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HEPATOPROTECTIVE EFFICACY OF *CUCUMIS CALLOSUS* (ROTTL.) COGN. (CUCURBITACEAE) FRUIT AGAINST CCL_4-INDUCED HEPATIC DAMAGE IN RATS

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ABSTRACT

To evaluate the hepatoprotective activity of methanol extract of *Cucumis callosus* fruit (MECC) against carbon tetrachloride (CCl_4)-induced hepatotoxicity. Hepatotoxicity was induced in male Wistar rats by intraperitoneal injection of CCl_4 (0.1 ml/kg/day for 10 days). Methanol extract of *C. callosus* fruit (200 and 400 mg/kg/day, p.o) were administered to the experimental rats for 16 days. The hepatoprotective effect of MECC was evaluated by the assay of serum biochemical parameters viz. serum glutamine oxaloacetate transaminase (SGOT), serum glutamine pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), total serum protein, total bilirubin content; and liver biochemical parameters such as thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) content, superoxide dismutase (SOD), catalase (CAT) and histopathological studies of the liver. In methanol extract-treated animals, the toxic effect of CCl_4 was controlled significantly ($P < 0.05$) by restoration of the levels of serum bilirubin, protein and enzymes as compared to the normal and the standard drug silymarin-treated groups. Histology of the liver sections of the animals treated with the extracts showed the presence of normal hepatic cords, absence of necrosis and fatty infiltration, which further evidenced the hepatoprotective activity. Methanol extract of the fruit of *C. callosus* possesses significant hepatoprotective activity.

Key words: Lipid peroxidation, Glutathione, Biochemical, Silymarin.

INTRODUCTION

Liver being the key organ of metabolism and excretion is often exposed to variety of xenobiotics and therapeutic agents. Hepatotoxicity is mainly caused due to infections,autoimmune conditions, chemical agents (certain antibiotics, aflatoxin, carbon tetrachloride, etc.).Excess consumption of alcohol is another major threat to the liver (Chaturvedi M *et al.,* 2010). Conventional drugs used in the treatment of liver diseases are often inadequate in healing the liver and are also having certain shortfalls. This has insisted us to look for an alternative source of treatment which includes safe, effective and inexpensive compounds from plant source to overcome these drawbacks.

Cucumis callous (Rottl.) Cogn. (Cucurbitaceae) commonly called as 'Bitter cucumber'(English), 'Kachri'(Hindi), is a highly branched very common prostate, perennial herb, distributed throughout India in the arid zones. The fruits are smooth, ovoid, ellipsoid, green variegated stripes and have bitter pulp (Rathore M, 2009 and Raut M, 1959). *C. callosus* is essentially a warm season crop and a long period of warm and humid climate is required. Fruit pulp of *C. callosus* is bitter, acrid, thermogenic, anthelmintic, liver tonic, cardio tonic, appetizer, expectorant and intellect promoting. Roots are used as emetic and purgative (Kirtikar KR and Basu BD,

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1984). Traditionally the fruits and seeds are used as memory enhancer, in controlling vertigo, as cooling and astringent and in bilious disorder (Rahman AHMM, 2013). The plant is used in treating diabetes mellitus by Srilankan ayurvedic and traditional physicians (Ediriweera ERHSS and Ratnasooriya WD, 2009). The leaf extract is topically used in wound healing (Patil DA *et al*., 2010).

The aqueous and alcoholic extract of *C. callosus* (seed) has been reported for its antioxidant activity (Chand T *et al*., 2012). The tribals use the fruits in worship and for curing diabetes, jaundice, epilepsy and diarrhoea (Patil DA *et al*., 2010). Hence, the present study was aimed at evaluating the hepatoprotective activity of the methanol extract of fruits of *C. callosus*.

MATERIALS AND METHODS

Collection of plant

The fruits of *C. callosus* were collected in the month of July 2014 from village area of Kendrapara and Balasore district, Odisha (India). The plant was authenticated by M. S. Mondal, Botanical Survey of India, Kolkata, India, and a voucher specimen (CNH/1-1(196)/2007/Tech-II/160) has been preserved in the Pharmacology Research Laboratory, Jadavpur University, Kolkata for future reference.

