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HEPATOPROTECTIVE ACTIVITY OF *ELYTRARIA ACAULIS* IN CCL_4 INDUCED HEPATO TOXIC ALBINO RATS

G. Venkat Raji Reddy[1]*, R.Vijay Kumar[1], V.Rama[1], M. Krishna Reddy[1] and Y. Narsimha Reddy[2]

[1]Department of Zoology, Reproductive Physiology Unit, Kakatiya University, Warangal – 506009, Telangana State.

[2]University College of Pharmasceutical Sciences, Kakatiya University, Warangal – 506009, Telangana State.

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***Correspondence for Author**
G. Venkat Raji Reddy
Department of Zoology, Reproductive Physiology Unit, Kakatiya University, Warangal – 506009, Telangana State.
Mobile No. 09989292009.

ABSTRACT

Historically, the nations like the Indians, the Greeks, the Chinese, and the Egyptians have a rich account of using medicinal plants. The bulk of the population in many developing as well as developed countries still use traditional medicines, which have not been fully discovered or appreciated in modern science-based therapy. The present experiment was conducted for 07 days to evaluate the hepatoprotective activity of plant *Elytraria acaulis* in CCl_4 (1ml/kg) treated rats. The extracts were prepared by the *Elytraria acaulis* whole plant extracts in ethanol 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 (hydroalcoholic solvent 200mg/kg and aqueous 200mg/kg). On the 08th day blood was collected for the study of serum enzymes like SGOT (Serum Glutamate Oxaloacetic Transaminase), SGPT (Serum Glutamate Phosphate Transaminase), serum total protein, albumin and bilirubin.

KEYWORDS: SGOT, SGPT, Alkaline Phosphate, Total protein, Albumin, Bilirubin.

INTRODUCTION

Plants and plant based medicaments have been employed since dawn of civilization for prolonging life of man by combating various ailments. Ancient ethnic communities around the world have learnt to utilize their neighborhood herbal wealth for curative purpose. Throughout Asia, the Ayurveda, Unani, and Chinese medical systems have developed and refined treatments based purely on preparations made from available natural resources.

Ayurveda was probably developed much earlier than the Unani and Chinese medicine systems. The oldest existing literature on this form of treatment is the ***Rigveda***, the classic Hindu text, which according to legend was written in the years 4500-1600 B.C. Other important Ayurvedic medical texts include the ***Charak Samhita*** (1000-800 B.C.) and ***Susruta Samhita*** (800-700 B.C.). The present experiment aimed at conducted for 07 days to evaluate the hepatoprotective activity of plant *Elytraria acaulis* in CCl_4 (1ml/kg) treated rats. The extracts were prepared by the *Elytraria acaulis* whole plant extracts in hydroalcoholic solvent 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 (hydroalcoholic solvent 200mg/kg and aqueous 200mg/kg). On the 08th day blood was collected for the study of serum enzymes like SGOT (Serum Glutamate Oxaloacetate Transaminase), SGPT (Serum Glutamate Phosphate Transaminase), ALP (Alkaline Phosphate), serum total protein, albumin, bilirubin and then the separated liver is processed for the histological studies. The decreased levels of SGOT, SGPT, ALP, serum total protein, albumin and total bilirubin in the treated rats were an indication of the hepatoprotective activity of whole plant extracts of *Elytraria acaulis*.

MATERIAL AND METHODS

Plant material

The whole plant of *Elytraria acaulis* were brought from the forest area of the Gudur Village, Warangal district, Telangana State. The plants materials are generally practiced by the village tribal people for various ailments. The whole plant was used in treatment of abscess of mammary glands, boils, burns, colic, diarrhea, rickets, throat compliments and tonsillitis.[16] The plant's infusion is prescribed as a remedy for cough; Leaves of *E. acaulis* are used to cure fever, venereal diseases[6], kidney stone and urticaria. Roots of *E. acaulis* are claimed to have therapeutic benefits in treating stomach ache, migrane [17], tooth ache, snake bite, asthma, expulsion of guinea worms[17], leucorrhoea, piles, mammary tumors, pneumonia and infantile diarrhea.[18] The collected plants were authenticated, given voucher number (RPU/ZOO/EA/2012) and preserved in the laboratory.

Hydroalcholic Extract

The whole plant was dried under shade and the powder was prepared from extracts. The powder (50 gr) was kept in the hydroalcoholic (250 ml) solvent (70% of ethanol, 30% of distilled water) and allowed for 24 hrs with the random shaking. Then the filtrate-I was

collected and the marc again allowed in 250 ml of hydroalcoholic solvent for 6 hrs and collected the filtrate-II. Then the filtrates (I&II) were performed distillation to get extracts and stored in refrigerator prior to treatment.

