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Research Article

EVALUATION OF *IN VIVO* ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY OF *CASSIA OCCIDENTALIS* LINN. AGAINST PARACETAMOL -INDUCED LIVER TOXICITY IN RATS

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ABSTRACT

The present study was aimed at evaluating the protective role of methanol fraction and its pure compound chrysophanol from *Cassia occidentalis* Linn. Against paracetamol-induced hepatotoxicity in rats. Adult male albino Wistar rats, weighing 110-150g, were randomized into control and experimental group. Test group of animals were treated with paracetamol (2g/kg bw on 8th day). Liv-52 was used as a standard reference. Chrysophanol (50 mg/kg bw) and methanol fraction (*COLMF*) (200 mg/kg bw) were administered to the paracetamol treated rats for seven days. Oral administration of chrysophanol and *COLMF* significantly normalized the values of SOD, CAT, GPx, GSH, Vit-C and Vit-E. And the elevated serum enzymatic levels of AST, ALT, ACP and ALP were significantly restored towards normalization by pre-treatment with Chrysophanol and *COLMF* ($p>0.05$). The histopathological studies also confirmed the hepatoprotective nature of the extracts. The results of this study strongly indicate that *Cassia occidentalis* has potent hepatoprotective action against paracetamol induced hepatic damage in rats.

Keywords: *Cassia occidentalis*, Chrysophanol, Hepatoprotective, Paracetamol.

INTRODUCTION

Liver is responsible for metabolism of chemicals and foods for the regulation of internal environment. The major functions of the liver are carbohydrate, protein, fat metabolism, detoxification, secretion of bile and storage of vitamins[1]. Excess consumption of certain toxic chemicals such as antibiotics, chemotherapeutics, peroxidised oils, acetaminophen, aflatoxin, carbon-tetrachloride, chlorinated hydrocarbons, alcohol etc leads to infections and autoimmune disorder. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for the treatment of liver disorders[2]. For a long time, medicinal plants and their extracts were widely used in the treatment of liver diseases like hepatitis, and liver cirrhosis. There are numerous plants and polyherbal formulations claimed to have hepato-protective activity. Nearly 150 phytoconstituents from 101 plants have been claimed to possess liver protecting activity[4].

In the traditional system of medicine, many medicinal plants have been reported to possess the potential to treat liver diseases. *Cassia occidentalis* Linn. is extensively used in the indigenous and folklore medicine systems to treat hepatotoxicity. In Unani medicine it is used as an antidote of poisons, blood purifier, expectorant, anti-inflammatory agent and a remedy for the treatment of liver diseases[5]. It is also an important ingredient of several polyherbal formulations marketed for liver diseases[6]. Its roots, flowers, seeds and leaves have been employed in herbal medicine around the world for a variety of purposes such as laxative, expectorant, anti-malarial[7], hepatoprotective[8], relaxant[9] and anti-inflammatory[10], and for wound healing[11]. This study was aimed at investigating the antioxidant and hepatoprotective effect of *COLMF* and Chrysophanol against paracetamol induced hepatotoxicity in rats.

MATERIALS AND METHODS

Biochemical Estimations

Aspartate transferase, alanine aminotransferase, acid phosphatase and alkaline phosphatase kits were procured from Futura Chemicals, Italy. The activities of superoxide dismutase (SOD)[12], catalase (CAT)[13], glutathione peroxidase (GPx) [14], reduced glutathione[15], α–tocopherol (vit E)[17], ascorbic acid (vit C)[16] were estimated. For histopathological examination liver sections were stained with haematoxylin and eosin using standard protocol and then analyzed by light microscope.

