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Protective effect of *Alysicarpus monilifer L.*, Against CCl_4 induced hepatotoxicity in albino rats.

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ABSTRACT

Alysicarpus monilifer L. is a widely used plant in the north coastal districts of Andhra Pradesh, India, has been used in indigenous system of medicine. The roots are used for the treatment of leprosy and urinary troubles. The decoction of roots is prescribed for cough. The boiled leaves are used as purgative. The herb is credited with anti- pyretic, anti- periodic and expectorant properties, febrifuge and also recommended for cutanious scabies and boils and to cure pain. Acute toxicity tests were conducted as per OECD guidelines on *Alysicarpus monilifer L.* whole plant. The hydro-alcoholic extract of the aerial parts at 200 mg/kg, 400 mg/kg and 800 mg/kg b.w., was tested in Carbon tetrachloride (CCl_4) induced hepatotoxicity of rats followed by histopathological examination of the isolated livers of the control and the treated groups . The potential effects in protecting liver function by reducing the elevated levels of various serum biochemical parameters in a dose dependent manner, reducing oxidative stress, and histopathological alterations in the rat model of CCl_4 – induced liver damage was demonstrated. The results showed significant protective effect against CCl_4 induced hepatotoxicity in albino rats. The study on qualitative phytochemical screening also identified some important bioactive phytochemical principles such as steroids, triterpenoids, saponins, flavonoids, tannins, carbohydrates and glycosides in this plant which were also validated as antioxidants and biologically active phytoconstituents. This report of hepatoprotective activity of *Alysicarpus monilifer L.* throws light on attenuation of hepatotoxic effects of CCl_4 challenged rats by membrane stabilization through antioxidation.

KEYWORDS

Alysicarpus monilifer L., Carbon tetrachloride, Hepatotoxicity, Hepatoprotective activity, antioxidant, Histopathological study.

INTRODUCTION

Alisicarpus monilifer Linn. (Fabaceae) grows throughout in India, Pakisthan and Ethiopia in sandy and sub-sandy soils and in lawns especially along the coast (Nasir and Ali,1977; Varadarajan, 1985). The plants are erect or prostrate seasonal herbs, leaves unifoliate, flowers produced in simple recemes, fruits constricted between seeds. *Alysicarpus monilifer* has been used in indigenous system of medicine. Eather and ethnolic extracts of leaves of *Alysicarpus veginalis* showed antiproliferation activity against tumor cells (Rathi et al., 2010). The leaves are used to treat jaundice (Sankarnarayan, 1988). Analgesic activity of methanolic extract of the aerial parts of *A. monilifera* was evaluated and found to be significant (Purvi et al., 2011).

The plant is being used by the local people and tribal folk of north coastal districts of Andhra Pradesh for liver ailments. In view of the increasing incidence of liver disorders , availability of not so effective modern allopathic medicine (Seeff and Ghany,2010) and to fill the lacuna in literature regarding the scientific basis for the hepatoprotective activity in this unexplored medicinal herb, the present study was undertaken to evaluate the protective effect of

methanolic extract of *Alysicarpus monilifer* whole plant on CCl_4 –induced hepatotoxicity and to elucidate the mechanism underlying the protective effects in rats which has not been reported earlier in this plant.

Materials and Methods

Plant material

The whole plant of the *Alysicarpus monilifer* was collected from the surroundings of Visakhapatnam, Andhra Pradesh and it's identity was confirmed by the department of Botany, Andhra University, Visakhapatnam. The herbarium specimen of the plant was deposited in the department of Botany, Andhra University with the Voucher no: VPJ/DOB/AM2509.

Preparation of extracts

The shade dried plants of about 500 g were subjected to size reduction to coarse powder. The powder was then extracted with 80%methanol using Soxhlet apparatus till exhaustion for about 48 hours. Later it was concentrated under vacuum to get the residue. The percentage yield was found to be 8% (w/w). The preliminary phytochemical screening showed the presence of steroids, terpenoids, saponins, flavonoids, carbohydrates and glycosides.

