Hepatoprotective Effect of Curcumin and Vitamin C against Cisplatin Induced Oxidative Stress and Toxicity in Albino Rats

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Abstract: Curcumin, a biologically active compound from turmeric and vitamin C act as a natural antioxidant and potent chemopreventive agent. The objective of the study was to investigate whether the combined pretreatment with curcumin and vitamin C offers more beneficial effects than that provided by either of them alone in reversing cisplatin (Cis) - induced hepatotoxicity. This was done through studying the effects of cisplatin and its combination with either curcumin or vitamin C and their combinations on some liver function parameters like liver enzymes, total protein, albumin, and globulin as well as antioxidant enzymes (SOD, CAT, GSH and MDA) in liver homogenates. For this purpose, 90 male Wistar albino rats were divided into nine groups (n = 10). The 1st (normal control group) received distilled water. The 2nd (Saline group) injected interperitoneal with physiological saline. The 3rd (Gum acacia group). The 4th (Curcumin treated group), rats were given curcumin (20 mg/kg b.wt). The 5th (Vitamin C treated group), received vit. C (100 mg/kg b.wt). The 6th (Cisplatin treated group), rats were intraperitoneal (i.p.) injected with cisplatin(0.4 mg/kg body weight b.wt). The 7th (Cis + vitamin C treated group), The 8th (Cis + Cur treated group) and The 9th (Cis + vitamin C + Cur treated group), rats were pre-treated with a single dose of vitamin C (100 mg/kg b.wt), curcumin (20 mg/kg b.wt) and combined vitamin C with curcumin, respectively, for 20 minutes prior the administration of cisplatin. After 60 days of first injection, blood samples and liver specimens were collected. Liver function parameters and antioxidant enzymes were investigated. Results showed Cisplatin revealed a significant increase of hepatic malondialdehyde (MDA) levels and a significant reduction of hepatic superoxide dismutase (SOD), catalase activities and GSH level compared to the saline group. It elicited a marked increase of the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels and decreasing in total protein, albumin and globulins levels. Pre-treatment with combined curcumin and vitamin C improved the liver enzymes, lipid peroxidation and antioxidant biomarkers.
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Keywords: Cisplatin, Oxidative stress, Curcumin, vitamin C, Hepatotoxicity
Abbreviations: Cis, cisplatin; Cur, curcumin; Vit.C, vitamin C; AST, aspartate transaminase; ALT, alanine transaminase; MDA, Malondialdhyde enzyme; SOD, Superoxide dismutase; CAT, Catalase; GSH, Glutathione reduced.

1. Introduction

Cisplatin, (*cis*-diammine-dichloroplatinum II, CP) a platinum co-ordinate complex, is a widely used antineoplastic agent for the treatment of metastatic tumors of the testis, metastatic ovarian tumors, lung cancer, advanced bladder cancer and many other solid tumors ***(Sweetman, 2002)***. The cytotoxic action of the drug is often thought to be associated with its ability to bind DNA to form cisplatin–DNA adducts ***(Goldstein and Mayor, 1983).***

In spite of its beneficial antitumor action, it has serious side effects on nontumor cells, including free radical generation ***(Masuda et al., 1994).*** In addition, dose related nephrotoxicity and hepatotoxicity limits its application in clinical oncology ***(Antonio et al., 2002).*** Cisplatin-induced liver injury and its mechanism in causing hepatotoxicity not fully understanding. There is a suggestion that the drug accumulates in significant amounts, in hepatic tissue particularly when injected in high doses ***(Liu et al., 1998)***. Although the mechanism of cisplatin-induced adverse effect is still unclear, however several evidences have shown that its hepatotoxicity is believed via reactive oxygen species (ROS) generation-mediated oxidative stress dependent mechanism (***Liao et al., 2008)***.

To prevent oxidative stress, there are several molecules that play a role to scavenge ROS called antioxidant which derived from both exogenous and endogenous sources.

Medicinal plants are good sources of exogenous antioxidants which might be considered as the new alternative approach to ameliorate pathological alterations in oxidative stress-related pathology. Curcumin (CUR) [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6 heptadiene-3, 5-dione (diferuloylmethane)] is a dietary antioxidant derived from turmeric (*Curcuma longa*, *Zingiberaceae*), it has

been demonstrated to possess antioxidant activity *in vivo* ***(Khopde et al., 1999)***. It has been demonstrated to prevent several pathologies related to oxidative damage such as the inorganic arsenic-induced hepatotoxicity ***(Gao et al., 2013)***. We hypothesized that treatment with combined curcumin and vitamin C may have higher antioxidant ability than treatment with curcumin or vitamin C alone.

Vitamin C (Ascorbic acid) is the most important vitamin in fruits and vegetables, and has been regarded as the most potent natural antioxidant. Although most of the higher animals can synthesize vitamin C in their liver or kidneys, in humans; the terminal enzyme in its synthetic pathway is absent and thus, vitamin C has become an essential dietary component for human survival ***(Vissers et al., 2011)***. Vitamin C can directly metabolize reactive oxygen species. Pathogenic dysfunction of tissues owing to cell death via apoptosis is one of the important outcomes of oxidative stress that could be diminished by vitamin C ***(Santos et al., 2009)***.

There are multitudes of reports available on the protective e□ects of curcumin, vitamin C individually against various xenobiotics induced oxidative stress in experimental animals. Still to date the reports are scanty regarding the combined alleviated e□cacy of curcumin in combination with vitamin C on cisplatin induced hepatotoxicity in rats. As well as there are some controversies over the combined administration of curcumin in combination with vitamin C. In view of the above considerations, the present study was designed to evaluate the protective e□cacy of curcumin in combination with vitamin C on cisplatin induced oxidative damages in the liver of rats.

