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HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF *CNIDOSCOLUS CHAYAMANSA* AGAINST D-GALACTOSAMINE INDUCED LIVER DAMAGE IN RATS

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ABSTRACT

In the deficiency of dependable liver-protective drugs in contemporary medicine, a large number of medicinal preparations are recommended for treatment of liver disorders. *Cnidoscolus chayamansa* Mc Vaugh (Euphorbiaceae) is a medicinal and edible plant known as Chaya, is commonly used as an antiinflammatory, antiprotozoal, antibacterial agent and as a remedy for respiratory illness, gastrointestinal disorders and vaginal infections related with the inflammation process. Although *C. chayamansa* is one of most used and valued medicinal plants, only few studies on documenting its pharmacological properties can be found. The antioxidant and hepatoprotective activities of ethanolic extracts of *C. chayamansa* leaves are evaluated here. Ethanolic extract of *C. chayamansa* (EECP, 200and400mg/kg,p.o.) was evaluated for its hepatoprotective and antioxidant activity in d-galactosamine (d-GalN)-induced hepatotoxicity in rats. Biochemical and histopathological studies were performed to assess hepatoprotective activity. d-GalN administration induced hepatotoxicity in rats which was manifested by increased levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), Gamma-glutamyl transpeptidase(GGTP) and decrease levels of Total protein(TP) and Total albumin(TA).In liver homogenate, there was significant decrease in SOD, CAT and GPx levels and increase in LPO levels were observed in animals treated with galactosamine as compared to normal control group. Pretreatment with EECP significantly protected the liver in d-GalN administered rats. EECP significantly elevated antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase and decreased lipid peroxidation levels in liver. Histological studies showed that EECP at 400 mg/kg attenuated the hepatocellular necrosis in d-GalN intoxicated rats. From this study, it can be concluded that the ethanolic extract of *C. chayamansa* is not only an effective hepatoprotective agent, but also possesses significant antioxidant activity.

Keywords: *Cnidoscolus chayamansa*, Hepatoprotective, Antioxidant activity, D-galactosamine, Biochemical and histopathological studies.

INTRODUCTION:

Impairment of vital organs like liver leads to serious consequences for the health of an individual and, in the majority of cases, is life threatening. Management of liver diseases is still a challenge to the modern medicine [1]. Modern medicines have little role to alleviation of hepatic diseases and the plant-based preparations which are chiefly available medicines employed for the treatment of liver disorders (Raju et al., 2008). The effectiveness of these plant products must be proved so as to identify newer medicaments acting against hepatic injury. In the absence of a reliable liver protective drug in the modern system of medicine, a number of medicinal plants in Ayurveda are recommended for the treatment of

liver disorders. Natural treatments from medicinal plants are considered to be effective and safe medicaments for hepatotoxicity[2]. Liver injury can be caused by different agents, such as viruses, chemicals, alcohol and auto-immune disease[3]. D-Galactosamine (D-GalN) is a well-established hepatotoxicant, it induces a diffuse type of liver injury closely resembling human viral hepatitis [4] and acute self-limiting hepatitis with necrosis, inflammation and regeneration, resembling drug-induced diseases in humans [5]. The toxicity of D-GalN is mainly related to the depletion of uridine pools that are associated with limited ribonucleic acid (RNA) and protein synthesis, thus altering hepatocellular function [6]. *Cnidoscolus chayamansa* Mc Vaugh, (Euphorbiaceae), called Chaya in South Texas, is popular in Mexico and Central America and has been introduced into the United States and now presently available in and around Southern part of India, for potential uses as a leafy vegetable and or as a medicinal plant. The edible parts of *C. chayamansa* plant which taste such as spinach when cooked, provide important nutritional sources for proteins, vitamins (A and C), minerals (calcium, iron, phosphorus), niacin, riboflavin, and thiamine. Among populations that cannot afford expensive foods rich in these nutrients [7]. *C. chayamansa* traditionally has been recommended for a number of ailments including diabetes, obesity, kidney stones, hemorrhoids, acne and eye problems [8]. *C. chayamansa* shoots and leaves have been used as a laxative, diuretic, circulation stimulant to improve digestion to stimulate lactation and to harden the finger nails [9]. The leaves contain mineral constituents such as K, Ca, Mg, Na, Fe, Mn, Zn, and Cu, flavonoids such as amentoflavone, Astragatin, kaempferol-3o-ruttinoside and dihydromyricetin. Leaves also contain hydrocyanic glycosides, a toxic compound easily destroyed by cooking, even though some people tend to eat raw *C. chayamansa* leaves, it is unwise to do so while the nutritional value of *C. chayamansa* has been demonstrated [10]. But still, no scientific investigation has so far been reported in the literature regarding its action on the liver. Therefore, the present study was aimed at evaluating the hepatoprotect.