Extraction

The fruits of *C. callosus* were shade dried and then powdered with a mechanical grinder. The powder (500 g) was defatted with petroleum ether at 40–60°C in a Soxhlet extraction apparatus (16-24 hours) and then extracted similarly with methanol at 60-80°C for 16-24 hours. The solvents were evaporated to dryness in vacuo (at 35 °C) in a Buchi evaporator, R-114 to obtain a dry mass. The yields of the petroleum ether and methanol extracts were found to be 2.8% and 9.00%, w/w, respectively. The extracts were stored in vacuum desiccators for further use (Dolai N *et al*., 2012).

Acute toxicity study

The acute oral toxicity of MECC in Swiss albino mice was performed as per OECD guideline 425 (OECD, 2008).

Animals

Adult male Wistar albino rats weighing 150–200 g were used for the present investigation. They were housed in a clean polypropylene cage and were fed with a standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory conditions for 1 week prior to the experiment. All procedures described were reviewed and approved by the university animal ethics committee (367001/C/ CPCACA).

Drugs and chemicals

Bovine serum albumin: Sigma Chemical Co., St. Louis, USA; Trichloroacetic acid (TCA): Merck Ltd. Mumbai, India; Thiobarbituric acid (TBA), Nitroblue tetrazolium chloride (NBT): Loba Chemie, Mumbai, India; 5,5'-dithio *bis*-2-nitro benzoic acid (DTNB), Phenazonium methosulphate (PMS), Nicotinamide adenine dinucleotide (NADH) and reduced glutathione (GSH): SISCO Research Laboratory, Mumbai, India. Silymarin: Ranbaxy Laboratories, Indore, India. All the other reagents used were of analytical reagent grade obtained commercially.

Treatment schedule

The rats were divided into five groups (n = 6). The animals from Group I served as the vehicle control and received the vehicle 0.25% sodium carboxymethylcellulose at a dose of 1 ml/ kg/day, p.o. for 16 days. Groups II–V received 0.1 ml/kg/ day, i.p. of CCl_4 (E-Merck, Mumbai, India) for 10 days. Group II served as CCl_4 control (Jaiprakash B *et* al., 2003). Groups III and IV were treated with methanol extract of *C. callosus* in the doses of 200 and 400 mg/kg/day, p.o. for 16 days respectively. On the basis of higher LD_{50} value (Up to 2000mg/kg the drug did not show any kind of toxicity) and lower toxicity the dose of 200 and 400mg/kg were selected. The standard drug Silymarin (Ranbaxy Lab.) was administered to Group V animals in the dose of 100 mg/kg/ day, p.o. for 16 days. After 24 h of last dose, blood was collected from overnight fasted rats of each group by cardiac puncture, for estimation of serum biochemical parameters. Then the rats were sacrificed by cervical dislocation for the study of liver biochemical and histopathological parameters.

Serum biochemical estimations

Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP) and total bilirubin content were estimated by using commercially available kits (Span Diagnostic Ltd., Surat, India). Serum total protein was estimated according to the (Lowry OH *et al*., 1951).

Liver biochemical estimations

The levels of lipid peroxidation i. e. thiobarbituric acid reactive substances (TBARS) in the liver tissue were measured as per (Chakraborty M *et al*., 2015a). The levels of lipid peroxides were expressed as µmoles of malondialdehyde (MDA)/g of liver tissue. The reduced glutathione (GSH) content of liver tissue was determined as per (Ellman GL, 1959) and expressed as µg/g of liver tissues. The superoxide dismutase (SOD) and catalase (CAT) activity in liver tissue were assayed as per the (Chakraborty M *et al*.,2015a; Kakkar P *et*

al.,1984) respectively. The SOD activity was expressed as unit/mg of liver tissue and CAT was expressed in terms of µmol of hydrogen peroxide decomposed/min/mg of liver tissue.

Histopathological study

For histopathological study the fresh liver tissues were collected and immediately fixed in 10% formalin, dehydrated in gradual ethanol (50-100%), cleared in xylene and embedded in paraffin. Sections (4-5 µm) were prepared and then stained with hematoxylin-eosin dye for photomicroscopic observations as per (Haldar PK *et al*., 2011).

