinusoid were cleared in areas. Moreover, the majority hepatocytes within normal limit seen (Fig. 8), while focal areas of hepatic necrosis with extravagated R.B.C.s and pyknotic nuclei were accompanied by kupffer cells were cleared in all animals scattered small areas of fatty vacuolated hepatocytes were prominent in G8 (fig. 9).

DISCUSSION

The objectives of the present study were to examine the prophylactic effect of water extracts of *Curcuma longa*, *Chicorium intybus*, and *Petroselinum sativum* against cisplatin-induced hepato- and nephro-toxicity in comparative with silymarin as antioxidant standard. The effect of CPN on body and liver weight was affected by decreasing percentage values; this effect was ameliorative after plant extract treatments. It was found that the body weight loss after CPN treatment is associated with cisplatin nephrotoxicity **(Sekine et al., 2007).**

Our results revealed that under the effect of CPN, lipid peroxidation was increased by MDA concentration enhancement, and GSH was reduced as well as the total antioxidant capacity (TAC) also was altered to the reversible effect by MDA decreased, GSH content and TCA were increased by variable values according to the type of each extracts and the presence of SN in combination with each extract. These results are in agreement with Ahmed (2010).

Table 1. Effect of water plant of *Curcuma longa*, *Chicorium intybus* and *Petroselinum sativum* alone or in combination with silymarin on liver weight/body weight in rat liver

Treatments	Body wt. (g)	Liver wt (g)	Liver wt/body wt
CONTROL	222.0 ± 5.5	7.62 ± 0.28	0.034
CPN	209.0 ± 8.4	6.26 ± 0.60	0.03 **↓ 11.8 %***
CL+CPN	217.3 ± 8.6	6.90± 0.29	0.032 **↓ 5.9 %***
CI + CPN	220.5 ± 4.1	6.95 ± 0.39	0.032 **↓ 5.9 % ***
PS + CPN	225.6 ± 4.30	6.85 ± 0.20	0.030 **↓ 8.8 % ***
SN+CPN	226.8 ± 6.5	6.80 ± 0.22	0.030 **↓11.8 % ***
CL+ CPN+ SN	230.9 ± 3.9	7.50 ± 0.25	0.032 **↓ 5.9 %***
CI +CPN +SN	236.3 ± 3.9	7.80 ± 0.19	0.033 **↓ 2.9 %†**
PS +CPN + SN	240.7 ± 5.02	7.85± 0.41	0.033 **↓ 2.9%†**

Data are expressed as mean± S.E.M. of eight rats per group. Significant different from Control group at $P < 0.05$, † **Insignificant** at $P > 0.05$.

Table 2. Effect of water *Curcuma longa*, *Chicorium intybus* and *Petroselinum sativum* alone or in combination with silymarin on different parameters in rat whole blood and plasma.

Treatments	GSH mg %		TAC mmol/L	MDA nmol/ml	
	Initial	End of Experiment		Initial	End of Experiment
CONTROL	73.4± 0.016	94.0 ± 0.09	3.41 ± 0.001	6.2± 0.010	8.2 ± 0.014
CPN	56.0 ± 0.006	37.0 ± 0.0042 a ↓60.6 %*	2.27 ± 0.004 a ↓33.4%*	6.1 ± 0.017	15.5 ± 0.025 a ↑89.0 %*
CL + CPN	45.0 ± 0.003	62.1 ± 0.021 b 67.8%↑*	3.68 ± 0.0035 b 62.1%*	6.0 ± 0.0027	9.5 ± 0.012 b ↓38.7%*
CI + CPN	45.0 ± 0.028	109.5 ± 0.118 b 195.9%↑*	3.80 ± 0.010 b 67.4%*	5.2 ± 0.023	9.2 ± 0.001 b↓40.6 %*
PS + CPN	51.0 ± 0.015	120.6±0.091 b 225.9% ↑*	3.90± 0.02 b 71.8%*	5.5 ± 0.0062	8.9 ± 0.002 b↓42.6 % *
SN + CPN	37.2 ±0.004	214.0 ± 0.083 b 478.4%↑*	4.98 ± 0.0074 b 119.4%*	4.0 ± 0.011	8.5 ± 0.011 b↓45.2 %*
CL + CPN+ SN	57.0 ± 0.014	161.1 ± 0.037 b 335.4%↑*	4.50 ± 0.0041 b 98.2%*	4.5 ± 0.012	8.3 ± 0.023 b↓ 46.5 %*
CI + CPN +SN	43.0 ± 0.016	126.2 ± 0.030 b 241.1%↑*	4.74 ± 0.013 b 108.8%*	5.6 ± 0.0096	8.4 ± 0.011 b↓45.8%*
PS + CPN + SN	55.0 ± 0.002	155.2 ± 0.077 b 319.5%↑*	4.94 ± 0.001 b 117.6%*	6.5 ± 0.015	8.6 ± 0.0074 b↓44.5 %*

