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Influence of L-arginine on Cisplatin-Induced Hepatotoxicity in both normally Fed and Protein Malnourished Rats

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Abstract: *Liver has great capacity to detoxify toxic substances and synthesized useful principles. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences. Protein malnutrition produces profound effects on biochemistry and physiology of the body as well as growth failure of most body organs. Cisplatin is a member of anticancer drugs that elicits many hepatotoxicity. The study was carried out to investigate the possible hepato-curative and hepato-protective effect of l-arginine either alone or in combination with silymarin against hepatotoxicity resulted from cisplatin (7.5 mg/kg i.p.) treatment in normally fed and protein malnourished male albino rats. All treatments were administered for 5 consecutive days (l-arginine, 200 mg/kg i.p.) and (silymarin, 100 mg/kg i.p.) after and before cisplatin injection. In the cisplatin group, the results revealed significant decrease in the body weight and increase in alanine aminotransferase and aspartate aminotransferase, as well as liver body weight ratio. Antioxidant status was suppressed as manifested by significant decline in reduced glutathione content and total protein level along with decreased enzymatic activity of super oxide dismutase and increased lipid peroxidation, nitric oxide, an effect that was enhanced by combination with protein malnutrition. Administration of l-arginine was effective in decreasing cisplatin hepatotoxicity that restore of anti-oxidant machinery and blunting of mounted malondialdehyde levels. Moreover, histological examination demonstrated that l-arginine significantly reduced cellular infiltration, congestion blood vessels, degenerative changes in hepatocytes and fatty changes. It is concluded that the combined administration of l-arginine with silymarin represents a promising strategy to restrain the cisplatin hepatotoxicity.*

Keywords: *Antioxidant status, Cisplatin, Hepatotoxicity, L-arginine, Protein malnutrition.*

I. Introduction

Liver, the key organ in maintenance of homeostasis as well as metabolism and excretion, has an important task of detoxifying xenobiotics and chemotherapeutic agents [1]. Its role in transforming and clearing chemicals renders it susceptible to damage from these agents. Generally, this usually goes unnoticed because of the considerable capacity of hepatocytes to regenerate toxic chemicals and certain drugs has been recognized as a toxicological problem and linked to the occurrence of oxidative stress [2]. Free radical's generation, arising from oxidative stress, is a common mechanism underlying hepatotoxicity caused by deleterious effect of drugs and toxicants. Oxidative stress has also been implicated in the pathogenesis of cellular damage caused by a number of toxic agents including arsenic, diclofenac, carbon tetrachloride (CCl4), cisplatin (CDDP), rifampicin and acetaminophen [3].

Protein malnutrition (PM) has been identified as a major health problem in developing countries [4]. It could be defined as a state of energy, protein or other specific nutrient deficiency which produces measurable changes in body function. It is associated with specific types of illness and is characterized by being reversible by nutritional support [5]. Also, protein malnutrition can occur throughout the lifespan from fetal life through adulthood. As dietary protein is an important source of essential amino acids that can serve as intracellular antioxidant, its restriction may lead to an increase in oxidative damage by diminishing antioxidant defenses of tissue [6]. So that, PM produces profound effects on biochemistry and physiology of the body as well as growth failure of most body organs [7].

Cis-diamminedichloridoplatinum(II) (CDDP, cisplatin) is one of the most effective and widely used chemotherapeutic agents in the treatment of various human solid tumors. Some studies have suggested that hepatotoxicity is also a major dose-limiting side effect of CDDP-based chemotherapy [8,9]. It is widely used for the treatment of several human malignancies, including head and neck cancers [10] , testicular cancer [11], small-cell [12] and non-small cell lung cancer [13], ovarian cancer [14]. CDDP has serious side effects, such as ototoxicity, nephrotoxicity and hepatotoxicity [15,16]. In spite of its undesirable side effects yet its clinical use depend on its significant anti-cancer effect. Although the mechanism underlying the side effects induced by

CDDP are not understood clearly, it was considered to be attributed to the combination of multi-ways [17-19], such as the generation of reactive oxygen species (ROS), which could interfere with the antioxidant defense system and result in oxidative damage in different tissues [20-22]. Indeed, other studies have suggested that oxidative stress plays an important role in CDDP-induced liver damage [8,21,22]. Hence, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidants in order to balance the ROS in human body.

Silymarin (Sly) is a flavonoid extracted from *Silybum marianum* (L.). It is also commonly used in herbal therapy especially for treating liver diseases partly due to its antioxidant activity. *Silybum marianum* has been used for long time in the treatment of liver diseases that caused by drug and chemical oxidative stress [23-25].

L-arginine is an essential amino acid that plays pivotal roles in maintaining body homeostasis. It is beneficial in the treatment of hepatic injury, hepatic cirrhosis, fatty liver degeneration [26]. Endothelial nitric oxide synthase (eNOs) produces nitric oxide (NO) from l-arginine. NO can rapidly react with reactive oxygen species to form peroxynitrite which generally relief the oxidative stress (antioxidants) [69]. Although, l-arginine is synthesized in the body, it is not produced in sufficient quantities to support growth or meet metabolic requirements during the periods of PM [27]. Therefore, this study was designed to investigate the possible curative and protective role of l-arginine on CDDP-induced hepatotoxicity in normally fed (NF) and (PM) rats.

II. Materials And Methods

1. Chemicals and reagents

Cisplatin: (cisplatyl 50 mg, Laboratoire Roger Bellon, France) (CDDP) was purchased from NCI drug store. Silymarin (Sly) was purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). L-arginine was purchased by Sigma–Aldrich Chemical Company (St. Louis, MO, USA).

Commercially available kits (Bio-Diagnostic, Egypt) were used for determining superoxide dismutase (SOD) activity and levels of alanine transaminase (ALT), aspartate transaminase (AST), albumin, reduced glutathione (GSH), malondialdehyde (MDA) and nitric oxide (NO). All other chemicals were obtained from Sigma (St. Louis, MO, USA).

