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Possible Protective Effects of Garlic, Ginkobiloba and Silymarin on Cisplatin Hepatotoxicity in Protein-Malnourished Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Background: Protein malnutrition (PM) is one of the major public health problems in developing countries. Cisplatin (CDDP) is an effective anticancer drug that elicits many hepatotoxicity. CDDP hepatotoxicity restricts its clinical use under long term treatment.
Objectives: The study was carried out to determine the possible protective effects of fresh garlic homogenate (FGH), Ginkobiloba extract (GBE) or silymarin (Sly) on cisplatin hepatotoxicity in protein malnourished rats.
Methods: Sprague-Dawley rats were divided into NF set and PM set. Each set divided into control group and seven treated groups received cisplatn and FGH, GBE or Sly and its combinations with cisplatin. Biochemical changes, reactive oxygen species (ROS) and superoxide dismutase (SOD), Malondialdehyde (MDA) and glutathione (GSH) parameters were evaluated. Liver samples were examined for histopathological changes
Results: Cisplatin increased ALT and AST, as well as liver body weight ratio. ROS

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parameters showed a significant increase in MDA and nitric oxide (NO) and decrease in glutathione and SOD. PM potentiates cisplatin side effects. FGH, GBE or Sly attenuate cisplatin toxicity and liver histopathological changes.
Conclusion: PM potentiates cisplatin toxicity. FGH, GBE or Sly has partial protective effects against the cisplatin- induced toxicity induced in NF and PM rats.

Keywords: Cisplatin; protein malnutrition; garlic; ginkobiloba; silymarin; hepatotoxicity; rats.

1. INTRODUCTION

Protein malnutrition (PM) has been identified as a major health problem in developing countries [1-2]. The majority of cancer cases exhibit malnutrition [3]. PM in patients with cancer occurs as a consequence of an imbalance between the nutritional needs of the patient, the demands of the tumour and the availability of nutrients in the body [4-5]. The global incidence of PM in cancer ranges from 30% to 85% and is most prevalent in patients with gastric, pancreatic, lung, prostate and colon cancer [6-7]. PM has been associated with a decreased quality of life, considerable morbidity and mortality, reduced response to chemotherapy and an increased risk of chemotherapy-induced adverse effects and toxicity [6]. PM causes growth failure in the liver of laboratory animals and humans [1]. It alters liver morphology and causes a marked decrease in the cytoplasmic eosinophilic content and nuclear shrinkage in hepatocytes, with a decrease in the glutathione (GSH) level [8]. PM was found to alter cisplatin hepatotoxicity [9].

Most anticancer agents cause toxicity in various organs, including the liver, by disturbing the oxidant/antioxidant balance [10]. Cis-diamminedichloroplatinum II (CDDP, cisplatin) is one of the most potent chemotherapeutic antitumour agents. Various agents, including antioxidants, attenuate the hepatotoxicity of this compound [11]. CDDP is one of the most effective and widely used chemotherapeutic agents in the treatment of various human solid tumours [12]. Recent studies have suggested that hepatotoxicity is also a major dose-limiting side effect of CDDP-based chemotherapy [13-14].

Garlic has a higher concentration of sulphur-containing compounds than any other member of the Allium species. This feature underlies the pungent odour of garlic and conveys the antioxidant properties of garlic preparations and many of its medicinal effects[15]. The main sulphur compound in both raw garlic and garlic powder is alliin; in addition, a small amount of oil-soluble sulphur is present [16-17]. The sulphur compounds found in fresh garlic appear to be almost 1000 times more potent as antioxidants than those found in aged garlic extracts [18]. Garlic extract and allicin efficiently scavenged exogenously generated hydroxyl radicals in a dose-dependent manner [19]. Other garlic constituents, such as S-allyl cysteine, also exhibited significant antioxidant effects in vitro [20].

The ginkgo biloba extract (GBE) is an extract of the leaves of the ginkgo biloba tree that is composed mainly of flavonoid glycosides and terpenoids (ginkgolides and bilobalides) and exerts various pharmacological actions) [21]. It is known as an antioxidant and shows potential in the treatment of cerebrovascular dysfunctions and peripheral vascular disorders [22-24]. GBE is expected to be effective as an antioxidant for preventing liver fibrosis [25]. GBE has been reported to be a potent scavenger of superoxide [26], hydroxyl [27] and peroxyl radicals [28] and nitric oxide (NO) [29]. It can counteract the function of reactive oxygen species (ROS) and reduce ROS-induced cell apoptosis in vitro [30].

Silymarin (Sly) is a flavonoid extracted from milk thistle seeds. These flavonoids are phenolic compounds of plant origin with antioxidant properties24. These properties seem to exist because of their ability to scavenge free radicals and to chelate metal ions [31-32]. Sly is frequently used in the treatment of liver diseases and in the prevention of liver injuries induced by various chemicals or toxins [33-34]. It also protects liver cells directly by stabilizing membrane permeability by inhibiting lipid peroxidation (LPO) [35] and preventing liver glutathione depletion [36].

The purpose of the present study was to evaluate whether FGH, GBE and Sly induce protective effects against CDDP-induced hepatotoxicity in normally fed (NF) and protein-malnourished rats.