Experimental animals

Healthy Wistar-Albino rats of either sex, weighing 150-250g, obtained from Ghosh Enterprises, Kolkotha were used in the study. The animals were given access to food and water they were fed with standard pellet diet and water *ad libitum*. All procedures were performed according to the Institutional Animal Ethics Committee's approval.

Toxicity studies

Acute toxicity study was performed for methanolic extract according to the acute toxic classic methods (as per OECD guidelines) on Albino rats. The animals were administered plant extract orally at different doses and observed for 14 days for mortality. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. Accordingly the doses of the extract tested for acute toxicity were selected for evaluation of hepatoprotective activity, i.e., 200, 400 and 800 mg/kg

Procedure

The Wistar albino rats of either sex were divided into six groups of six animals (n=6) each. Group-I served as normal control and received vehicle (Sodium CMC) + olive oil suspension in the ratio of 1:1 (1 ml/kg. p. o) once daily for 3 days. Group –II served as hepatotoxin treated group (negative control), received vehicle on 1st and 2nd day and CCl_4 (1ml/kg s.c. suspended in olive oil in the ratio of 1:1) on the third day. Group-III, (positive control) received Silymarin (50mg/kg. i. p. suspended in sodium CMC) once daily for 3 days and Carbon tetrachloride (CCl_4)1ml/kg.,sub cutaneous (s.c.) on the third day. The three test groups (IV – VI) received oral administration of 80% methanolic extract of *Alysicarpus monilifer* whole plant at doses of 200, 400 and 800mg/kg body weight in sodium CMC suspension once daily for 3 days followed by CCl_4 (1ml/kg s.c) on the third day as per Kurma and Mishra, (1997); Suresh kumar and Mishra,(2005) with slight modification. 24 h after CCl_4 treatment, blood was collected from all the groups, and allowed to clot for the separation of serum. The blood was centrifuged at 3000rpm for 15 min to separate the serum. The serum was used for estimation of biochemical parameters such as serum Glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transminase (SGPT), alkaline phosphatase (ALKP) and total bilirubin (TBL). All the determinations were carried out using standard kits by an auto analyzer.

Histopathological studies

One animal from each of the treated group showing maximum activity as indicated by improved biochemical parameters was used for this purpose. The rats were sacrificed by cervical dislocation and the abdomen was cut open to remove the livers. The

liver samples of gross lesion were excised, washed thoroughly with saline water and the weight and volume of the wet liver was estimated. The livers were then fixed in 10% neutral buffered formalin solution for 24 hours and embedded in paraffin using conventional methods (Galighor and Kozloff, 1976). Later they were cut into 5µm thick sections and stained using haematoxylin eosin dye and finally mounted in di-pheny xylene (DPX). The sections were examined under light microscope for histopathological changes in liver architecture and their photomicrographs were taken.

Statistical analysis

The mean values ±S.E.M. are calculated for each parameter. For determining the significant inter-group differences, each parameter was analyzed separately and 1-way analysis of variance (ANOVA)(Gennaro,1995) was carried out and the individual comparisons of the group mean values were done using Dunnet's procedure (1964).

RESULTS

Acute toxicity studies were performed for the extract according to the toxic classic methods as per guidelines - 423 prescribed by OECD. The methanolic extract did not cause any mortality up to 2000mg/kg and hence considered as safe (OECD, 1996).

The methanolic extract of *Alysicarpus monilifer* at dose levels of 200 mg/kg , 400 mg/kg and 800mg/kg b.w., was tested in CCl_4 induced hepatotoxicity rats. The results of serum biochemical parameter levels have been presented as mean ±SEM. The percentage decrease or increase was calculated by considering the enzyme level difference between hepatotoxin treated and control rats as 100% level of reduction, the results were recorded in Table 1. The comparative efficacy of the extract tested for its hepatoproctective activity, the relationship between dose and percentage reduction in each case was depicted in the form of a bar diagram as shown in figure: 1.

