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HEPATOPROTECTIVE ACTIVITY OF *ENICOSTEMMA AXILLARE* (LAM) Raynal IN *LANTANA CAMARA* LINN INDUCED HEPATOTOXICITY

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ABSTRACT

Hepatotoxicity is a common condition of liver damage due to chemical entities or any other substances. The present study aims to explore the hepatoprotective activity of aqueous and alcoholic extracts of aerial parts of *Enicostemma axillare* (Lam.) Raynal against *Lantana camara* Linn induced hepatotoxicity in albino rats. Silymarin (100mg/kg) is used as a reference standard. The aqueous and alcoholic extracts of aerial parts of *Enicostemma axillare* at about the doses of 100mg/kg, 200mg/kg and 400mg/kg are used for the study. Both aqueous and alcoholic extracts have shown very significant hepatoprotection against *Lantana camara* induced hepatotoxicity in male wistar rats by reducing Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) levels and increasing the serum total albumin and protein levels. However the alcoholic extract is found to be more potent than aqueous extract at about the dose of 400 mg/kg. Thus we can claim that, the drug *Enicostemma axillare* is a hepatoprotective agent suitable for veterinary category, since *Lantana camara* is a common hepatotoxic agent among the veterinary categories.

Keywords: *Enicostemma axillare*, hepatotoxicity, *Lantana camara* and Silymarin

INTRODUCTION

Enicostemma axillare (Lam.) Raynal was a perennial herb found throughout India and common in coastal areas, belonging to the family Gentianaceae. The plant was used in folk medicine to treat diabetes mellitus, rheumatism, abdominal ulcers, hernia, swelling, itching and insect poisoning.[1] The plant was found to be a potent anti-inflammatory, [2] hypoglycemic[3-5] and anticancer[4] drug. Various phytoconstituents such as swertimarin, alkaloids, steroids, triterpenoids, saponins, flavonoids, xanthones, and phenolic acid were isolated from the plant[6] and these compounds are found to posses various protective actions.[7] Liver diseases remains one of the serious health problems. Herbs play a major role in the management of various liver disorders. A number of plants possess hepatoprotective property.[8] *Lantana camara* Linn, also known as Spanish Flag or West Indian Lantana, was a species of flowering plant in the verbena family, Verbenaceae. The plant was very well known for its hepatotoxicity among the veterinary categories such as goats and cows. The foliage of it contains pentacyclic triterpenoids which cause hepatotoxicity and photosensitivity in grazing animals such as sheep, goats, bovines and horses.[9] The present study was designed with an aim to assess the hepatoprotective activity of the aqueous and alcoholic extract of aerial parts of *Enicostemma axillare* (Lam.) Raynal against *Lantana camara* induced hepatotoxicity.

EXPERIMENTAL METHODS

Collection of plant material: The aerial parts of *Enicostemma axillare* were collected during the month of august 2009. The specimen was

authenticated by Dr. N. Ravichandran, Assistant Professor, Department of CARISM, SASTRA University. A voucher specimen has been in Department of CARISM, SASTRA University, India (Voucher specimen No: 103).

Preparation of extract: The collected materials were shade dried and powdered. The powdered plant material was extracted using 95% ethanol in a soxhlet apparatus by using hot continuous extraction technique and the percentage yield of the extract was found to be 18.7%w/w. Aqueous extract was prepared by cold maceration technique with a percentage yield of 21.3 % w/w. All the chemicals and reagents used for the study were used from Ranbaxy chemicals. Alcoholic extract of *Lantana camara* was prepared using the hot continuous extraction technique using a soxhlet extractor. The prepared extract was used for the induction of hepatotoxicity. All the extracts were dissolved in water prior to administration and used for the study.

Animals: Healthy male albino rats of wistar strains weighing between 150-300gms were selected for the studies. The animals were kept in standard plastic animal cages in a group of six to eight in each cage, at standard conditions, with 12 hours of light and dark cycle, in an institutional animal house. The animals were fed with the standard rodent diet and with water *ad libitum*. After one week of acclimatization the animals were used for further experiments.

Acute Toxicity studies: Acute toxicity study was performed as per OECD 425 guidelines in albino rats. Based on this one medium, low and high doses were selected for the study as 100mg/kg b.wt, 200mg/kg b.wt, 400mg/kg b.wt and a recovery group with 400mg/kg b.wt. *Lantana camara* at about a dose of 50mg/kg was used for the induction of hepatotoxicity, based on the preliminary studies of trial and error method.

Procedure: The animals were divided into nine groups of six animals each. The animals received the following treatment for 14 days.