2. MATERIALS AND METHODS

2.1 Animals

Sprague-Dawley rats (250±30g) were obtained from the animal house of the National Organization for Drug Control and Research, Egypt. The animals were maintained in cages with free access to food and tap water. All animals were treated daily at a fixed time, as described for the experimental groups. Two weeks prior to drug administration, rats were fed two types of diet according to the casein content: standard protein diet (20% casein) [37] or low-protein diet (5% casein) [38]. Each 1000g of the standard protein diet contained casein (200g), a mixture of sucrose and starch (700g), a salt mixture (40g), oil and oil-soluble vitamins (250g), a vitamin mixture in starch (6g) and a choline-starch mixture 1:3 (4g). The low-protein diet contained the same constituents of the low protein diet with the exception of the amount of casein, which was 50g per 1000g; the difference in weight (150g) was replaced by a sucrose–starch mixture.

2.2 Experimental Design

The animal utilization protocols were in accordance with the guidelines provided by the Experimental Animal Laboratory, and the study was approved by the Ethics Council of the General Division for Basic Medical Science, NODCAR (12-06-10).

Rats were randomly divided into two sets: NF (set I) and protein-malnourished (set II) sets. Each set was divided into eight groups of eight rats each, as follows: (1) control group: rats were injected intraperitoneally (i.p.) with isotonic saline solution (vehicle of CDDP) and oral saline (vehicle of protective agents); (2) FGH group: animals received FGH orally for 4 days, followed by saline (1 mg/kg, i.p.) on the fourth day; (3) GBE group animals received GBE orally for 4 days, followed by saline (1 mg/kg, i.p.) on the fourth day; (4) Sly group: animals received Sly orally for 4 days, followed by saline (1 mg/kg, i.p.) on the fourth day; (5) CDDP group: animals received a single dose of saline for 4 days, followed by CDDP on the fourth day; (6) FGH + CDDP group: animals received FGH orally for 4 days, followed by CDDP on the fourth day; (7) GBE + CDDP group: animals received GBE orally for 4 days, followed by CDDP on the fourth day and (8) Sly + CDDP group: animals received Sly orally for 4 days, followed by CDDP on the fourth day. FGH was administered orally at a dose of 400 mg/kg [39]. GBE was administered orally at a dose of 100 mg/kg [40]. Sly was administered orally at a dose of 200 mg/kg [41]. CDDP was administered at a dose of 15 mg/kg (i.p.) [42].

Twenty-four hours after the last treatment, the animals were reweighed. Serum was collected from the retro-orbital plexus and used for the determination of AST and ALT levels. Subsequently, the animals were sacrificed and the liver was removed, washed thoroughly with ice-cold saline (0.9% sodium chloride) and weighed. A piece of the right lobe of the liver of each animal was frozen and stored at -80ºC for ROS and antioxidant determination, whereas the rest of the organ was maintained in 10% formalin for histopathological studies. The mean ratio between the weight of the liver and total body weight was calculated in each group of animals.

Serum AST levels were determined calorimetrically using the method described by Reitman and Frankel [43]. The colour of the product shows maximum absorbance at 580 nm against the blank. Serum ALT levels were determined calorimetrically using the method described by Husdan et al. [44]. Colour absorbance was measured at 520 nm and was proportional to creatinine concentration. Malondialdehyde (MDA) levels in liver tissues were determined using the method of Buege and Aust [45]. Colour absorbance was measured at 535 nm (UV detector). GSH levels in liver tissues were determined according to the modified method of Beutler et al. [46], and colour absorbance was measured at 412 nm. NO levels in liver tissues was determined by measuring nitrite according to the photometric method of Green et al. [47]. SOD levels in liver tissues were determined according to the pyrogallol method of Marklund and Marklund [48], and colour absorbance was measured at 420 nm.

Liver samples were collected, rinsed in 10% formalin, dehydrated, cleared, impregnated, blocked and embedded in paraffin according to standard histological techniques. Six-micrometre-thick sections were cut through the liver. The sections were stained with hematoxylin and eosin for light microscopic examination [49].

2.3 Statistical Analysis

The results are expressed as mean ± SD. Data were statistically analyzed using the Neuman–Keuls test to evaluate the comparisons between means, with significance set at $P< 0.001$.

3. RESULTS

To confirm the role of the antioxidant system in CDDP-induced toxicity in NF and protein-malnourished rats, parameters of oxidative stress in both sets of animals were evaluated. The antioxidant status is a potential biomarker of changes in the effects of the physiological state of cells, tissues or organs.

Table 1 show that administration of a single dose of CDDP (15 mg/kg, i.p.) caused a significant increase in relative liver weight by 28.04% in the NF rats and 80.2% in the protein-malnourished rats. In contrast, administration of FGH, GBE and Sly for 3 days and prior to CDDP administration significantly reduced the relative liver weight by 9.5%, 4.4% and 7.2% in the NF rats and by 15.7%, 9.9% and 18.1% in the protein-malnourished rats, respectively, compared with administration of CDDP alone. Administration of FGH, GBE or Sly for 3 days and before CDDP administration significantly reduced serum ALT and AST levels by 2.5%, 15.0% and 22.5% in the NF rats and by 29.2%, 21.3% and 29.8% in the protein-malnourished rats, respectively compared with CDDP-treated alone ($P< 0.001$) (Table 1).