2. Materials and Methods

2.1. Drugs

2.1.1 cisplatin (cis-diamminedichloroplatinum II):

Cisplatin (Cis) represents a class of antineoplastic drug containing a heavy metal, platinum. The drug was purchased from the local pharmacy. It is manufactured by EIMC United Pharmaceuticals Egypt. Each vial contains 50mg/50ml, vial were then dissolved in physiological saline (0.9 % sodium chloride), then the drug was injected interaperitoneal in a dose level of (0.4 mg/kg b.wt) was selected on the basis of literature ***(Pratibaha et al, 2006)***. The dose administration was as follow: (0.2mg of cisplatin was equivalent to 1 ml of prepared solution).

2.1.2 Vitamin C:

(vitacid C) simply ascorbate (the anion of ascorbic acid), is an essential nutrient for humans and certain other animal species. Effervescent tablets Single vitamin (ascorbic acid) was purchased from the local pharmacy. It is manufactured by CID Company. Each tablet contains 1g ascorbic acid. Each tablets was then dissolved in 100 ml distilled water then the drug was given orally in a dose level of (100mg/kg b. wt.) was selected on the basis of literature ***(Rana and Ahmad, 2012)***. The dose administration was as follow: (10mg of vitamin C was equivalent to 1 ml of prepared solution).

2.2. Plants extract:

Curcuma longa **extract (curcumin)** was purchased from National Bio Lab (Medical laboratory) 15El Nour St, Floor 1, Dokki, Giza Egypt. Curcumin was suspended in 0.05% gum acacia solution then it was given orally at dose level of (20 mg/kg b.wt) was selected on the basis of literature ***(Xu et al., 2007)***. The dose administration was as follow: (2.5mg of curcumin was equivalent to 1 ml of prepared solution).

2.3. Animals

The present study was carried out at Zoology Department, Faculty of Science - Zagazig University, using (ninety) 90 clinically healthy mature adult male albino rats (*Rattus norvegicus*). The animals were obtained from the Animal House of Faculty of Veterinary Medicine, Zagazig University, Their weights ranged from 200-250gm each.

The animals were housed in standard conditions, where the animals were housed in metal cages and bedded with wood shavings and kept under standard laboratory conditions of aeration and room temperature at about 25^{o}C. The animals were allowed to free access of standard diet and water *ad libitum*. The animals were accommodated to the laboratory conditions for two weeks before being experimented.

2.4. Experimental design

After the period of acclimation, animals were divided into nine groups with 10 animals in each as:

I) The 1st normal Control group:

Animals received distilled water orally daily for 60 successive days.

II) The 2nd control group:

Animals were received 0.05% gum acacia solution orally daily for 60 successive days using metallic stomach tube.

III) The 3rd control group:

Animals were injected interaperitoneal with physiological saline (0.9 % sodium chloride) for 60 successive days using metallic stomach tube.

IV) The 4th curcumin treated group:

Animals were received curcumin orally at a dose of (20mg/kg) suspended in.0.05% gum acacia solution for 60 successive days using metallic stomach tube.

V) The 5th vitamin c treated group:

Animals were given orally single dose of vitamin c (100mg/Kg) dissolved in distilled water for 60 successive days using metallic stomach tube.

VI) The 6th cisplatin treated group:

Animals were Injected interaperitoneal with a single dose of (0.4 mg /kg) cisplatin dissolved in physiological saline for 60 successive days**.**

VII) The 7th cisplatin + curcumin treated group:

Animals were received curcumin (20 mg/kg) and after 20 minute animals were Injected interaperitonealy with a single dose of (0.4 mg/kg) cisplatin dissolved in physiological saline for 60 successive days.

VIII) The 8th cisplatin + vitamin C treated group:

Animals were received vitamin C (100 mg/kg) and after 20 minutes animals were Injected interaperitonealy with a single dose of (0.4 mg/kg) cisplatin dissolved in physiological saline for 60 successive days.

XI) The 9th cisplatin + curcumin + vitamin c treated group:

Animals were received curcumin (20 mg/kg) as well as vitamin C (100 mg/kg) and after 20 minute animals were Injected interaperitonealy with a single dose of (0.4 mg/kg) cisplatin dissolved in physiological saline for 60 successive days.

2.5. Biochemical Assays

Blood samples were collected after the end of the experiment from the retro-orbital vein, which is a simple, convenient and successful procedure that allows bleeding of the same animal more than one time with minimal stress ***(Scherners, 1967)***. After the last administration of the drug at the end of 8th week, individual blood samples were drawn by orbital puncture (from eye plexus) using microhematocrit capillary tubes (Lancer, Athy, County-Kildare, Republic of Ireland), Serum was harvested from blood without EDTA and then serum samples were transferred into Eppendorf tubes and subsequently used for the determination of (Aspartate amino transferase (AST), Alanine amino transferase (ALT) Albumin, Globulin and Total protein).

2.5.1 Determination of serum Aminotransferase enzymes activities:

Activities of AST and ALT in the serum were determined colorimetrically by using bio Merieux kit (France), using method adopted by **(*Reitman and Frankel, 1957)***.

2.5.2 Determination of serum albumin:

Albumin estimation depends on the dye binding, as an essential method and according to ***(Doumas and Watson, 1971)***. The method depends on using citrate buffer and reading the final color that indicate albumin content in serum. The kits were obtained from Stanbio Laboratory Inc., (USA).