Data are expressed as mean± S.E.M. of eight rats per group. Significant different from control group at $P < 0.05$, a Relative change for CPN group from Normal control. b Relative change for the treatments from CPN group.

Table 3. Effect of *Curcuma longa*, *Chicorium intybus* and *Petroselinum sativum* alone or in combination with silymarin on ALT, AST in serum and total protein TP) in plasma.

Treatments	ALT (U / ml)		AST (U / ml)		TP in plasma (g / dl)	
	Initial	At the end of experiment	Initial	At the end of experiment	Initial	At the end of experiment
CONTROL	40.03±0.007	36.6±0.005	87.3±0.002	78.4±0.002	8.74 ± 0.029	8.1 ± 0.033
CPN	39.1±0.006	46.8±0.0014 a↑28.7%*	82.3±0.0007	153.5±0.004 a↑95.8%*	8.68 ± 0.030	4.4 ± 0.015 a ↓45.7 %*
CL + CPN	61.8±0.008	39.1±0.004 b↓16.5%*	125.8±0.006	94.5±0.004 b↓38.4%*	8.28 ± 0.068	8.0 ± 0.030 b↑81.8 %*
CI + CPN	59.5 ±0.003	40.8±0.002 b↓12.8%*	106.8 ±0.001	96.8±0.006 b↓37.0%*	7.90 ± 0.044	9.0 ± 0.032 b↑104.5 %*
PS + CPN	46.7±0.003	41.9±0.006 b↓10.5%*	107.7±0.004	119.6±0.015 b↓22.1%*	8.04 ± 0.030	7.8 ± 0.051 b↑77.3 %*
SN + CPN	51.7±0.004	41.6±0.002 b↓11.1%*	91.0±0.011	104.0±0.018 b↓32.2%*	9.5 ± 0.052	8.5 ± 0.036 b↑93.2 %*
CL + CPN + SN	53.3±0.002	36.4±0.002 b↓22.2%*	96.6±0.009	107.7±0.020 b↓29.8%*	8.5 ± 0.036	8.2 ± 0.013 b↑86.4 %*
CI + CPN +SN	47.8±0.004	38.2±0.001 b↓18.4%*	93.7±0.005	109.1±0.013 b↓28.9%*	8.8 ±0 .0069	9.6 ± 0.029 b↑118.2 %*
PS + CPN+SN	39.5±0.003	37.5 ±0.003 b↓19.9%*	103.1±0.009	101.2 ±0.016 b↓34.1%*	8.5 ± 0.024	7.9 ± 0.045 b↑79.5 %*

Data are expressed as mean± S.E.M. of eight rats per group. Significant different from control group at $P < 0.05$, a Relative change for CPN group from Normal control. b Relative change for the treatments from CPN group.