2. Animals

All animal procedures and experimental protocols were carried out in accordance with the Guide for the Care and Use of Laboratory Animals, Faculty of Pharmacy, Al-Azhar University. The protocol was approved by the committee on the Ethics of Animal Experiments of the Faculty of Pharmacy-University of Al-Azhar (Permit Number: 11-2015). The surgery was performed under light ether anesthesia and scarified using cervical dislocation method, and all efforts were made to minimize suffering. Male albino rats weighting 100-120g were obtained from the Egyptian Organization for Biological Products and Vaccines (Vacsera, Egypt) and housed under controlled temperature (25 ± 1°C) on a 12 h light/dark cycle. Food and water were allowed and *ad libitum* during the study period. Two weeks prior to drug administration, rats were fed two types of diets were utilized depending on the amount of casein: Normal protein diet containing 20% casein [28] or low protein diet containing 8% casein [29]. 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.

3. Pharmacological treatment

One hundred and twenty eight adult male albino rats adult male albino rats were randomly divided into two sets: (set I) normally fed (NF) and protein malnourished (PM) rats (set II) sets. Each set was divided into eight groups of eight rats each, as follows: (1) Control group: rats were injected intraperitoneal (i.p.) with isotonic saline solution (vehicle of CDDP), (2) CDDP group: rats were administered single dose of CDDP (7.5 mg/ kg i.p.), [30] on the 1st day, (3) Sly group (curative): rats were administered single dose of CDDP (7.5 mg/ kg ip) on the 1st day and administered Sly at a dose (100 mg/ kg i.p.), [31], for 5 consecutive days, (4) L-arginine group (curative) : rats were administered single dose of CDDP (7.5 mg/ kg i.p.) on the 1st day and administered l-arginine (200mg/ kg i.p.) [26], for 5 consecutive days. (5) Co-administered Sly with l-arginine group (curative): rats were administered single dose of CDDP (7.5 mg/ kg i.p.) on the 1st day and co-administered Sly with l-arginine with the same previously mentioned regimen, (6) Sly group (protective): rats were administered Sly at a dose (100mg/ kg i.p.) for 5 consecutive days and administered single dose of CDDP (7.5 mg/ kg i.p.) on the 5th day, (7) L-arginine group (protective): rats were administered l-arginine at a dose (200mg/ kg i.p.) for 5 consecutive days and administered single dose of CDDP (7.5 mg/ kg i.p.) on the 5th day,

(8) Co-administered Sly with l-arginine group (protective): rats were administered Sly with l-arginine with the same previously mentioned regimen and administered single dose of CDDP (7.5 mg/ kg i.p.) on the 5th day.
On 6th day in the control group and on day 6 after CDDP administered, curative and protective group, the animals were reweighted and scarified for the evaluation of all parameters.

4. Sample collection

Blood samples were collected on EDTA (for plasma) or without EDTA (for serum) through the orbital sinus, under light ether anesthesia, centrifuged at 1000 X g for 15 min. Serum, plasma and erythrocyte lysate samples were separated and stored at -80°C. After scarification 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, whereas the rest of the organ was maintained in 10% formalin for histo-pathological studies.

5. Weight measurement

The animal's weights were measured at the final day before scarification.

6. Liver ratio (relative liver weight to body weight)

Liver was removed and weight immediately. Liver ratio was calculated as the following formula, liver ratio was calculated as the following formula, organ ratio (%) = organ weight (g) × 100/ body weight (g).

7. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST)levels in serum

ALT and AST levels were estimated according to the method of Reitman and Frankel [32]. Colorimetric determination of ALT or AST was estimated by measuring the amount of pyruvate or oxaloacetate produced by forming 2, 4-dinitrophenylhydrazine. The color of which was measured at 520 nm using colorimeter (6051 JENWAY designed and manufactured in the EU by Bar lowered scientific Ltd. Dunmow. Essex CM63LB, model 6051, voltage 230/115V, frequency 50/60 Hz and power 20VA).

8. Albumin level in serum

Albumin level was measured according to the method of [33].

9. Lipid per-oxidation level in serum

Lipid per-oxidation was a good way for evaluating oxidative stress-induced damage to tissues. Hence levels of malondialdehyde as thiobarbituric acid-reactive substances were measured in serum by the method of [34]. Briefly, thiobarbituric acid reacts with malondialdehyde in acidic medium at temperature of 95°C for 30 min to form colored substances. The resultant pink color representative of thiobarbituric acid-reactive substances was measured at 534 nm using colorimeter (6051 JENWAY designed and manufactured in the EU by Bar lowered scientific Ltd. Dunmow. Essex CM63LB, model 6051, voltage 230/115V, frequency 50/60 Hz and power 20VA).

10. Superoxide-dismutase activity in erythrocyte lysate

Erythrcyte lysate was prepared from different samples according to standard protocol [35]. Activity of the antioxidant enzyme superoxide-dismutase was measured in the erythrocyte lysate using standard spectrophotometric assay. Briefly, Superoxide-dismutase activity in the erythrocyte lysate was determined by generating superoxide radicals by the photochemical reduction of phenazine methosulphate, which reduced nitro blue tetrazolium into a blue-colored compound, formazone. Superoxide-dismutase quenches free oxygen radicals and inhibited reduction of nitrobluetetrazolium, which was measured at 560 nm using colorimeter (6051 JENWAY designed and manufactured in the EU by Bar lowered scientific Ltd. Dunmow. Essex CM63LB, model 6051, voltage 230/115V, frequency 50/60 Hz and power 20VA) [36].

11. Reduced glutathione in liver tissue

Glutathione is a key antioxidant and is used as an indicator of the reduction capacity of the tissue. Glutathione was determined by the spectrophotometric method, which was based on the use of Ellman’s reagent. Results were expressed in micromoles of glutathione per gram tissue [37].

