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Investigation of hepatoprotective activity of roots & rhizomes of *Antigonon leptopus* Hook against carbon tetrachloride-induced hepatotoxicity in rats

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ABSTRACT

Extracts from *Antigonon leptopus* Hook (Polygonaceae) roots & rhizomes are well known in India as garden creeper, commonly called as Picchibatani (telugu), Mexican creeper (English). The Hepatoprotective activity of ethyl acetate and methanol extracts of *Antigonon leptopus* (AL) Stem against carbon tetrachloride (CCl_4) induced liver damage in Wistar albino rats. Ethyl acetate and methanolic extracts of AL (100, 200mg & 400/kg.p.o.), were administered respectively, Silymarin (50 mg/kg.p.o.) was given as reference standard. The extracts were effective in protecting the liver against the injury induced by CCl_4 in animals. This was evident from significant reduction in serum enzyme, SGOT, SGPT, ALP and Total bilirubin (TB).Various pathological changes like centribular necrosis and vacuolization were observed in CCl_4 treated rats, which were significant protective activity in groups treated with AL and silymarin. It was concluded from the study that ethyl acetate and methanolic extracts of AL possess hepatoprotective activity against CCl_4 induced hepatotoxicity in rats.

Keywords: Hepatoprotective, *Antigonon leptopus,* Carbontetrachloride, Silymarin.

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INTRODUCTION

Antigonon leptopus Hook (polygonaceae) are well known in India as garden creeper, picchibatani in Telugu. A. leptopus is a tender perennial vine can easily grow to 30-40 ft. in length. The coral vine has attractive green heart shaped leaves [1,2]. The coral vine is found in Indian Ocean and coastal areas [3]. Previously isolated compounds 2-anthocyanins, Pelargonin, Malvin, quercetin, rhamnetin and quercetin-3-o-β-D-glucopyranoside [4]. Very recently the methanol extract of *A. leptopus* was found to possess Antithrombin activity [5], Antidiabetic [6] and Consumed as food [7] reported. The present studies were performed to assess the hepatoprotective activity against CCl_4 induced hepatotoxicity in rats as hepatotoxin to prove its claim in the folklore practices against liver disorders.

MATERIALS AND METHODS

Plant Material Collection

Antigonon leptopus roots & rhizomes were collected from the Andhra University campus area, Visakhapatnam in the month of November 2006 and authenticated by the taxonomist, Dept. of Botany, Andhra University and the specimen voucher No. AUCP/BGR/2006/A52 was preserved in the Department.

Acute toxicity studies

Acute toxicity studies were performed for extracts of selected plant according to the toxic classic method as per guidelines. None of these extracts showed mortality even at a dose of 1000mg/kg and therefore considered safe. Toxicological studies were conducted in mice (N=6) for all the extracts as per the Irvin's method [8] at the doses of 100, 300 and 1000 mg/kg, no mortality was observed.

Materials

All the materials used for this experiment are of Pharmacopoeial grade. Carbon tetrachloride (E. Merck), silymarin (Sigma Chemical Co.,) and olive oil were purchased from the local supplier. Diagnostic kits for the estimation of SGOT, SGPT, SALKP and serum bilirubin were purchased from local supplier (Sai Chemicals) manufactured by Ranbaxy Diagnostics Ltd., New Delhi, India. Water represents the double distilled water, standard orogastric cannula was used for oral drug administration.

Animals

Wistar albino rats of either sex weighing between 200-250 gm were obtained from M/s. Mahavir Enterprises, Hyderabad, Andhra Pradesh, India. The animals were housed under standard environmental conditions (temperature of 22 ± 1°C with an alternating 12 h light–dark cycle and relative humidity of 60 ± 5 %), one week before the start and also during the

experiment as per the rules and regulations of the Institutional Animal Ethics committee and by the Regulatory body of the government (Regd no. 516/01/a/CPCSEA). They were fed with standard laboratory diet supplied by M/s. Rayans biotechnologies Pvt. Ltd., Hyderabad, Andhra Pradesh, India. Food and water was allowed *ad libitum* during the experiment.