Table 4. Effect of *Curcuma longa*, *Chicorium intybus* and *Petroselinum sativum* alone or in combination with silymarin on MDA, TP, SOD and CAT in rat liver

Treatments	MDA nmol/ml	TP mg/ml	SOD U/mg protein	CAT U/mg protein
CONTROL	3.36 ±0.014	80.0 ± 0.027	3.720 ± 0.0033	400.9±0.007
CPN	6.80 ± 0.04 ↑102 %*	35.4 ± 0.011 ↓55.8%*	1.422 ±0.0033 a↓62%*	180.5±0.005 ↓55%*
CL + CPN	3.43 ± 0.036 b↓49.6%*	56.10 ± 0.031 b↑58.5%*	2.088 ± 0.0009 b↑47%*	260.2±0.007 b↑44%*
CI + CPN	3.42 ± 0.049 b↓49.7%*	63.4 ± 0.023 b↑79.1%*	2.0 ± 0.0009 b↑41%*	251.3±0.002 b↑39%*
PS + CPN	4.68 ± 0.033 b↓31.2%*	69.3 ± 0.008 b↑95.8%*	1.988 ± 0.0014 b↑40%*	246.1±0.007 b↑36%*
SN + CPN	2.70 ± 0.013 b↓60.3%*	57.90 ± 0.01 b↑63.6%*	2.220 ± 0.0003 b↑56%*	260.1±0.002 b↑44%*
CL + CPN + SN	2.87 ± 0.021 b↓57.8%*	79.30 ±0.114 b↑124%*	2.420 ± 0.0007 b↑70%*	271.4±0.002 b↑50%*
CI + CPN +SN	2.60 ± 0.023 b↓61.8%*	66.7 ± 0.051 b↑88.4%*	2.388 ± 0.0007 b↑68%*	286.1±0.003 b↑59%*
PS + CPN+SN	2.26 ± 0.015 b↓66.8%*	77.9 ± 0.024 b↑120.1%*	2.440 ± 0.0003 b↑72%*	298.3±0.002 b↑65%*

Data are expressed as mean± S.E.M. of eight rats per group. Significant different from control group at $P < 0.05$, a Relative change for CPN group from Normal control. b Relative change for the treatments from CPN group.

Table 5. Effect of *Curcuma longa*, *Chicorium intybus* and *Petroselinum sativum* alone or in combination with silymarin on MDA, TP, SOD and CAT in rat kidney.

Treatment	MDA nmol/ml	TP mg/ml	SOD U/mg protein	CAT U/mg protein
CONTROL	3.56 ± 0.0146	65.5 ±0.034	3.90±0.002	190.1±0.001
CPN	8.80 ± 0.038 a↑147%*	34.01±0.04 a↓48*	1.75±0.001 a↓55*	96.7±0.096 a↓49%*
CL + CPN	4.35 ± 0.020 b↓50.6%*	60.65±0.02 b↑78.3%*	2.40±0.001 b↑37%*	126.0±0.001 b↑30%*
CI + CPN	3.50 ± 0.021 b↓60.2%*	59.35±0.04 b↑74.5%*	2.29±0.001 b↑31%*	120.8±0.005 b↑25%*
PS + CPN	3.56 ± 0.015 b↓59.6%*	60.20±0.03 b↑77%*	2.23±0.004 b↑27%*	113±0.004 b↑17%*
SN + CPN	4.39 ± 0.018 b↓50.1%*	64.59±0.02 b↑89.9%*	2.52±0.006 b↑44%*	135.6±0.003 b↑40%*
CL + CPN + SN	3.10 ± 0.0046 b↓64.8%*	76.71±0.13 b↑125.6%*	2.71±0.001 b↑55%*	150.4±0.001 b↑56%*
CI + CPN +SN	2.42 ± 0.013 b↓72.5*	60.72±0.02 b↑78.5%*	2.82±0.001 b↑66%*	140.8±0.001 b↑46%*
PS + CPN+SN	2.58 ± 0.013 b↓70.7%*	95.5±0.144 b↑180.8%*	2.79±0.001 b↑59%*	160.4±0.001 b↑65%*

Data are expressed as mean± S.E.M. of eight rats per group. Significant different from control group at $P < 0.05$, a Relative change for CPN group from Normal control. b Relative change for the treatments from CPN group.