12. Determination of tissue nitric oxide

Nitrite concentration was measured in tissue samples as indicator of nitric oxide production using Griess reaction. Briefly, in acid medium and in the presence of nitrite, the formed nitrous acid diazotizes sulphanilamide and the product was coupled with N-(1–naphthyl) ethylenediamine. The resulting purple azo dye was measured at 540 nm.

13. Histo-pathological examination

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 (H&E) for light microscopic examination [38].

14. Statistical analysis

The quantitative data of continuous variables were expressed as mean ± S.E.M. Statistical significance was tested by one way analysis of variance (ANOVA) followed by *Bonferroni post hoc* test. All statistical tests were performed using the Statistical Package for Social Sciences, version 19 (SPSS Software, SPSS Inc., Chicago, USA), and the differences were considered significant when $P \leq 0.05$.

III. Results

1. Effect on body weight

The protein-malnourished rats showed a significant decrease in body weight compared with NF rats. In addition, body weights were compared among all groups on day 5- post CDDP administration (7.5 mg/kg). Current results showed that administration a single dose of CDDP caused a significant decrease in body weight by 6.6% in the NF rats and 7.6% in the PM rats when compared to corresponding control group. In contrast, administration of Sly, l-arginine and their combination for 5 days after CDDP administration significantly increased the body weight by 8.56%, 4.6% and 7.8% in the NF rats and by 6.6%, 6.2% and 8% in the PM rats, respectively, compared with administration of CDDP alone. Administration of Sly, l-arginine and their combination for 5 days and prior CDDP administration significantly increased the body weight by 8.5%, 8.84 and 7.26 in the NF rats and by 10.4%, 11.5% and 14.24 in the PM rats, respectively, compared with administration of CDDP alone *($P \leq 0.05$)* (Table 1).

2. Effect on liver ratio (relative liver weight to body weight)

The protein-malnourished rats showed a significant decrease in relative liver weight compared with the NF rats. Comparing liver ratio on day 5 post intra peritoneal injection of CDDP successfully induced liver toxicity as was evident by a significant increase ($P \leq 0.05$) in liver ratio by 57.5% in the NF rats and 80% in the PM rats compared with corresponding control group. Administration of Sly, l-arginine and their combination for 5 days after CDDP significantly decreased the liver ratio by 35.3%, 38.7% and 43.6% in the NF rats and by 44.7%, 41.5% and 40.1% in the PM rats, respectively, compared with administration of CDDP alone. Also, administration of Sly, l-arginine and their combination for 5 days and prior CDDP administration significantly decreased the liver ratio by 36.4%, 36.4% and 35.4% in the NF rats and by 35.14%, 37.2% and 44.1% in the PM rats, respectively, compared with administration of CDDP alone *($P \leq 0.05$)* (Table 2).

3. Effect on serum ALT and AST level

Serum ALT and AST levels were determined on days 5 after administration CDDP that induced hepatotoxicity. The CDDP-treated alone group showed a significant increase *($P \leq 0.05$)* in ALT and AST enzyme activity by (≈1 fold), in the NF and the PM rats, compared with corresponding control group. Administration of Sly, l-arginine and their combination for 5 days after CDDP significantly decreased in ALT serum levels by 42.1%, 38.4%, 46.3% and AST by 44.4%, 39.9%, 42.5% Also, their administration for 5 days before CDDP showed significantly decreased in ALT serum levels by 37.3%, 37.5%, 39.5% and AST by 39.8%, 42.5, 45.1% in the NF rats, respectively, compared with administration of CDDP alone. Interestingly, a regression in ALT and AST serum levels was observed in curative and protective regimen in the PM rats, yet remained higher than normal level (Fig. 1, 2).

4. Determination of serum albumin level

The administration of CDDP (7.5 mg/kg) resulted in a significant decrease of serum albumin level by 54.7% in the NF rats and by 61.3% in the PM rats, respectively, compared to corresponding control group. Individual treatment and the individual pretreatment with Sly, l-arginine and their combination significantly partially increased the serum albumin level by 49.1%, 60.8%, 85.4%, 55.3%, 86.5% and 111.7% in the NF rats and by 90.9%, 100%, 152.5%, 72.7%, 92.9% and 100% in PM rats compared to CDDP treated alone. Individual pretreatment with l-arginine, its combination with Sly in curative and protective group successfully normalized albumin level in the NF rats. Furthermore, the combination of l-arginine with Sly in curative group successfully normalized albumin level in the PM rats (Fig. 3).

5. Effect on lipid per-oxidation

To study the differential and the combined effect of Sly and l-arginine on lipid peroxidation induced by CDDP in the NF rats and PM rats, serum MDA levels were evaluated in different treatment groups. A significant ($P \leq 0.05$) increase in serum MDA levels were observed in CDDP-treated alone group in the NF rats and PM rats, compared to corresponding control group. The individual treatment as curative and protective with Sly, l-arginine or their combination blunted the mounted levels of MDA by 31.6%, 33.2% , 40.9%, 30.2%, 43.6 and 40.4% in the NF rats and by 23.8%, 31.75% , 36.5, 25.8%, 30.2% and 32.1% in the PM rats indicating a partial protection against lipid peroxidation in all treated groups (Fig. 4).

6. Effect on superoxide-dismutase

On the other hand, the activity of the anti-oxidant enzyme SOD significantly decreased in the erythrocytes lysate of CDDP treated-alone in NF and PM rats ($P \leq 0.05$) when compared to corresponding control group, indicating attenuation of anti-oxidative defense. The treatment with Sly, l-arginine, their combination after CDDP administration and the pretreatment with the same regimen prior to CDDP administration resulted in a significant increase in SOD by 30.9%, 28.3%, 49.7%, 45.8%, 39.9% and 46.3% in the NF rats and by 69.4%, 65.2%, 84.8%, 68%, 72.6% and 86.6% in the PM rats, respectively when compared to CDDP treated alone (Fig. 5).

