

Role of vitexin and isovitexin in hepatoprotective effect of *Alysicarpus monilifer* Linn. against CCl₄ induced hepatotoxicity

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Received: 24th May 2012, **Revised:** 7 June 2012, **Accepted:** 7 June 2012

Abstract

Acute toxicity tests were conducted as per OECD guidelines on *Alysicarpus monilifer* Linn., a widely used plant in the north coastal districts of Andhra Pradesh, India, to treat various liver disorders and other common ailments. The methanolic extract of the whole plant at dose levels of 200 mg/kg, 400 mg/kg and 800 mg/kg b.w., was tested in CCl₄ induced hepatotoxicity 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 (SGOT, SGPT, ALP & T. Bil.) in a dose dependent manner, reducing oxidative stress, and histopathological alterations in the rat model of CCl₄-induced liver damage was demonstrated. This first report of hepatoprotective activity of *Alysicarpus monilifer* throws light on attenuation of hepatotoxic effects of CCl₄ challenged rats by membrane stabilization through antioxidantation

Keywords: *Alysicarpus monilifer*, Carbon tetrachloride, Hipatotoxicity;

Introduction

Alysicarpus monilifer Linn. (Fabaceae) grows throughout in India, Pakistan 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 racemes, fruits constricted between seeds. *Alysicarpus monilifer* has been used in indigenous system of medicine. In India, the roots are used for the treatment of leprosy and urinary troubles. The decoction of root is being used for cough and boiled leaves are used as purgative. Ether and ethanolic extracts of leaves of *Alysicarpus vaginalis* showed antiproliferation activity against tumor cells (Rathi et al., 2010). The herb is credited with antipyretic, antiperiodic and expectorant properties (Varadarajan, 1985). The leaves are

used to treat jaundice (Sankarnarayan, 1988). Its paste is used for coetaneous problems (Rahmatullah et al., 2010). In Indonesia the leaves are used to cure pain in lion and in Philippine, the decoction of stem is used to cure stomach pain (Baquar,1984). Analgesic activity of methanolic extract of the aerial parts of *A. monilifera* was evaluated and found to be significant (Purvi et al., 2011).

Now a days, liver diseases have become common and frequent occurrence. Current medical treatments for these liver diseases are often ineffective medications (Seeff and Ghany, 2010). Developing pharmacologically effective agents from natural products has become a new trend by virtue of their safe toxicity or levels marginal side effects. Herbs have recently become attractive as health-beneficial foods (physiologically functional foods) and as a source material for the development of drugs. Herbal medicines derived from plant extracts are being utilized increasingly to treat a wide variety of clinical diseases, with relatively little knowledge regarding their modes of action (Matthews, 1981)

Carbon tetrachloride (CCl₄) is a potent hepatotoxin producing centrilobular hepatic necrosis, which causes liver injury. CCL₄-induced liver injury depends on a toxic agent that has to be metabolized by the liver NAPDH- cytochrome P450 enzyme system to a highly reactive intermediate. It was reported that the changes associated with CCl₄-induced liver damage are similar to that of acute viral hepatitis (Rubinstein, 1962), drug/chemicals-induced hepatopathy and oxidative stress (Recknagel et al., 1989; Kadiiska et al., 2005), therefore, CCl₄-induced hepatotoxicity model is frequently used for the investigation of hepatoprotective effects of drugs and plant extracts.

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 CCl₄-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 its 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 perce-

ntage 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 maintained at standard housing conditions (room temperature 23-25° C, relative humidity 55%). A controlled 12 h light / 12 h dark cycle was maintained. 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). Albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose of 400mg/kg and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If the mortality was not observed, the procedure was repeated for further higher dose, i.e., 200mg/kg. 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

CCl₄-induced hepatotoxicity

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 CCl₄ (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 CCl₄ (1ml/kg 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 800 mg/kg p.o in sodium CMC suspension once daily for 3 days followed by CCl₄ (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 CCl₄ 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 transaminase (SGPT), alkaline phosphatase (ALKP) and total bilirubin (TBL). All the determinations were carried out using standard kits by an autoanalyser.

Physical Parameters

Determination of wet liver weight and volume:

Livers isolated from the animals were washed with saline solution and dried with filter paper strips and weighed on an electric balance (Dhona, Calcutta) and were expressed with

respect to their body weight i.e. g/100g. After recording the liver weights, the livers were individually dropped into a measuring cylinder containing a fixed volume of distilled water and the volume displaced was recorded and expressed as ml/100g body weight (Parmar et al., 2009).

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 5 μ m thick sections and stained using haematoxylin eosin dye and finally mounted in di-phenyl 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 \pm 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).