Table 6. Effect of *Curcuma longa*, *Chicorium intybus* and *Petroselinum sativum* alone or in combination with silymarin on DNA concentrations of rat liver

Treatments	Purity A_{260}/A_{280}	DNA concentration (µg/ml)	% variation
CONTROL	1.887	535 ± 0.125	-
CPN	1.900	711.7 ± 0.0051	a↑33%*
CL + CPN	2.067	601.7 ± 0.035	b↓16.0%*
CI + CPN	1.996	613.3 ± 0.069	b↓14.0%*
PS + CPN	1.951	701.7 ± 0.026	b↓14.0%*
SN + CPN	2.153	481.7 ± 0.133	b↓32.0%*
CL + CPN + SN	2.117	480.0 ± 0.124	b↓33.0%*
CI + CPN +SN	2.084	426.7 ± 0.145	b↓40.0%*
PS + CPN+SN	2.227	501.7 ± 0.249	b↓30.0%*

Data are expressed as mean± S.E.M. of eight rats per group. Significant different from control group at $P < 0.05$, a Relative change for CPN group from Normal control. b Relative change for the treatments from CPN group.

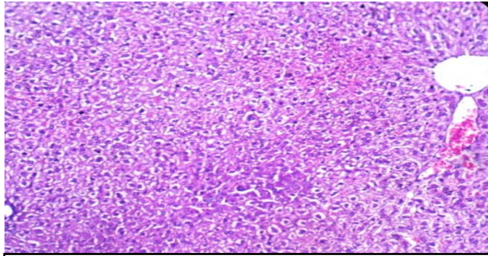

Figure (1a, G_1): Liver section of control (vehicle) showing normal hepatic section Architecture, showing central vein and sinusoid (H & E. x 300)

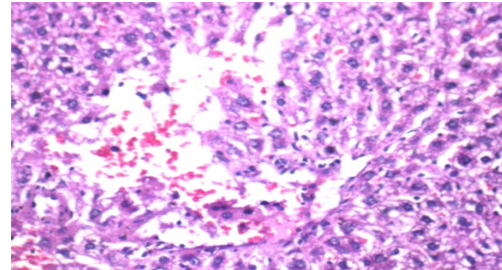

Figure (1b, G1): Photomicrograph of liver control section of control (vehicle), show normal hepatocytes with vesicular nuclei (H & E. x 150).

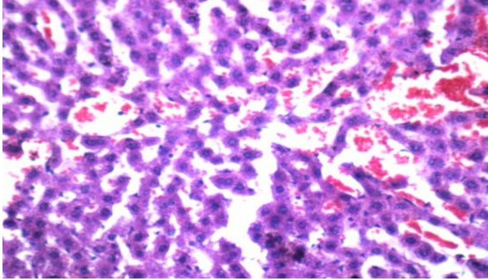

Figure (2a, G2) Liver section of CPN treated rats showing hepatic necrosis with extravagated R.B.C.s in some areas. A mild dilated congested sinusoid (H & E. x 300).

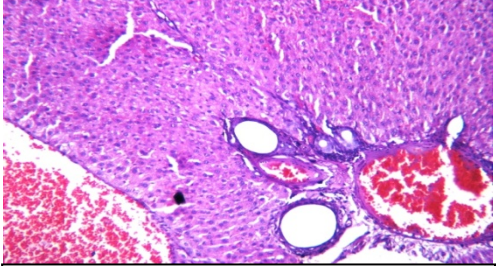

Figure (2b, G2) Liver section of CPN treated rats showing dilated congested blood vessels with thickened hyalinized in their wall and bile ducts proliferated (H & E. x 150).

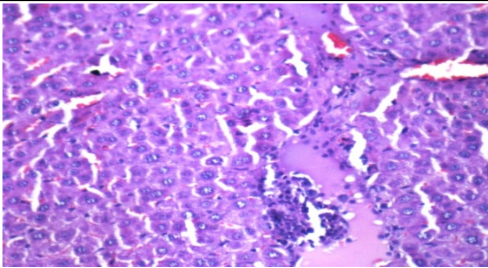

Figure (2c, G2) Liver section of CPN treated rats showing moderate inflammatory cells aggregated in portal areas with esinophilic material was also seen (H & E. x 300).