2.5.3 Determination of total serum globulin:

Serum globulin concentration was calculated by subtracting serum albumin concentration from serum total protein ***(Oser, 1971).***

2.5.4 Determination of serum total protein concentration:

Serum total proteins were determined by Biuret method, using the Diamond kit ***(Henry, 1974).*** According to this method, protein forms a colored complex with cupric ions in an alkaline medium.

2.6. Preparation of Tissue Homogenate:

The remainder tissues of liver were used for the analysis of oxidative stress parameters. They were washed with saline and distal water for the removal of blood, and later the fatty parts were removed and blotted over a piece of filter paper. Prior to dissection, tissue was perfused with a 50 mM (sodium phosphate buffer saline (100 mM Na_2HPO_4 / NaH_2PO_4) (pH 7.4) in an Ice containing medium containing 0.16 mg / ml heparin or containing 0.1 mM ethylene diamine tetra acetic acid (EDTA)to remove any red blood cells and clots. Then tissues were homogenized in 5 – 10 ml cold buffer per gram tissue and Centrifuged at 5000 r.p.m for ½ hours. The resulting supernatant was transferred into Eppendorf tubes, and preserved at (-80) C in a deep freezer until used for various biochemical Assays ***(Habig et al., 1974)***.

2.6.1 Determination of Lipid peroxide (Malondialdhyde) activity:

Malondialdehyde (MDA) was determined by using Biodiagnostic kit (Biodiagnostic Company, Dokki, Giza, Egypt), according to the method of ***Satoh, (1978)*** **and** ***Ohkawa et al., (1979).***

2.6.2 Determination of Catalase activity:

Catalase (CAT) activity was determined by biodiagnostic kit method (Biodiagnositc Company, Dokki, Giza, Egypt), according to the method of **Aebi, (1984).**

2.6.3 Determination of reduced glutathione (GSH).

Reduced glutathione was determined in tissue supernatant by colorimetric method using biodiagnostic kit, according to **Beutler *et al.* (1963).**

2.6.4 Determination of Superoxide dismutase activity:

Superoxide dismutase (SOD) activity was determined by biodiagnostic kit (Biodiagnositc Company, Dokki, Giza, Egypt), according to the method of *Nishikimi,* ***et al., (1972).***

2.7. Statistical analysis

Data were collected, arranged and reported as mean ± standard error of mean (S.E.M) of nine groups (Each group was considered as one experimental unit), summarized and then analyzed using the computer program SPSS/ version 15.0) The statistical method was one way analyzes of variance ANOVA test (F-test), and if significant differences between means were found, Duncan's multiple range test (Whose significant level was defined as (P<0.05) was used according to ***Snedecor and Cochran, (1982)*** to estimate the effect of different treated groups.

3. Results

3.1. Effect of Cisplatin, Curcumin, Vitamin C and their combinations on ALT:

Serum alanine transferases (ALT) levels were markedly elevated after 8th week post cisplatin administration to normal rats when compared with saline treated group and other control groups. Meanwhile, significant decrease in serum ALT level as compared with cisplatin treated group was recorded in normal rats in response to administration of curcumin and vitamin C and their combinations. Whereas cisplatin plus curcumin and vitamin C treated group afforded the best safer treatment comparing with cisplatin plus curcumin and cisplatin plus vitamin C treated groups as it showed no significant changes in serum ALT level as shown in table (1) and fig (1).

3.2. Effect of Cisplatin, Curcumin, Vitamin C and their combinations on AST:

Table (1) and Fig (1) showed that Cisplatin treated group afforded highly significant increase in AST level as compared with saline treated group and other normal control groups. Meanwhile, Cisplatin plus curcumin and cisplatin plus vitamin C treated groups elucidate significant decrease in AST level as compared with Cisplatin treated group. At the same time, Cisplatin plus curcumin and Vitamin C treated group elucidate non significant change in AST level as compared with normal control group.

A significant decrease in AST level was recorded in vitamin C treated group as compared with normal control group, at the same time curcumin treated group afforded non significant decrease as compared also with control group.

3.3. Effect of Cisplatin, Curcumin, Vitamin C and their combinations on Albumin:

Cisplatin treated group afforded a significant decrease in albumin level as compared with saline group and other control groups. Meanwhile, Cisplatin plus curcumin, cisplatin plus vitamin C treated groups elucidate significant increase in albumin level as compared with Cisplatin treated group only while they also, afforded significant decrease in total protein level as compared with normal control group, but the effect was much less intense as compared with cisplatin treated group. At the same time, Cisplatin plus curcumin and vitaminC treated groups elucidate non significant change in albumin level as compared with saline control group as shown in table (1) and Fig (2).

3.4. Effect of Cisplatin, Curcumin, Vitamin C and their combinations on Globulin:

Cisplatin treated group exhibited significant decrease in serum globulin level as compared with saline treated group and other control groups. Meanwhile, Cisplatin plus curcumin treated group elucidate significant increase in globulin level as compared with Cisplatin treated group only while it also, afforded significant decrease in globulin level as compared with normal control group, but the effect was much ameliorated as compared with cisplatin treated group Table (1) and Fig (2). At the same time, Cisplatin plus curcumin and vitamin C treated group elucidate non significant decrease in globulin level as compared with normal control group.

3.5. Effect of Cisplatin, Curcumin, Vitamin C and their combinations on total protein:

Table (1) and Fig (2) demonstrated that treatment of normal rats with cisplatin exhibited a marked decrease ($P<0.05$) in total protein level after the end of the experiment when compared with saline treated group and other control groups. Meanwhile, Cisplatin plus curcumin and cisplatin plus vitamin C treated groups elucidate significant increase in total protein level as compared with Cisplatin treated group only while they also, afforded significant decrease in total protein level as compared with normal control group, but the effect was much less intense as compared with cisplatin treated group. At the same time, cispatin plus vitamin C and Cisplatin plus curcumin and vitamin C treated groups elucidate non significant decrease in total protein level as compared with normal control group.