Statistical analysis

All results were expressed as the mean ± standard error of mean (SEM). The results were analyzed for statistical significance by one-way ANOVA followed by Dunnett's *post hoc* test of significance. $P < 0.05$ was considered as statistically significant.

RESULTS

Acute toxicity

Oral administration of MECC in mice, at doses from 100 to 2000 mg/kg, did not produce any significant change in behaviour, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects. During the 24 h experimental period, no deaths occurred in any of the groups. These results indicate that LD_{50} of MECC is higher than 2000 mg/kg and presented a considerable safety margin, being apparently devoid of acute toxicity for male and female mice.

Serum biochemical parameters

Biochemical parameters like SGOT, SGPT and SALP, in CCl_4 control group were significantly ($P< 0.05$) elevated as compared to normal control group. Treatment with MECC at a dose of 200 and 400 mg/kg significantly ($P< 0.05$) brought their levels towards normal values in a dose dependent manner. Total protein was found to be significantly decreased in CCl_4 control group as compared with normal control group ($P< 0.05$). The administration of MECC increased total protein content in animals of treated group significantly ($P< 0.05$) as compared with CCl_4 control group (Table 1).

Liver biochemical parameters

The TBARS content increased significantly in CCl_4 control group as compared to normal control group. Treatment with MECC at 200 and 400 mg/kg significantly ($P< 0.05$) reduced TBARS levels when compared with CCl_4 control animals in dose related manner. The GSH, CAT and SOD levels in liver was drastically reduced in CCl_4 control group but it was effectively restored to nearly normal level by supplementation with MECC (Table 2).

Histopathological study

Histopathological study of livers of saline control group showed normal hepatocellular architecture (Fig.A). Livers challenged with CCl_4 showed disarrangement of normal hepatic cells with massive interlobular necrosis, inflammatory infiltration of lymphocytes and fatty changes (Fig. B). The MECC (400 mg/kg) treated rats exhibited significant protection against CCl_4 intoxication as evident by presence of normal hepatic cords and absence of necrosis with minimal inflammatory conditions around the central vein (Fig. D). However, moderate protection was observed in case of low dose (200 mg/kg) group animals (Fig.C).

Histopathological observations (liver sections stained with Haematoxylin and Eosin, magnification x 100) showing the effects of MECC extract on CCl_4 - induced histopathological changes in mouse liver. A) Normal, (C) CCl_4 + MECC extract (200 mg/Kg) (D) CCl_4 + MECC (400 mg/kg, p.o.) (E) CCl_4 + Sylimarin (100 mg /kg, p.o.).shows near to similar hepatic architecture with distinct hepatic cells, sinusoidal spaces and a central vein; (B) CCl_4 -treated group shows severe centrilobular hepatic necrosis, fatty changes, degeneration. In pictures C,D and E, only mild inflammation and lymphocyte infiltration are observed.

Table 1. Effect of Methanol extract of *C. callosus* (MECC) on serum enzyme levels and Total protein of CCl_4 intoxicated rats.

Treatment	SGOT(IU/L)	SGPT(IU/L)	SALP(IU/L)	Total Bilirubin(mg/dl)	Total Protein (mg/dl)
Normal Control (5 ml/kg)	22.67±1.39*	24.88±0.55	112.1±1.02*	0.73±0.15	8.93±0.42*
CCl_4 Control (0.5 ml/kg)	68.95±3.83	79.53±1.59	257.3±1.55	2.77±0.38	4.83±0.53
CCl_4 + MECC (200 mg/kg)	41.12±1.35*	39.58±1.04*	217.4±1.42*	2.05±0.61*	5.96±0.65*
CCl_4 + MECC (400 mg/kg)	34.36±1.59*	36.13±1.86*	190.9±1.58*	1.05±0.11*	7.71±0.54*
CCl_4+Sylimarin(100mg/kg)	32.62±0.53*	36.89±1.12*	143.5±0.95*	0.92±0.06*	7.88±0.75*