Materials and Methods

Chemicals

All chemicals were of analytical grade and purchased from Himedia Lab Limited, India. D-Galactosamine (d-GalN) was purchased from Merck India Ltd.,Mumbai, India. Biochemical estimations were carried out using kits purchased from Ecoline Merck Limited, India.

Plant collection and extraction

The leaves of *C. chayamansa* were collected from in and around Bhopal District, Madhya Pradesh. The leaves of the plant *C. chayamansa* were dried in the shade, milled into coarse powder by a mechanical grinder and about 500gm of dry powder packed into Soxhlet apparatus and extracted with petroleum

ether, chloroform and ethanol at 75-79°C for 72hrs. The extract obtained was evaporated at 45°C, then dried and stored in airtight container. Finally the percentage yields were calculated of the dried extracts. The yield of the petroleum ether, chloroform and ethanol extracts was found to be 3.6, 5.2 and 8.4% (w/w), respectively.

Acute toxicity study

This study was carried out as per OECD test guideline 423 (OECD, 2001) in Wistar albino rats. The Animal Ethics Committee of the institution approved the study protocol. The extract fell under class 4 (LD50 > 2000 mg/kg). One-tenth and one-fifth of this dose was selected as the therapeutic dose for the evaluation of hepatoprotective activity [11].

Experimental animals

Male/female albino Wistar rats weighing 200 ± 20 gm used in this study were obtained from the Animal House of PBRI, Bhopal (). The animals were maintained under standard laboratory conditions of constant temperature (24 ± 2^0C), relative humidity (50% ± 15%), 12 h light: 12 h dark cycle, and allowed free access to food and water. Animal care and handling was done according to the guidelines set by the World Health Organization, Geneva, Switzerland and approved by the Committee for Animal Care at the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority (AEA).

Experimental design

In the present study animals were divided into five main groups with six rats in each group:

Group 1: Served as vehicle control.

Group 2: Toxic control received 25mg/kg of D-galactosamine through I.P for 21 days

Group 3: Standard control received 25mg/kg of vitamin E orally for 21 days

Group 4: The treatment control received 200mg/kg of EECP for 21 days

Group 5: The treatment control received 400mg/kg of EECP for 21 days

On day 22 after 24 hrs of Galactosamine administration animals in all the groups were humanely sacrificed using Ketamine HCl and 4ml of blood was withdrawn by cardiac puncture and allowed to clot for 30mins at room temperature. The serum was separated by using cooling centrifuge and used for the assay of marker enzymes viz AST, ALT, ALP, TB, GGPT and total albumin [12]. On the same day, rats were sacrificed by cervical decapitation liver samples were dissected out and homogenates (10% w/v) were prepared and centrifuged. The obtained supernatant was used for the estimation of total protein (TP)[13], superoxide dismutase (SOD) [14], catalase (CAT) [15], glutathione peroxidase (GPx) [16] and lipid peroxidation (LPO) [17]. For histopathological studies, liver sections were prepared, stained with alum hematoxylin and eosin, examined microscopically for histopathological changes.

ive and antioxidant activity of *C. chayamansa* leaves on rat liver damage induced by d-GalN.

