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RESEARCH ARTICLE

PRELIMINARY HEPATOPROTECTIVE ACTIVITY OF MEDICINAL PLANT EXTRACTS AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN ALBINO RATS

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ABSTRACT

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight. But it is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol and prescribed and over-the-counter drug which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there is not much drug available for the treatment of liver disorders. Therefore, many folk remedies from plant origin are tested for its potential hepatoprotective liver damage in experimental animal model. Carbon tetrachloride (CCl_4) induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts.

INTRODUCTION

The *Elytraria acaulis* whole plant hydroalcholic and aqueous extracts were studied for hepatoprotective activity against Swiss albino rats with liver damage induced by carbon tetra chloride (CCl_4). The present experiment was conducted for 07 days to evaluate the hepatoprotective activity of plant *Elytraria acaulis* in CCl_4 (1ml/kg) treated rats. It was found that the hydroalcholic and aqueous extracts of *Elytraria acaulis* at a dose of 200 mg/kg body weight (the extracts were prepared by the *Elytraria acaulis* whole plant extracts in hydroalcholic and aqueous solvents through maceration technique. The 6 groups were maintained as control, CCl_4 induced, CCl_4+ Liver tonic, CCl_4+*Elytraria acaulis* extracts (hydroalcholic (ethanol 70% + aqueous 30%), 200mg/kg and aqueous 200mg/kg) exhibited moderate protective effect by lowering the serum levels of alanine amino transferase (ALT) or Serum Glutamate Pyruvate Transaminase (SGPT), aspartate amino transferase (AST) or Serum Glutamate Oxaloacetate Transaminase (SGOT) and Total cholesterol, HDL, LDL, Triglycerides, liver glycogen and liver protein to a significant extent. The highest activity of observed for the *Elytraria acaulis* whole plant hydroalcholic and aqueous extracts at a dose of 200 mg/kg body weight. The hepatoprotective activity was also supported by histopathological studies of liver tissue. Since results of biochemical studies of blood samples of carbon tetrachloride treated rats showed significant increase in the levels of serum enzyme activities (Ahsan *et al.*, 2009), reflecting the liver injury caused by CCl_4 and blood samples from the animals treated with the *Elytraria acaulis* whole plant hydroalcholic and aqueous extracts showed significant decrease in the levels of serum markers, indicating the protection of hepatic cells, the extracts of *Elytraria acaulis* whole plant could afford significant dose dependent protection against CCl_4 induced hepatocellular injury.

MATERIAL AND METHODS

Plant material

Whole plant of *Elytraria acaulis* were brought from the forest area of the Gudur Village, Warangal district, Telangana State, India. An ethno botanical survey was conducted by interviewing traditional healers in each locality using the local language. Each interview followed a semi structured questionnaire designed to obtain the following information, local names, habit, parts of plant used, ailments treated. The plant materials used in this study were collected from the above

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mentioned area randomly during the months of June - December. The collected plants were identified by Professor V. S Raju, Department of Botany, Kakatiya University. The collected whole plants were authenticated, given voucher number and preserved in the laboratory.

The plants materials are generally practiced by the village tribal people for various ailments. *Elytraria acaulis* is for asthma, migraine, snake bite, mammary tumor etc. The *Elytraria acaulis* plant is already proved its anti hyperglycemic activity.

Preparation of extracts

The whole fresh plant were washed, shade dried, the powdered using the electric blender and stored in the air tight bottles.

Extraction process

The maceration technique was employed in extraction process

50gr of dried powder of selected whole plant was added to 250ml of ethanol solvent [hydroalcohol (ethanol 70% + aqueous 30%), aqueous] and allowed for 24 hrs with the random shaking. Then the filtrate-I was collected and the marc again allowed in 250 ml of same solvent for 24 hrs and collected the filtrate-II. Then the filtrates (I&II) were performed distillation to get extracts. It was stored in refrigerator prior to use.

Animal models

Albino rats (Wistar strain - *Rattus norvegicus*) weighing between 200 to 230gr (for the hepatoprotective studies) were brought from Mahaveer Enterprisers, Hyderabad. The protocol approved by the Institutional Animal Ethical Committee (IASC/03/UCPSc/KU/10). *The animals were kept in polypropylene cages (three in each) under standard conditions (temperature 25-29^0 C, 12hrs light 12hrs darkness cycles and 55-65% relative humidity). Animals were fed with pelleted standard rat diet (HYPRO Amrut Rodent Diet (Hypro premium) Feeds Ltd-PUNE.) and water was provided ad libitum. The study was conducted in accordance with the recommendations from the declaration of WHO on guiding principles in care and use of animals.*

The husk for the purpose of keeping as a bed to the animals was cleaned and autoclaved. Before the animals were kept, the polypropylene cages were sterilized along with the water feeding bottles.