7. Effect on reduced glutathione

Parallel to SOD activity, GSH level was found to be significantly ($P \leq 0.05$) deceased in the tissue sample of CDDP-treated alone rats when compared to corresponding control group. Individual treatment and pretreatment with Sly, l-arginine and their combination significantly up-regulated GSH levels by 64.3%, 43%, 74.2%, 58.8%, 53.6% and 69% in the NF rats and by 64.4%, 85.2%, 95.7%, 95.7%, 76.6%, 74.8% and 80.9% in the PM rats, respectively, compared with administration CDDP alone (Fig. 6).

8. Effect on tissue nitrite concentration

An effect of CDDP was observed on oxidative stress parameters. CDDP significantly elevated tissue nitrite concentration level in the NF rats and PM rats. The treatment with Sly, l-arginine and their combination for 5 consecutive days after and before CDDP administration resulted in a significant decreased in nitrite concentration by approximately 75.3%, 34.55, 64.4%, 69.2%, 52.4% and 66.6% in the NF rats and by 55.6%, 35.8%, 48.5%, 54.2%, 37.8% and 49.6% in the PM rats, respectively, compared with administration of CDDP alone. Interestingly, the curative and the protective effect of l-arginine revealed minimum regression in nitrite concentration yet remained higher than normal level in the NF rats and PM rats (Fig. 7).

9. Histopathological examination

Histopathological examination revealed a normal liver histology in the control group (Fig. 8 a), in the NF rats but showed fatty changes in the hepatocytes of the control group (Fig. 9 a), in the PM rats. But in the histo-pathological examination of the liver tissue on day 6 after CDDP injection revealed severe mononuclear cellular infiltration around the congested blood vessels and different degenerative changes in hepatocytes and loss of hepatocytes (Fig. 8 b), in the NF rats and the liver of CDDP treated group rat of the PM set showed sever mononuclear cellular infiltration around the congested blood vessels and band of fibrous tissue scattered fatty changes in the hepatocytes, (Fig. 9 b).

In addition, the individual treatment as curative and protective with Sly reduced mononuclear cellular infiltration, congestion blood vessels, degenerative changes in hepatocytes and fatty changes to less extent than the treatment with CDDP alone in the NF and PM rats, (Fig. 8 c, 8f; Fig. 9 c, 9 f) respectively.

Interestingly, liver sections of rats in the groups of individual treatment and pretreatment with l-arginine after CDDP administration showed that reduction mononuclear cellular infiltration, congestion blood vessels, degenerative changes in hepatocytes and fatty changes to lesser extent than the treatment with CDDP alone but showed marked dilatation in the sinusoids in the NF and PM rats, (Fig. 8 d, 8 g; Fig. 9 d, 9 g) respectively.

Furthermore, the combination treatment as curative and protective with l-arginine and Sly after CDDP administration showed nearly the same histological profiles as in the control group with marked sinusoidal dilation in the NF and PM rats, (Fig. 8 e, 8 h; Fig. 9 e, 9 h), respectively.

IV. Discussion

Nutritional stress in the form of deficient protein condition is quite prevalent in developing countries [39]. Protein-deficient diet intake has been shown to influence the activity of drug metabolizing enzymes as well as antioxidant enzymes [40].

In this study, a model of protein malnutrition (PM) was successfully established by feeding rats a low protein diet (5%) according to the study of [41,42] where the PM rats showed a significant decrease in relative

liver weight compared to NF rats. These results are in agreement with Bozzetti study. who reported that the growth rate during PM was significantly lower than that during normal conditions [43]. The decrease in body weight observed in the PM rats may be due to a reduction in the levels of protein and food consumption [44]. Moreover, the present study revealed that the PM rats showed a significant increase in serum ALT and AST levels compared to the NF rats. These results supported by the study of Obima [45]. The elevation in serum AST and ALT levels due to PM may be caused by up regulation of the tumor 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 [46]. This study showed that a significant increase in serum MDA and reduction in SOD, GSH and albumin levels were observed in the PM rats compared to the NF rats. These findings are in accordance with those of the studies of [47,48]. These findings suggest an altered protein and antioxidant status in protein-energy malnutrition.

Cisplatin can induce hepatotoxicity after having administered at high doses [49,50]. To better understand the mechanism by which CDDP succeeded in the induction of hepatotoxicity, we analyzed serum levels liver enzymes and oxidative stress markers.

In the present study, CDDP-treated NF and PM rats induced liver impairment was evidenced by an increase in serum ALT, AST, MDA and tissue nitrite concentrations level and in relative liver weight accompanied with significant decrease in serum albumin levels, GSH and SOD levels compared to corresponding control group. These results are in agreement with the study reported by Naqshbandi et al [51]. This observation is consistent with a recent report by Yadav, (2015) that showed CDDP administration induced significant increase in serum ALT, AST and significant decrease in serum total protein, and albumin levels [30]. The ability of CDDP to cause alterations in the activity of these enzymes could be a secondary event following CDDP-induced liver damage with the consequent leakage from hepatocytes. It has been shown previously that oxidative stress due to the formation of free radicals is one of the pathogenic mechanisms underlying the adverse effects of CDDP in the liver [56]. 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. Moreover, depletion of GSH may contribute to CDDP-induced lipid peroxidation. Thus, an alteration in the enzymatic antioxidant status accompanied by an increase in lipid peroxidation and tissue nitrite concentrations level indicates that the enzymes play an important role in combating free radical-induced oxidative stress in tissues [58].