Statistical analysis

Values are expressed in mean ± SD for six rats in each group. P value was calculated using one way ANOVA followed by Newmann Keul's multiple range tests. Values of $p<0.01$ were considered significant in all cases.

Results

In the acute toxicity studies, EECP did not show any toxic signs or mortality at 2000 mg/kg dose. The elevated levels of AST, ALT, ALP, GGPT and TB in d-GalN intoxication were significantly reduced in the rats pre-treated with EECP and increased levels of TP and TA significantly (Table 1). Pre-treatment with EECP (400 mg/kg) exhibited significant hepatoprotective activity which was comparable with the standard drug Vitamin E. Table 2 also depicts there is a decrease in the levels of SOD, CAT, GPx and increase in LPO in d-GalN treated rats liver in comparison to vehicle treated group. EECP pre-treatment dose dependently caused a significant increase in the levels of antioxidants and decreased the LPO levels in comparison to d-GalN treated rats. Table 3 shows the levels of non-enzymatic antioxidants such as reduced glutathione, Vitamin C and Vitamin E in the tissues (liver) of D-galactosamine hepatotoxic and control rats. The levels of non-enzymatic antioxidants in D-galactosamine hepatotoxic rats significantly decreased. EECP both doses administered rats showed significantly increased levels of these non-enzymic antioxidants as compared with untreated hepatotoxic rats. Histology of liver sections of normal control animals (Group I) showed normal liver architecture with were brought out central vein, were preserved cytoplasm and prominent nucleus and nucleolus. The liver sections of galactosamine treated animals (Group II) showed hepatic cells with serum toxicity characterized by inflammatory cell collection, scattered inflammation across liver parenchyma, focal necrosis and swelling up of vascular endothelial cells. Vitamin-E (Group-III) exhibited protection from galactosamine induced changes in the liver. EECP pretreatment at a dose of 200mg and 400mg/kg (group IV and V) appeared to significantly prevent the galactosamine toxicity as revealed by the hepatic cells with were preserved cytoplasms. EECP pretreatment also caused marked decrease in inflammatory cells (Fig 1).

Table.1 Effect of EECP and Vitamin E pre-treatment on biochemical parameters of the rats intoxicated with D-Galactosamine

Group. No.	Treatment	AST (IU/ml)	ALT (IU/ml)	ALP (IU/ml)	TP (gm/dl)	TB (mg/dl)	GGTP (mg/dl)	TA (g/dl)
I	Normal control 10ml/kg	44.40±1.52	30.09±1.49	23.68±1.30	5.15±0.08	1.92±0.08	96.90±2.75	3.80±0.16
II	*a Toxic control D-galactosamine 25mg/kg	105.90± 2.40	94.49±1.05	144.10±2.35	3.16±0.22	4.40±0.26	173.42±2.90	2.20±0.07
III	*b Standard control Vitamin E 25mg/kg	60.10±1.20	40.56±1.06	56.4±1.70	3.90±0.08	2.8±0.15	122.20±1.95	2.90±0.05
IV	*b Treatment control EECP 200mg/kg	68.65±1.46	54.82±2.72	65.86±2.30	4.60±0.25	3.30±0.20	136.30±3.04	2.54±0.04
V	*b Treatment control EECP 400mg/kg	62.45±1.15	47.94±0.97	58.50±1.95	4.05±0.26	2.95±0.18	130.94±1.23	2.30±0.09

Values are given as mean ± SD from six rats in each group; Values are found out by using one way ANOVA followed by Newmannkeul's multiple range tests; *a-values are significantly different from Normal control at P< 0.01;*b-values are significantly different from Toxic control (G2) at p< 0.01.

Table2 Effect of EECP and Vitamin E pre-treatment on biochemical liver parameter in D-Galactosamine induced hepatotoxicity.