Extraction, separation and purification of the compound

Fresh leaves of *C. occidentalis* L. were collected from the premises of Loyola College Chennai, and it was authenticated by Dr. M. Ayyanar, Taxonomist, Entomology Research Institute, Chennai and a voucher specimen (ALC DB-27) was preserved. The leaves were shade dried and coarsely powdered for extraction. The dried leaf material (3kg) was extracted with methanol (w/v 1:3) three times at room temperature for a week. The methanol crude extract was combined and concentrated to yield a residue (140 g) which was subjected to successive solvent partitioning to give chloroform (26g), methanol (114g) soluble fractions. The methanol fraction showed some activity. Thus, the methanol extract (100g) was chromatographed on a silica gel column (100-200 mesh) using a gradient solvent system of chloroform: methanol (100: 0, 95:5, 90:10, 85: 15…….0: 100) to give 25 fractions. The fractions were combined based on the TLC profile. Finally 7 fractions were obtained. The fractions were screened for hepatoprotective activity. Fourth fraction showed hepatoactivity and its yield was 10.6 g. It was further purified by column chromatography using chloroform & methanol solvent system. Fraction 2 showed single (yellow colour) spot on TLC over silica gel with $CHCL_3$: methanol (9:1) as the developing solvent system. The yield was 4.2 g. The spot turned to pink color on exposure to ammonia vapour or spraying with 5% alcoholic sodium hydroxide. It indicated the presence of anthraquinones. The pure compound was subjected to IR ^1H and ^{13}C NMR and MASS spectrum analyses. Tetra Methyl Saline (TMS) was used as standard, which showed chemical shift value at zero on the δ scale. ^1H and ^{13}C NMR spectra were recorded with a JEOL 500 MHz FT NMR spectrometer (H^1), 500 MHz (C^{13}) and chemical shifts were recorded in ppm. Solvents used for IR spectra were $CHCl_3$- and Ccl_4. Liquid samples are taken in KBr crystals and solid samples were grinded with KBr or Nujomull. IR spectra were recorded in Shimadszu by KBr pellet method. IR spectra were taken on a Perkin Elmer FT-IR (Spectrum One) spectrophotometer. EI-MS were taken on a JEOL-GC mate spectrum in Indian Institute of Technology, Chennai.

Drugs and chemicals

The drugs and fine chemicals were purchased from Sigma Aldrich (USA) and Ranbaxy (India). All other chemicals and solvents were obtained from local firms and were of analytical grade.

Phytochemical screening

The *COLMF* was screened for the presence of various phytoconstituents (Anthraquinones, Sitosterols,Tannins, Xanthorin, Saponin, and Flavonoids) using well established methods reported in literature[11,12].

Acute toxicity studies

A separate experiment was conducted to know whether any toxic effect was produced by *COLMF* and Chrysophanol over a period of 72 h by the method of Lorke[13]. Behavioral changes (irritation, restlessness, respiratory distress, abnormal locomotion and catalepsy) were observed over a period of 72 h for sign of acute toxicity. Rats fasted for 12 h were randomly divided into drug treated 'test' groups and vehicle treated 'control' group. Totally five groups of six rats were used. *COLMF* 150, 300, 600, 900 and 1200 mg/kg bw and chrysophanol 50, 150, 300, 600, and 900 mg/kg b.w were administered orally to the rats in each of the test groups, respectively. Each of the rats in the control groups was treated with vehicle alone. The animals were given food and water, and behavioral changes were observed over a period of 72 h for sign of acute toxicity. The number of mortality caused by the compound/*COLM*F within this period of time was observed.

Animals

Male Albino Wistar rats weighing approximately 110-150 g were obtained from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. They were acclimatized to animal house conditions, fed with commercial pellet rat chow (Hindustan Lever Ltd., Bangalore, India) and had free access to water. All the animal experiments were conducted according to the ethical norms.

Animal treatment

Group I	Control animals treated with vehicle (carboxymethyl cellulose), orally
Group II	Animals treated with Paracetamol (2g/kg) orally on 8th day
Group III	Animals treated with *COLMF* (250 mg/kg) for 7 days
Group IV	Animals treated with Chrysophenol (50mg/kg) for 7 days
Group V	Animals treated with Liv-52 (10 ml/kg) for 7 days

All the animals received respective treatments by forced oral administration for 7 days. Paracetamol 2g/kg b.wt (dissolved in 10 ml saline water) was administered to the animals of group II, III, IV and V in a single dose on the eighth day. Animals in all the groups were sacrificed 24 h after paracetamol administration and various biochemical and histopathological parameters were assessed.

