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Protective Effect of *Coccinia grandis* on Cyclophosphamide Induced Liver Damage

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Abstract: Liver is the key organ regulating homeostasis in the body. Cyclophosphamide (CP) is commonly used to treat cancers, systemic vasculatures and kidney diseases. Although the therapeutic effect of CP has many adverse effects such as hepatoyoxicity. *Coccinia grandis* (CG) has been tested to be an excellent antioxidant, thereby can play a role in the prevention of hepatotoxicity. The study was conducted to examine the hepatoprotective effect of CG on cyclophosphamide-induced liver damage. Fifty male albino rats have been divided into five equal groups. Group 1 was held as a non-treated group (negative control) Group 2, which was held as a toxic group (positive control), groups (3), (4) and (5) received orally the leaf extract of CG in doses of two hundred, four hundred and six hundred mg/kg/day respectively, for 59 days. At day 60 groups from 2 to 5 received one dose of CP (two hundred mg/kg i.p) 1 h before CG. Blood was collected for biochemical analysis. The liver was also examined histopathologically. The results illustrated that the CG leaves extract at doses of four hundred and six hundred mg/kg B.wt/day significantly decreased serum levels of liver enzymes and MDA but there were an increased in levels of GSH, SOD and serum inflammatory cytokines (TNF-α and IL-1α) compared to control rats. There was also an improvement in histopathological changes observed in the liver of hepatotoxic rats. Therefore, the administrations of CG leaf extract have antioxidant and hepatoprotective effects in hepatotoxic rats.

Key words: Hepatotoxicity · *Coccinia grandis* · Cyclophosphamide · Rats · Cytokines · Histopathological

INTRODUCTION

Cyclophosphamide (CP), as an immunosuppressive drug, is commonly used in the treatment of various cancer, it is a cytotoxic alkylating agent with potent antineoplastic properties [1]. However, CP usefulness is often limited due to its toxicity in heart [2], liver [3], testes [4], urinary bladder [5] and renal [6].

Researchers do their best to explore the mechanism (s) of CP-induced hepatotoxicity. However, to date, the mechanisms of CP-induced hepatotoxicity have not been reached, but growing evidence suggests that oxidative stress and intense inflammatory reactions are likely to play the greatest role [7, 8].

Management of liver diseases is still a challenge to the modern scientific community. Unfortunately, drugs have little to offer alleviation of hepatic ailments. Thus given rise to research involved in identification of safe, inexpensive and available alternatives from natural resources. Recent studies revealed the plants belonging to *Cucurbitaceae* family showed significant hepatoprotective properties [9].

Coccinia grandis Linn (*Cucurbitaceae* family) leaves water and ethanolic extracts have been widely studied for its pharmacological properties. It plays a functional role against many diseases. Extract of *Coccinia grandis* leaves has comparable anticancer property as reference drug (vinblastine) [10], mutagenic effect on Neurospora crassa [11], anti-inflammatory against formaldehyde induced-paw edema in rats [12], antibacterial [13], anthelmintic [14] activities. It produced significant antiulcer activity in pylorus ligation and ethanol induced ulcer models in rats [15]. Also, it has hypoglycemic and hypolipidemic activities [16 -17], as well as stimulated gluconeogenesis and inhibited glycogenolysis in the diabetic rat [18]. The extract can be used for management of obesity [19]. Some studies reported that *Coccinia grandis* leaves extract has hepatoprotective effect against paracetamol [20] and carbon tetrachloride in rats [9] induced hepatic damage in experimental animals.

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However, to the best of our knowledge, this is the first study aims to assess the potential hepatoprotective role of CG leaves extract against CP-induced acute hepatic toxicity. Furthermore, the underlying mechanism will be explored on the inflammation and oxidative stress.

MATERIALS AND METHODS

Plant Material: *Coccinia grandis* (CG) was obtained from the local market, Jeddah, Saudi Arabia.

Drugs and Chemicals: Cyclophosphamide (CP) was purchased from a local pharmacy (Jeddah). All chemicals and kits with high analytical grade were purchased from Sigma-Aldrich Co., USA.