L-arginine is an essential amino acid that has numerous functions in the body and plays an important role in cell division, healing of wounds and in immune function [59,60]. Results of the present study showed that the weight loss by CDDP could be improved by l-arginine treatment in the NF and PM rats. In harmony with our results, Saleh & El-Demerdash, (2005) reported that l-arginine ameliorates weight loss induced by CDDP in male rats. Also, l-arginine may diminish the weight loss induced by obstructive jaundice in male rat and the increased in the body weight was attributed to modulation of immune function [61], an effect that was enhanced by combination with Sly. In the current study, Sly, l-arginine and their combination-treated rats exhibited a significantly increased hepatic antioxidant activity (SOD and GSH levels), serum albumin levels compared to CDDP group in the NF and PM rats. Moreover, the serum indices of hepatotoxicity (ALT, AST), and lipid peroxidation level were significantly decreased compared to CDDP group in the NF rats and PM rats. In the line with our results Moustafa & Badria, (2010) who reported that l-arginine was significantly decreased serum levels of ALT, AST, lipid peroxidation and OH radicals when compared to hepatotoxic group, due to their antioxidant effect and their ability to act as a free radical scavenger and showed that severe decrease in albumin by CDDP injection tended to be normalized by the effect of l-arginine. It has been shown previously that l-arginine treatment have anti-oxidant effect in carbon tetrachloride-induced (CCl4) hepatic injury in mice [62].The current work was supported by the results of [26], in which l-arginine can ameliorate the kidney dysfunction and that was related to the improvement of the functional status of the liver . In addition to, l-arginine administration resulted in reversed alterations in antioxidant defense system components in liver from rats with low resistance to hypoxia [63].

Interestingly, although l-arginine is an NO donor, It was showed a significant decreased in nitrite concentration compared to CDDP treated group in the NF and PM rats that it is attributed to NO inhibits inducible nitric oxide synthase (iNOS) own gene expression, at the level of transcription, by down-regulating NF-KB expression in rat hepatocytes and primary human hepatocyte iNOS mRNA, protein, and NO release were markedly suppressed in the presence of either endogenously produced NO or an NO donor as levels of NO increase, feedback regulation begins and NO negatively modulates NF-KB DNA binding activity and enzyme activity to tightly regulate the amount of NO produced. This data identifies a novel negative feedback loop whereby NO down-regulates iNOS gene expression possibly to limit over production during pathophysiologic conditions [68]. This would explain why l-arginine revealed minimum regression in nitrite concentration yet remained higher than normal level in the NF rats and PM rats.

Some researches about the role of NO in the regulation of hepatic microcirculation have shown that the l-arginine-derived iNOS activation supports vasodilatation and inhibition of platelet functions [64,65].

The effect of Sly is consistent with a recent report by Ezzeldin et al., (2014) who reported that Sly increased the activities of antioxidant enzymes, albeit to different extents, when accompanied by CDDP administration. These results indicate a marked protection afforded by natural products against CDDP-induced oxidative damage to liver tissues. Furthermore, Sly has the ability to scavenge free radicals and ROS and of strengthening the antioxidant system [66]. These results were also in agreement with those of study of [67], who reported that the protective action of Sly is associated with its anti-oxidant properties.

According to pathological finding in this study the combination of Sly and l-arginine further significant reduction in the total histo-pathological changes and was confirmed by normalization of all previously mentioned changes and resuming normal histological architecture with sinusoidal dilatation in the NF and PM rats, respectively. These marked sinusoidal dilatation due to NO vasodilatation effect [68] indicating the enhancing effect of l-arginine on anti-hepato-toxicity activities of Sly. In harmony with our results, Nahavandi et al. was found that histo-pathological changes were partially but significantly prevented by exogenous l-arginine in obstructive jaundiced rats where l-arginine played a protective role by inducing iNOS expression and NO synthesis [69].

V. Figures And Tables

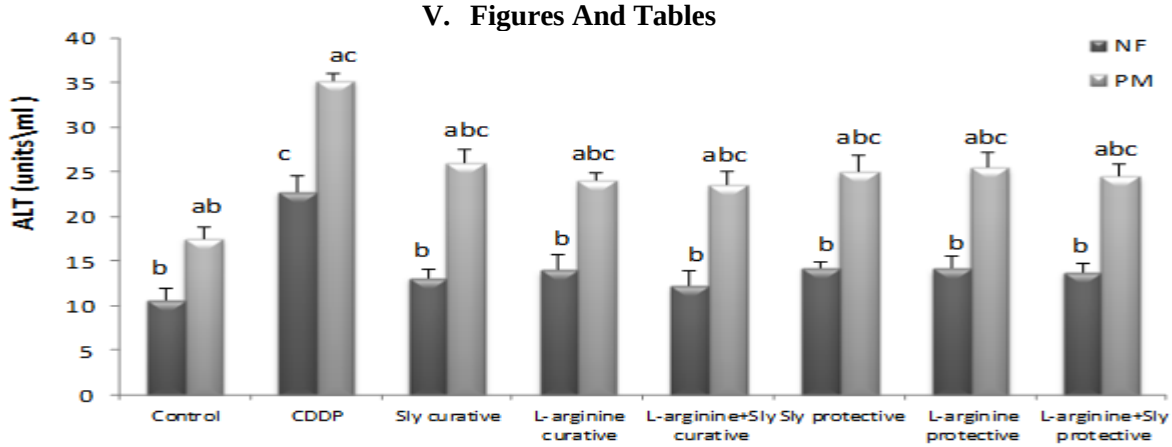

Figure 1: Effect of administration of silymarin (100mg/kg), l-arginine (200mg/kg) and their combination on ALT enzyme activity in cisplatin-induced liver toxicity in normally fed and in protein malnourished rats. ALT serum levels of rats were measured and expressed as mean ± S.E.M. All data were analyzed using ANOVA followed by *Bonferroni post hock* test. a P≤0.05 with respect to corresponding normally fed group, b P≤0.05 with respect to corresponding cisplatin treated group, c P≤0.05 with respect to corresponding control group.