Aqueous Extract

The whole plant was dried under shade and the powder was prepared from extracts. The powder (50 gr) was kept in the aqueous (250 ml) extracts and allowed for 24 hrs with the random shaking. Then the filtrate-I was collected and the marc again allowed in 250 ml of aqueous extracts for 6 hrs and collected the filtrate-II. Then the filtrates (I&II) were performed distillation to get extracts and stored in refrigerator prior to treatment.

Animal models

Albino rats (Wistar strain - Rattus norvegicus) weighing between 200 to 230gr 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

Hydroalcholic Extract

To study the toxicology of hydroalcohoic whole plant extracts of *Elytraria acaulis*, the doses (150,200,250,300 mg/ kg) were administered to the rats (5 groups – 8 animals in each group) and put under observation for 72 hrs.[6] There was no toxic effect observed to the rats and the 200 mg / kg were selected for the treatment.

Aqueous Extract

To study the toxicology of aqueous extract whole plant extracts of *Elytraria acaulis*, the doses (150,200,250,300 mg/ kg) were administered to the rats (5 groups – 8 animals in each group) and put under observation for 72 hrs.[6] There was no toxic effect observed to the rats and the 200 mg / kg were selected for the treatment.

Experimental Design

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

Group-1. Treated with dist. water for 15 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.[3]

Group-3 Administered with liver tonic (5ml/kg) daily for 7 days and on 5th day the CCl_4 is induced through i. p. (1 ml / kg).

Group - 4, 5 were treated with *Elytraria acaulis* hydro alcoholic whole plant extract-EAHE, aqueous extract-EAAE (200mg/kg, 200 mg/kg) for 7 days, CCl_4 is administered on the 5th day.[3]

On the 08th day, all rats were sacrificed and the blood collected, centrifuged and the collected serum samples were studied for SGOT, SGPT and bilirubin (through commercially available 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

Body, Liver weights

The final body weight of group-I rats were increased, whereas the rats weight was decreased in group II. The weights of rats of group IV, V were increased than to the group II. The elevated levels of the weights were also seen in the group III. (Table-1, 1.1 and chart 1, 2).

SGPT, SGOT, ALP and Bilirubin

The results were observed that the serum parameters like SGPT values were increased in the CCl_4 induced rats (104.02±3.12). The decreased level of SGOT, SGPT, ALP were observed in the group III (CCl_4 + Liv 52 group), group-IV, V (Table 2, chart 3).

SGOT, bilirubin values were also indicating the damage of the liver in the CCl_4 induced rats (155.21±2.16), (1.31±0.21) respectively. The values of SGOT (66.19±2.45), SGPT (51.41±2.71), ALP (285.46±4.61) and bilirubin (0.82±0.18) were noted in the group CCl_4 + Liver tonic.

The decreased levels of SGPT, SGOT, ALP and bilirubin levels were seen in the CCl_4+ EAHE 200 mg/kg (99.12±2.14, 61.52±3.56,222.31±2.52, 0.93±0.12), EAAE 200mg/kg (83.31±3.42, 59.23±2.61, 213.73±3.68, 0.91±0.16) respectively (Table – 2,3). The bilirubin levels were increased in the group II than to the group-I and they were decreased in group IV, V (EAHE, 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 (EAHE, EAAE). The bilirubin levels were decreased in the group III and also in treated groups (Table -3, chart – 4).

TABLE- 1- Weights of Liver

GROUP	NAME	Liver Weight (g)
I	CONTROL	9.05±1.50
II	CCl_4 INDUCED	$6.12±1.45^{a}$
III	CCl_4 + LIVER TONIC	$9.01±1.19^{n}$
IV	CCl_4 + EAHE 200 mg/kg	$7.69±1.32^{n}$
V	CCl_4 + EAAE 200mg/kg	$8.93±1.39^{n}$

All values were expressed in mean ± SD With n=8, the values were analyzed with one way ANOVA followed by Dunnett multiple comparison Test. n= not significant when compare to Group-I, a=p<0.05 compare to Group-I.

TABLE- 1.1- Body weights

GROUPS	NAME	Initial (gr)	Final (gr)
GROUP-I	CONTROL	214.5±11.45	230.20±10.20
GROUP-II	CCl_4 INDUCED	222.12±11.17	205.12±10.08**
GROUP-III	CCl_4+LIVER TONIC	228.31±9.36**	236.42±10.82*
GROUP-IV	CCl_4 + EAHE 200mg/ kg	$219.31±10.12^{b}$	$227.27±11.18^{n}$
GROUP-V	CCl_4 + EAAE 200mg/kg	$216.62±12.14^{b}$	232.15±11.17 *

All values expressed in mean ± SD, With n=8, * = p<0.05, b=p>0.05, ** = p<0.01 compare to control, n= not significant when compare to Group-I. The values were analyzed with one way ANOVA followed by Dunnett multiple comparison Test.