Dose determination

COLMF at a dose of 250 mg/kg b.wt and chrysophanol at a dose of 50 mg/kg b.wt showed significant hepatoprotection in the preliminary investigation, and this dose was fixed as effective dose.

RESULTS

The results of the oral acute toxicity of chrysophanol and *COLMF* indicate that, there was no mortality or any toxic reaction until the end of the study. Therefore the LD_{50} value of the *COLMF* was fixed as 1200 mg/kg b.w in *COLMF* and 900 mg/kg b.w in chrysophanol.

Identification of compounds based on spectral evidence

The purified compound was subjected to IR, ^{1}H and ^{13}C NMR and MASS spectral analysis. The compound was obtained as a yellow powder .The EI-MS spectrum showed a molecular ion peak at m/z (rel. int.): 253 $[M+1]^{+}$: 234,223, 194, 177, 166, 149, 136, 124, 113, 102. IR spectrum showed chelated hydroxyl (3435 cm^{-1}), chelated carbonyl (1627 cm^{-1}), unchelated carbonyl (1676 cm^{-1}), and aromatic system (3055,1606, 1568,1475 and 1453, 903,868, 839,815 and 753 cm^{-1}): The 1H-NMR spectrum showed δ H-2-7.05 (1H,brs), H-4-7.59 (1H,brs), H-5-7.77 (1H,brdJ=7.5 Hz),H-6-7.63 (1H.t, J=7.6 Hz),H-7-7.25 (1H,br d J= 8.5 Hz),CH3-2.44(3H, S), 2X-OH-11.5 (1H,br s) · ^{13}C NMR: δC Found: 162.65 (1), 124.32 (2), 149.32 (3), 121.31 (4), 133.56 (4a), 119.89 (5), 136.91 (6), 124.51 (7), 162.36 (8), 115.80 (8a), 192.42 (9), 113.66 (9a), 181.85 (10), 133.19 (10a), 22.24 (CH3). These spectral data suggested that the compound was an anthraquinone derivative. The structure of the compound was determined ase 1,8- dihydroxy-3-methyl anthraquinone, chrysophanol (Fig. 1). The NMR spectral and physical data of compound were in good agreement with those reported in a previous paper[19].

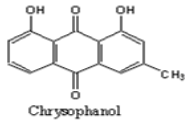

Fig. 1: Chrysophanol isolated from *C. occidentalis*

The levels of SOD and CAT recorded a significant decrease and the level of lipid peroxidation and GPx were increased in paracetamol-treated animals when compared with control group of animals (Table 1), while in chrysopahnol and *COLMF* treated rats the activities of these enzymes attained a near-normalcy ($p<0.05$).

Rats treated with a single dose of paracetamol developed significant hepatic damage as observed from the decreased enzymatic levels of GSH, Vit-C and the elevated levels of Vit-E ($P<0.001$) when compared to control group (Table 2). Pre-treatment with chrysopahnol, and *COLMF* normalized the GSH, Vit-C and Vit-E levels to near normal ($P<0.05$)

Table 1: Effect of *COLMF* and Chrysophanol on Lipid peroxidation (n moles) and Antioxidant enzymes (U/mg protein) in experimental rats

Groups	Lipid peroxidation	SOD	CAT	GPX
I	8.93 ± 1.69	558.51 ± 1.06	105.90 ± 1.47	11.61 ± 1.19
II	13.23 ± 1.49	314.57 ± 1.94	63.33 ± 1.68	19.47 ± 2.42
III	10.16 ± 1.73	465.78 ± 71.69	93.07 ± 1.35	12.44 ± 1.30
VI	9.90 ± 1.18*	495.53 ±48.90*	97.31 ± 5.46*	12.34 ± 1.11*
V	9.04 ± 2.17	557.93 ± 1.27	106.21 ± 1.33	11.88 ± 1.46

Values are expressed as mean ± S.D (n=6). Statistically significant alterations are expressed as *p<0.05. Group III, IV and V are compared with group II.