Carbon tetrachloride (1ml/kg s.c.) intoxication in normal rats produced significantly elevated levels of serum biochemical parameters SGOT (86.07± 1.83 to 550.48± 12.33 IU/L), SGPT (46.00± 0.35 to 456.18 ± 8.38 IU/L), ALP (160.08 ± 1.60 to 375.66 ± 5.46 IU/L) and TB (0.31 ± 0.06 to 1.86 ± 0.14 mg/dl). The liver showed significant increase in its weight indicating acute hepatocellular damage and biliary obstruction. The percentage reduction of various serum biochemical parameters in case of standard drug silymarin (50 mg/kg. i.p.) in CCl_4 intoxicated rats revealed a significant reduction ($p<0.01$) in the levels of SGOT (91.27%), SGPT (88.92%), ALP(83.80%) and TB(96.77%) (Table 1).

Treatment with methanolic extract of *Alysicarpus monilifer* whole plant (200, 400 and 800 mg/kg p.o doses) on CCl_4 intoxicated rats revealed a significant dose dependant reduction ($p<0.01$) in the levels of SGOT, SGPT, ALP, TB (Table 1 ; Fig: 1&2), compared to that of CCl_4 intoxicated group.

Histopathological studies of liver section of the control group showed normal cellular architecture with distinct hepatocytes with well preserved cytoplasm, prominent nucleus, nucleolus, sinusoidal spaces and central vein. There was no sign of inflammation, fatty change or necrosis in these animals (Fig: 2A). The liver section of CCl_4 intoxicated group showed complete disarrangement of normal hepatic cells with intense centrilobular necrosis, vacuolization, neutrophile infilteration, fatty changes and sinusoidal haemorrhages and dilatation(Fig:2B). The liver sections of silymarin treated rats at 50mg/kg dose showed apparently normal liver lobule with no sign of necrosis in centrizonal area and portal vein but only a few inflammatory cells were observed in the centrizonal area. They showed a normal hepatic architecture with normal hepatocytes, sinusoidal spaces, less vacuole formation, absence of necrosis and less visible changes as compared to control (Fig: 2C).

The histopathological examination of rats administered with methanolic extract of *Alysicarpus monilifer* whole plant (200,400 and 800mg/kg p.o doses) intoxicated with CCl_4 showed fatty changes

with sinusoidal dilatation and absence of necrosis and with higher dose 800mg/kg p.o showed significant attenuation of inflammatory and necrotic changes and cellular architecture of liver was preserved indicating a marked protective activity similar to that observed in silymarin treated rat liver sections, and the effect was found to be dose dependant and at the dose of 400mg/kg itself the protective effect was very significant(Fig:2D)

Table:1 Effects of Methnolic extract (80%) of *Alysicarpus monilifer* whole plants against CCl4 induced hepatotoxicity in albino rats in terms of serum biochemical parameters

S.No	Treatment group	Serum biochemical parameters			
		SGOT (1U/L)	SGPT (1U/L)	ALP(1U/L)	TB(mg/dl)
1	Control (olive oil 1ml/kg p.o)	86.07± 1.83	46.0± o.35	160.08± 1.60	0.31± 0.02
2	Toxic- CCl_4 (1ml/kg s.c.)	550.48± 12.33	456.18± 1.38	375.66± 2.46	1.86± 0.01
3	Standard- Silymarin (50 mg/kg i.p.)	126.57± 1.18 (91.27)*	91.43±1.52 (88.92)*	195.68± 1.98 (83.80)*	0.36± 0.03 (96.77)*
4	AMME (200 mg/kg p.o)	257.33± 1.25 (63.12)*	237.91± 1.42 (53-21)*	265.06± 1.06 (51.30)*	0.67± 0.02 (75.31)*
5	AMME (400mg / kg p.o)	213.66± 1.38 (72.52)*	168.01± 1.86 (70.25)*	243.11± 1.25 (61.48)*	0.58± 0.01 (81.01)*
6	AMME (800 mg/kg p.o)	158.09± 1.70 (84.49)*	150.22± 1.53 (74.59)*	225.16± 1.44 (69.81)*	0.50± 0.03 (86.07)*