3.6. Effect on Antioxidant enzymes:

3.6.1 Effect of cisplatin, curcumin, vitamin C and their combinations on (MDA) activity:

The MDA content of the liver was significantly elevated ($P<0.05$) in response to treatment of normal male rats with cisplatin for 8 weeks compared with saline treated group and other control groups. Meanwhile, Cisplatin plus curcumin and cisplatin plus vitamin C treated groups elucidate significant decrease in MDA level as compared with Cisplatin treated group only while they also, afforded significant increase in MDA level as compared with normal control group, but the effect was much less intense as compared with cisplatin treated group. At the same time, Cisplatin plus curcumin and Vitamin C treated group elucidate non significant change in MDA level as compared with normal control –groups as shown in table (2) and fig (3).

3.6.2 Effect of cisplatin, curcumin, vitamin C and their combinations on Catalase (CAT) activity:

Liver catalase level was highly decreased after 8thweek post cisplatin administration to normal rats when compared with saline treated group and other control groups. Meanwhile, group treated with vitamin C afforded the highly significant increase value of CAT level as compared with other treated groups. At the same time significant increase in CAT level as compared with cisplatin treated group was recorded in

cisplatin plus curcumin and cisplatin plus vitamin C treated groups. At the mean time, cisplatin plus curcumin and vitamin C treated group afforded a slight significant decrease in CAT level as compared with normal control group so it was the best safer treatment comparing with cisplatin plus curcumin and cisplatin plus vitamin C treated groups as shown in table (2) and fig (4).

3.6.3 Effect of cisplatin, curcumin, vitamin C and their combinations on Glutathione reduced (GSH) activity:

Treatments of normal rats with cisplatin exhibited a highly significant decrease (P<0.001) in liver GSH level after the end of the experiment when compared with saline treated group and other control groups. Meanwhile, cisplatin plus vitamin C and cisplatin plus curcumin treated groups afforded significant increase in GSH level as compared with cisplatin treated group.

Whereas, cisplatin plus curcumin and vitamin C treated group elucidate slight significant decrease in GSH level as compared with control groups but afforded significant increase in GSH level as compared with cisplatin treated group as shown in table (2) and fig (5).

3.6.4 Effect of cisplatin, curcumin, vitamin C and their combinations on Superoxide dismutase (SOD) activity:

Table (2) and Fig (6) showed that Cisplatin treated group afforded highly significant decrease in liver SOD level as compared with saline treated group and other normal control groups. Meanwhile, Cisplatin plus curcumin and cisplatin plus Vitamin C treated groups elucidate significant increase in SOD level as compared with Cisplatin treated group only while they also afforded significant decrease in SOD level as compared with normal control group. At the same time, Cisplatin plus curcumin and vitamin C treated group elucidate non significant change in SOD level as compared with normal control group.

Table (1): Effect of Cisplatin (0.4mg/kg), Curcumin (20mg/kg), Vitamin C (100mg/kg) and their combinations on Liver function parameters.

Groups	ALT (U/ml)	AST (U/ml)	Albumin (g/dl)	Total protein (g/dl)	Globulin (g/dl)
Normal control group	51.65±2.34^{d}	64.73±4.30^{cd}	3.96±0.04^{a}	10.40±0.32^{ab}	6.30±0.25^{ab}
Gum acacia treated group	57.50±1.85^{cd}	69.33±3.24^{cd}	3.95±0.04^{a}	10.74±0.01^{ab}	6.13±0.32^{ab}
Saline treated group	52.26±3.40^{d}	65.13±2.88^{cd}	3.75±0.10^{ab}	10.32±0.53^{ab}	5.93±0.37^{bc}
Curcumin treated group	50.23±3.48^{d}	63.20±4.79^{de}	3.80±0.26^{ab}	10.63±0.67^{ab}	6.26±0.22^{ab}
Vitamin C treated group	51.93±0.96^{d}	56.88±2.34^{e}	3.71±0.10^{ab}	11.06±0.38^{a}	6.96±0.45^{a}
Cisplatin treated group	118.86±5.88^{a}	113.91±5.31^{a}	2.56±0.16^{d}	6.65±0.38^{e}	4.85±0.29^{d}
Cis +Cur treated group	77.53±4.16^{b}	94.21±3.79^{b}	3.15±0.09^{c}	8.03±0.24^{d}	4.19±0.35^{c}
Cis +Vit. C treated group	84.98±4.54^{b}	94.80±3.47^{b}	3.10±0.04^{c}	8.60±0.52^{cd}	5.50±0.38^{bc}
Cis+Cur+Vit.C treated group	67.05±3.82^{c}	76.40±2.94^{c}	3.40±0.14^{bc}	9.74±0.09^{bc}	6.06±0.44^{ab}

Means within the same column in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