Rats: Male Sprague Dawley rats (180± 10 g) were provided from King Fahd Research Center, KAU. They were kept in standard laboratory conditions, fed on a standard AIN-93 diet [21]. They were kept accordance to the standard guide for the care and use of laboratory animals in King Fahd Research Center.

Plant Material and Extraction: *Coccinia grandis* leaves (1 kg) were washed, properly dried in shade for 4-6 days. After drying, the plant materials were minced to powder and then it was defatted with petroleum ether and exhaustly extracted with 70% of methanol by cold medium for 72 hours. The extract was separated by the filtration and concentrated on vacuum evaporator and a greenish-black material was obtained (yield 9.5% w/w). It was stored at 4°C until used [22].

Experimental Design: The fifty male Wister rats weighing about 180 ± 10 g were used in the study. The rats were fed standard diet and water ad libitum in a steady environment (room temperature 22 ± three °C, room humidity 50 ± five %) with a fourteen-h mild and 10h dark cycle. The animals were kept under observation for one week before the beginning of the experiment. After the acclimatization period, the rats were distributed randomly into five groups of ten rats each.

Group 1 (control negative -ve): Rats were fed on a basal diet for 60 days and given orally saline.
Group 2 (control positive + ve): Rats were fed on the basal diet for 59 days and given the CP (200 mg/kg i.p) at day 60 according to Ademola *et al*. [23].
Group 3: Rats were received oral gavages of CG 200 mg/kg/ day for 59 days and given one dose of CP (200 mg/kg i.p) at day 60 1 h before CG.
Group 4: Rats were received oral gavages of CG 400 mg/kg/ day for 59 days and given one dose of CP (200 mg/kg i.p) at day 60 1 h before CG according to Shyam *et al*. [24].
Group 5: Rats were received oral gavages of CG 600 mg/kg/ day for 59 days and given one dose of CP (200 mg/kg i.p) at day 60 1 h before CG.

After the 60 days of experimental time, rats for each group were sacrificed under light ether anesthesia. Blood samples were collected by heparinized capillary tubes, kept for couple hrs and centrifuged at 3000 rpm for 15 min. Then the separated serum was stored at -20°C for subsequent followed analyzes. The liver was removed for histopathological examination.

Determination of Serum Biomarkers: Activities of liver enzymes in serum (aspartate aminotransferase (AST), alanine aminotransferase (ALT) determined according to Reitman and Frankel [25] and alkaline phosphatase (ALP)) were assessed by Belfied and Goldberg [26]. Oxidative stress biomarkers glutathione superoxide dismutase (SOD), reduced glutathione (GSH) and malondialdehyde (MDA) were tested chemically according to Giannopolitis and Ries [27], Spitz and Oberley [28] and Beutler *et al*. [29], respectively. Anti-inflammatory cytokines Interleukin– 1α (IL- 1α) and tumor necrosis factor-α (TNF-α) were tested according to Piguet *et al*. [30] and Dinarello [31], respectively.

Histopathological Examination: The liver tissues from all experimental groups were fixed in 10% neutral formalin, dehydrated and embedded in paraffin wax. Fixed tissues were cut at 5 μm sections and then stained with hematoxylin-eosin by routine procedures [32].

Statistical Analysis: The analysis of resulted data will be done by SPSS software Version 24. Values were expressed as mean ± SDM and analyzed by one-way variance (ANOVA) followed by comparison test (t-test). The results were considered as statistical significance at $P \leq 0.05$. according to Snedecor and Cochron [33].

RESULTS

From information recorded in Table (1) it might be recognized that rats acutely intoxicated by CP (200 mg/kg i.p) at the end of the experimental period had significant ($P < 0.05$) increases in the serum activities of AST, ALT and ALP enzymes as compared to control (-ve) group. Administration of CG extract at doses of 400 and 600 mg/kg/ day for 59 days before intoxicated with a

Table 1: Hepatoprotective effect of *Coccinia grandis* extract on serum liver enzymes AST, ALT and ALP in intoxicated rats with cyclophosphamide

Groups	AST (U/L)	ALT(U/L)	ALP (U/L)
Negative control (-ve)	51.24±2.23 c	46.33± 0.44 c	56.56±3.34 c
Positive control (+ve)	139.28±2.56a	126.53±0.34a	130.26±1.35a
CG (200 mg/kg/ day)+ CP	125.14±3.76a	119.44±1.43a	123.56±2.65a
CG (400 mg/kg/ day)+ CP	71 .49±2.35 b	59.37±0.39 b	70.54±1.91 b
CG (600 mg/kg/ day) + CP	57. 34±1.48c	49.67± 0.54 c	61.78 ± 1.23c

- Values are presented as mean ± SDE.