Group1- Normal control (only food and water ad libitum)
Group2- Diseased control (alcoholic leaf extract of *Lantana camara* 50mg/kg, p.o)
Group3- Standard control (alcoholic leaf extract of *Lantana camara* 50mg/kg + Silymarin 100mg/kg, p.o)
Group4- *Enicostemma axillare* aqueous extract low dose (alcoholic leaf extract of *Lantana camara* 50mg/kg + aqueous extract of *Enicostemma axillare* 100mg/kg, p.o)
Group5- *Enicostemma axillare* aqueous extract medium dose (alcoholic leaf extract of *Lantana camara* 50mg/kg + aqueous extract of *Enicostemma axillare* 200mg/kg, p.o)
Group6- *Enicostemma axillare* aqueous extract high dose (alcoholic leaf extract of *Lantana camara* 50mg/kg + aqueous extract of *Enicostemma axillare* 400mg/kg, p.o)
Group7- *Enicostemma axillare* alcoholic extract low dose (alcoholic leaf extract of *Lantana camara* 50mg/kg + alcoholic extract of *Enicostemma axillare* 100mg/kg, p.o)
Group8- *Enicostemma axillare* alcoholic extract medium dose (alcoholic leaf extract of *Lantana camara* 50mg/kg + alcoholic extract of *Enicostemma axillare* 200mg/kg, p.o)
Group9- *Enicostemma axillare* alcoholic extract high dose (alcoholic leaf extract of *Lantana camara* 50mg/kg + alcoholic extract of *Enicostemma axillare* 400mg/kg, p.o)

After 24hrs of the last treatment, blood samples were collected by cardiac puncture. The blood samples thus collected were immediately centrifuged at 2200rpm for 15minutes. The separated serum was analyzed for SGPT, SGOT, albumin and protein levels using reagent kits from SIGMA Reagent Kit.[10&11]

Statistical Analysis: Results were expressed as mean ± SEM. The means were analyzed using unpaired test and one way ANOVA. Differences with values of $p<0.05$ were considered statistically significant.[12]

RESULTS

The present studies were performed to evaluate the hepatoprotective potential of *Enicostemma axillare* against *Lantana camara* 50mg/kg induced hepatotoxicity at about 100mg/kg, 200mg/kg and 400mg/kg both for alcoholic and aqueous extracts. Hepatoprotective ability of the extracts was evaluated by means of determining the SGPT, SGOT, Protein and Total albumin level. The results were tabulated in table1. From the table 1, it was clearly seen that both aqueous and alcoholic extracts of *Enicostemma axillare* dose dependently prevent the severity of the hepatotoxicity induced by the hepatotoxin *Lantana camara*. The elevated levels of SGOT, SGPT, albumin and protein's were tends to revert back to the near normal level in the extract treated groups with the highest dose. Histopathological examination of the liver of group 1 shows normal cellular pattern and arrangements. The control group or group 2, exhibits

large cell necrosis along with inflammation. Group 3 exhibits near about normal cellular pattern and were very similar to that of the group 1. In group 4, 5 and 6, cell necrosis level and inflammation level was markedly decreased, however the maximum preventive effect was observed with group 6 only. Alcoholic extract treated groups 7, 8 and 9 also exhibits a similar pattern to that of aqueous extract groups. However, the level of cell necrosis and inflammation was much lesser than group 6. Thus both the extracts exhibits preventive effects dose dependently with the maximum effect being with group 9, i.e., alcoholic extract treated group of about 400mg/kg.