Toxic study of the extracts

To study the toxicology of whole plant extracts of *Elytraria acaulis*, the doses (200 mg/ kg) of plant were administered to the rats (5 groups – 8 animals in each group) and put under observation for 72 hrs. (Kavitha *et al.*, 2011). There was no toxic effect observed to the rats and the 200 mg / kg was selected for the treatment.

Experimental design for Hepatoprotecive activity

The animals were divided into 5 groups of 8 rats in each.

- ***Group-1***- Treated with dist. water for 7 days (Control).
- ***Group-2***- CCl_4 (Carbon tetra chloride) was given intra peritonially (1ml/ kg) with 1:1 dilution of coconut oil on the 5th day (Chaudhari *et al.*, 2009).
- ***Group-3***- Administered with liver tonic (5ml/kg) daily for 7 days and on 5th day the CCl_4 is induced through intra peritonially (1 ml / kg).
- ***Group-4***- Was treated with *Elytraria acaulis* whole plant ethanol extract-EAEE (200mg/kg) for 7 days, CCl_4 is administered on day 5. (Chaudhari *et al*., 2009).
- ***Group-5***- Was treated with *Elytraria acaulis* whole plant aqueous extract-EAAE (200mg/kg) for 7days, CCl_4 is administered on day 5. (Chaudhari *et al*., 2009).

On the 8th day, all rats were sacrificed and the blood collected, centrifuged and the collected serum samples were studied for Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT) and bilirubin (through kits) tests for the study of the toxic effect of CCl_4 and also the therapeutic effect of the plant extracts. The livers were fixed in the fixative (Bouin's fluid) for the histological study. The results were analyzed by one way ANOVA and Dunnet multiple comparison test with the significant level at $p<0.05$.

RESULTS

SGPT, SGOT, ALP and Bilirubin

The results were observed that the serum parameters like SGPT values were increased in the CCl_4 induced rats. The decreased level of SGOT, SGPT, ALP were observed in the group III (CCl_4 + Liv 52 group), group-IV, V (Table 1).

SGOT, bilirubin values were also indicating the damage of the liver in the CCl_4 induced rats respectively.

The decreased levels of SGPT, SGOT, ALP and bilirubin levels were seen in the CCl_4+ EAEE 200 mg/kg, EAAE 200mg/kg respectively (Table – 1, 2). The bilirubin levels were increased in the group II than to the group-I and they were decreased in group IV, V (EAEE, EAAE).

Albumin, Total Protein and Total Bilirubin

The serum levels of albumin and total protein were decreased in the group II compare to the treated and control group. The albumin, total protein levels were normalized in the group III including group IV, V (EAEE, EAAE). The bilirubin levels were decreased in the group III and also in treated groups (Table -2).

Total cholesterol, LDL, HDL and Triglycerides

Total cholesterol levels were elevated in the group II (178.12± 3.45). They were reduced in the CCl_4 + Liv 52 group and also in EAEE, EAAE treated groups (148.26±2.71, 134.87±2.53).

The LDL (Low Density Lipoproteins) values were also increased in the group II (33.63±3.16). The values of HDL (High Density Lipoproteins) were declined in the group II (21.24±2.13). The reduced levels of cholesterol and LDL were seen in the group IV, V (148.26±2.71, 134.87±2.53; 27.52±2.49, 22.63±2.41). The increased levels of HDL were observed in the group IV, V (23.75±2.31, 31.76±4.76) compare to the group II (21.24±2.13). The results were significantly comparable to group III. The triglycerides were elevated in group- II than to the group-I. Triglycerides were decreased in the group- III, IV and V (Table - 3).

Glycogen content in Liver

The group IV,V liver glycogen levels also observed to be mostly similar to the group III. (Table -4).

Protein content in Liver

The protein levels of liver were also declined in the group II than to the group I. They were elevated in the treated groups of IV, V. The group III rats also observed the normalized liver protein levels like control. (Table - 4).

Histological observations

The histological sections of control rat liver were observed with developed hepatocytes, central vein etc. (Fig. 1). The damaged hepatic cells, necrosis of liver were seen in group II due to lipid peroxidation (Fig. 2). The rejuvenated hepatocytes seen in the group III group IV and group V. The regeneration of cells of liver was increased in group V than to the group IV. (Fig.3 - 5).