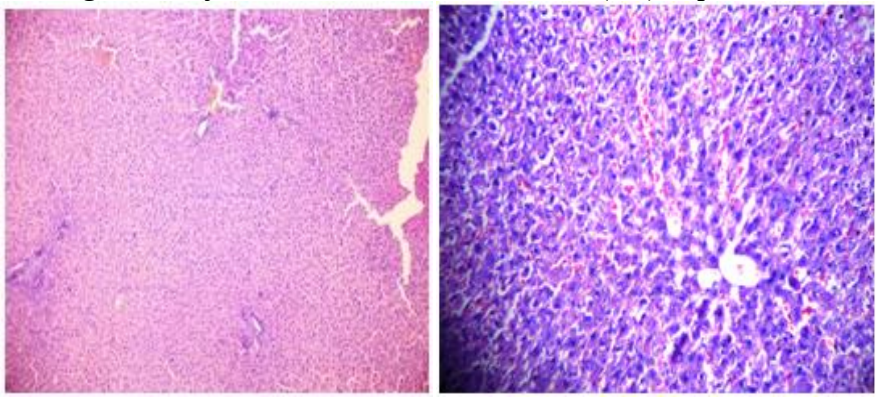
Group. No.	Treatment	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	LPO (nmol of NDA/mg protein)
I	Normal control 10ml/kg Normal saline	132.25±2.40	290.40±2.40	1.10±0.05	3.90±0.17
II	*aToxic control D-galactosamine 25mg/kg	68.20±1.65	190.75±2.70	0.40±0.02	7.40±0.12
III	*bStandard control Vitamin E 25mg/kg	118.05±2.80	260.45±1.92	0.85±0.02	4.50±0.14
IV	*b Treatment control EECP 200mg/kg	96.50±1.60	230.05±1.80	0.55±0.02	5.60±0.28
V	*b Treatment control EECP 400mg/kg	105.65±2.62	240.75±2.65	0.74±0.02	4.80±0.08

Values are given as mean ± SD from six rats in each group; Values are found out by using one way ANOVA followed by Newmannkeul's multiple range tests; *a-values are significantly different from Normal control at P< 0.01;*b-values are significantly different from Toxic control (G2) at p< 0.01.

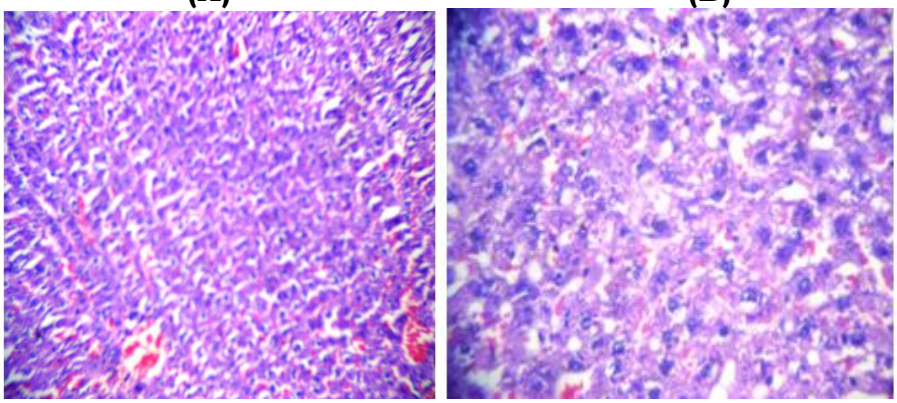
Table 3 Effect of EECP on non enzymatic antioxidants in the liver tissue of d-galactosamine-hepatotoxic and control rats

Groups	Glutathione mg/100g Tissue	Vitamin-C mg/100g Tissue	Vitamin-E mg/100g Tissue
Normal control 10ml/kg	132.60±3.45	0.82±0.08	5.92±0.60
*a Toxic control D-galactosamine 25mg/kg	73.55±1.70	0.30±0.02	2.40±0.30
*b Standard control Vitamin E 25mg/kg	110.32±2.70	0.74±0.07	5.60±0.55
*b Treatment control EECP 200mg/kg	98.05±2.16	0.60±0.04	4.92±0.50
*b Treatment control EECP 400mg/kg	91.90±1.95	0.69±0.06	5.02±0.48