Table 1. Protective effect of FGH, BGE and Sly on AST, ALT and relative liver body weight cisplatin-induced liver toxicity in normally fed and in protein malnourished rats

Groups	AST (U/L)		ALT (U/L)		Relative liver weight (g)	
	NF	PM	NF	PM	NF	PM
Control	12.50±1.46	18.00±0.76*	10.00±0.68	14.17±1.64*	4.1±0.17	2.02±0.08*
FG	12.17±1.17	17.50±0.95*	10.20±0.48	13.83±1.66*	4.13±0.13	2.18±0.11*
GBE	12.67± 1.28	17.83±0.89*	9.33±0.56	13.33±1.76*	4.27± 0.19	2.23±0.12*
Sly	12.50±1.34	17.67±0.80*	9.80±0.60	13.50±1.63*	4.22±0.14	2.15±0.09*
CDDP	23.50±1.71^{+}	28.00±1.95^{*+}	20.00±1.67^{+}	24.00±1.81^{*+}	5.25±0.18^{+}	3.64±0.23^{*+}
CDDP+FG	15.80±1.85$^{\alpha}$	21.00±1.53$^{*\alpha}$	15.00±1.57$^{\alpha}$	17.00±1.86$^{*\alpha}$	4.75±0.25$^{\alpha}$	3.07±0.10$^{*\alpha}$
CDDP+GBE	18.50±1.82$^{\alpha}$	24.43±1.67$^{*\alpha}$	17.00±1.81$^{\alpha}$	20.00±1.57$^{*\alpha}$	5.02±0.15°	3.28±0.10$^{*\alpha}$
CDDP+Sly	16.50±1.71$^{\alpha}$	22.00±1.97$^{*\alpha}$	15.50±1.67$^{\alpha}$	17.83±1.88$^{*\alpha}$	4.87±0.12$^{\alpha}$	2.98±0.11$^{*\alpha}$

*: Significant comparison to corresponding normally fed group, P<0.001, $_{a}$: Significant comparison to cisplatin treated group, P<0.001, $_{+}$: Significant comparison to corresponding control group, P<0.001, CDDP: Cisplatin, FGH: fresh garlic homogenate, GBE: Ginkobiloba extract, Sly: silymarin, PM: Protein malnourished, NF: Normally fed

LPO was evaluated in terms of the MDA levels. Protein deficiency significantly elevated MDA and NO levels by 23.0% and 19.7%, respectively, and decreased GSH and SOD levels by 27.4% and 32.0%, respectively (Table 2). An effect of CDDP was observed on oxidative stress parameters. CDDP elevated LPO and significantly altered antioxidant enzymes in a differential manner (Table 2). MDA levels significantly increased by 40.55% and 44.1% and NO levels increased by 33.75% and 35.2% in the NF and protein-malnourished rats, respectively. Pretreatment with FGH, GBE or Sly for 4 consecutive days prior to CDDP administration resulted in a significant increase in GSH by 63.7%, 39.2% and 47.3% in the NF rats and by 30.0%, 71.3% and 29.0% in the protein-malnourished rats, respectively. In contrast, SOD levels were increased by 39.1%, 27.2% and 35.0% in the NF rats and by 48.7%, 31.4% and 41.6% in the protein-malnourished rats, respectively, compared with administration of CDDP alone (Table 2). Moreover, MDA levels were decreased by 33.1%, 21.3% and 26.9% in the NF rats and by 38.4%, 31.4% and 35.6% in the protein-malnourished rats, respectively. NO levels were decreased by 27.1%, 22.7% and 25.7% in the NF rats and by 30.3%, 23.2% and 29.0 in the protein-malnourished rats, respectively, compared with administration of CDDP alone (Table 2).

Histopathological examination revealed a normal liver histology in the control (Fig. 1a), FGH (Fig. 2a), GBE (Fig. 3a) and Sly (Fig. 4a) groups in set I and showed fatty changes in the hepatocytes of the control (Fig. 1b), FGH (Fig. 2b), GBE (Fig. 3b) and Sly (Fig. 4b) groups in set II. The liver of the NF rats injected with CDDP showed congestion of the central veins and degenerative changes and mainly fatty changes (Fig. 5a). The liver of the protein-malnourished rats injected with CDDP showed fatty changes in hepatocytes located at the periphery of the hepatic lobules (Fig. 5b) and marked congestion of the central veins and hepatic sinusoids. Treatment with FGH, GBE or Sly before CDDP administration yielded fatty changes, congestion of the central veins and degenerative changes in the surrounding hepatocytes, albeit to a lesser extent than treatment with CDDP alone, as illustrated for FGH (Fig. 6a,b), GBE (Fig. 7a,b) and Sly (Fig. 8a,b) in the NF and protein-malnourished rats, respectively.