The hepatoprotective activity of the extract was improved from group- II to group-III which observed in all tests.

DISCUSSION

In addition to the changes in liver, significant changes were also noticed in the serological parameters like SGPT, SGOT, ALP and bilirubin. Similar results were achieved in rats treated with aqueous extract of *Capparis decidua* **(**Ali *et al.,* 2011). In the present investigation these parameters were decreased for EAEE and EAAE in treated rats.

In the present investigation the effect of EAEE and EAAE on the serological parameters was determined in albino rats. The levels of above serological parameters increased highly in EAAE treated rats as compared to CCl_4 treated rats. These results also similar to wound healing activity of the leaves of *Solanum nigrum* **(**Asif Mir *et al.,* 2010).

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 (Cameron *et al,* 1936). The present study revealed significant decrease in the levels of serum bilirubin in EAEE and EAAE treated rats when compared to CCl_4 treated group rats. Decreased serum bilirubin may arise from liver protection. Similar results were achieved in rats treated with aqueous extract of *Argemon mexicana* leaf extract by (Das *et al.,* 2009). The albumin and total proteins of serum were decreased in the group II rats.

The liver failure results the drastic come down of albumin levels of serum, the reduced levels of serum proteins also because of the hepatotoxicity. The normalized values of albumin and protein were seen in the group III, IV and V, which was because of the rejuvenating or repairing of liver. The EAEE and EAAE extract may have the capability to reform the liver cells by increasing protein values of serum. The similar results were observed in the poly herbal tablet treated CCl_4 induced rats. (Chaterrjee, 2000).

The total cholesterol levels were increased in group II because of the cholestasis of the liver which generally occurs due to elevated levels of ALP. The elevated LDL levels reduced HDL cholesterol levels were also seen in the group II rats. The reduced total cholesterol, LDL levels were seen in the group III. The EAEE, EAAE treated rats also observed having decreased levels of LDL and total cholesterol. The increased HDL levels were seen in the group III, IV, V. The increased levels of HDL explain the capacity of the extract to fight against atherosclerosis and other coronary artery diseases. The similar reduced levels of serum cholesterol and increased levels of HDL were also found in the *Polygala arvensis* treated CCl_4 rats. (Dhanabal *et al.,* 2006).

The normalized levels of cholesterol were also observed in the work of Gupta *et al.,* 2006 (*Chamomile capitula).* When he studied the hepato-protective activity of some medicinal plant extracts on CCl_4 induced hepatoroxic rats.

The triglycerides of serum observed to be elevated in the group II. It may be because of the cardiovascular disturbances. The recovery of the triglycerides was seen in the group III, IV and V.

The depleted levels of the glycogen content of liver were seen in group II. The reduced glycogen levels were because of the decreased enzymatic activity of hexokinase (Hewawasam *et al.,* 2004).

The reduced levels of liver protein content were seen in the group II. The reduced liver protein levels were because of the damage of liver tissue due to the CCl_4. The GSH (reduced glutathione). CAT (catalase), superoxide dismutase (SOD) stores may be decreased because of the toxic free radicals of CCl_4. (Jamshidzadeh *et al.,* 2006).

CCl_4 damages the liver by its metabolite $CCl_3^{\cdot}$ free radical, with which the damage of cellular membranes occur through the lipid peroxidation (Kalpana Patila *et al.,* 2011). The serum parameters like Serum Glutamic Oxaloacetate Transaminase (SGOT) or Aspartate Transaminase (AST), Serum Glutamic Phosphate Transaminase (SGPT) or Alaline transaminase (ALT), including the bilirubin content also elevated because of their release into the blood in the CCl_4 induced hepatotoxic rats (Kavita Suryawanshi *et al.,* 2011).

The EAEE and EAAE also possess the flavonoids, which has the potential anti-oxidant activity. It is also found that the plants having tannins and flavonoids can have the capacity of hepatoprotection (Manjunath *et al.,* 2008). Several plants proved for its anti-oxidant property due to the presence of phenolic acids (Patel *et al.,* 2009).

Investigation on various plant phytochemicals revealed that the presence of flavonoids, phenolic compounds has the potential free radical scavenging activity (Patil Prakash *et al.,* 2011). The phytochemicals like phenols, flavonoids, and tannins may be the reason for the hepato-protective activity through which the antioxidant enzymes may be raised and oxidative stress may be plugged.