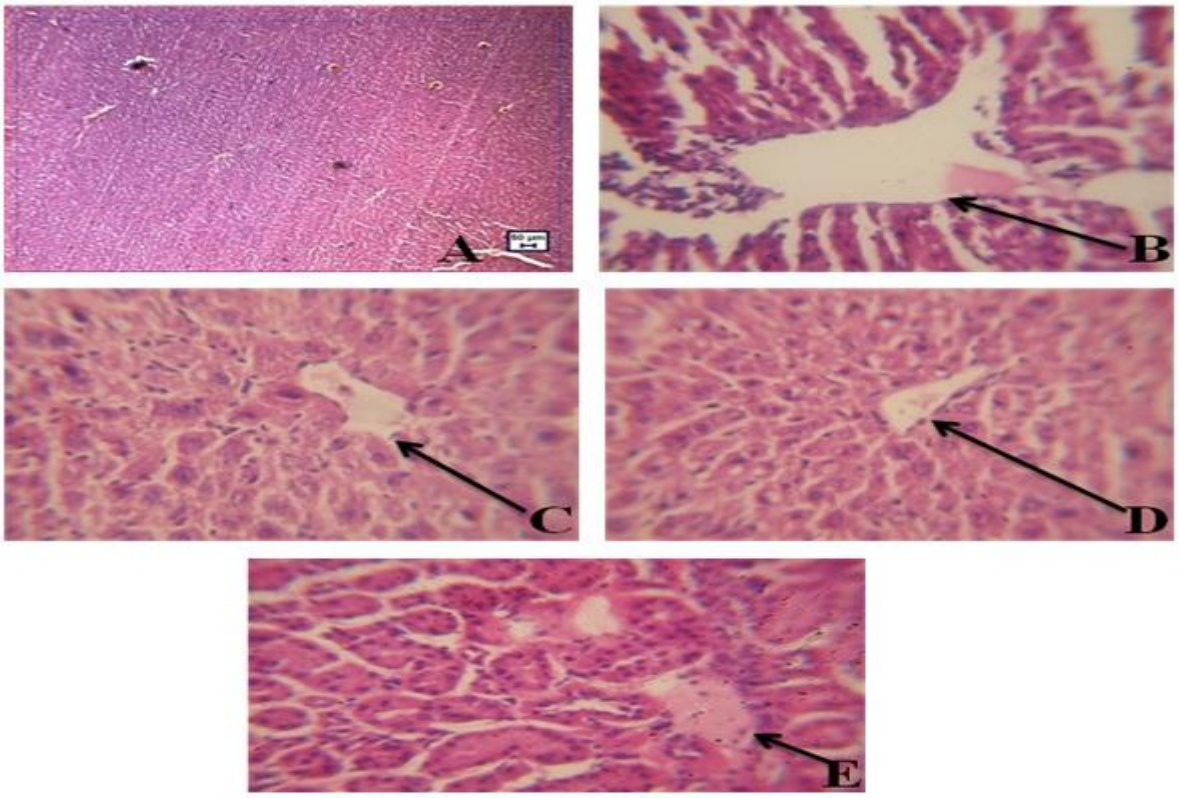
Values are expressed as mean ± SEM from n = 6, * $p < 0.05$ compared with CCl_4- control group. Statistically analysis was evaluated by Dunnett's vs. control.

Table 2. Effect of Methanol extract of *C. callosus* (MECC) on liver biochemical parameters of CCl_4 intoxicated Rats.

Treatment	TBARS(µmoles/g)	GSH (µg/g)	CAT(µmol/min/m)	SOD (unit/mg)
Normal Control (5 ml/kg, p.o.)	1.06±0.10	43.30±2.05*	71.35±2.10	8.95±0.33*
CCl_4Control (0.1 ml/kg , i.p.)	1.81±0.15	27.89±2.79	31.27±2.25	3.35±0.89
CCl_4+ MECC (200 mg/kg, p.o.)	1.48±0.11*	33.96±1.37*	44.72±1.13*	5.81±0.31*
CCl_4 + MECC (400 mg/kg, p.o.)	1.24±0.08*	37.75±1.91*	54.84±3.25*	6.97±0.29*
CCl_4 + Sylimarin (100 mg /kg, p.o.)	1.12±0.12	40.13±0.98*	59.82±1.27*	7.99±0.35

Values are expressed as mean ± SEM from n = 6, * $p < 0.05$ compared with CCl_4- control group. Statistically analysis was evaluated by Dunnett's vs. control.