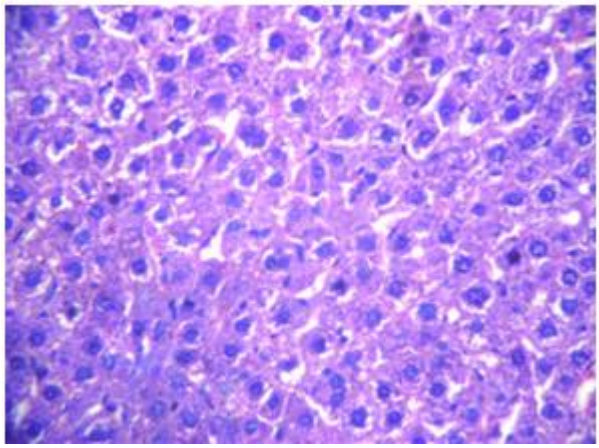
Values are given as mean ± SD from six rats in each group; Values are found out by using one way ANOVA followed by Newmannkeul's multiple range tests; *a-values are significantly different from Normal control at P< 0.01;*b-values are significantly different from Toxic control (G2) at p< 0.01.



(A) **(B)**



(C) **(D)**



(E)

Fig. 1(A): Magnified view of liver from normal control group (B): Toxic Control group (C): Standard Drug control (D): (*C. Chayamansa* 200 mg/kg/rat) (E) (*C. Chayamansa* 400 mg/kg/rat) Data were presented as mean±SD, No. of animals (n) =6

DISCUSSION

Elevated levels of ALT, AST, ALP enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [18]. Pre-treatment with EECP decreased the level of hepatic enzymes towards their respective normal value is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by d-GalN. The 400mg/kg dose had a better effect than the low dose of EECP (200mg/kg). The higher concentration might have resulted in the production of more by products that would have interfered with the activity. Treatment with EECP significantly decreased these enzyme activities, indicating that EECP has a hepatoprotective effect against a D-galactosamine- induced liver injury. An increase in TB and ALP reflects the pathological alteration in biliary flow [19]. EECP mediated suppression of the increased TB level suggests the possibility of the extract being able to stabilize biliary dysfunction. These biochemical findings were further substantiated by histopathological studies. D-galactosamine-induced oxidative damage is generally attributed to the formation of the highly reactive hydroxyl radical (OH·), the stimulator of lipid peroxidation and the source of destruction and damage to the cell membrane [20]. D-galactosamine toxicity enhanced lipid peroxidation and reduced antioxidants were reported in the kidney [21]. The previous studies show that D-galactosamine-induced rats significantly increased thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes in liver and kidney [22,23]. In the present study, we observed an increase in the levels of thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes in the tissues of D-galactosamine-hepatotoxic rats. Increased lipid peroxidation in various tissues has long been known to