- Values with different superscript letters within a column are significantly different at P< 0.05.

Table 2: Hepatoprotective effect of *Coccinia grandis* extract on serum MDA and GSH levels in intoxicated rats with cyclophosphamide

Groups	MDA (nmol/g protein)	GSH (µg/mg protein)	SOD (U/mg)
Negative control (-ve)	11.43±1. 26c	5.72±.32a	59.43 ± 1.23a
Positive control (+ve)	23.73±1. 41a	2.78±0.07c	25. 64 ± 1.54c
CG (200 mg/kg/ day) + CP	21.32±. 78 a	3.16±0.17c	32.72 ± 2.79c
CG (400 mg/kg/ day) + CP	14.43±. 92b	4.75±0.09b	45.41±0.54b
CG (600 mg/kg/ day) + CP	12.42±1. 04c	5.24±0.11a	57.62±1.87a

- Values are presented as mean ± SDE.

- Values with different superscript letters within a column are significantly different at P< 0.05

Table 3: Hepatoprotective effect of *Coccinia grandis* extract on serum IL-1 and TNF-α level in intoxicated rats with cyclophosphamide.

Groups	IL-1 pg/ml	TNF-α pg/ml
Negative control (-ve)	21.18 ± 0.34 c	10.01 ± 1.52 c
Positive control (+ ve)	55.43± 0.55 a	25. 13 ± 0.98 a
CG (200 mg/kg/ day) + CP	45.68± 1.05 a	21.15 ± 0.12a
CG (400 mg/kg/ day) + CP	28.43± 0.34b	15.28±1.69 b
CG (600 mg/kg/ day) + CP	22.76± 1.21 c	11.07 ± 1.73 c

- Values are presented as mean ± SDE.

- Values with different superscript letters within a column are significantly different at P< 0.05

CP induced significant decreases (P<0.05) in all the elevated serum marker levels of AST, ALT and ALP, when compared to the hepatotoxic rats (control +v group), while there was no significant decrease found in group of rats given 200 mg/kg/ day CG extract compared to the positive control group.

Data illustrated in Table (2) showed that rats intoxicated by a CP (200 mg/kg i.p) at the last day of the experimental period had significant (P < 0.05) increases in the serum activities of MDA accompanied with significant reduction in GSH and SOD enzymes activity compared to control (-ve) group. Administration of CG extract at doses of 400 and 600 mg/kg/ day for 59 days before intoxicated with a CP showed remarkably amelioration the elevation of MDA level and the reduction in both GSH and SOD enzymes activity when compared with the hepatotoxic rats (control+ve group), Prevention with CG (200 mg/kg/ day) showed non-significant difference when compared with the positive control group.

Anti-inflammatory cytokines IL-1?? and TNF-?? serum levels are significantly (?? < 0.05) increased in rats intoxicated by a CP (200 mg/kg i.p) compared to the control negative group. Administration of CG extract in a dose of 400 and 600 mg/kg/ day results in a significant decrease in serum IL-1?? and TNF-?? compared to hepatotoxic group. Administration of CG extract in a dose of 200 mg/kg results in a non-significant decrease in serum IL-1?? and TNF-?? compared to hypatotoxic group. The high dose (600mg/kg) of CG extract is the most effective compared to the other groups as shown in Table (3).

Liver sections of normal control rats showed normal histological structure of hepatic lobule with normal hepatocytes and hepatic sinusoids (Fig. 1). Injection of CP to rats induced coagulative necrosis of hepatocytes and dilated ventral vein (Fig. 2). Liver sections of rats given orally the small dose (200mg/kg) of CG showed vacuolar degeneration of hepatocytes (fatty change) (Fig. 3). Examination of liver of rats pretreated with the large doses 400 and 600 mg/kg of CG revealed almost normal histological architecture of hepatic lobule (Figs. 4 and 5 respectively).