The restoration of these anti-oxidant enzymes in the EAEE, EAAE treated group IV, V and liver tonic treated group III could be the reason for the elevated (normalized) liver protein levels.

Whereas, the EAEE, EAAE treated rats serum parameters revealed the significant decrease in the SGOT, SGPT and bilirubin levels compare to the CCl_4 induced rats. These enzymatic values were also decreased in the liver tonic treated rats.

The hepatoprotection of the drug depends on the reduced effects of toxic levels of the CCl_4 in the damaged liver (Rajeswari *et al.,* 2011). The results that decreased levels of SGOT, SGPT and bilirubin in the EAEE, EAAE treated rats against CCl_4 were observed similar to the results of the hepatoprotective activity of poly herbal drug against CCl_4 damaged liver (Rusu *et al.,* 2005).

The histological sections are also revealed that the hepatocellular damage in the CCl_4 induced hepatotoxic group (group- 2) (figure-2). The EAEE + CCl_4 (200mg/kg) i.e., group-4 were showed the rearrangement of damaged cells. The regeneration of liver cells was seen in the group –4(figure 4). The EAAE + CCl_4 group - 5 (200mg/kg) (figure 5) were also shown recovery of the tissue compare to the hepatocytes of the CCl_4 damaged group. The histology can be easily comparable with the liver tonic+ CCl_4 group rats.

The dose EAEE 200mg/kg indicating that serum enzymes are decreased more than to the dose of EAAE 200mg/kg in the animals treated with both plant extracts. The hepatoprotective activity is increased as the administration of extracts dose increased. The results that observed were supporting the protective activity of the liver though they were damaged by the CCl_4. The plant extracts of EAEE (*Elytraria acaulis* ethanol extract) and EAAE (*Elytraria acaulis* aqueous extract) are shown more protective activity.

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Table1 Serological tests- SGOT, SGPT and ALP

Group	Name	Sgot (iu/l)	Sgpt (iu/l)	Alp (iu/l)
I	CONTROL	42.14±2.12	34.23±2.11	184.61±1.26
II	CCl_4 INDUCED	152.11±1.12**	103.01±1.13**	282.34±3.40**
III	CCl_4 + LIVER TONIC	64.16±1.23**, a	50.30±1.60**, a	190.22±1.42**, a
IV	CCl_4 + EAEE 200 mg/kg	97.10±1.13**, a	60.41±2.45**, a	220.21±1.41**, a
V	CCl_4 + EAAE 200mg/kg	82.20±2.31**, a	58.12±1.50**, a	212.62±2.57**, a

Table2 Serological tests- Total Bilirubin, Total Protein and Albumin

Group	Name	Sgot (iu/l)	Sgpt (iu/l)	Alp (iu/l)
I	CONTROL	42.14±2.12	34.23±2.11	184.61±1.26
II	CCl_4 INDUCED	152.11±1.12**	103.01±1.13**	282.34±3.40**
III	CCl_4 + LIVER TONIC	64.16±1.23**, a	50.30±1.60**, a	190.22±1.42**, a
IV	CCl_4 + EAEE 200 mg/kg	97.10±1.13**, a	60.41±2.45**, a	220.21±1.41**, a
V	CCl_4 + EAAE 200mg/kg	82.20±2.31**, a	58.12±1.50**, a	212.62±2.57**, a

Table 3 Serological tests- Total Cholesterol, HDL, LDL and Triglycerides

GROUP	NAME	Total Bilirubin (mg/dL)	Total Protein (g/dL)	Albumin (g/dL)
I	CONTROL	0.61±0.18	5.11±0.11	3.57±0.61
II	CCl_4 INDUCED	1.20±0.11**	2.12±0.40**, a	1.10±0.33**
III	CCl_4 + LIVER TONIC	0.81±0.17n, a	5.47±0.34n, a	2.21±0.50**,a
IV	CCl_4 + EAEE 200 mg/kg	0.92±0.10n, a	3.14±0.48**, a	1.41±0.56**
V	CCl_4 + EAAE 200mg/kg	0.90±0.15n, a	3.86±0.52a	2.52±0.34*, a

Table4 Biochemical tests- Liver Glycogen and Liver Protein

GROUP	NAME	Liver Glycogen (µg/100mg)	Liver Protein (mg/gm)
I	CONTROL	310.14±6.23	174.01±7. 45
II	CCl_4 INDUCED	156.42±5.23**	95.54±5.23**,a
III	CCl_4 + LIVER TONIC	280.65±7.74**, a	170.57±6.51n,a
IV	CCl_4 + EAEE 200 mg/kg	262.52±8.42**,a	145.14±6.18**,a
V	CCl_4 + EAAE 200mg/kg	269.58±7.61**,a	156.72±8.43**,a

All values were expressed in mean ± SD With n=8, the values were analyzed with one way ANOVA followed by Dunnett multiple comparison Test. **=p<0.01compare to Group-I and a=p<0.01 compare to Group- II. n= not significant when compare to Group-I.