Table 2: Effect of *COLMF* and Chrysophanol on Non enzymatic anti oxidant levels (mg/dl) in experimental rats

Groups	GSH	Vit-C	Vit-E
I	17.74 ± 1.25	2.90 ± 0.09	21.43 ± 0.01
II	10.38 ± 1.16	1.73 ± 0.09	14.26 ± 0.03
III	14.87±1.11	2.56±0.10	19.37±3.97
VI	15.51±1.40*	2.67±0.07*	19.75±0.02*
V	17.68±1.58	2.81±0.07	21.34±0.07

Values are expressed as mean ± S.D (n=6). Statistically significant alterations are expressed as *p<0.05. Group III, IV and V are compared with group II.

Table 3: Effect of *COLMF* and Chrysophanol on Serum hepatic marker enzymes (IU/L) in experimental rats

Groups	AST	ALT	ACP	ALP
I	62.98 ± 5.60	26.40 ± 2.92	16.59 ±1.73	169.42±0.38
II	85.50 ± 8.32	4 1. 90 ± 4.16	25.76 ± 2.84	230.67 ± 0.03
III	67.02 ± 5.93	29.19 ± 3.22	1 9.09 ± 2.07	180.35 ± 0.14
VI	66.18 ± 4.05 *	28.62 ± 2.14*	18.10 ± 1.79*	179.27 ± 0.02*
V	63.01 ± 6.14	26.17 ± 2.17	16.89 ± 1.89	170.47 ± 0.04

Values are expressed as mean ± S.D (n=6). Statistically significant alterations are expressed as *p<0.05. Group III, IV and V are compared with group II.

The levels of AST, ALT, ACP and ALP increased significantly (P<0.05) in paracetamol alone treated animals. Treatment with chrysophanol decreased the elevated marker enzyme levels (P<0.05) to near normal. *COLMF* decreased the serum marker enzyme levels only to some extent (Table 3).

Recovery in these variables could be further corroborated by histopathological studies. The histopathological examination revealed that the liver of control rats showed normal histology (Fig. a). Paracetamol-treated animals showed alterations in the antioxidant status of the tissues, which is manifested as an abnormal histopathology like cloudy swelling, centrilobular fatty changes, steatosis, fatty vacuolization and individual hepatocytic necrosis of hepatic cells (Fig. b). *COLMF* treated animals showed mild focal hepatocytic damage and necrosis (Fig.c). Chrysophanol restored all these changes (Fig. d). The liver section of rat treated with Liv 52 showed normal histology (Fig.e).