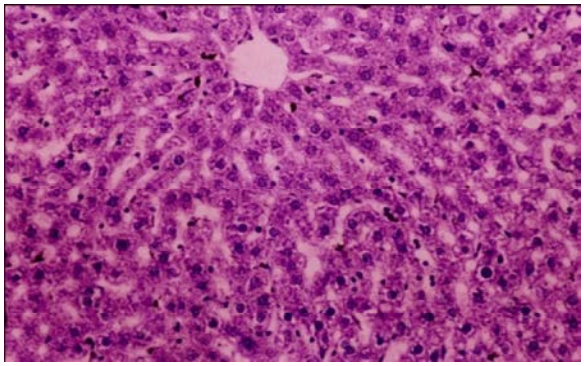

Fig. 1: Liver section of control rats (-ve) showed normal histological structure of hepatic lobule with normal hepatocytes and hepatic sinusoid

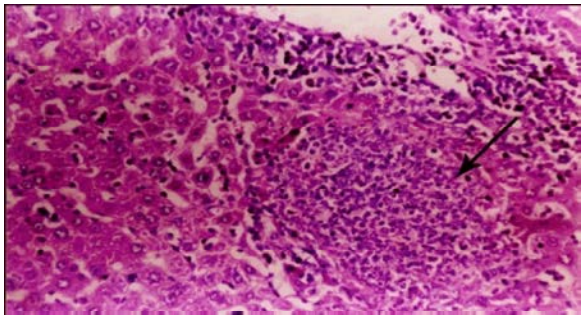

Fig. 2: Liver section of CP intoxicated rats showed coagulative necrosis of hepatocytes and dilated ventral vein (H&E stain x200)

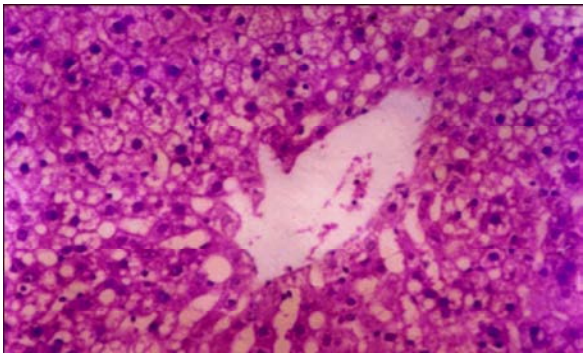

Fig. 3: Liver sections of pretreated intoxicated rats with CG in a dose of 200mg/kg showed vacuolar degeneration of hepatocytes (fatty change). (H&E stain x200)

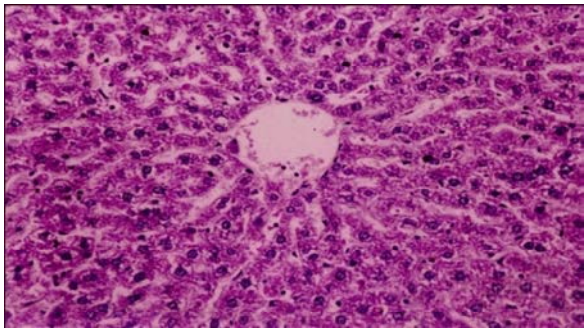

Fig. 4: Liver sections of pretreated intoxicated rats with CG in a dose of 400 mg/kg showed no histopathological changes. (H&E stain x200)

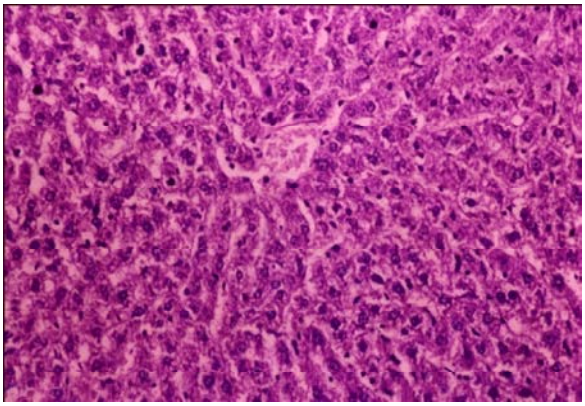

Fig. 5: Liver sections of pretreated intoxicated rats with CG in a dose of 600 mg/kg showed normal histological structure. (H&E stain x200)