Table 2. Effects of FGH, BGE and Sly on MDA, GSH, NO and SOD in cisplatin-induced liver toxicity in normally fed and in protein malnourished rats

	MDA (nmol/g tissue)		GS (µg/g tissue)		NO (µg/g tissue)		SOD(µg/g tissue)	
	NF	PM	NF	PM	NF	PM	NF	PM
Control	8.67±0.21	11.25±0.59*	5.65±0.20	4.10±0.19*	3.3±0.10	3.95±0.21*	79.17±1.10	53.83±2.36*
FG	8.47±0.62	10.95±0.44*	5.43±0.33	3.88±0.21*	3.42±0.09	3.98±0.25*	81.50±1.95	54.50±2.63*
GBE	8.38±0.37	11.38±0.40*	5.57±0.34	3.77±0.22*	3.10 ±0.11	4.10±0.26*	81.17±2.1	54.83±2.47*
Sly	9.01±0.38	11.10±0.37*	5.55±0.39	3.95±0.19*	3.39±0.09	3.92±0.26*	80.0±1.75	55.67±2.60*
CDDP	14.57±0.92^{+}	20.12±1.29^{*+}	2.73±0.20^{+}	2.41±0.23^{*+}	4.98±0.34^{+}	6.10±0.41^{*+}	46.22±3.32^{+}	31.20±2.47^{*+}
CDDP+FG	9.75±0.45$^{\alpha}$	12.40±0.75$^{*\alpha}$	4.47±0.12$^{\alpha}$	3.35±0.15$^{*\alpha}$	3.63±0.12$^{\alpha}$	4.25±0.17$^{*\alpha}$	64.29±4.56^{a}	46.39±3.49$^{* a}$
CDDP+GBE	11.47±0.57$^{\alpha}$	13.80±0.63$^{*\alpha}$	3.80±0.18$^{\ddot{}}$	2.97±0.18$^{*\alpha}$	3.85±0.15$^{\alpha}$	4.75±0.20$^{*\alpha}$	58.78±4.97^{a}	41.00±3.77$^{* a}$
CDDP+ Sly	10.65±0.58$^{\alpha}$	12.95±0.72$^{*\alpha}$	4.02±0.17$^{\alpha}$	3.17±0.17$^{*\alpha}$	3.7±0.11$^{\alpha}$	4.33±0.16$^{*\alpha}$	62.83±4.3^{a}	44.17±3.19^{*a}

*: Significant comparison to corresponding normally fed group, P<0.001. $_{a}$: Significant comparison to cisplatin treated group, P<0.001.
CDDP: Cisplatin, FGH: fresh garlic homogenate, GBE: Ginkobiloba extract, Sly: silymarin PM: Protein malnourished, NF: Normally fed, MDA: Malondialdehyde, GS: glutathione, NO: nitric oxide, SOD: superoxide dismutase

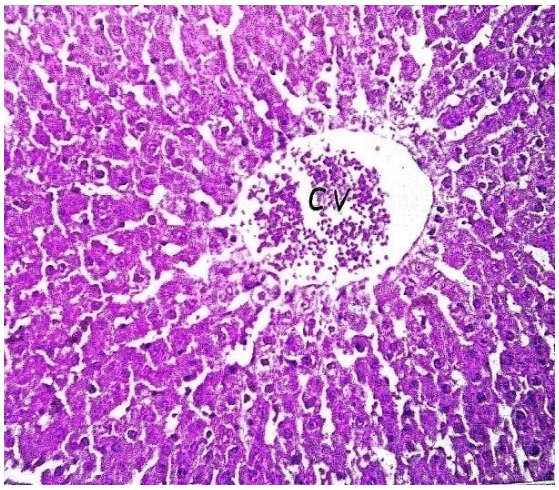

Fig. (1a). Liver of Control group rat of NF set showing no histopathological alterations (H&E stain, X100)

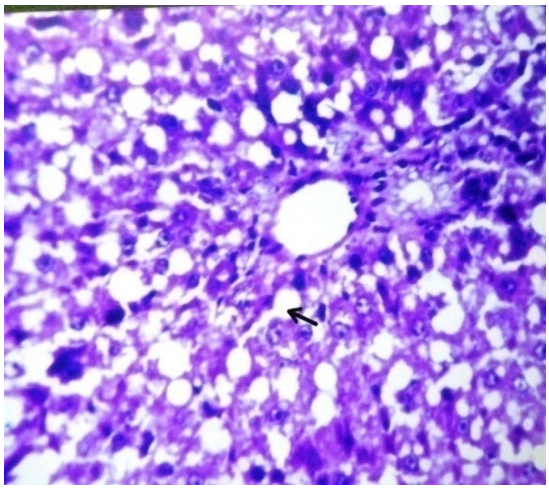

Fig. (1b). Liver of Control group rat of PM set showing some fatty changes in hepatocytes (H&E stain, X200)

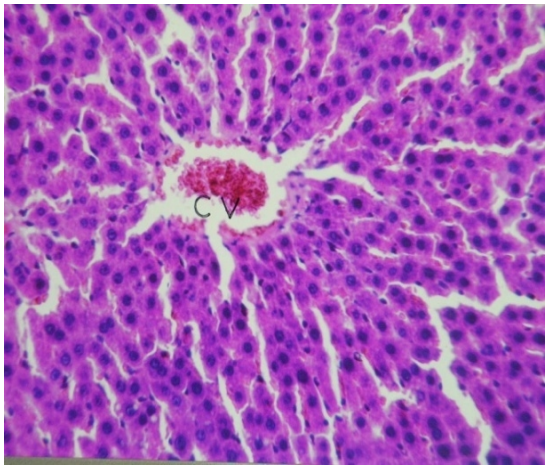

Fig. (2a). Liver of FGH group rat of NF set showing no histopathological alterations (H&E stain, X100)