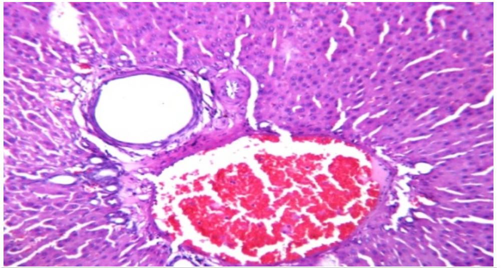

Figure (3a, G3) Liver section of CPN + CL treated rats showing dilated congested blood vessels with thickened hyalinized in their wall, with central vein, portal area and mild fibrous tissues were seen accompanied by degenerative changes in individual number of hepatocytes (H & E. x 150).

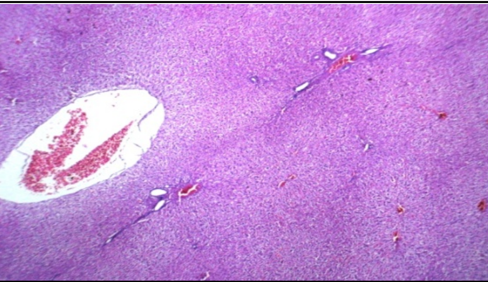

Figure (3b, G3) Liver section of CPN +CL treated rats showing scattered dilated congested blood vessels with ballooning hepatocyte and bile ducts proliferation (H & E. x 60).

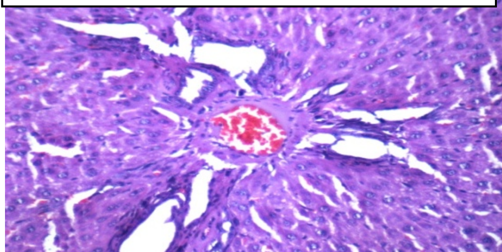

Figure (4a, G_4) Liver section by CNP+CI treated rats revealed thickened hyalinized in their wall, fibrotic area, central vein, and bile ducts proliferation (H & E. x 300).

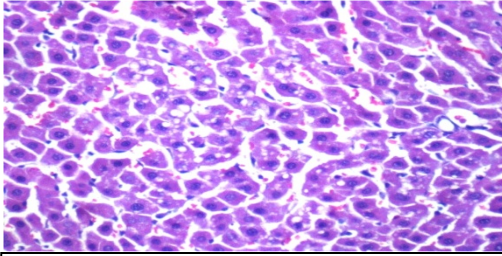

Figure (4b, G_4) Liver section by CPN+CI treated rats revealed showing loss in architecture in some liver tissue fibrotic area, and fatty vacuolated were also seen (H & E. x 300).

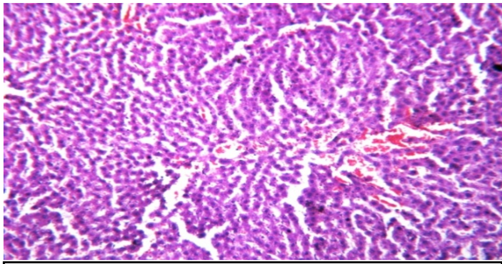

Figure (6a, G6) Liver section of treated rats by SN+CPN showing focal areas of hepatic necrosis with extravagated R.B.C.s, portal vein and moderate liver cells loss their cells boundaries (H & E. x 150).

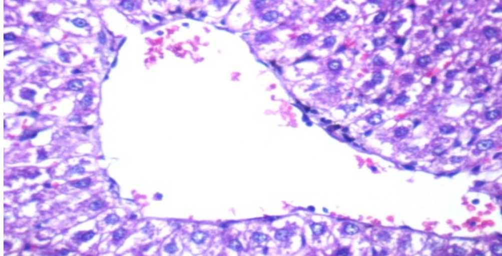

Figure (7, G_7) Liver section of treated rats by CL+SN+CPN revealed swollen, vacuolated hepatocytes, normal liver architecture and dilated central vein (H & E. x 300).