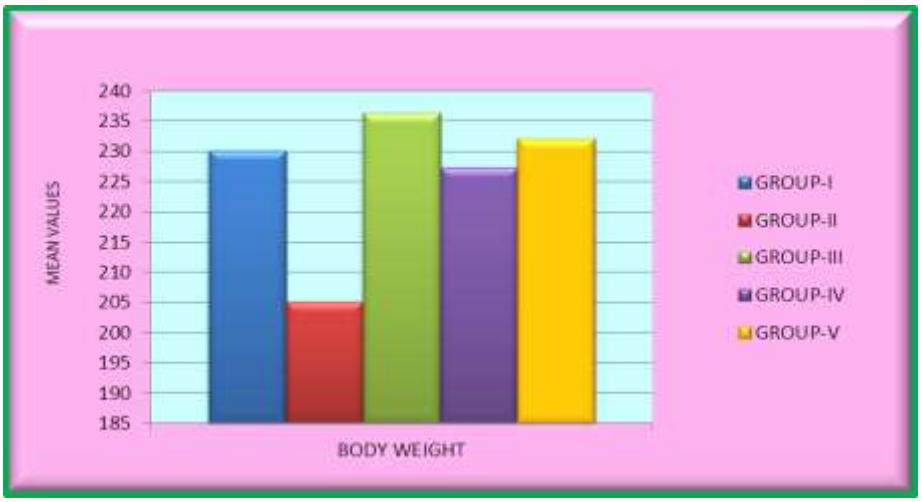


CHART-1-Body weights

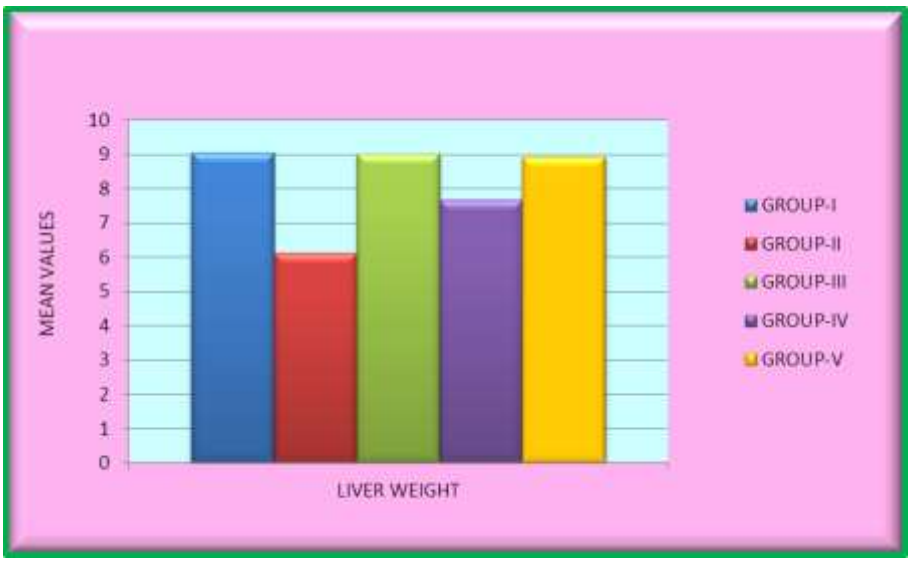


CHART-2- Liver weight

TABLE- 2- Serological tests- SGOT, SGPT and ALP

GROUP	NAME	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
I	CONTROL	44.24±3.22	36.45±3.42	186.71±2.38
II	CCl_4 INDUCED	155.21±2.16**	104.02±3.12**	285.46±4.61**
III	CCl_4 + LIVER TONIC	66.19±2.45**, a	51.41±2.71**, a	199.23±3.42**, a
IV	CCl_4 + EAHE 200 mg/kg	99.12±2.14**, a	61.52±3.56**, a	222.31±2.52**, a
V	CCl_4 + EAAE 200mg/kg	83.31±3.42**, a	59.23±2.61**, a	213.73±3.68**, 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.