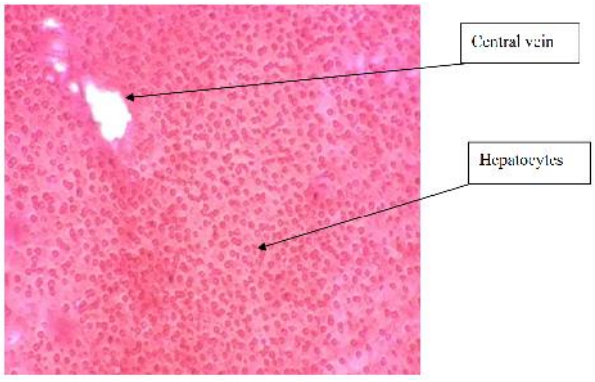

Fig.1 Liver Cross Section (Control)

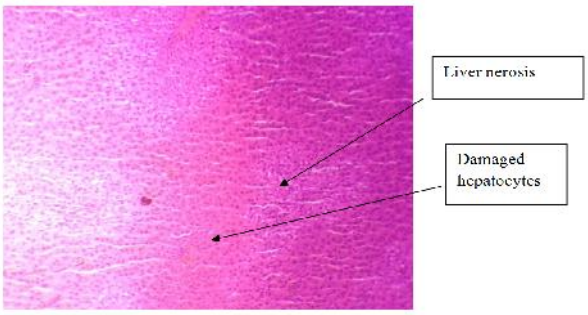

Fig 2 Liver Cross Section (Ccl_4 Induced Rat)

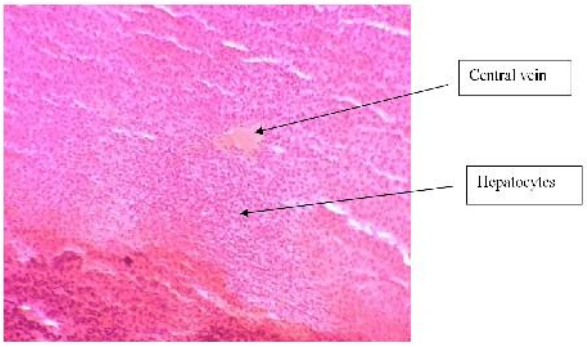

Fig.3 Liver Cross Section (Ccl_4 +Liver Tonic)

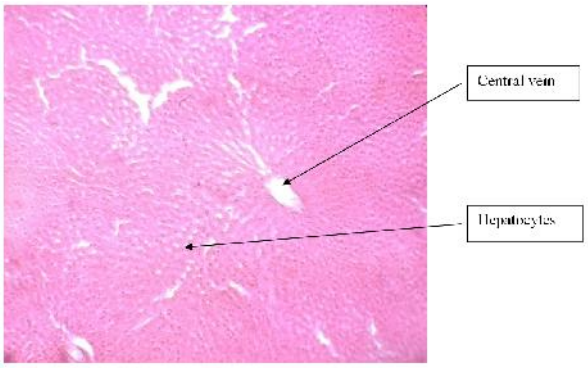

Fig4 Liver Cross Section (Ccl_4 +Eaae 200mg/Kg)

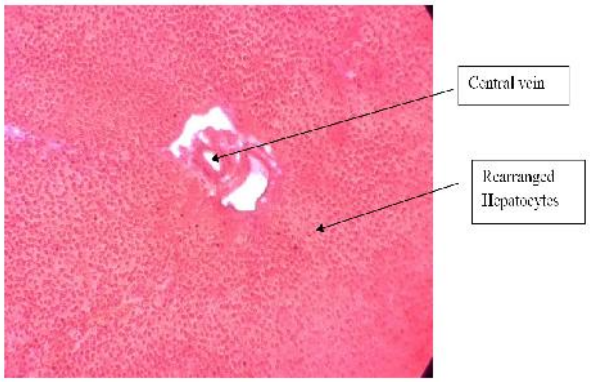

Fig.5 - Liver Cross Section (Ccl_4 +Eaee 200mg/Kg)

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