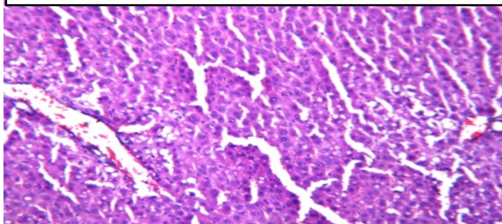

Figure (9, G_9) Liver section of treated rats by PS+CPN+SN revealing focal areas of fatty vacuolation regenerating hepatocytes, less normal tissue and dilated blood vessels (H & E. x 150).

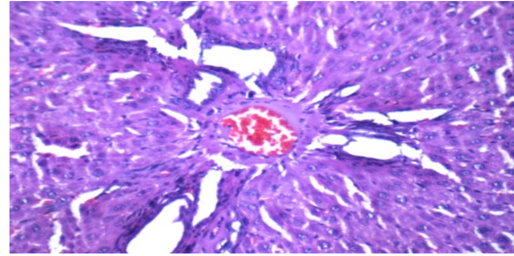

Figure (5, G_5) Liver section of treated rats By CPN+PS showing fatty vacuolation, regenerating hepatocyte, ballooning hepatocytes, and prominent kupffer cells (H & E. x 600).

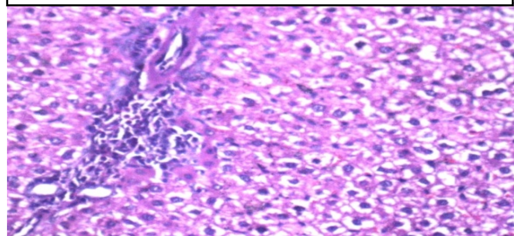

Figure (6b, G6) Liver section of treated rats by SN+CPN revealed moderate to marked aggregation of inflammatory cells in portal areas with hyalinized wall of portal vessels (H & E. x 300).

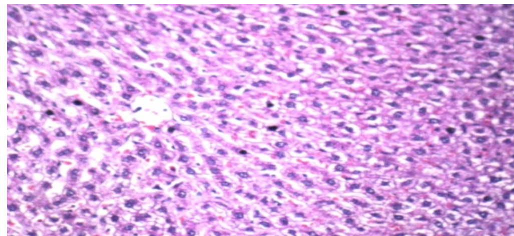

Figure (8, G_8) Liver section of treated rats by CI+CPN+ SN revealed most hepatocytes were quite changes in some areas with central vein and portal area (H & E. x 150).

Glutathione reduced is thought to be an important factor in cellular function and defense against oxidative stress. It was found that dietary GSH suppresses oxidative stress in-vivo in prevention of diabetic complications **(Osawa and Kato, 2005 and Ahmed and Nashwah, 2009).** Plant containing flavonoids and phenolics are known to possess strong antioxidant properties **(Tripathi et al. 1996). Cersosimo (1993)** reported that a significant depletion in GSH levels with subsequent loss in enzymatic activity and increased lipid peroxidation in liver after CPN treatment, which is suggestive of increased oxidative stress on the tissues. It was found that the significant decrease in reduced glutathione levels can thus by justified and correlated with significant reduction in glutathione reductase. Lipid peroxidation showed a significant increase in CPN group. The simultaneous reduction in oxidized glutathione can be contributed to increased lipid peroxidation **(Pratibha et al., 2006).**

It had been investigated that glutathione might be utilized in biotransformation reactions for conversion of CPN to CPN-glutathione complex which is then exported out of cell as CPN-glutathione complex **(Hanigan, 1998).** The glutathione uptake is one of the primary mechanisms in tubular cells to maintain intracellular thiol redox status **(Visarus et al., 1996).** Thus, it is possible to speculate that a positive regulation in the biosynthesis of glutathione may contribute to an increase in its intracellular contents. Thus, the ability of cells to increase glutathione synthesis may be an important protective mechanism against toxic agents **(Iseri et al., 2007).**