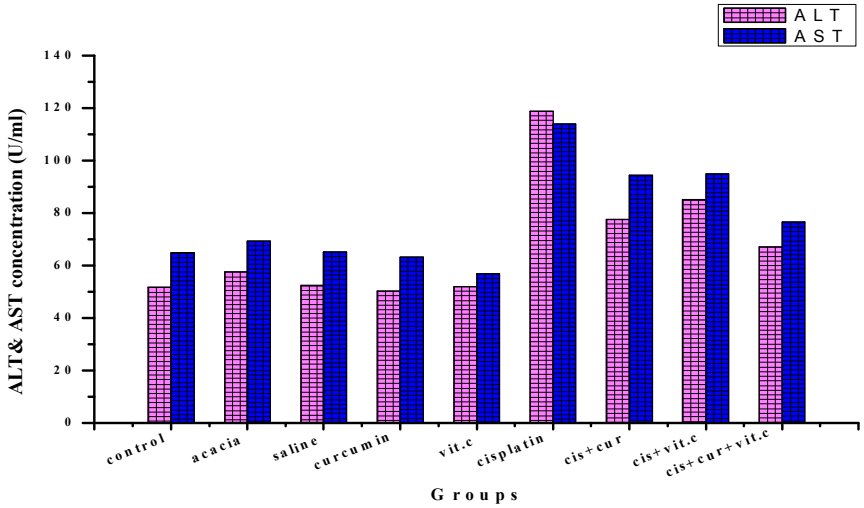

Fig. (1): Effect of Cisplatin (0.4mg/kg), Curcumin (20mg/kg), VitaminC (100mg/kg) and their combinations on ALT & AST (U/ml)in male albino rats.

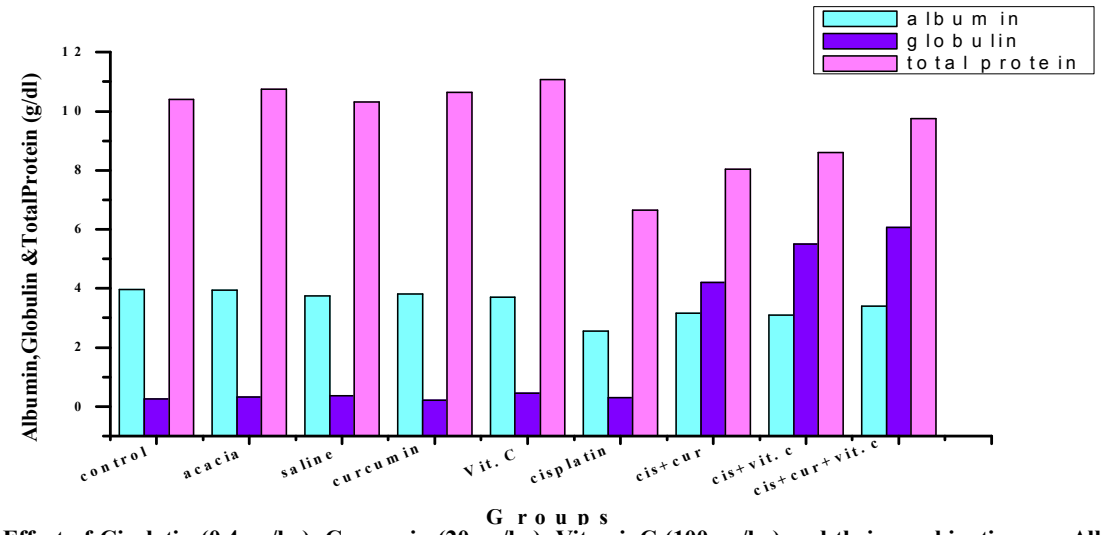

Fig.(2): Effect of Cisplatin (0.4mg/kg), Curcumin (20mg/kg), VitaminC (100mg/kg) and their combinations on Albumin, total protein & globulins (g/dl)in male albino rats.

Table (2): Effect of Cisplatin (0.4mg/kg), Curcumin (20mg/kg), Vitamin C (100mg/kg) and their combinations on Antioxidants activity.

Groups	MDA (U/g)	CAT (U/g)	GSH (U/g)	SOD (U/g)
Normal control group	13.96±0.564^{d}	0.866±0.014^{ab}	1.02±0.052^{a}	331.7±4.80^{cd}
Gum acacia treated group	15.05±0.484^{d}	0.829±0.008^{ab}	0.93±0.050^{ab}	311.1±9.34^{d}
saline treated group	14.29±0.643^{d}	0.867±0.011^{ab}	0.92±0.034^{ab}	312.7±16.99^{d}
curcumin treated group	14.39±0.812^{d}	0.860±0.005^{ab}	0.99±0.025^{ab}	327.0±7.01^{b}
Vitamin C treated group	14.13±0.491^{d}	0.892±0.002^{a}	1.00±0.024^{a}	356.0±6.23^{a}
cisplatin treated group	27.73±0.849^{a}	0.495±0.049^{f}	0.30±0.014^{e}	233.6±11.59^{g}
Cis +cur treated group	20.67±0.441^{b}	0.663±0.016^{e}	0.58±0.030^{d}	270.9±8.08^{f}
Cis +vit. C treated group	18.70±0.723^{c}	0.709±0.040^{d}	0.61±0.060^{d}	273.6±7.63^{ef}
Cis+cur+ vit. C treated group	15.70±0.562^{d}	0.797±0.019^{cd}	0.84±0.053^{c}	311.0±6.88^{d}

Means within the same column in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