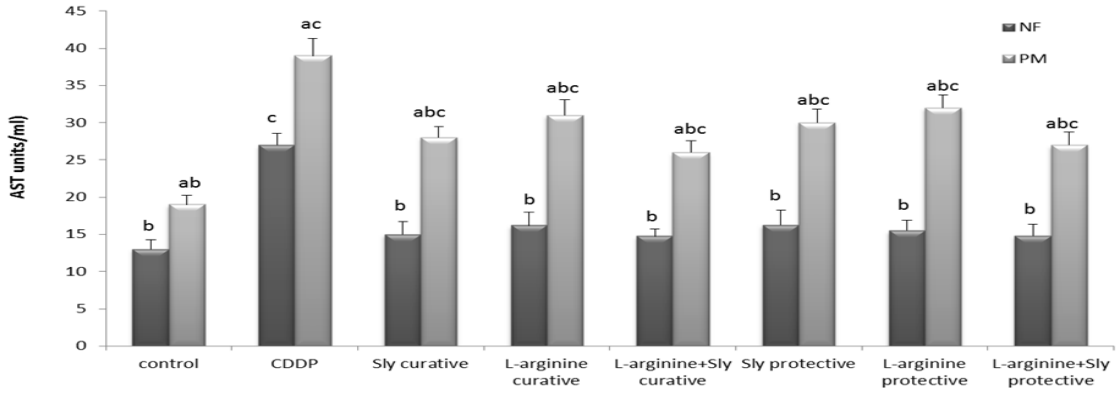

Figure 2: Effect of administration of silymarin (100mg/kg), l-arginine (200mg/kg) and their combination on AST enzyme activity in cisplatin-induced liver toxicity in normally fed and in protein malnourished rats. AST serum levels of rats were measured and expressed as mean ± S.E.M. All data were analyzed using ANOVA followed by *Bonferroni post hock* test. a P≤0.05 with respect to corresponding normally fed group, b P≤0.05 with respect to corresponding cisplatin treated group, c P≤0.05 with respect to corresponding control group.

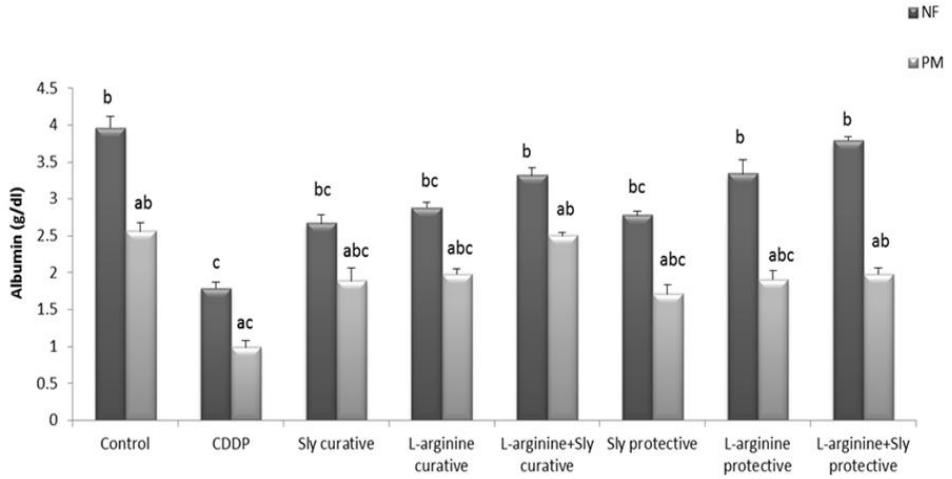

Figure 3: Effect of administration of silymarin (100mg/kg), l-arginine (200mg/kg) and their combination on the albumin levels in cisplatin-induced liver toxicity in normally fed and in protein malnourished rats were evaluated spectrophotometrically. Values are expressed as mean ± S.E.M. All data were analyzed using ANOVA followed by *Bonferroni post hock* test. a P≤0.05 with respect to corresponding normally fed group, b P≤0.05 with respect to corresponding cisplatin treated group, c P≤0.05 with respect to corresponding control group (n=8).

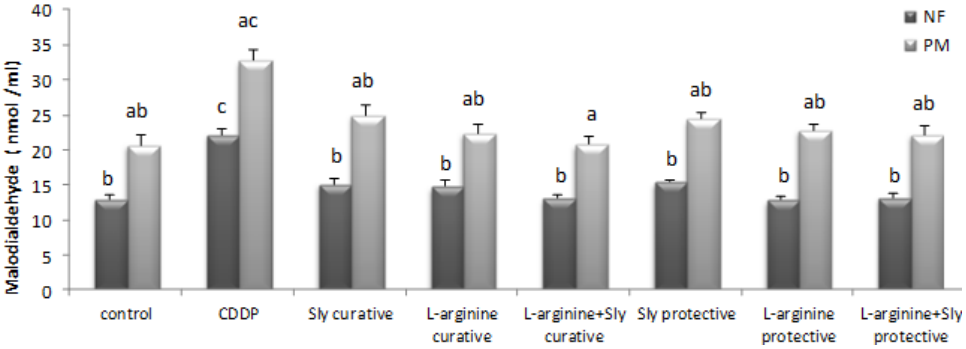

Figure 4: Effect of administration of silymarin (100mg/kg), l-arginine (200mg/kg) and their combination on lipid peroxidation in cisplatin-induced liver toxicity in normally fed and in protein malnourished rats. Serum levels of malondialdehyde as thiobarbituric acid-reactive substances were measured spectrophotometrically and expressed as mean ± S.E.M. All data were analyzed using ANOVA followed by *Bonferroni post hock* test. a P≤0.05 with respect to corresponding normally fed group, b P≤0.05 with respect to corresponding cisplatin treated group, c P≤0.05 with respect to corresponding control group (n=8).