cause functional degradation; thus, the degradation of vital tissue leading to complications may be indirectly due to increased oxidative stress. Treatment with EECP and Vitamin-E showed a significant reduction which might be due to the antioxidant ability of these compounds and the consequent reduction in lipid peroxidation. EECP possesses antioxidative and free-radical scavenging effects. Oxidative stress is an imbalance between reactive oxygen species and the antioxidant defense mechanisms of a cell or tissue, which leads to lipid peroxidation, DNA damage and the inactivation of many enzymes [24]. The enzymatic antioxidant defense system is the natural protector against lipid peroxidation that includes superoxide dismutase, catalase and glutathione peroxidase. Reduced activities of these enzymes in the tissue of D-galactosamine- hepatotoxic rats were observed in our study. Superoxide dismutase protects against the superoxide radical ($O2\cdot-$), which damages the membrane and its biological structure. Catalase primarily decomposes hydrogen peroxide to H_2O at a much faster rate, sharing this function with glutathione peroxidase. Glutathione peroxidase may play an important role in the removal of lipid hydroperoxides. The balance between these enzymes is important for the efficient removal of oxygen radicals from tissues [25]. Therefore, reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and H_2O_2. Significant increases in the activities of these enzymes were observed on EECP administration. The second line of defense consists of the non-enzymic scavenger's glutathione, ascorbic acid, and α-tocopherol, which scavenge residual free radicals escaping from decomposition by the antioxidant enzymes. Moreover, enzymic antioxidants are inactivated by the excessive levels of free radicals and hence the presence of non-enzymic antioxidants is presumably essential for the removal of these radicals [26]. Glutathione a major non-protein thiol in living organism's plays a central role in coordinating the antioxidant defense process. Glutathione reacts directly with reactive oxygen species and electrophilic metabolites, protects the essential thiol group from oxidation, and serves as a substrate for several enzymes including glutathione peroxidase [27]. The lowered glutathione in D-galactosamine induced rats represents the increased utilization of glutathione as a result of oxidative stress. Perturbation in the redox status of glutathione not only impairs cellular defense against toxic compounds but also results in enhanced oxidative stress and oxidative injury [28]. Apart from glutathione, α-tocopherol and ascorbic acids are important free-radical scavengers which protect cell membrane against toxic agents. Both vitamins C and E have a synergistic action in scavenging oxygen-derived free radicals [29]. Vitamin C functions as a free-radicals cavenger of oxygen radicals and successfully prevents detectable oxidative damage under all types of oxidative stress. Ascorbic acid appears to trap the peroxyl radical in the aqueous phase with a rate large enough to lipids and dehydroascorbate is produced in this reaction. A thiol cycle converts the dehydroascorbate into

ascorbate. The thiol cycle consists of a GSSG/GSH couple [30]. Thus glutathione in blood keeps up the cellular levels of the active form of vitamin C. When there is a reduction in glutathione, the cellular level of ascorbic acid is also lowered. The observed decrease in the levels of α-btocopherol and ascorbic acid in the D-galactosamine rats might be due to an antioxidant defense against increased ROS or due to a decrease in glutathione levels in D-galactosamine-hepatotoxic rats. In this respect, reported that ascorbic acid and α-tocopherol decreased in liver diseases, particularly in D- galactosamine- hepatotoxic rats. Our study observed increase the levels of these antioxidants in EECP and Vitamin-E administered rats. The ability of EECP to enhance the levels of antioxidants along with its anti lipid peroxidative activity suggests that this compound might be potentially useful in counteracting free-radical-mediated tissue damage caused by hepatotoxicity. Studies on the antioxidative potency of various flavonoids have confirmed the importance of the distribution and quantity of the hydroxyl groups. In general, the antioxidative properties of polyphenols depend on hydroxylation of ring B. The present results corroborate the protective action of EECP in D- galactosamine intoxication of rats, particularly noticeable with the high dose used by us (400 mg/kg body weight). Since *C. chayamansa* is rich in flavonoids and phenolics, the possibility of the mechanism of hepatoprotection of *C. chayamansa* extract may be due to its antioxidant action.

Conclusion

Our findings demonstrated that EECP at both doses possesses hepatoprotective and antioxidant activity, which is evidenced by lowered serum hepatic marker enzyme activities. Among the two dosages tested, 400mg/kg/body weight showed more promising hepatoprotective and antioxidant activity, and is comparable to the standard drug Vitamin-E. The hepatoprotective effect is documented by the biochemical and histopathological data obtained. It may be speculated that the constituents of *C. chayamansa* leaf are to be responsible, at least in part, for the observed hepatoprotective effect. A possible mechanism of *C. chayamansa* extract as hepatoprotective may be due to its antioxidant effect which impairs the activation of D-galactosamine into the reactive form, it may be speculated that the high flavonoid content of *C. chayamansa*, was responsible for the observed protective effects. Further studies need to be done on *C. chayamansa* extract to identify the active constituent(s) responsible for the hepatoprotective activity and elucidate the mechanism of action.

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