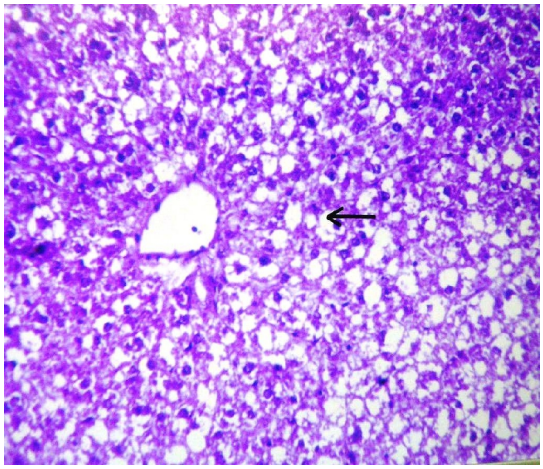

Fig. (2b). Liver of FGH group rat of PM set showing degenerative changes and few fatty changes in hepatocytes (H&E stain, X200)

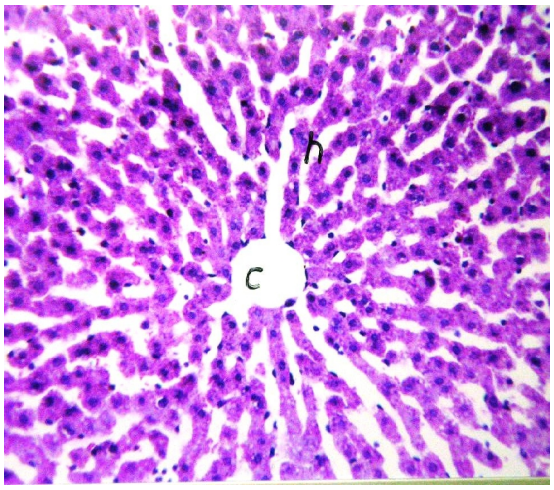

Fig. (3a). Liver of GBE group rat of NF set showing no histopathological alterations (H&E stain, X100)

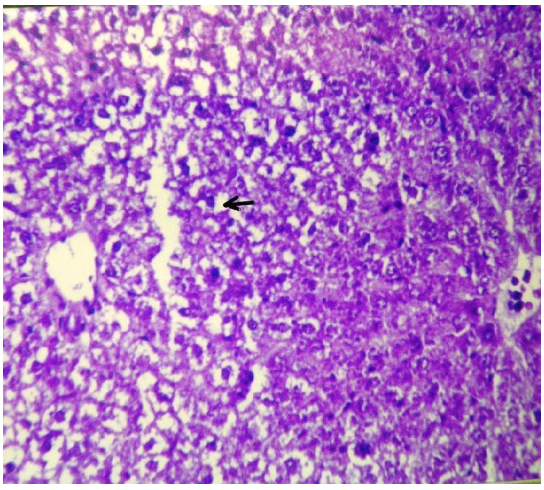

Fig. (3b). Liver of GBE group rat of PM set showing some degenerative changes in some hepatocytes located at the periphery of the hepatic lobules, as well as fatty changes in hepatocytes (H&E stain, X200)

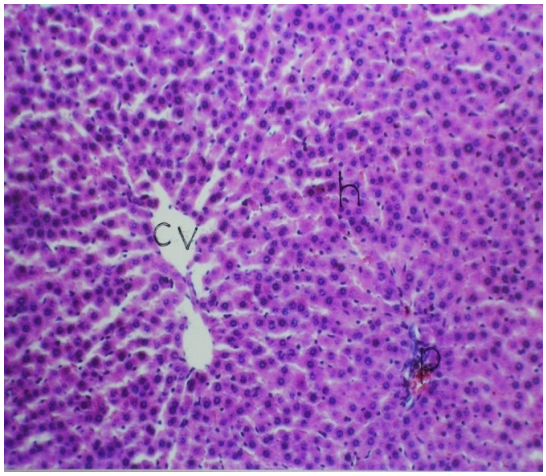

Fig. (4a). Liver of Sly group rat of NF set showing no histopathological alterations (H&E stain, X100)

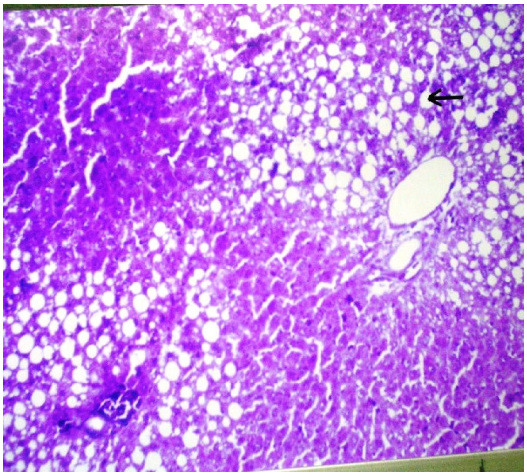

Fig. (4b). Liver of Sly group rat of PM set showing, congestion of the central vein and fatty changes in hepatocytes (H&E stain, X200)