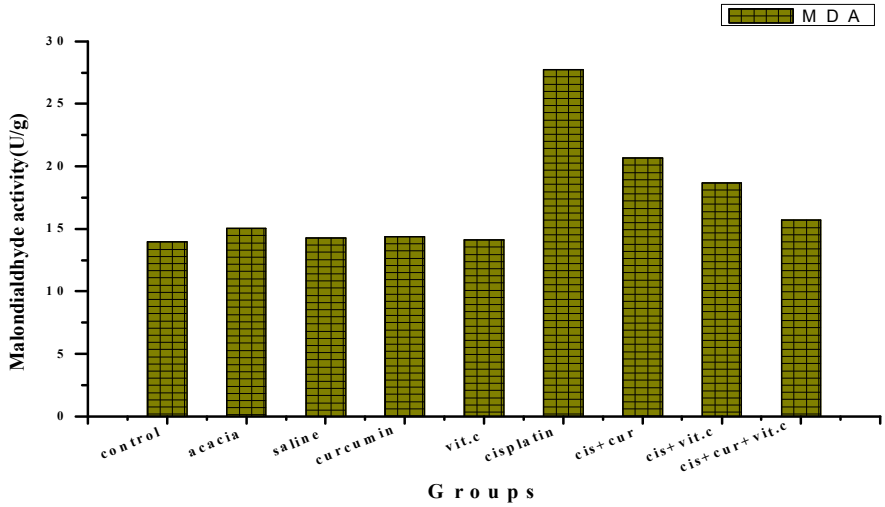

Fig.(3): Effect of Cisplatin (0.4mg/kg), Curcumin (20mg/kg), Vitamin C (100mg/kg) and their combinations on Malondialdhyde (MDA) activity (U/g) in male albino rats.

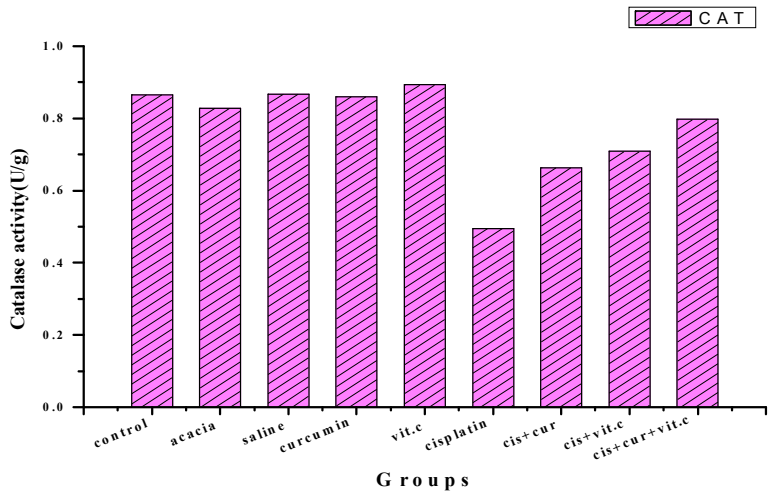

Fig.(4): Effect of Cisplatin (0.4mg/kg), Curcumin (20mg/kg), Vitamin C (100mg/kg) and their combinations on Catalase activity (U/g) in male albino rats.

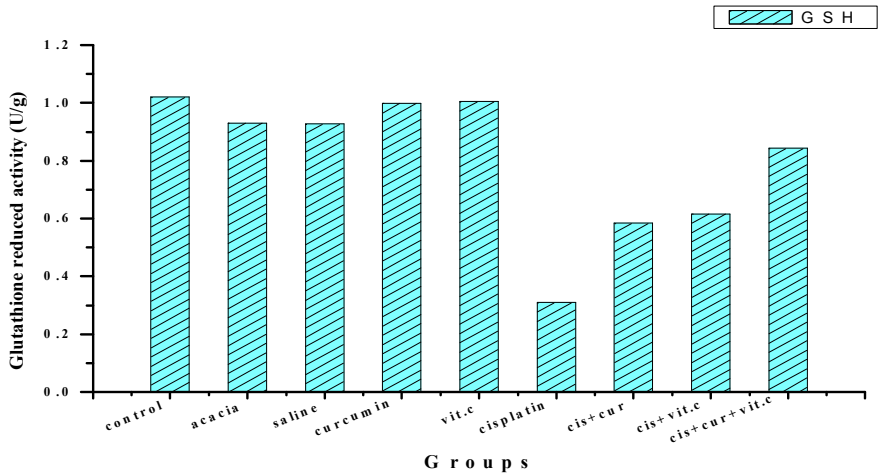

Fig. (5): Effect of Cisplatin (0.4mg/kg), Curcumin (20mg/kg), VitaminC (100mg/kg) and their combinations on Glutathione reduced activity (U/g) in male albino rats.

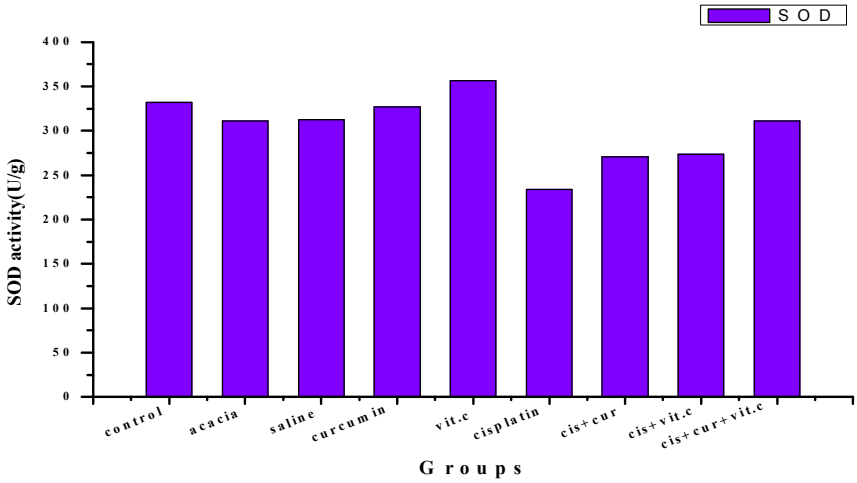

Fig. (6): Effect of Cisplatin (0.4mg/kg), Curcumin (20mg/kg), VitaminC (100mg/kg) and their combinations Superoxide dismutase (SOD) activity in male albino rats

4. Discussion:

Chemotherapy is the use of drugs to kill cancer cells by stopping their ability to grow and divide. Because cancer cells generally grow and divide faster than normal cells, they are more susceptible to the action of these drugs. However, damage to healthy cells is unavoidable, and this damage accounts for the side effects linked to these drugs ***(Patricia et al., 2006)*.**

Cisplatin is platinum coordinated complex based anticancer drug used against many human cancers including and many other solid tumors ***(Gottfried et al., 2008)***. In spite of its effective anticancer behavior, it exerts many unwanted side effects including nephrotoxicity, hepatotoxicity, ototoxicity, myelosuppression and spermiotoxicity ***(Tarladacalisir et al., 2008)***. Hence, these major side effects limit the clinical use of the drug.