P<0.01 when compared to toxic (CCl4 treated) group; n=6

AMME- *Alysicarpus monilifer* Methanolic extract

*Percentage reduction of various serum biochemical parameters due to treatment with Methnolic extract(80%) of *Alysicarpus monilifer* whole plants against CCl4 induced hepatotoxicity in albino rats

Fig:1 Hepatoprotective activity of methanolic extract (80%) of *Alysicorpius monilifer* whole plant against CCl_4 induced hepatotoxicity in albino rats showing Percentage reduction of various serum biochemical parameters

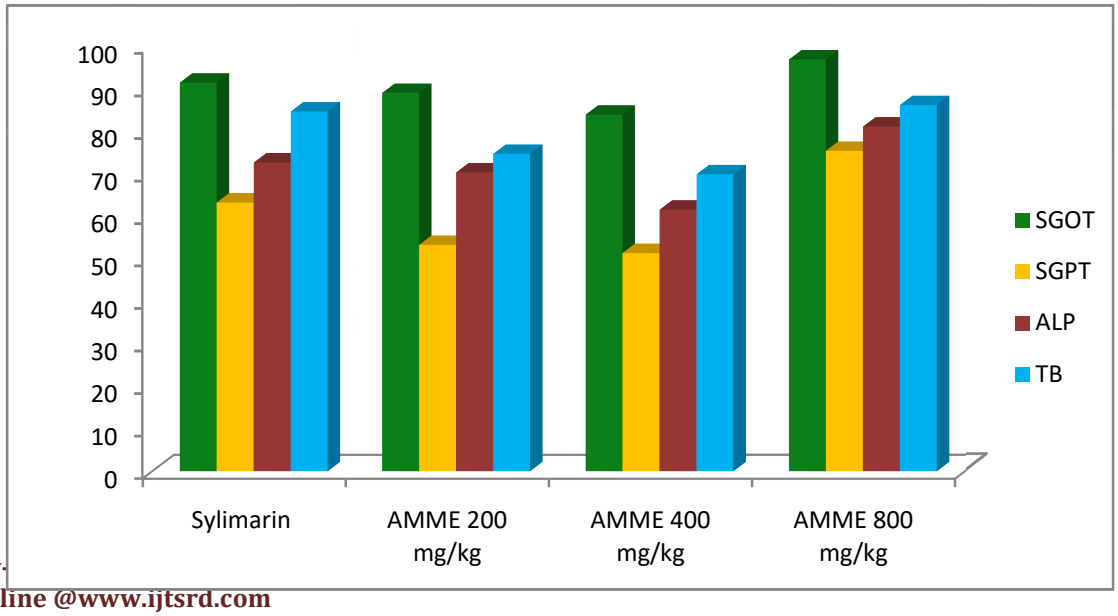
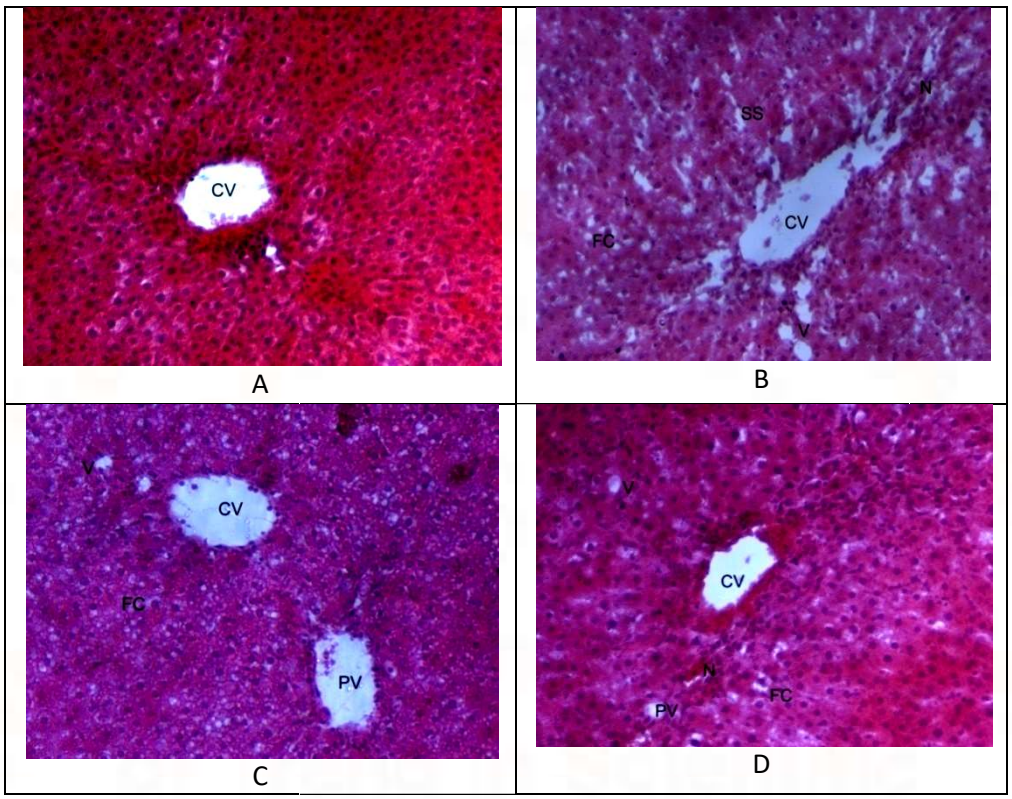