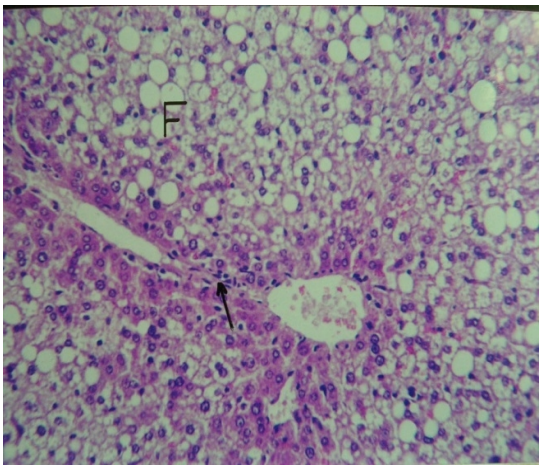

Fig. (5a). Liver of CDDP-treated group rat of NF set showing congestion of the central and portal veins and different degenerative changes and mainly fatty changes in hepatocytes (H&E stain, X 400)

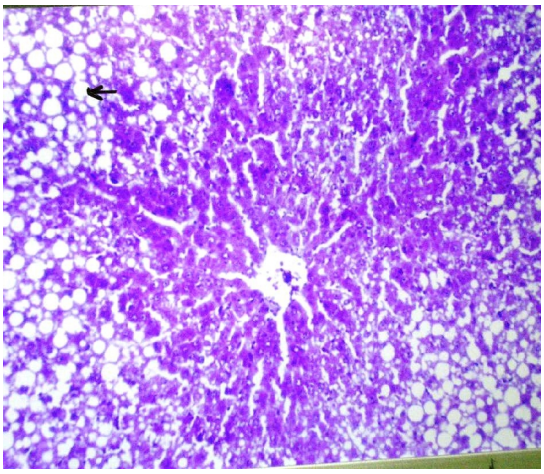

Fig. (5b). Liver of CDDP-treated group rat of PM set showing changes in hepatocytes located at the periphery of hepatic lobules (H&E stain, X200)

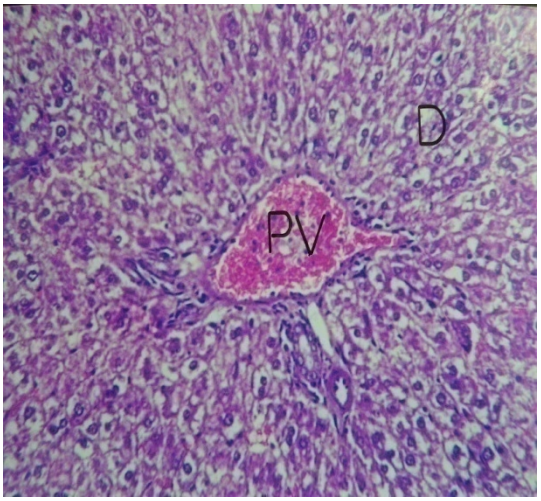

Fig. (6a). Liver of FGH group rat of NF set treated with CDDP showing congestion of the portal vein (PV) and degenerative changes in the surrounding hepatocytes (D) (H&E stain, X200)

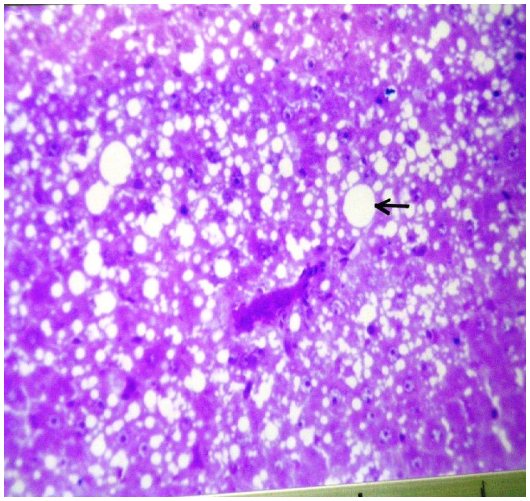

Fig. (6b). Liver of FGH group rat of PM set treated with CDDP showing fatty changes in hepatocytes and congestion of the portal vein (H&E stain, X200)

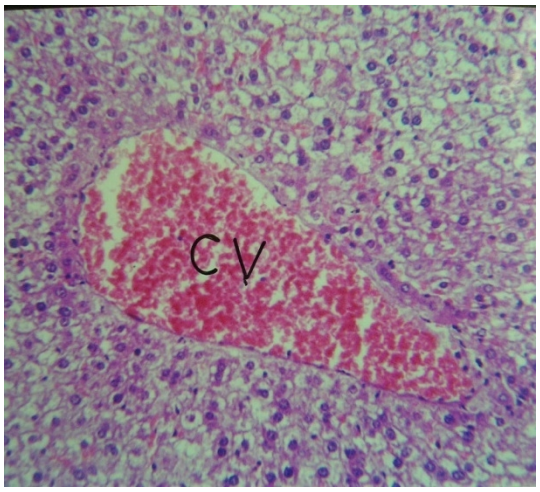

Fig. (7a). Liver of GBE group rat of NF set treated with CDDP showing congestion in portal vein (PV), diffuse proliferation of the Kupffer cells in between the degenerated hepatocytes (D) (H&E stain, X200)