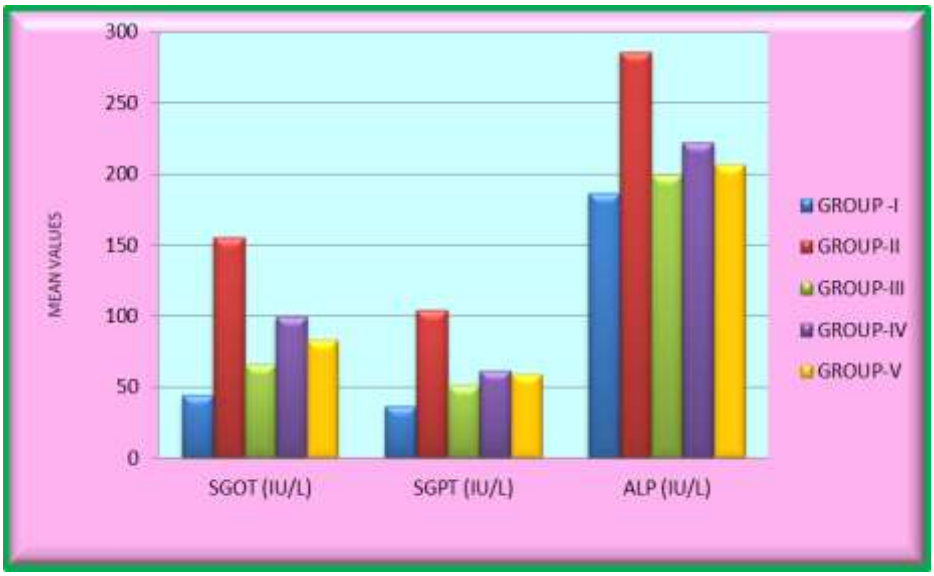


CHART-3- Serological tests- SGOT, SGPT and ALP

TABLE- 3- Serological tests- Total Bilirubin, Total Protein and Albumin

GROUP	NAME	Total Bilirubin (mg/dL)	Total Protein (g/dL)	Albumin (g/dL)
I	CONTROL	0.64±0.19	5.12±0.12	3.58±0.64
II	CCl_4 INDUCED	1.31±0.21**	2.42±0.41**, a	1.15±0.34**
III	CCl_4 + LIVER TONIC	0.82±0.18n, a	5.48±0.35n, a	2.33±0.51**,a
IV	CCl_4 + EAHE 200 mg/kg	0.93±0.12n, a	4.15±0.49**, a	1.42±0.57**
V	CCl_4 + EAAE 200mg/kg	0.91±0.16n, a	4.89±0.57a	2.53±0.39*, 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.

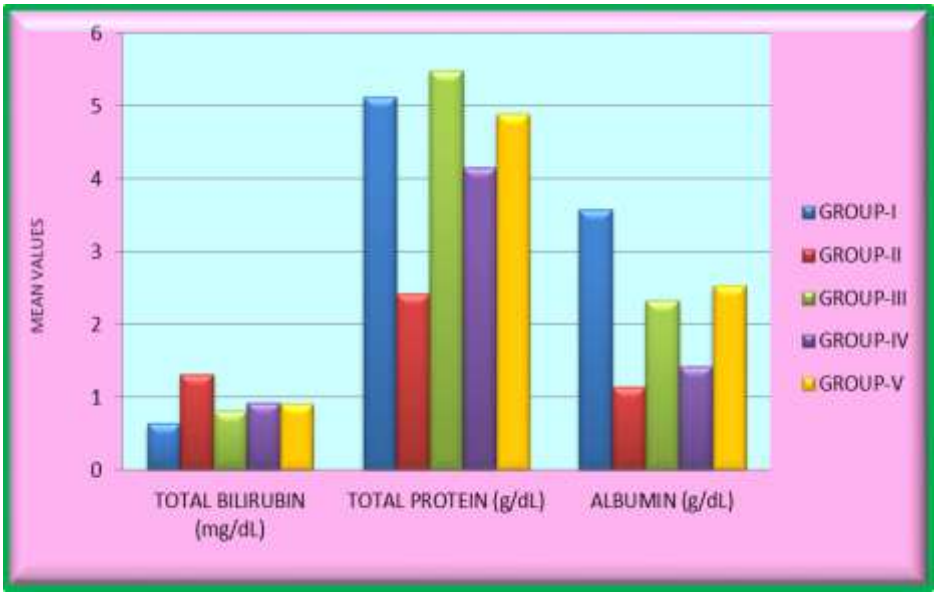
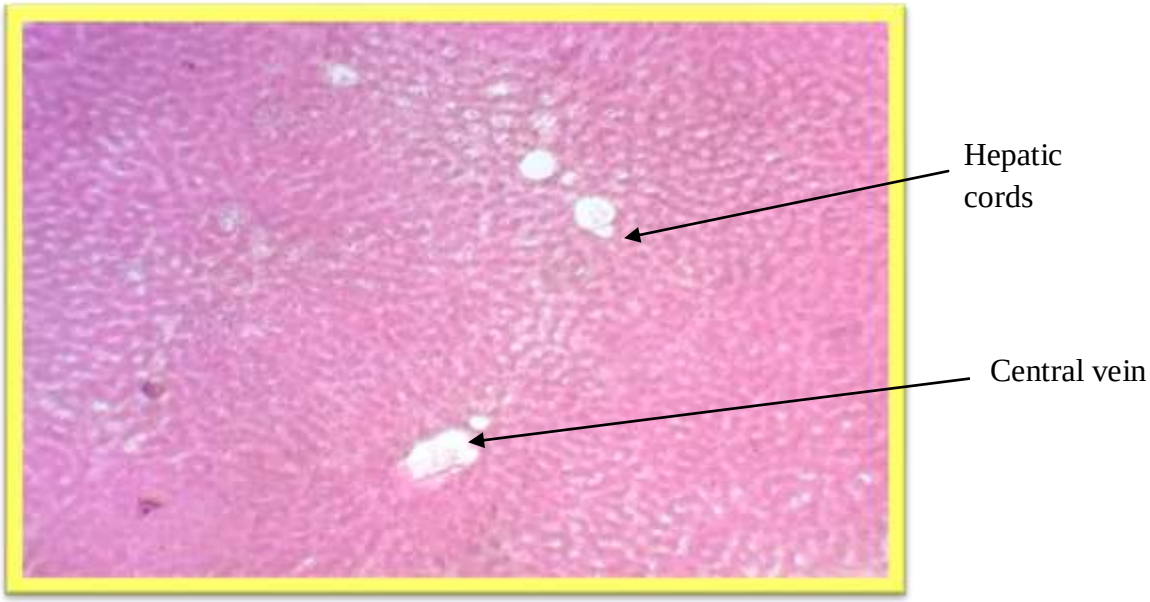
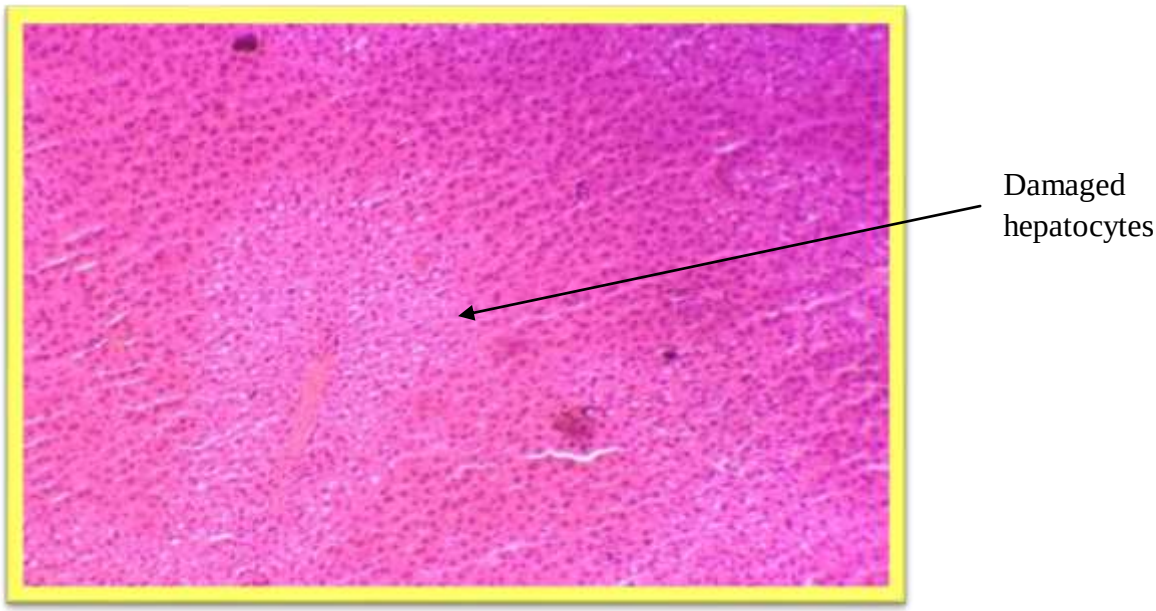