Carbontetrachloride-induced hepatotoxicity:

The animals were divided into nine groups of six animals each. Group-I served as normal control received 5% acacia mucilage (1 ml/kg.p.o) daily once for 7 days. Group-II served as toxic control and received CCl_4 (1 ml/kg i.p) daily once for 7 days [9]. Group-III was treated with the standard drug Silymarin (50 mg/kg .p.o) and followed by CCl_4 (1 ml/kg i.p) daily once for 7 days[10]. Groups IV-VI were treated with ethyl acetate extract of A. *leptopus* roots & rhizomes at doses of 100, 200 & 400mg/kg p.o. in acacia mucilage respectively followed by CCl_4 (1 ml/kg i.p) daily once for 7 days. Groups VII-IX were treated with methanol extract of A. *leptopus* roots & rhizomes at doses of 100, 200 & 400mg/kg p.o, in acacia mucilage respectively followed by CCl_4 (1 ml/kg i.p) daily once for 7 days. After completion of treatment blood was collected, serum was separated and used for determination of biochemical parameters.

Collection of blood samples

All the animals were sacrificed on 7 th day under light ether anesthesia.The blood samples were collected separately in sterilized dry centrifuge tubes by puncture retro-orbital plexes and allowed to coagulate for 30 min at 37 °C .The clear serum was separated at 2500rpm (Microcentrifuge) for 10min and subjected to biochemicalinvestigationviz..,serumglutamic oxaloacetate transe aminase (SGOT), serum glutamic Pyruvate transe aminase (SGPT), Alkaline phosphatase (ALP) and Total Bilirubin (TB).

Assessment of liver function

The Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were estimated by UV kinetic method in which both SGOT and SGPT were assayed based on enzyme coupled system; where keto acid formed by the aminotransaminase reacts in a system using NADH.The coenzyme is oxidized to NAD and the decrease in absorbance at 340 nm for SGOT malate dehydrogenase (MDH) reduces to malate with simultaneous oxidation of NADH to NAD.The rate of oxidation of NADH is measured, where as SGPT [11] the pyruvate formed in the reaction is converted to lactate by lactate dehydrogenase.Estimation of Alkaline phosphate (ALKP)[12] involves hydrolysis of P-nitrophenyl phosphate by alkaline phosphatase to give P-nitrophenol, which gives yellow color in alkaline solution. The increase in absorbance due to its formation is directly proportional to alkaline phosphate (ALKP) activity. Estimation of total bilirubin (TB) [13] involved the reaction of bilirubin with diazotized sulphanic acid to form an azocompound, the color of which is measured at 546 nm .All the estimations were carried out using standard kits in semi auto analyzer Screen Master 3000.

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Statistical analysis

Results of biochemical estimation were reported as mean ±SEM for determination of significant inter group difference was analyzed separately and one-way analysis of variance (ANOVA) was carried out[14].Dunnet's test was used for individual comparisons [15].

RESULTS

Serum levels of SGOT, SGPT, SALKP and total bilirubin were significantly increased ($p<0.01$) in carbon tetrachloride treated Group-2 rats. Group-3 rats treated with Silymarin produced significant reduction ($p<0.01$) in SGOT, SGPT, SALKP and total bilirubin levels.
In Groups: 4-6, treated with ethyl acetate extract of *Antigonon leptopus*, at doses of 100, 200 and 400mg/kg; p.o respectively, there is significant decrease in SGOT, SGPT, SALKP and total bilirubin levels when compared to Group-2 rats. The results were given in Table-(1).

The comparative efficacy of the extracts tested for their hepatoprotective activity, the relationship between dose and percentage reduction in each case were depicted in the form of a bar diagram as shown in Fig-1.

Carbontetrachloride (1ml/kg.i.p) intoxication in normal rats produced elevated levels of serum biochemical parameters significantly SGOT(160.5 ± 0.62, 295.5 ± 0.39), SGPT(96.95 ± 1.34, 269.5 ± 1.8), ALKP(179.5 ± 0.99, 296.5 ± 1.45), T.B(0.82 ± 0.06, 2.02 ± 0.03) indicating acute hepatocellular damage and biliary obstruction. The percentage reduction of various serum biochemical parameters in case of standard drug Silymarin(50mg/kg.p.o) in CCl_4 intoxicated rats revealed a significant reduction ($p<0.01$) in the levels of SGOT(89.63%), SGPT(93.8%), ALKP(92.99%) and T.B(94.16%) respectively.