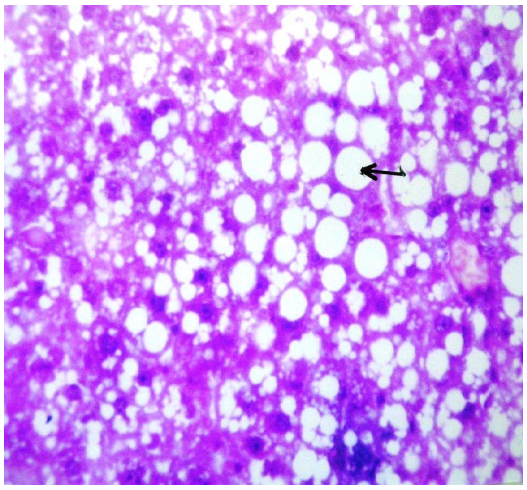

Fig. (7b). Liver of GBE group rat of PM set treated with CDDP showing fatty change in the hepatocytes (H&E stain, X400)

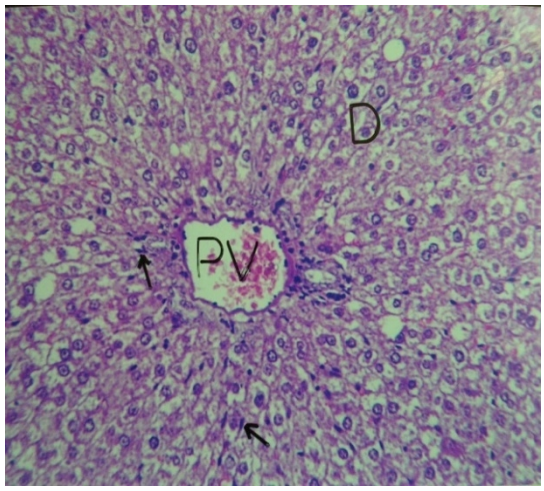

Fig. (8a). Liver of Sly group rat of NF set treated with CDDP showing congestion in portal vein (PV), diffuse proliferation of the Kupffer cells in between the degenerated hepatocytes (D) (H&E stain, X200)

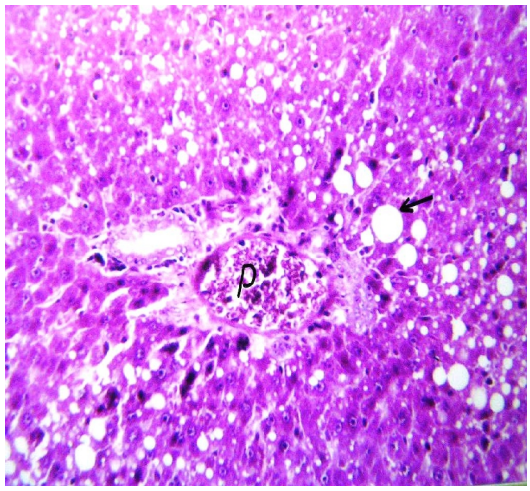

Fig. (8b). Liver of Sly group rat of PM set treated with CDDP showing congestion in the central vein and Fatty change in the hepatocytes, (H&E stain, X200)

4. DISCUSSION

A model of PM was successfully established by feeding rats a low-protein diet (5%) according to the study of Oumi et al. [50]. The protein-malnourished rats showed a

significant decrease in relative liver weight compared with the NF rats. These results are in agreement with those of the study of Bozzini et al. [51] who reported that the growth rate during PM was significantly lower than that during normal conditions. The decrease in body weight observed in the PM rats may be due to a reduction in the levels of protein and food consumption [52].

The present study revealed that the protein-malnourished rats showed a significant increase in serum ALT and AST levels compared with the NF rats. These results support those of the studies of Güler et al. [53] and Obimba [54]. The elevation in serum AST and ALT levels due to PM may be caused by upregulation of the tumour necrosis factor (TNF) in the serum and of the absolute amounts of TNF secreted by cells, leading to an increase in the rate of cell damage as a consequence of PM [55]. In addition, it was reported that PM reduced the level of cell proliferation and increased the level of DNA damage [56], which was accompanied by an impaired capacity to repair this damage [57].

A significant increase in serum MDA levels was observed in the protein-malnourished rats and was associated with a decrease in GSH and SOD levels. These findings are in accordance with those of the studies of Bosnak et al. [58] and Sidhu et al. [59]. These effects may be due to the fact that protein-malnourished rats are more susceptible to oxidative stress. The reduction in SOD and GSH levels observed in the protein-malnourished rats confirmed this assumption.

CDDP-treated NF and protein-malnourished rats showed a significant increase in AST and ALT levels and in relative liver weight. These results are in agreement with those of the study of Naqshbandi et al. [60]. A significant increase in MDA and NO levels and a significant decrease in GSH and SOD levels were observed after CDDP administration. PM potentiated these effects of CDDP. Our results confirmed those of the studies of Kadikoylu et al. [61] and Saad et al. [62].

It is known that CDDP produces hepatotoxicity [63]. It is provokes several responses including membrane peroxidation, dysfunction of mitochondria, inhibition of protein synthesis and DNA damage [64-65]. Oxidative stress due to the formation of free radicals is one of the pathogenic mechanisms underlying the adverse effects of CDDP in the liver [66]. CDDP induces mitochondrial dysfunctions, particularly the inhibition of the electron transfer system, resulting in enhanced ROS production and tissue damage [67].