It was investigated that an increase of oxidative stress in systemic sclerosis, total antioxidant capacity (TAC) wasn't decreased as anticipated. Moreover, it was even higher than in healthy controls **(Firuzi et al., 2006).** The lack of significant changes in TAC may lie in some as yet unknown feedback mechanism being a response to increased oxidative stress **(Prior and Cao, 1999).**

Previous findings suggest that oxidative stress is more pronounced in the later stages of the disease. It includes oxidation of proteins (signified by reduced sulfhydryl groups), deterioration of antioxidant defenses (decreased TAC) and also lipid peroxidation. Nevertheless, the finding of very low sulfhydryl groups and elevated carbonyl groups indicates a serious oxidative injury, particularly in proteins.

The analysis on antioxidant status and biomarkers along with lipid peroxidation in rat after cisplatin treatment was investigated **(Pratibha et al., 2006).** The investigation revealed a significant increase in lipid peroxidation status and decrease in glutathione level in hepatic tissue of rat after cisplatin treatment, which indicates that it might cause inactivation of enzymes.

Several flavonoids as Apigenin, kaempferol, quercetin hesperetin and luteolin and polyphenols have been isolated from these plants extract (Parsley, Curcumin and Chicory) as well as SN was containing a flavonolignan **(Shi et al., 2006).**

Our results exerted that cisplatin caused significant increases in serum ALT and AST, while total protein in plasma appeared to be decreased. This result was in agreement with the results of **Iseri et al. (2007).** They found that CPN caused a marked reduction in liver function by increase the activity of transaminases. After treatment with each plant extract the enzyme activity was decreased by different percentage values, this due to the antioxidant activity of CL, CI and PS and also SN as a higher flavolignan contents **(Shi et al. 2006).**

Cisplatin is one of the most active cytotoxic agents in the treatment of cancer. Toxic effects, as nephrotoxicity and neutrotoxicity and less frequent toxic effects as hepatotoxicity was generally observed after administration of high doses of cisplatin **(Koc et al., 2005).** CPN has been demonstrated to generate active oxygen species, such as superoxide anion and hydroxyl radical **(Baliga et al., 1998)** and to stimulate lipid peroxidation in the kidney tissues **(Sadzuka et al., 1992).**

Curcuma longa is a naturally potent antioxidant occurring phenolic compound. It has been reported to possess a variety of biological and pharmacological activities, including antioxidative, anti-inflammatory anti-carcinogenic activities **(Wei et al., 2006).**

Also, it was reported that, free radicals exert their toxic effects by acting on DNA, membrane proteins and lipids **(Bhattacharya et al., 2003).** It has been investigated by **Romero et al. (1994)** that the protein content was reduced in the liver.

Our results revealed that administration of CPN to the rats caused a significant decrease in the level of SOD and CAT either in liver or in kidney tissues. Also, the level of TBARS in these organs was significantly increased, when compared to normal control ($P<0.05$). A significant reversal of the alterations towards the CPN group was observed by the administration of water plant extracts of CL, CI and PS**,** as well as SN in both liver and kidney ($P<0.05$). Also, the total protein was decreased under the effect of CPN in either liver or kidney tissues; this effect was increased after plant extracts treatment. It was suggested that cisplatin treatment is associated with its efficacy in treating various cancers and also associated with increased risk of causing long-term toxicities such as nephrotoxicity,

hepatototoxicity and neurotoxicity **(Kharbhangar et al., 2000).**

Lipid peroxidation is found to be an important pathophysiological event in a variety of diseases including aging cancer, diabetes, cardiovascular disorders and rheumatoid arthritis **(Ajitha and Rajnarayana, 2001).** The lipid peroxidative degradation of the biomembrane is one of the principle causes of toxicity of CCl_4 **(Kaplowitz et al., 1986)**. This is evidenced by the elevation of TBARS and decrease in the activity of free radical scavenging enzymes viz., SOD and CAT in the CCl_4 treated animals **(Badami et al., 2003).** SOD is the key enzyme in scavenging the superoxide radicals. Catalase (CAT) is also another key enzyme in the scavenging, which helps in cleaning the H_2O_2 formed during incomplete oxidation.