In addition, Cisplatin generates oxidative and nitrosative stresses ***(Xiao et al., 2003)*** because of depletion or inhibition of antioxidant enzymes and proteins which results into nephrotoxicity and hepatotoxicity as major side effects of the drug ***(Iseri et al., 2007)***.

Various treatment-strategies and curing agents have been tried and used to monitor or control its side effects since its discovery. Herbal medicines are commonly used for alleviating the side- effects of chemotherapy or radiotherapy and for improving the quality of life in patients with cancer

Curcumin is a potent anticancer agent ***(Kuttan et al., 1985)***. Curcumin is considered to be an effective antioxidant against oxidative tissue damage. It can significantly inhibit the generation of reactive oxygen species (ROS) both in vitro and in vivo ***(Joe and Lokesh, 1994)***

Vitamin C is considered as highly effective antioxidant and an enzyme cofactor for the bio synthesis of many important biochemicals. It activates some enzymes which have important role in protein, carbohydrates and fat metabolism. It exerts positive effect on lipid and iron metabolism ***(Victoret and Ropper, 2001)*** and promote immune function.

Effect on some liver functions:

The present data suggests that cisplatin can cause liver function impairment via elevation of liver function biomarker levels including ALT and AST. And these results seems to be conceivable with that obtained by ***Naqshbandi et al., (2012), Khaled et al., 2013 and Sarawoot and Chuchard, (2013).*** They reported that cisplatin administration afforded a toxic effect on liver.

Serum aminotransferases (ALAT & ASAT) are cytosolic enzymes of hepatocytes; an increase in their activities reflecting an increase in the plasma membrane permeability of hepatocyte which in turn associated with cell death ***(Rosen & Keeffe, 2000)***.

One or more mechanism could explain the cisplatin-induced hepatic disorder. Cisplatin may result in mitochondrial membrane rigidification and energetic metabolism impairment through the oxidation of a diverse set of hepatic mitochondrial components, including protein sulfhydryl groups. This interference is due to cisplatin ability to increase mitochondrial inner membrane permeability ***(Martins et al., 2008)***. This may be due to lipid peroxidation induced by cisplatin as that reported by ***(Baliga et al., 1999). Jordan and Carmo-Fonseca, (2000)*** they also reported that formation of free radicals leading to oxidative stress has been shown to be one of the pathogenic mechanisms of the adverse effects of Cis in liver.

With respect to control rat group, significant decrease in serum total protein albumin and globulin levels were indicated as aconsequence of cisplatin intoxication. This indication is consistent with the report of ***Kharbangar et al., (2000) and Shibayama et al., (2007)***.

In addition, ***Heminger et al., (1997)*** reported that cisplatin administration caused a significant decrease in serum proteins level by its impaired synthesis in liver and this could be in turn ascribed to inefficiency of hepatocytes and/or decreased level of blood amino acid.

Serum proteins were lost more rabidly from circulation owing to changes, in distribution between the extra and intravascular space or more pronounced extra-hepatic catabolism. Moreover, the decrease observed in total serum protein was probably concordant with some disorder related with building new proteins by the liver ***(Al-Badry et al., 1988)***. Furthermore, the protein decline may be due to some faulty reabsorption in the kidney.

In addition, the inhibition effect of cisplatin on serum proteins may be associated with the impact of the drug on RNA synthesis and arrangement, as well as mRNA degradation ***(Stoll et al., 1976)***. This was probably due to inhibition of amino acid reabsorption across the basolateral cell membranes of the renal tubules as a result of the direct action of the drug at the site ***(Foulkes and Blank, 1991)***.

The reversing of hepatotoxic effect induced by cisplatin, herewith observed after pretreatment with vitamin C which evaluated by significant decreasing in liver marker ALT, AST and serum proteins comparing with cisplatin only treated group. And these results seem to be conceivable with that obtained by ***Mossa et al., (2011), Omar et al., (2012).*** They reported that Vitamin C was able to attenuate hepatic damage induced by some chemical agents especially in animals. Vitamin C normalized levels of alanine

aminotransferase, aspartate aminotransferase and alkaline phosphatase.

These may be attributed mainly to the ameliorative effect of vitamin C against the oxidative stress induced by cisplatin as vitamin C has reported to have antioxidant activity and these findings supported by ***(El-Gendy et al., 2010)*** they revealed that The mechanism by which vitamin C decrease the hepatotoxicity induced by cisplatin, is embodied in the fact that vitamin C might ameliorate the oxidative damage by decreasing lipid peroxidation and altering antioxidant defense system or by denoting electrons to free radicals and quenching their reactivity ***(Bendich, 1990)***.