The toxic effects of CDDP are extended via the induction of ROS generation [68,69]. A major cellular defence against ROS is provided by SOD, which converts superoxide radicals first to hydrogen peroxide and then to molecular oxygen and water. However, oxidative stress can occur as a result of an increase in ROS generation and/or a decrease in the antioxidant enzyme system. These antioxidant enzymes protect the cell against cytotoxic ROS. In agreement with the studies of Naqshbandi et al. [60] and Khan et al. [70] and, the present study showed that CDDP enhanced LPO, an indicator of tissue injury, and depleted protein thiols. The ability of CDDP to cause alterations in the activity of these enzymes may be an event that results from CDDP-induced liver damage, resulting in leakage from hepatocytes.

CDDP is also involved in altering the thiol status of tissues, with concomitant alterations in enzymatic antioxidants. GSH and SOD levels were significantly decreased after CDDP therapy. This effect may be a secondary event following CDDP-induced increase in free radical generation and/or decrease in LPO-protecting enzymes. CDDP can lead to the generation of oxygen free radicals, such as hydrogen peroxide, superoxide anions and

hydroxyl radicals. The hydroxyl radical can abstract a hydrogen atom from polyunsaturated fatty acids in membrane lipids to initiate LPO. These radicals can evoke extensive tissue damage by reacting with macromolecules, such as membrane lipids, proteins and nucleic acids [71-72]. Moreover, depletion of GSH may contribute to CDDP-induced LPO71. Thus, an alteration in the enzymatic antioxidant status accompanied by an increase in LPO and NO indicates that the enzymes play an important role in combating free radical-induced oxidative stress in tissues.

In contrast, the consumption of natural products (FGH, GBE and Sly) increased the activities of antioxidant enzymes, albeit to different extents, when accompanied by CDDP administration 3 days of after pretreatment with the natural agents. Moreover, the decline in SOD and GSH activities were reduced significantly compared with those after administration of CDDP alone. These results indicate a marked protection afforded by natural products against CDDP-induced oxidative damage to liver tissues.

Treatment with FGH, GBE or Sly before CDDP administration to rats caused significant increases in SOD and GSH levels, which were accompanied by lower LPO values in liver tissues. The protection against CDDP afforded by these natural products can be attributed to their intrinsic biochemical and natural antioxidant properties. It appears that FGH, GBE and Sly enhanced resistance to the free radical attack generated by CDDP administration.

The protective effects of garlic can be attributed to its ability to increase GSH levels and to protect endothelial cells by reducing oxidative stress [73]. MDA levels were significantly reduced in the FGH group. The protective effects of garlic can also be attributed to allicin, which decreased lipid peroxide significantly and has been reported to be a very good hydroxyl radical scavenger [74]. Garlic is also a rich source of highly bioavailable selenium, which is thought to account for, in part, the antioxidant and cancer-preventive effects of garlic [75].

The present investigation showed that GBE administration restored the control values of oxidative stress markers. This study provided evidence that the antioxidant properties of GBE may contribute to its ability to restore SOD levels and reduce MDA levels. The antioxidant activity of GBE may be attributed to its active components, namely flavono glycoside and terpene lactones [20].

Sly has antihepatotoxic activity against hepatotoxicity induced by some drugs in albino rats. Sly protects against an increase in serum ALT and AST levels[76] and induced mitochondrial Ca2+release [76]. It has been proposed that because of its hydrophobic character, Sly produces an alteration in the lipidic medium of the inner membrane that is conducive to the inhibition of the electron transport in the respiratory chain, as well as to the loss of energy-dependent accumulated Ca2+ [77].

In the present study, Sly administration for 3 days and before CDDP administration significantly attenuated CDDP-induced increase in both ROS and nitrogen species. Sly has the ability to scavenge free radicals and ROS and of strengthening the antioxidant system [78]. These results were also in agreement with those of study of Lee et al. [79], probably because of the association between the antioxidant activity of Sly and the reduction in hepatic NO protein content. The protective action of Sly is associated with its antioxidant properties because it possibly acts as a free radical scavenger, an inhibitor of LPO and a plasma membrane stabilizer [80]. It also acts as a preservative of liver GSH content and prevents LPO. This effect may be due to its antioxidant activity [80-82].

5. CONCLUSION

We concluded that CDDP elicited harmful hepatotoxic effects may be by causing severe damage to the plasma membrane, mitochondria and other organelles by suppressing the antioxidant defence mechanism. These effects were potentiated by PM; however, they were improved by supplementation with natural products (FGH, GBE and Sly). Thus, the results of the present study support the hypothesis that pre-treatment with either FGH, GBE or Sly is effective in maximizing the clinical use of CDDP in the treatment of various malignancies, by minimizing its hepatotoxicity. The results of this study showed that the toxicity induced by a high dose of CDDP was decreased by pre-treatment and concurrent treatment with natural products, such as FGH, GBE and Sly. Further studies are needed to investigate the exact mechanisms that underlie the prophylactic effects of FGH, GBE and Sly on CDDP-induced hepatotoxicity. Moreover, studies of the effects of the natural products on the pharmacokinetics and efficacy of CDDP are needed.

CONSENT

Not applicable.

ETHICAL APPROVAL

The animal utilization protocols were in accordance with the guidelines provided by the Experimental Animal Laboratory, and the study was approved by the Ethical Committee and Council of the General Division for Basic Medical Science, NODCAR (01-03-10).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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