CHART- 4- Serological tests- Total Bilirubin, Total Protein and Albumin



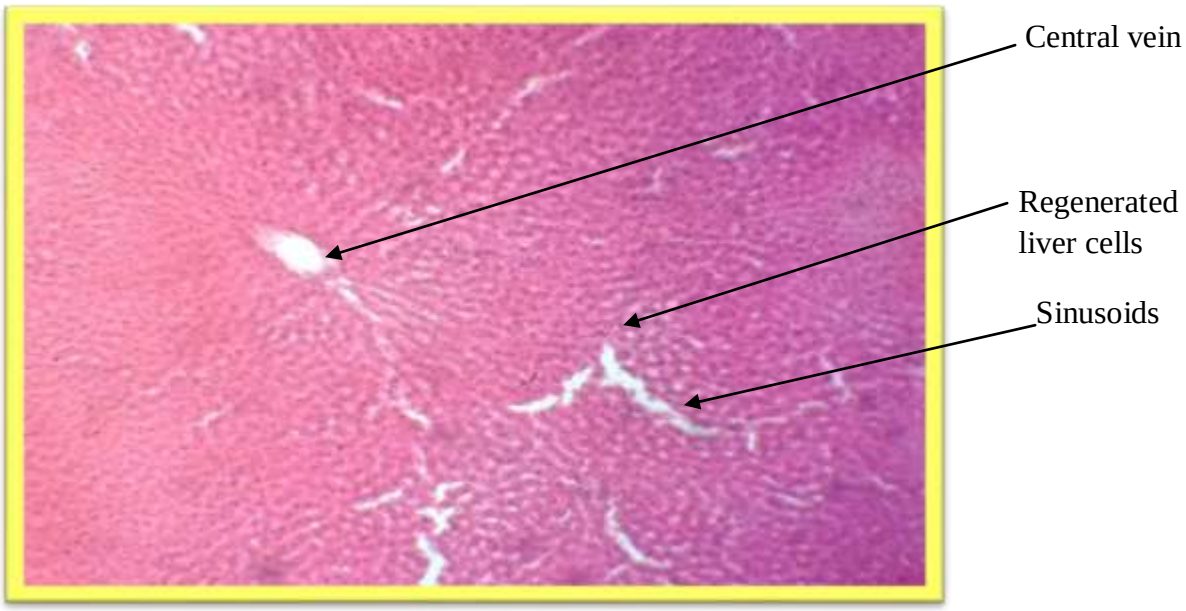
Liver section shows the normal histology with central vein, sinusoids and hepatocytes.