Fig 1. Photomicrographs of Liver sections stained with hematoxylin and eosin (magnification 100X). (A) Normal control, (B) CCl_4 control, (C) CCl_4 + MECC(200 mg/kg), (D) CCl_4 + MECC (400mg/kg), (E) CCl_4 + Sylimarin (100mg/kg).



DISCUSSION

The present studies were performed to assess the hepatoprotective activity in rats, against Carbontetrachloride as hepatotoxin to prove its claim in folklore practice against liver disorder.

Carbon tetrachloride (CCl_4) has been widely used hepatotoxin for inducing experimental hepatic damage due to free radical formation during its metabolism by hepatic microsome (Chakraborty M *et al*., 2015b). The hepatotoxicity induced by CCl_4 is due to its metabolite $CCl_3^{\bullet}$, a free radical that binds to lipoprotein and damage cellular membranes through the lipid peroxidation (Chakraborty M *et al*., 2015b).

Elevated levels of SGOT, SGPT and ALP are useful quantitative marker of the extent and type of hepato cellular damage (Mitra SK *et al*., 1998; Yue M *et al*., 2006). The enhanced levels of these serum marker enzymes were observed in CCl_4-treated rats in our experiment. Restoration of levels of these enzymes to/towards near normal values in the *C. callosus* extract and silymarin treated animals is a clear manifestation of hepatoprotective effect of *C.callosus* extract and silymarin. Serum bilirubin is the protein with the highest concentration in plasma and it is synthesized by the liver. It transports many small molecules in the blood (for example calcium and progesterone). It also prevents the fluid in the blood from leaking out into the tissues. Increase in serum bilirubin was reflected the depth of jaundice, which was attenuated in *C. callosus* extract and silymarin treated groups, indicating its hepatoprotective

effect further (Gnanadesigan M *et al*., 2011). The lowered level of total protein in CCl_4 challenged animals is attributed to the initial damage produced and localized in the endoplasmic reticulum which results in the loss of CYP_{450} leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver (Recknagel RO *et al*.,1989; Takate SB *et al*., 2010).

The trichloromethyl (CCl_3) free radical in turn reacts with molecular oxygen & gets converted to trichloromethyl peroxy radical. This radical forms covalent bonds with sulfhydryl group of several membrane molecules like GSH (reduced glutathione) leading to their depletion & causes lipid peroxidation. The peroxidation initiates a cascade of reactions leading to tissue necrosis (Chakraborty M *et al*., 2015b). Lipid peroxidation is usually measured through its catabolite malondialdehyde (MDA) as a marker of oxidative stress. MECC showed ability to prevent CCl_4 induced elevation of MDA level as compared to standard drug, which suggest that MECC may inhibit hepatic lipid peroxidation in CCl_4 intoxicated rats. This implies the reduction in free radical production and subsequent decrease in damage to the hepatocellular membranes.

Superoxide dismutase (SOD) and catalase (CAT) are endogenous enzymatic antioxidants present in all oxygen metabolizing cells involved in the clearance of superoxide and hydrogen peroxide. The CAT and SOD stores may be decreased because of the toxic free radicals of CCl_4 (Jamshidzadeh A *et al*., 2006). The administration of MECC significantly recovered the SOD and CAT activities towards normal in a dose dependent manner as compared to standard drug.

MECC also restored the GSH level towards normal and standard drug in a dose dependent manner.

In conclusion, treatment with MECC could reduce damage induced by CCl_4. The mechanisms of protection include the inhibition of lipid peroxidation, increasing the content of GSH, elevating the expression of antioxidant enzymes, all of which result in the recuperation of biological parameters and the integrity of the tissue. Furthermore, the high content of polyphenols, alkaloids and saponins in MECC contributes free radical scavenging and antioxidant activities (Muriel P *et al*., 1992).

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CONFLICT OF INTEREST

No Interest.

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