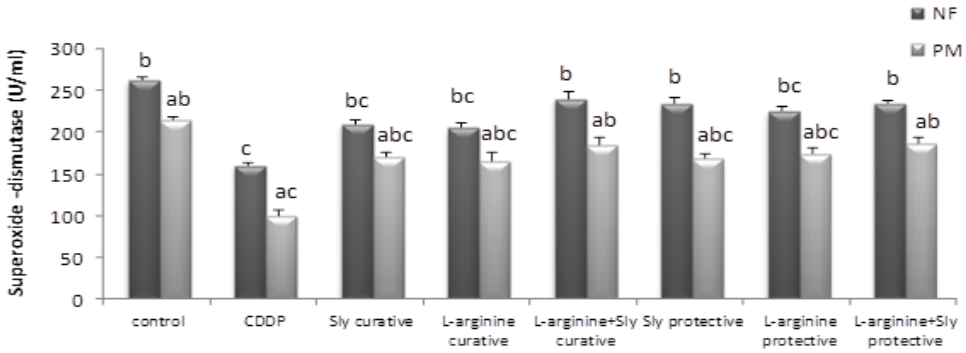

Figure 5: Effect of administration of silymarin (100mg/kg), l-arginine (200mg/kg) and their combination on the evaluation of enzymatic antioxidant (superoxide-dismutase) in cisplatin-induced liver toxicity in normally fed and in protein malnourished rats were evaluated spectrophotometrically and expressed as mean ± S.E.M. All data were analyzed using ANOVA followed by *Bonferroni post hock* test. a P≤0.05 with respect to corresponding normally fed group, b P≤0.05 with respect to corresponding cisplatin treated group, c P≤0.05 with respect to corresponding control group (n=8).

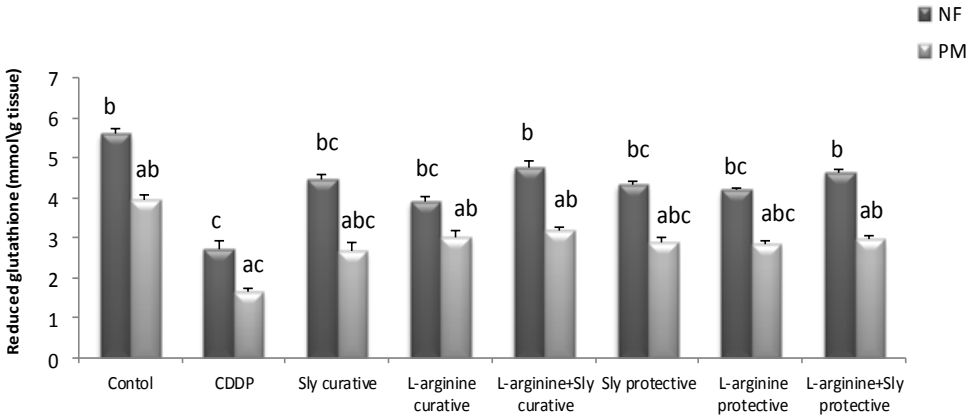

Figure 6: Effect of administration of silymarin (100mg/kg), l-arginine (200mg/kg) and their combination on the evaluation of non-enzymatic antioxidants (reduced glutathione) in cisplatin-induced liver toxicity in normally fed and in protein malnourished rats were evaluated spectrophotometrically. Values are expressed as mean ± S.E.M. All data were analyzed using ANOVA followed by *Bonferroni post hock* test. a P≤0.05 with respect to corresponding normally fed group, b P≤0.05 with respect to corresponding cisplatin treated group, c P≤0.05 with respect to corresponding control group (n=8).

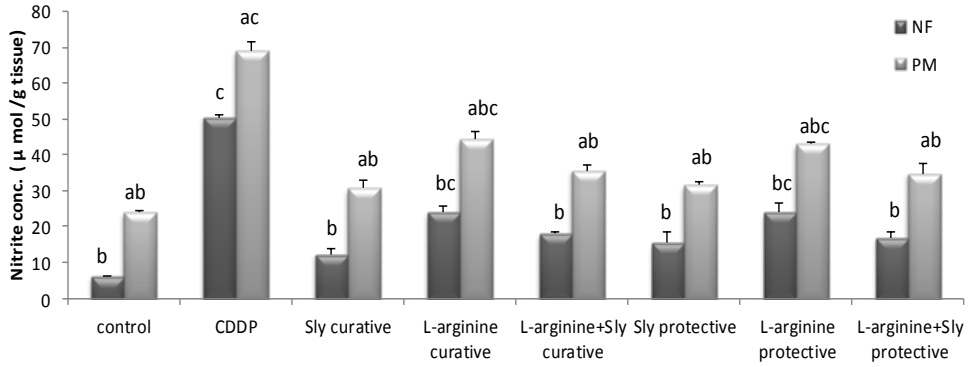

Figure 7: Effect of administration of silymarin (100mg/kg), l-arginine (200mg/kg) and their combination on the nitrite concentrations in cisplatin-induced liver toxicity in normally fed and in protein malnourished rats were evaluated spectrophotometrically. Values are expressed as mean ± S.E.M. All data were analyzed using ANOVA followed by *Bonferroni post hock* test. a P≤0.05 with respect to corresponding normally fed group, b P≤0.05 with respect to corresponding cisplatin treated group, c P≤0.05 with respect to corresponding control group (n=8).

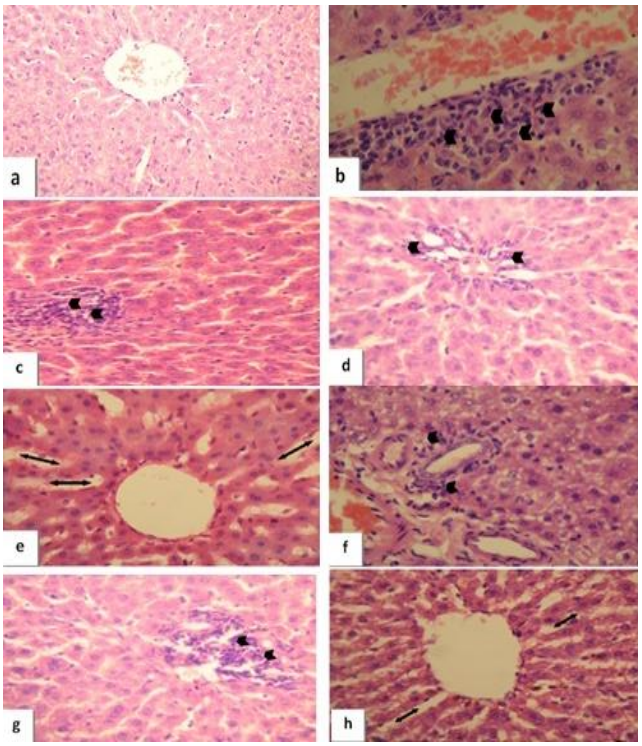

Figure 8: Photomicrographs represent changes in the liver of cisplatin induced hepatotoxicity in the NF set. (a) Control, (b) Cisplatin, (c) Silymarin curative, (d) L-arginine curative, (e) Silymarin and L-arginine curative , (f) Silymarin protective, (g) L-arginine protective, (h) Silymarin and L-arginine protective. Histological examination using H&E staining of the liver showed significant decrease in the histo-pathological alteration in comparison to cisplatin treated group (head arrow for and infiltration and double head arrow for vasodilatation) (X400) (n=8).