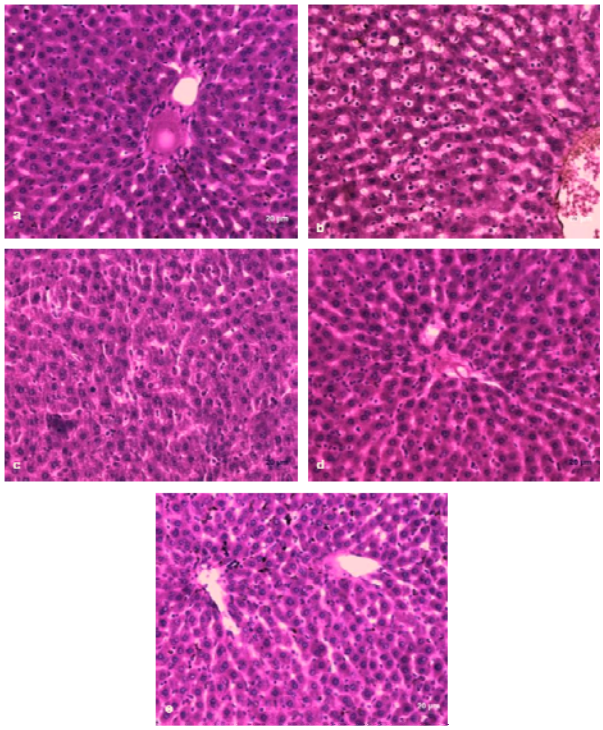

Fig. 2: Microscopic observations of liver

a. The liver section of control rats showing normal architecture. b. The liver section of paracetamol treated rat showing centrilobular necrosis, steatosis and fatty vacuolaization were seen with acute inflammatory cells infiltration sinusoids mainly in central zone. c. The liver section of *COLMF* treated rats showed normal lobular architecture and mild hepatic degeneration in the central vein. d. The liver section of chrysophanol treated rats showed normal lobular architecture and sinusoids. e. The liver section of rats treated with Liv-52 showed normal histology.

DISCUSSION

Paracetamol is a common antipyretic agent which is safe in therapeutic doses but can produce fatal hepatic necrosis in toxic doses[20]. However hepatoxicity of paracetamol has been attributed to the formation of toxic metabolites, when a part of paracetamol is activated by hepatic cytochrome P-450 to a highly reactive metabolite N-acetyl-p-benzoquinoneimine[21]. Generally these metabolites react with antioxidant enzymes such as catalase, glutathione peroxidase and super oxide dismutase.

The studies on lipid peroxidation, antioxidant enzymes (glutathione peroxidase, superoxide dismutase and catalase) and nonenzymatic antioxidants (glutathione, VitE and VitC) have been found to be of great importance in the assessment of liver damage [22].

Alterations in these enzyme levels was observed in paracetamol treated rats. SOD is very effective antioxidant enzyme and responsible for catalytic disputation of highly reactive and potentially toxic superoxide radicals to H_2O_2. The H_2O_2 is further metabolised either by catalase or peroxidase. The nonenzymatic antioxidants such as GSH, VitE and VitC act as scavengers[23]. Oral administration of Chrysophanol and *COLMF* significantly brought back the lipid peroxidation, antioxidant enzymes such as SOD, CAT and GPx and nonenzymatic antioxidants such as GSH, VitE and VitC values to near normal level. The potential activity exhibited by the chrysophanol, and *COLMF* action against acute paracetamol toxicity make them potential agents to treat liver diseases and oxidative stress. These results were similar to the protective effect of the aqueous extract of *Cassia fistula* Linn in albino wistar rats[24].

Paracetamol-induced hepatic injury is associated with a variety of biochemical abnormalities and attributed to the release of intracellular constituents into circulation, such as AST, ALT, ACP and ALP. Their estimations are useful quantitative markers for the extent of hepatocellular damage[25]. It is known that increased enzymatic activity of ALT and AST in the serum directly reflects a major permeability or cell rupture[26]. Stabilization of AST, ALT, ALP and ACP activities by treatment with *COLMF* and chrysophanol clearly indicated the improvement in the functional status of liver cells. These results were similar to the protective effect of the aqueous extract of *Hygrophila spinosa* and *C. occidentalis* in rats[8]. As few anthraquinones isolated from *Cassia tora*[28] and *Ventilago leiocarpa*[31] are reported to possess hepatoprotective activity, it is very likely that the anthraquinones present in *C. occidentalis* may be responsible for hepatoprotective activity.

CONCLUSION

It can be concluded that the herb is a potential antioxidant and attenuates the hepatotoxic effect of paracetamol by acting as an *in vivo* antioxidant and thereby inhibiting the initiation and promotion of lipid peroxidation. All the effects of chrysophanol and *COLMF* were comparable with Liv-52. The results of our study indicated that Chrysophanol and *COLMF* could protect liver against paracetamol induced hepatotoxicity.

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