At the same time, the present results revealed that pre-treatment with curcumin alone or/and in combination with vitamin C before cisplatin administration was able to normalize the levels of liver enzymes biomarker. These demonstrated by significant decrease in ALS, AST and significant increase in albumin, globulin and total protein as compared with cisplatin treated group. And these results were supported with that reported by ***Suresh et al., (2011), Essam, and Ashraf, (2013) and Palipoch et al., (2014)).*** They reported the protective effects of curcumin against chemically-induced hepatotoxicity are well documented, and have been attributed to its intrinsic antioxidant properties. It could be suggested that the leakage of enzymes because of liver injury is prevented by the liver cell membrane stabilizing action of curcumin ***(Yousef et al., 2008)***.

Akila et al. (1998) reported that administration of curcumin preserved the structural integrity of the hepatocellular membrane. This was evident from the reduction in the enzyme activities against the cisplatin induced rise in the enzyme levels in plasma.

The effect on lipid peroxidation and antioxidant status:

The present data showed that cisplatin significantly decreased the activities of SOD, catalase and GSH and enhanced LPO in the hepatic tissues indicating CP-induced oxidative damage. In contrast, a marked increase in the antioxidant enzyme activities was seen when rats were treated with vitamin C and/or curcumin alone and their combination before cisplatin administration.

In the same context, the inhibition of enzymatic antioxidant (CAT, and SOD) system and reduction of the non enzymatic antioxidant system such as GSH herewith, after cisplatin administration in full agreement with that reported by ***Pratibha et al. (2006) and Kart et al. (2010).***

In addition, ***Xiao et al. (2003)*** demonstrated that, increased production of reactive oxygen species (ROS) and free radicals has been implicated in mediating cisplatin induced toxicity. However, oxidative stress can occur as a result of either increased ROS generation and/or decreased antioxidant enzyme system ***(Fadillioglu et al., 2004).*** ***Kadikoylu et al. (2004)*** indicated the involvement of hydroxyl radicals in the mechanism of cisplatin induced oxidative damage in liver. Hydroxyl radicals are highly reactive oxygen species, capable of reacting with proteins and abstracting a hydrogen atom from polyunsaturated fatty acids in membrane lipids to initiate lipid peroxidation (evidenced here by the elevated hepatic MDA level) which in turn impairing the hepatocyte membrane permeability, eventually leakage of the enzymes.

Reduced glutathione (GSH) is considered as a sensitive oxidative stress marker because it helps to maintain the integrity of mitochondria and cell membrane. Its compromised level in the cells may deteriorate the membrane permeability and risks the cellular defense against ROS resulting in oxidative injuries ***(Younes and Siegers, 1981)***. Many glutathione based antioxidant enzymes and proteins are important to maintain redox status of the cells. All these enzymes utilize glutathione (GSH) in the reactions they catalyze which may lead to depletion of GSH in the living system in the condition of oxidative stress. This may be the possible reason for decreased level of GSH in target organs in cisplatin treated mice ***(Cayir et al., 2009)***.

In addition, the present results show that administration of cisplatin afforded a highly significant increase in MDA level comparing with control groups. This may attributed to the lipid peroxidation induced by cisplatin. And this finding in full agreement with that reported by ***Autunes et al., (2001) and Mora et al., (2003).*** They demonstrated that Lipid peroxidation (LPO) is crucial in the pathogenesis of cisplatin-induced organ injury.

Regarding the effect of vitamin C and curcumin, different strategies have been proposed to inhibit cisplatin-induced oxidative stress. In this study we investigated the effects of antioxidant activity of vitamin C and curcumin on cisplatin-treated adult rats. And these finding strongly supported by ***Bashandy and Alwasel (2011), Suhail et al. (2012) and Osama (2013)***. They reported that vitamin C had a protective effect against oxidative stress, and via its antioxidative property, vitamin C reduce the lipid preoxidation, normaliz glutation level and improve ulterations in biochemical markers. In addition, ***Lee et al. (2003)*** documented that vitamin C supplementation lower level of lipid peroxidation (MDA)

At the same time, this increase of the antioxidant enzymes SOD, catalase, GPx activities and decrease of lipid peroxides with administration of curcumin in cisplatin induced oxidative stress in the present study was in agreement with ***Suryanarayana et al. (2007)***

and Balamurugan et al. (2009). They reported that Pre-treatment with antioxidant curcumin decreased the generation of ROS thus prevents the cisplatin induced derangement in the activities of the antioxidant enzymes.

The antioxidant property of curcumin extract might be attributed to the presence of chemical groups like hydroxyl ***(Kurup et al., 2007)*** methoxy and 1,3-diketone conjugated diene system. Thus, from the *in vitro* and *in vivo* experimental results it can be derived that curcumin is able to reduce oxidative stress during inflammatory conditions by down regulation of nitric oxide formation, scavenging, and/or neutralizing free radicals (such as superoxide anions and H_2O_2) known to participate in oxidative chain reactions ***(Motterlini et al., 2000)***.

There is a synergistic effect between curcumin and vitamin C which appeared in the results obtained from their combination in the pretreatment in cisplatin induced hepatotoxicity, which in turn afforded the best ameliorative effect.

5. Conclusions

From the present results we could conclude that oxidative stress has been implicated in the pathogenesis of cisplatin induced hepatotoxicity by enhancing ROS generation through reducing activities of enzymatic antioxidants. These findings indicate that pretreatment with combined curcumin and vitamin C can protect cisplatin-induced hepatotoxicity including biochemical, physiological aspects. The study provides the evidence of combined curcumin and vitamin C as the new adjuvant of cisplatin to abrogate the hepatotoxicity upon cancer chemotherapy.

Recommendations

So we recommend the use of the combination of curcumin and vitamin C which is known as antioxidants compounds in order to ameliorate the hepatotoxic effect effects caused by cisplatin.

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