DISCUSSIONS

Plants always serve to be a rich source for treatment of various diseases and disorders of human system. In our day to life they are gaining much more importance because of their broad range of pharmacological actions. The current study deals with hepatoprotective activity of *Enicostemma axillare* in *Lantana camara* induced hepatotoxicity. Hepatoprotective activity of several herbal extracts had been previously tested using various hepatotoxicants such as paracetamol, Carbon tetra chloride, ethanol etc.,[13&14] However induction of liver damage using *Lantana camara* Linn was limited. *Lantana camara* was a rich source of hepatotoxin. *Lantana camara* induces hepatotoxicity by means of the presence of pentacyclic triterpenoids within them,[9] known to be as Lantadene A and B.[15] The ethanolic extract of *Lantana camara* 50mg/kg b.wt was used as the hepatotoxin for the induction of hepatotoxicity. Hepatoprotective effects of both alcoholic and aqueous extracts were evaluated at 100mg/kg, 200mg/kg and 400mg/kg b.wt. Liver is an important organ for metabolism and detoxification. SGPT and SGOT are markers of liver function.[16] Assessment of liver damage can be made by estimating the level of serum GOT, GPT, ALP and LDH, which were enzymes originally present higher concentration in cytoplasm. When there was a hepatic damage, these enzymes leak into the blood stream in conformity with the extent of liver damage.[17&18] From the table 1, it was clearly seen there was a huge increase of SGOT level in group II, which was treated only with the hepatotoxin. However, it was clearly seen in the extract treated groups, the enzyme level tends to revert back to the normal level with the maximum restoring at the highest dose of aqueous and alcoholic extracts. Biochemical parameters such as SGPT, SGOT, Albumin and Protein levels were used to assess the liver conditions in all the groups of rats. SGPT was taken as an index for biochemical estimation as it is more abundant in liver cells than in any other cells in the body. [19] The SGPT levels were found to be significantly increased by mean of administration of the hepatotoxin – *Lantana camara*. Both ethanolic and aqueous extracts of *Enicostemma axillare* exhibit hepatoprotective activity by means by bringing back the elevated SGPT level in the extract treated groups. The liver was an important organ which was actively involved in many metabolic functions and found to be as most frequent target for a number of toxicants. Hepatic damage was associated with distortion of these metabolic functions.[20] Reduction of serum albumin in only hepatotoxin treated group may be due to formation of protein adduct. The decrease in the level of albumin content implies the severity of the hepatic damage. Extract treated animal groups (IV- IX) showed marginal to higher increase in the albumin level indicating hepatoprotective potential of the extracts. All the extracts showed dose dependent activity with the maximum effect towards alcoholic extract at 400mg/kg b.wt. Measurement of protein level was mainly used to calculate the level of purity of a specific protein. Hepatotoxin's causes the depletion of proteins indicating the tissue damage. [21] Group II animals, which were treated with only hepatotoxin showed the decrease in total protein level indicating the liver damage. However, the extract treated groups exhibited recovery from the hepatotoxicity by bringing back the elevated protein levels. The histopathological studies were the direct evidence of efficacy of drug as protectant or not.[22] Histopathological examination of the liver indicates the severe cellular damage with large cellular necrosis in group 2 as compared to group 1 indicating the severity of the hepatotoxicity induced by the hepatotoxin. However, the aqueous extract treated groups along with the hepatotoxin tries to revert back the cellular damage to marginal normal level only, with maximum preventive effect is observed with the 400mg/kg aqueous extract. The alcoholic extracts showed decreased necrosis level but the inflammation around the central vein was persistent in group 7 and group 8. Group 9, exhibited very less necrosis level and the inflammation around the vein was also drastically reduced. Among all the alcoholic extract treated groups the maximum preventive effect was observed with group 9 only.

All the determined biochemical parameters and the histopathological studies confirm the hepatoprotective potential of *Enicostemma axillare*. The results were found to be significant and comparable to that of the standard drug silymarin at 100mg/kg b.wt.

CONCLUSIONS

From the present study it is clearly seen that *Enicostemma axillare* is a potent hepatoprotective agent which is confirmed from the restoring level of elevated biochemical parameters. The histopathological studies of the liver also confirm the same. However, the alcoholic extract of *Enicostemma axillare* is found to be more potent than the aqueous group, which may be due to the presence of lipophilic phytoconstituents. Isolation of the lipophilic phytoconstituents and evaluating the same against *Lantana camara* induced hepatotoxicity may leads to a novel hepatoprotective in the arena of veterinary field.

Table 1: Effect of aqueous and alcoholic extracts of *Enicostemma axillare* (Lam) Raynal on *Lantana camara* induced hepatotoxicity

Groups	SGOT U/L	SGPT U/L	Albumin mg/dl	Protein mg/dl
1	46 ± 0.3651***	41.67±0.4216	4.60±0.0365**	7.62± 0.0126***
2	114 ± 0.3651	70.00±0.3651	3.84 ± 0.0054	6.9 ± 0.0365
3	59.83 ± 0.6009	42.00±0.5774	3.96 ± 0.0057	7.84 ± 0.0058
4	102± 0.3651***	69.00±0.3651	3.65 ± 0.0030	6.92 ± 0.0042^{ns}
5	94 ± 0.5774***	68.00±0.2582	3.72 ± 0.0051	6.98 ± 0.0036^{ns}
6	78 ± 0.3651***	61 ± 0.3651	3.861±0.0001	7.01 ± 0.0052*
7	88.33±0.3333***	56 ± 0.5774	3.85 ± 0.0055	7.22± 0.0025***
8	80.83±0.4773***	51.17±0.4773	3.9 ± 0.0447	7.49± 0.0026***
9	65 ± 0.5164***	48.17±0.3073	3.96 ± 0.0030	7.59± 0.0056***

All the values are mean ± SEM and compared to diseased control
ns= Non significant *p<0.05 **p<0.01 ***p<0.001, n=6

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