DISCUSSION

Cyclophosphamide (CP) is commonly used in treatment of various types of cancers and autoimmune diseases [1]. However, its usefulness is often limited by a wide range of adverse effects, including hepatotoxicity [3]. Evidence suggests that oxidative stress and intense inflammatory reactions could be the mechanisms underline CP-induced hepatotoxicity [7, 8]. Also, antioxidants and anti-inflammatory agents were proved effective in attenuating CP hepatotoxicity [34, 35]. This study aimed to demonstrate the potential protective ability of CG leaves extract on liver damage induced by CP in rats.

In the present work, a single i.p injection of CP at a dose of 200 mg/kg caused significant increase of serum liver enzymes activity, MDA and a significant decrease in enzymatic antioxidant SOD and GSH activities, also induced significant increase in pro-inflammatory cytokines IL- 1α and TNF- α levels as compared to control group. This confirmed the destruction caused by CP on the liver cells by examining the pathological changes. Hepatotoxicity of CP caused by intermediary cytotoxic metabolites formed as depicted by elevation in serum liver enzymes level [36, 37].

The current study is in agreement with Rajasekaran *et al*. [38], Bhatia *et al.* [39] and Kouidhi *et al*. [40] who revealed that CP-induced hepatotoxicity involves induction of oxidative stress due to excessive formation of ROS which causes lipid peroxidation of the cellular membrane and reduction in the antioxidant enzymes.

Hepatotoxicants has been shown to induce an inflammatory response, which participated in the organ injury [41, 42]. The current study revealed a significant increase in serum pro-inflammatory cytokines of CP-injected rats when compared to control rats. Consistently, the pro-inflammatory cytokine implicated in the pathogenesis of CP [43, 44]. Destruction of hepatocellular architecture associated with inflammatory infiltration were comparable to many researches [45, 46].

The results of this study showed the hepatoprotective effect of CG extract at 400 and 600 mg/kg against CP-induced alteration in the liver enzyme activities (AST, ALT and ALP), reduction in oxidative stress as marked by decreased in MDA and increased in SOD and GSH and reduction in pro-inflammatory cytokines levels (IL- 1α and TNF- α) toward control level, as well as induced weakening the pathological damage induced by CP.

The current study is in agreement with Sunilson *et al*. [9] who revealed that the ethanolic extract of *Coccinia grandis* leaves possessed significant hepatoprotective effect in the CCl4 model of intoxication in rats. Hepatoprotective activity of the extract may be due to the antioxidant effects of flavonoids, triterpens and tannin present in CG, which may be interfere with free radicals formation [9, 47-48]. In this study a significant increase in antioxidant enzymes activities in groups pretreated with CG suggesting the protective mechanism of the CG in response to free radicals. These results are similar to the data of Umamaheswari and Chatterjee [20] who revealed that pretreatment of mice with CG increased the activities of enzymatic antioxidants and prevented the accumulation of excessive free radicals from paracetamol intoxication. The CG leaves extract showed potent antioxidant activity this attributed to the reducing power ability and hydrogen peroxide scavenging potential [49, 51].

Sunilson *et al*. [9] showed that liver sections of the rats pretreated with CG leaves extract and intoxicated with CCl4, the normal cellular architecture was retained as compared with silymarin, thereby confirming the protective effect of the extract which could be explained by active compounds as glucosides [52]. The presence of flavonoids, tannins, saponins and terpenoids in CG which are antioxidant and anti-inflammatory agents, thus explain its role in hepatoprotection by interfere with free radicals formation [51].

In conclusion, the results of the present study indicate that CG leaves extract significantly ameliorated CP- induced hepatotoxicity. The antioxidant and anti-inflammatory activities of CG could be considered the main factors which are responsible for the hepatoprotective effect. Therefore, CG extracts a potential therapeutic option to prevent hepatotoxicity induced by CP. Further studies are needed to assess its potential utility of this extract in clinical conditions associated with liver damage.

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