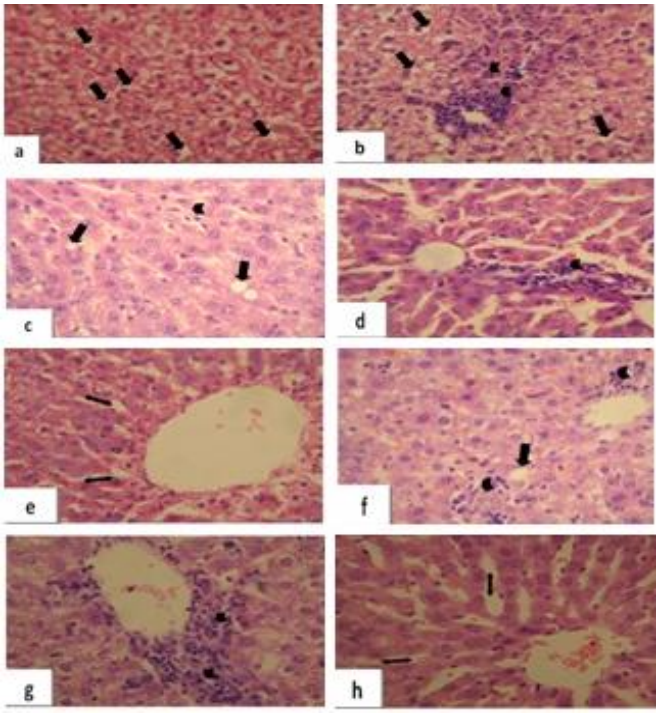

Figure 9: Photomicrographs represent changes in the liver of cisplatin induced hepatotoxicity in the PM set. (a) Control, (b) Cisplatin, (c) Silymarin curative, (d) L-arginine curative, (e) Silymarin and L-arginine curative , (f) Silymarin protective, (g) L-arginine protective, (h) Silymarin and L-arginine protective. Histological examination using H&E staining of the liver showed significant decrease in the histo-pathological alteration in

comparison to cisplatin treated group (head arrow for and infiltration, double head arrow for vasodilatation and arrow for fatty changes) (X400) (n=8).

Table 1. Effect of administration of silymarin (100mg/kg), l-arginine (200mg/kg)and their combination for 5 days on body weight of cisplatin-induced liver toxicity in normally fed and in protein malnourished rats. Body weight of rats were measured done on day 5 after the induction of liver toxicity expressed as mean ± S.E.M. All data were analyzed using ANOVA followed by *Bonferroni post hock* test. a P≤0.05 with respect to corresponding normally fed group, b P≤0.05 with respect to corresponding cisplatin treated group, c P≤0.05 with respect to corresponding control group.

body weight		
Groups	**NF**	**PM**
Control	110.5±2.42^{b}	97.25±3.45^{ab}
Cisplatin	103.2±4.75^{C}	89.85±3.58^{aC}
Silymarin curative	112.04±3.96^{b}	95. 8±3.83^{ab}
L-arginine curative	108.05±4.73^{b}	95.44±4.64^{ab}
L-arginine+ Silymarin curative	111.35±3.67^{b}	97.08±3.45^{ab}
Sly protective	112±4.34^{b}	99.2±4.94^{ab}
L-arginine protective	112.33±3.55^{b}	100.25±3.52^{ab}
L-arginine+ Silymarin protective	110.7±4.25^{b}	102.65±4.44^{ab}

Table 2. Effect of administration of silymarin(100mg/kg), l-arginine(200mg/kg) and their combination for 5 days on liver ratio of cisplatin-induced liver toxicity in normally fed and in protein malnourished rats. Liver ratio of rats were measured and expressed as mean ± S.E.M. All data were analyzed using ANOVA followed by *Bonferroni post hock* test. a P≤0.05 with respect to corresponding normally fed group, b P≤0.05 with respect to corresponding cisplatin treated group, c P≤0.05 with respect to corresponding control group.

liver ratio		
Groups	**NF**	**PM**
Control	4.03±0.13^{b}	1.75±0.05^{ab}
Cisplatin	6.37±0.25^{c}	3.51±0.05^{ac}
Silymarin curative	4.12±0.31^{b}	1.94±0.10^{ab}
L-arginine curative	3.9±0. 2^{b}	2.05±0.10^{ab}
L-arginine+ Silymarin curative	3.5±0.05^{b}	2.10±0.35^{ab}
Silymarin protective	4.05±0.75^{b}	2.28±0.14^{ab}
L-arginine protective	4.05±0.15^{b}	2.20±0.08^{ab}
L-arginine+ Silymarin protective	4.11±0.52^{b}	1.96±0.09^{ab}

VI. Conclusions

We concluded that CDDP elicited harmful hepatotoxic effects may cause severe damage to the plasma membrane, mitochondria and other organelles by suppressing the antioxidant defense mechanism. These effects were potentiated by PM; however, they were improved by l-arginine and Sly. Thus, the results of the present study support the hypothesis that treatment and pre-treatment with l-arginine, Sly and their combination is effective in maximizing the clinical use of CDDP in the treatment of various malignancies, by minimizing its hepatotoxicity. Current data propose l-arginine as a potential inhibitor of CDDP-induced hepatotoxicity.

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