Fig:2 Hepatoprotective activity of methanol extract of *Alysicarpus monilifer* whole plant against CCl_4 induced hepatotoxicity in albino rats



Photographs of liver sections stained with haematoxylin and eosin, taken using Nickon Trinocular microscope with image analyzer
CV- central vein, PV – portal vein, N - necrosis, SS – sinusoidal spaces, FC – fatty changes
(**A**) Normal control provided with olive oil showing normal liver architecture
(**B**) Negative control- treated with CCl_4+ vehicle (1:1)1ml/kg b.w.,s.c.. showing complete disarrangement of normal hepatic cells
(**C**) Positive control- liver tissue treated with Standard drug Silymarin (50mg/kg) and CCl_4 showing normal hepatic architecture
(**D**) Liver tissue treated with methanol extract of *Alysicarpus monilifer* whole plant(AMME- 400mg/kg b.w.,p.o.) and CCl_4 (1ml/kg b.w., s.c..) showing absence of necrosis and less fatty accumulation preserving cellular architecture of liver indicating a marked protective activity

DISCUSSION

As there was no report on the hepatoprotective activity of this plant, the present study indicates the potential hepatoprotective activity of *Alysicarpus monilifer* whole plant. Reduction in the levels of SGOT and SGPT towards the normal value is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by CCl_4. Reduction of ALKP levels with concurrent depletion of raise in bilirubin level suggests the stability of the biliary function during injury with CCl_4. The raise in protein and albumin levels suggests the stabilization of endoplasmic reticulum leading to protein synthesis. The protective effect exhibited by the methanolic extract is similar to silymarin treatment. Silymarin is a standard drug. In the present investigation 50mg/kg of silymarin showed significant difference compared to other extracts..

It was evident from the results that after the treatment with the plant extract, there was a significant reduction in the increased levels of serum biochemical parameters due to CCl_4 caused hepatotoxicity. The histopathological observations also showed that plant extract treated liver sections against CCl_4 induced hepatotoxisity revealed the absence of necrosis and well preserved cellular architexture . This is an indication that the cellular damage caused by hepatotoxin(CCl_4) was either prevented or repaired

by the bioactive phytoconstituents of the plant indicating their protective effect.

Therefore the bioassays with the methanolic extract of the whole plant *Alysicarpus monilifer* recorded significant hepatoprotective activity and the study not only provides helpful information for the application of herbal drugs in liver disease, but also promotes the understanding of the pharmacological mechanisms of action in the acute toxic liver injury

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