When compared to the CCl_4 toxic control group, the group treated with the ethyl acetate extracts of *Antigonon leptopus*, at doses of 100, 200 and 400mg/kg; p.o in CCl_4 intoxicated rats exhibited a significant reduction ($p<0.01$) of SGOT(40.18%, 49.85%, 78.96%), SGPT(39.80%, 49.42%, 80.24%), ALKP(76.92%, 84.61%, 86.41%) and T.B(56.66%, 62.49%, 79.99%) levels respectively.

In Groups: 7-9, treated with methanol extract of *Antigonon leptopus*, at doses of 100, 200 and 400mg/kg; p.o respectively, there is significant decrease in SGOT, SGPT, SALKP and total bilirubin levels when compared to Group-2 rats.

Carbontetrachloride (1ml/kg.i.p) intoxication in normal rats produced elevated levels of serum biochemical parameters significantly SGOT (160.5 ± 0.62, 295.5 ± 0.39), SGPT(96.95 ± 1.34, 269.5 ± 1.8), ALKP(179.5 ± 0.99, 296.5 ± 1.45), T.B(0.82 ± 0.06, 2.02 ± 0.03) indicating acute hepatocellular damage and biliary obstruction. The percentage reduction of various serum biochemical parameters in case of standard drug Silymarin(50mg/kg.p.o) in CCl_4 intoxicated rats revealed a significant reduction ($p<0.01$) in the levels of SGOT(89.63%), SGPT(93.8%), ALKP(92.99%) and T.B(94.16%).

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TABLE- 1: Effect of ethyl acetate and methanol extracts of *A. leptopus* on biochemical estimation of SGOT, SGPT, SALKP and total bilirubin of CCl_4 induced toxicity in rats.

Groups	SGOT (IU/ml)	SGPT (IU/ml)	SALKP (IU/ml)	Total Bilirubin (mg/dl)
Control 1ml/kg	160.5 ± 0.62	96.95 ± 1.34	179.5 ± 0.99	0.82 ± 0.06
CCl_4 1ml/kg	295.5 ± 0.39^{+}	269.5 ± 1.8^{+}	296.5 ± 1.45^{+}	2.02 ± 0.03^{+}
Silymarin 50mg/kg	174.8 ± 1.88***	107.5 ± 1.45***	187.7 ± 2.25***	0.89 ± 0.04***
ALEE 100 mg/kg	253.2 ± 4.12*	204.1 ± 2.52*	229.1 ± 3.20*	1.41 ± 2.01*
ALEE 200 mg/kg	208.0 ± 1.75**	171.7 ± 3.65**	212.5 ± 1.37**	1.24 ± 1.05**
ALEE 400 mg/kg	193.1 ± 4.15***	134.4 ± 3.08***	200.5 ± 4.05***	1.16 ± 2.25***
ALME 100 mg/kg	249.9 ± 2.84*	201.3 ± 0.80*	225.5 ± 1.45*	1.38 ± 0.02*
ALME 200 mg/kg	204.2 ± 3.60**	170.0 ± 2.84**	203.5 ± 2.31**	1.19 ± 0.07**
ALME 400 mg/kg	190.3 ± 1.05***	128.9 ± 1.18***	198.5 ± 0.75***	1.11 ± 0.03***

Values are mean ± SEM for six observations, P: $^{+}$<0.001 Compared to respective control group-1, P: *<0.05, **<0.01, *<0.001 Compared to respective control CCl_4 group-2, ALEE- Antigonon *leptopus* ethyl acetate extract, ALME- Antigonon *leptopus* methanol extract**

TABLE-1.1: Percentage reduction of various biochemical parameters due to treatment with ethyl acetate and methanol extracts of *A. leptopus* against CCl_4 induced hepatotoxicity in rats.

S.NO	TREATMENT	SGOT (IU/ml)	SGPT (IU/ml)	SALKP (IU/ml)	T. B (mg/dl)
1	Silymarin 50mg/kg	89.63	93.8	92.99	94.16
2	ALEE 100mg/kg	40.18	39.80	76.92	56.66
3	ALEE 200mg/kg	49.85	49.42	84.61	62.49
4	ALEE 400mg/kg	78.96	80.24	86.41	79.99
5	ALME 100mg/kg	31.40	23.87	42.73	44.99
6	ALME 200mg/kg	47.55	32.15	50.59	54.16
7	ALME 400mg/kg	61.55	45.88	55.72	64.99

ALEE- Antigonon *leptopus* ethyl acetate extract, ALME- Antigonon *leptopus* methanol extract

Figure-1: Effect of ethyl acetate and methanol extracts of *A. leptopus* on biochemical estimation of SGOT, SGPT, SALKP and total bilirubin of CCl_4 induced toxicity in rats.