It was reported by **Koo et al. (2004)** that water extract of CL having $O_2^{\cdot-}$ scavenging activity protected the cells from induced cell death. Also, it causes a marked decrease in malondialdehyde (MDA) and significantly elevated the antioxidant enzyme activities (glutathione peroxidase, and catalase).

Curcumin extract is known as flavonoid that can enter the blood cells and the metabolites inside the cell may also act as an antioxidant **(Jain et al., 2006).** In addition, curcumin by 30mg/kg b.w. ameliorated hepatic MDA, SOD, and GSH in hepatotoxic rats. Also, curcumin decreased significantly serum ALT, AST and LDH levels. Curcumin can significantly inhibited lipid peroxidation in rat liver **(Redly and Lokesh, 1994).** The protective effect of curcumin was attributed to its antioxidant properties by inhibiting free radical generation **(Manikandan et al., 2004).** Curcumin exhibits a variety of antioxidant effects and appears to have a significant potential in the treatment of multiple disease resulted from oxidative stress.

It has been investigated that the protective effect of PS extract due to its active ingredients such as flavones, apigenin, myristicin, coumarins, flavonoids **(Nielson et al., 1999).** It was found that MDA concentration in rat liver homogenate was inhibited by CL and PS ethanolic extracts **(Nermien, 2008).** These plant extracts was ameliorated the toxic effect of CCl_4-induced hepatotoxicity. **Badawi et al. (2003)** found that the root, fruit and foliage of PS contain flavonoids, carotenoids like β-carotene and vitamin C as antioxidant activity source. PS has received considerable attention because it had flavonoids as apigenin, kaempferol, quercetin, hespertin, luteolin polyphenol and tannins classified as flavones and flavonol. It is believed to be an excellent source of natural antioxidant, anti-inflammatory stimulant and may be particularly important in protecting cells against free radicals and chronic diseases **(Lugasi and Hovari, 2000).**

DNA concentration was reduced after the treatment of CPN, while the administration plant extract improved the concentration of DNA by different percentage values.

It was found by **Bhattacharya et al. (2003)** that the free radical exerted their toxic effect by acting on DNA, membrane proteins and lipids, while under the effect of plant extract, the genomic DNA concentration was less increased **(Ippoushi et al., 2003).** It is well known that malondialdehyde from lipid peroxidation reacts with DNA bases and induces mutagenic lesions **(Benamira, 1995). Pratibha et al. (2006)** showed that the activated oxygen species can in turn induce cellular events such as enzyme inactivation, DNA strands cleavage and also membrane lipid peroxidation.

The histological changes in the liver tissues of all groups were observed. CPN-treated rats showed different severe affection of hepatic tissue, cytoplasmic vascular degenerative change with swelling of cells and loss of architecture pattern **(Brenes et al., 2007).** In addition, it was investigated by **Koo et al. (2004)** that the water extract of *Curcuma longa* having superoxide anion scavenging activity protected the cells from induced cell death. PS contains antioxidant compounds as flavonoids which was believed to have an anti-inflammatory stimulant effect and may be important in protecting cells against free radicals and chronic disease **(Lugasi and Hovari, 2000).**

In summary, the present results demonstrated that the plant extracts of CI, CL and PS protect the liver and kidney tissues against toxic effects of cisplatin. The results of the present study concluded that the water extract of CI, CL, and PS alone and in mixture with SN revealed prophylactic effects on cisplatin-induced hepato-toxicity and nephro-toxicity in rats. The combination of each extract with SN could render more effective protection than each individual extract treatment. Our findings supported that the use of each extract may therefore be more effective for clinical purposes with antioxidant properties.

However, further investigations will be done to elucidate the mechanism of protection and potential usefulness of these plant extracts as a source of protective agents against drugs or xenobiotics toxicity in clinical trials.

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