FIG.1: LIVER CROSS SECTION (CONTROL)



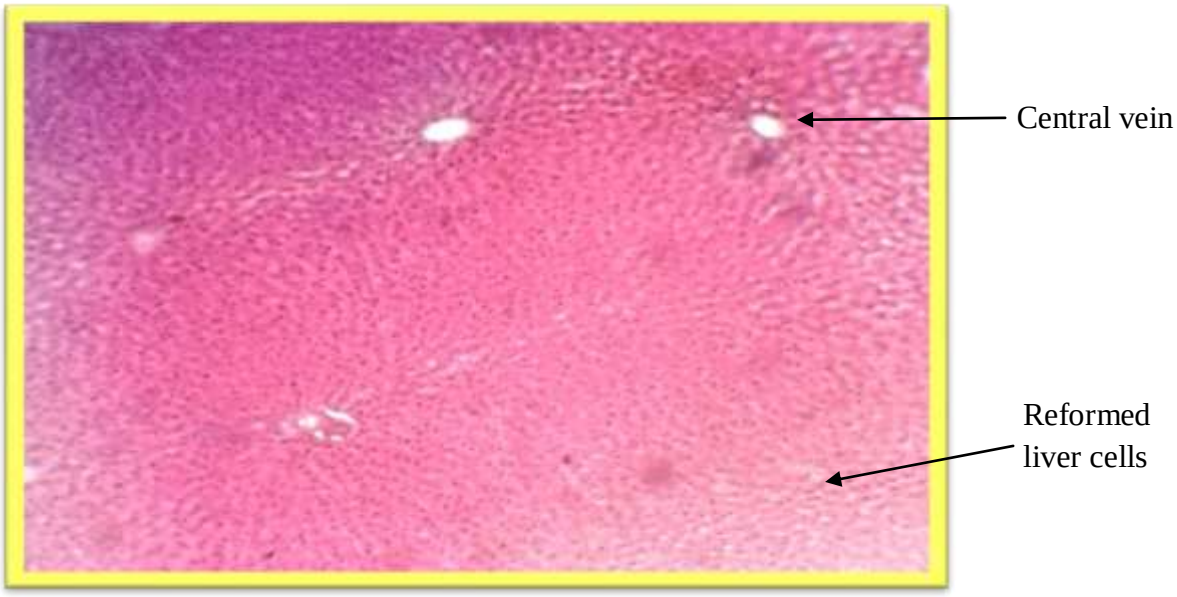
Liver section shows the histology with damaged hepatocytes.

FIG. 2: LIVER CROSS SECTION (CCl_4 INDUCED RAT)



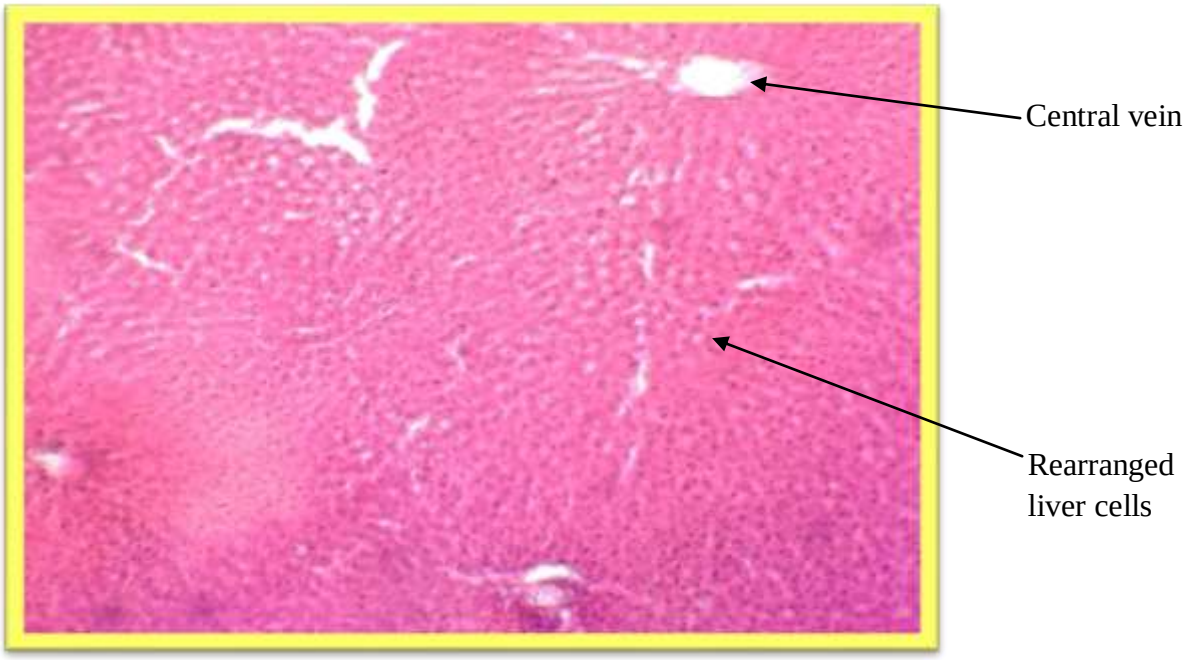
Liver section shows the histology with rearranged sinusoids and hepatocytes.