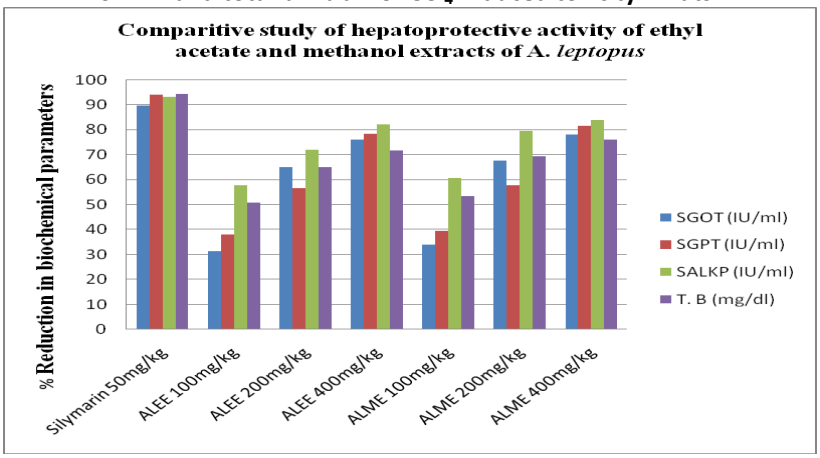

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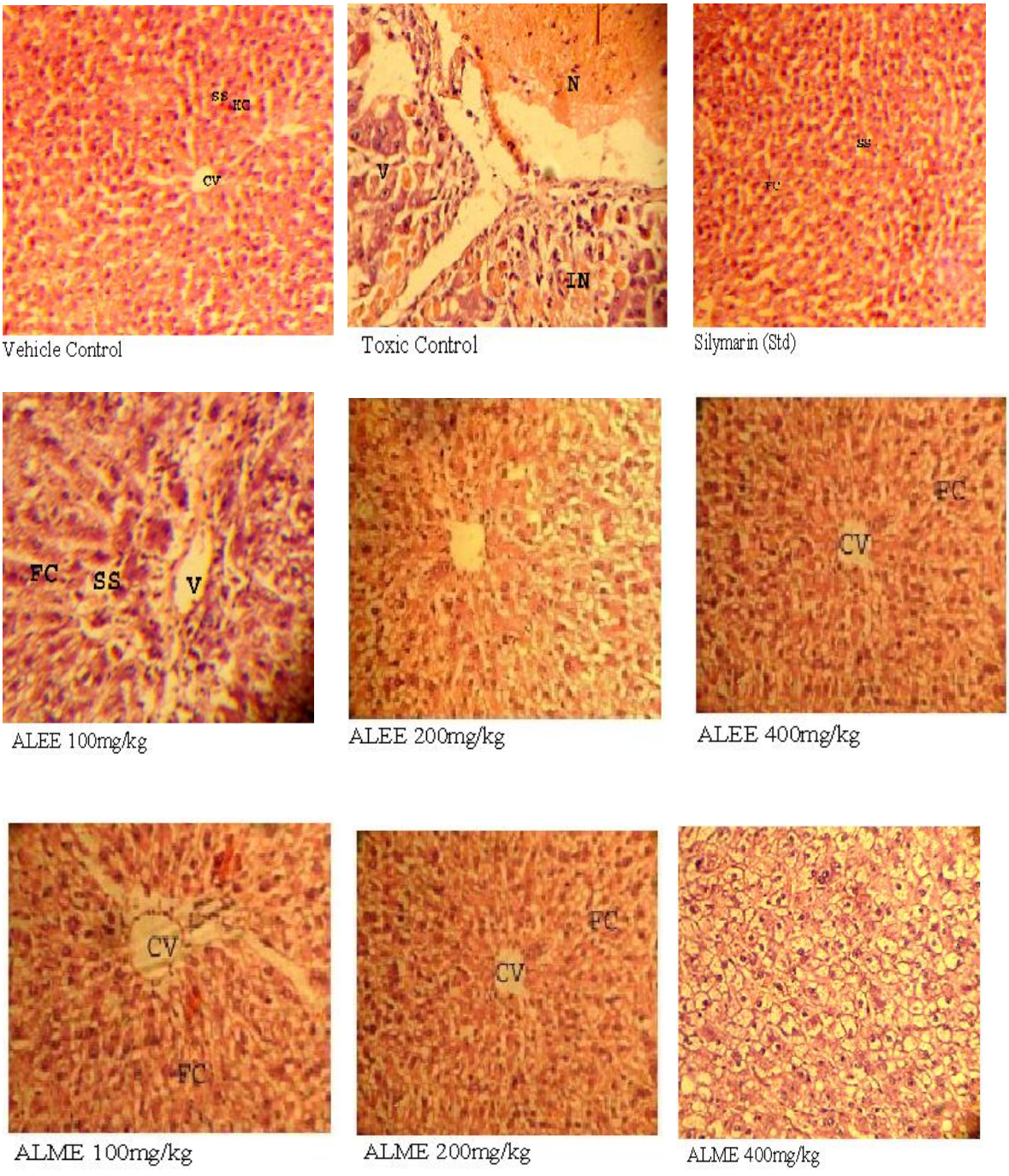
Figure1A: Representative photographs of histopathological changes showing effect of the test material on the rats intoxicated with carbon tetrachloride. ALEE- Antigonon *leptopus* ethyl acetate extract, ALME- Antigonon *leptopus* methanol extract



When compared to the CCl_4 toxic control group, the group treated with the methanol extracts of *Antigonon leptopus*, at doses of 100, 200 and 400mg/kg; p.o in CCl_4 intoxicated rats exhibited a significant reduction ($p<0.01$) of SGOT(31.40%, 47.55%, 61.55%), SGPT(23.87%,

32.15%, 45.88%), ALKP(42.73%, 50.59%, 55.72%) and T.B(44.99%, 54.16%, 64.99%) levels respectively.

Considering the enzyme level difference between hepatotoxin treated and control rats as 100% of level of reduction and recorded(Table-1.1)

Though both the extracts were recorded with significant hepatoprotective activity with same "p" value ($p<0.01$). The methanol extract was found to be more potent than ethyl acetate extract because of effect on percentage reduction in elevated levels of biochemical parameters and effect was dose dependant.

The effect of ethyl acetate and methanol extracts of stem on CCl_4 induced liver damage in rats with reference to biochemical changes in serum was shown. Percentage decrease or increase was calculated by Histopathology of liver tissues. Group I (vehicle control)—section shows central vein surrounded by hepatic cord of cells (normal architecture). Group II (toxic control)—section shows patches of liver cell necrosis with inflammatory collections, around central vein. Group III (standard silymarin)—almost near normal. Group IV (ALEE 100mg/kg)—inflammatory collections around central vein and focal necrosis. Group V (ALEE 200mg/kg)—inflammation decreasing around central vein. Group VI (ALEE 400mg/kg)—less inflammatory cells around central vein, absence of necrosis. Group VII (ALME 100mg/kg)—less inflammation around central vein. Group VIII (ALME 200mg/kg)—less inflammatory cellular infiltration. Group IX (ALME 400mg/kg)—minimal inflammatory cells Almost normal liver (Fig-1A).

CONCLUSION

The activity of the extracts is found to be dose dependant. In CCl_4 induced toxic hepatitis, toxicity begins with the changes in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures [16]. Administration of ethyl acetate and methanol extracts of *A. leptopus* showed recovery against the toxic effects of CCl_4. The hepatoprotective effect of the drugs was further concluded by the histopathological examinations of the liver sections which reveal that the normal liver shape was disturbed by hepatotoxin intoxication. In the liver sections of the rats treated with ethyl acetate extract and methanolic extract and intoxicated with CCl_4 the normal cellular shape was retained as compared to silymarin, thereby confirming the protective effect of the extracts of A. *leptopus*. The hepatoprotective activity of *A. leptopus* could be due to the presence of flavonoids which have hepatoprotective properties [17-19]. These results indicate that it is worth undertaking further studies on possible usefulness of the extracts of the roots and rhizomes of *A. leptopus* in hepatotoxicity.

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