FIG.3: LIVER CROSS SECTION (CCl_4 +LIVER TONIC)



Liver section shows the histology with rearranged sinusoids and hepatocytes.

FIG. 4: LIVER CROSS SECTION (CCl_4 +EAAE 200mg/kg)



Liver section shows the histology with rearranged sinusoids, hepatocytes and central vein.

FIG.5: LIVER CROSS SECTION (CCl_4 +EAHE 200mg/kg)

DISCUSSION

The final body weights of the group II rats were decreased than to the control group and other groups of treated rats. The decreased levels of the final body weights of the group II was because of the toxic effect of the CCl_4. The increased levels of body weights of group III may be because of the suppressing the toxic effect of CCl_4 by the syrup Liv 52. The EAHE, EAAE treated rats' weight of group IV; V also increased similar to the group III.

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 *Beta vulguris.*[19] In the present investigation these parameters were decreased for EAHE and EAAE in treated rats.

In the present investigation the effect of EAHE 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 *Argemone Mexicana.*[20] and *Solanum americanum.*[11]

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.[21] The present study revealed significant decrease in the levels of serum bilirubin in EAHE 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 *Psidium guajava* leaf extract[22] and *Momordica carantia.*[15] The albumin and total proteins of serum were decreased in the group II rats.

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.[1,2,4] The hepatoprotection of the drug depends on the reduced effects of toxic levels of the CCl_4 in the damaged liver.[12] The results that decreased levels of SGOT, SGPT and bilirubin in the EAHE, 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.[14,8,9] The histological sections are also revealed that the hepatocellular damage in the CCl_4 induced hepatotoxic group (group- 2) (figure-1). The EAHE + CCl_4, EAAE + CCl_4 (200mg/kg, 200mg/kg) i.e., group-4, group-5 were showed the rearrangement of damaged cells. The histology is more observed in the group – 5 (figure 3, 4). The histology can be easily comparable with the liver tonic+ CCl_4 group 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[5,10]. 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 EAHE 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.[23, 13]

CONCLUSION

The hydroalcoholic and aqueous extracts were revealed with the effective hepatoprotective activity against CCl_4 induced hepatotoxic rats. The reduced levels of SGPT, SGOT, Alkaline phosphate, Albumin and Bilirubin were the same of markers to explain the hepatoprotective activity. The results that observed were supporting the protective activity of the liver though they were damaged by the CCl_4. The plant extract of *Elytraria acaulis* are shown more protectiveness. Though the results are supporting the hepatoprotective activity further study is needed to confirm the activity.

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REFERENCES:

1. Brattin WJ, Glend FA Jr, Recknagel RO. (Pathological mechanism in carbon tetrachloride hepatotoxicity). Journal of Free Radical Boil Med, 1985; 1(1): 27-28.
2. Chaudhari BP, Chaware VJ, Joshi YR, Biyani KR. (Hepatoprotective activity of hydroalcoholic extract of *Momordica charantia* Linn. leaves against Carbon tetra chloride induced hepatopathy in rats). International J of Chem Tech Research, 2009; 2(3): 355-358.
3. Chowdhary GD, Kamboj P, Singh I, Kaila AN. (Herbs as liver savers – A review). Indian J. of Natural Products and Resources, 2010; 14(2): 397-408.
4. Durga Prasan Nayak SC, Dinda PK, Swain B, Kar VJ, Patro NM. (Hepatoprotective activity against CCl_4- induced hepatotoxicity in rats of Chenopodium album aerial parts). Journal of Phytotherapy and Pharmacology, 2012; 2(3): 33-41.
5. Jeong HG, You HJ, Park SJ, Moon A, Chung YC, Kang SK, Chun HK. (Hepatoprotective effects of 18-glycerrheinic acid on carbon tetra chloride induced liver injury: Inhibition of cytochrome P450 2E1 expression). Pharmacological Res, 2002; 46(1): 221-227.
6. Kavitha. BT, Shruthi SD, Padmalatha Rai S, Ramachandra**.** YL. (Phytochemical analysis and hepatoprotective properties of *Tinospora cordifolia* against carbon tetrachloride-induced hepatic damage in rats). Journal of Basic and Clinical Pharmacy, 2011; 2(6): 139-142.
7. Mascolo N, Sharma R, Jain SC, Capasso F. (Ethnopharmacology of *Calotropis Procera* Flowers). Journal of Ethnopharmacology, 1998; 2(4): 211-213.
8. Prabhat Kumar das, Prasanna Panda, Somya Ranjan Pani, Ranjan Sethi. (Hepatoprotective activity of Plant *Argemone mexicana* (Linn) against CCl_4 induced hepatotoxicity in rats). International J of Pharmaceutical Research and Development, 2009; 8(1): 1-20.
9. Rajesh Kumar, Sushil Kumar, Arjun Patra, Jayalakshmi.S. (Hepatoprotective activity of aerial parts of *Plumbago zeylanica* linn, against CCl_4 induced hepatotoxicity in rats). International J of Pharmacy Pharmaceutical Sciences, 2009; 1(9): 171-175.

10. Ruby K, Koshy, Raj Kapoor Band, Mohammad Azamthulla. (Anti Hyperglycemic Activity of *Elytraria acaulis* Lind. On Streptozotocin- Induced Diabetic Rats). Med Aromat plants, 2012; 1(2): 103-107.
11. Satheesh Kumar B, Sathyanarayana J, Krishna Reddy M and Prasad MSK. (Evaluation of hepatoprotective activity of aqueous extract from leaves of *Solanum americanum*). International J. of Plant Animal and Environmental Sciences, 2011; 1(3):150-154.
12. Siddhatha Singh, Archana Mehta and Pradeep Mehta. (Hepatoprotective activity of *Cajanus cajan* against CCl_4 induced liver damage). International J. of Pharmacy and Pharmaceutical Sciences, 2011; 3(3):146-147.
13. Venkateshalu V, Gopalan N, Pillai CR, Singh V, Chandrasekaran M, Senthilkumar A, Chanramouli N. (In vitro anti-plasmodial activity of some traditionally used medicinal plants against *Plasmodium falciparum*). Parasitol Res, 2012, 1(5): 497-501.
14. Vilas A, Arsul RO, Ganjiwale PG, Yeole. (Phytochemical and pharmacological standardization of polyherbal tablets for hepatoprotective activity against carbon tetrachloride induced hepatotoxicity). International J of Pharmaceutical Sciences and Drug Research, 2010; 2(1): 265-268.
15. Zahara K, Malik MA, Mughal MS, Arshad M, Sohail MI. (Hepatoprotective role of extracts of *Momordica charantia*.L. in Acetamainophan – induced to toxicity in rabbits). The Journal of Animal and Plant Sciences, 2012; 2(4): 273-277.
16. Jain Madhulika, Michael S, Evans, Jonathan King and Patricia Clark L. (Monoclonal Antibody Epitope Mapping Describes Tailspike b-Helix Folding and Aggregation Intermediates). Global Biogeochem, 2005; 2(1): 121-134.
17. Khadabadi SS and Bhajipale NS. (A Review on Some Important Medicinal Plants of *Abutilon spp*). Research J. of Pharmaceutical, Biological and Chemical Sciences, 2010; 4(1): 720-729.
18. Sikarwar RLS, Pathak B and Jaiswal A. (Some unique ethnomedicinal perceptions of tribal communities of Chitrakoot, Madhya Pradesh). Indian J. of Traditional Knowledge. 2008; **7**(1): 613–617.
19. Agarwal S, Srivastava M, Saxena VK, Kumar A. (Hepatoprotective activity of *Beta vulgaris* against CCl_4-induced hepatic injury in rats). Fitoterapia, 2006; 7(7): 91-93.
20. Patil MB, Jalalpure SS and Ali A. (Preliminary phytochemical investigation and wound healing activity of the leaves of *Argemone mexicana* Linn. Papaveraceae). Indian Drugs, 2001; 3(8): 288-293.

21. Kokate CK, Purohith AP and Gokhale SB. Pharmacognosy. Nirali Prakashan, Pune., 2005: 120.
22. Roy CK. (Hepatoprotectivity activity of *Psidium guajava* leaf extract). Indian J. of Experimental Biology, 2006; 1(2): 222-224.
23. Gagandeep Chaudhary, Sandeep Goyal, Priyanka Poonia. (*Lawsonia inermis* L. A Phytopharmacological Review). International J. of Pharmaceutical Sciences and Drug Research, 2010; 2(6): 91-98.