Phytochemical evaluation

Column chromatography was done by standard procedure using silica gel (Qualigens), 60-120 mesh. The column was eluted with n-hexane: ethyl acetate and ethyl acetate: methanol by step gradient. The bioactivity guided fractionation yielded three compounds, stigmaterol (**1**) from hexane-ethyl acetate fraction (95:5), two flavones glycosides Isovitexin (**2**) from hexane-ethyl acetate fraction (10:90) and vitexin (**3**) from ethyl acetate-methanol fraction (97:3) (Figure 1). The structures of the three compounds were elucidated with the help of ^1H NMR, ^{13}C NMR in general for all compounds, and 2D NMR HMBC and HSQC spectral analysis were carried out to determine vitexin (Manikya kumari, 2012).

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 CCl₄ induced hepatotoxicity rats. The analyzed biochemical parameters included serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin (T.Bil); physical parameters included wet liver weight and volume and histopathology of liver damage. The results of serum biochemical parameter levels have been presented as mean \pm SEM. The percentage decrease or inc-

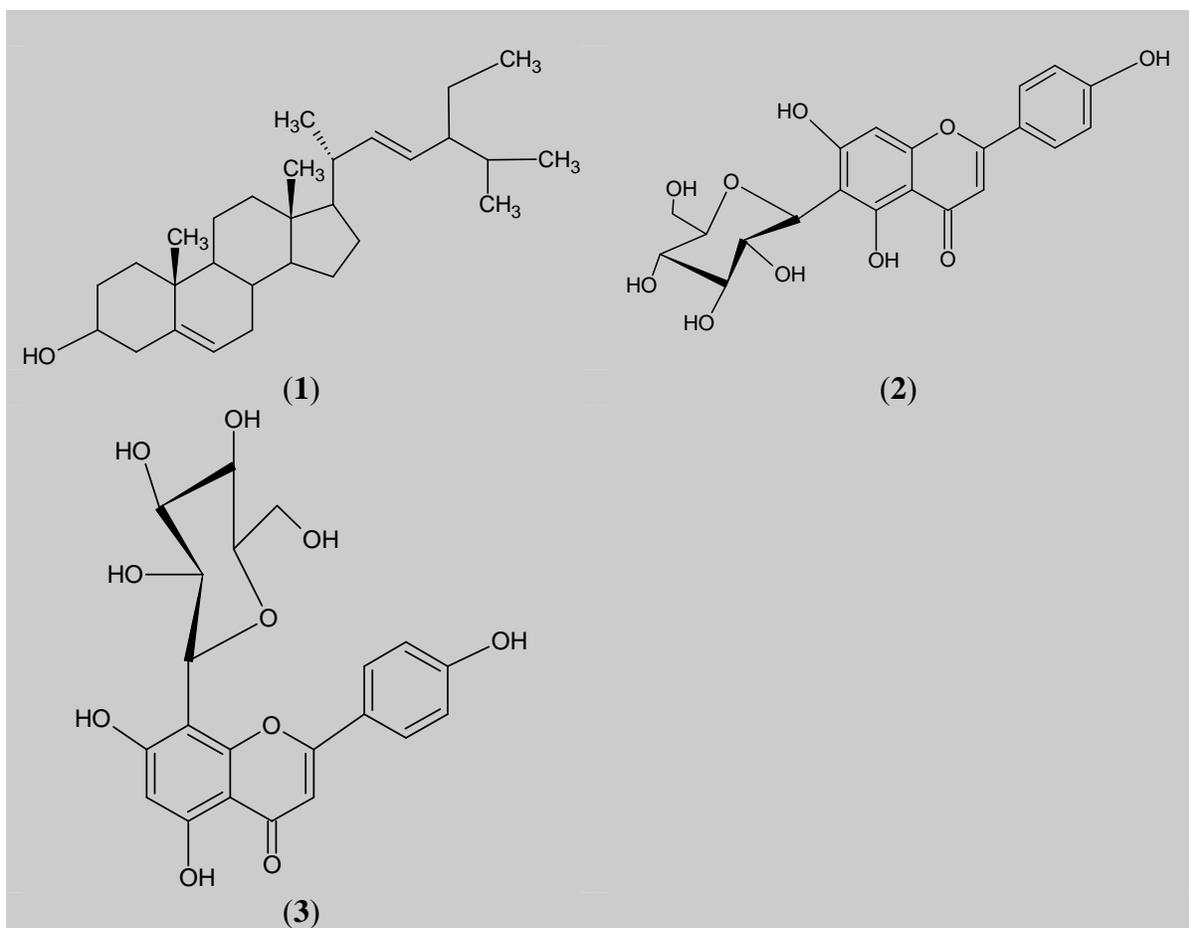


Figure 1. Chemical structures of isolated compounds

rease was calculated by considering the enzyme level difference between hepatotoxin treated and control rats as 100% level of reduction, the results were recorded in Tables 1 and 2. The comparative efficacy of the extract tested for its hepatoprotective activity, the relationship between dose and percentage reduction in each case was depicted in the form of a bar diagram as shown in figure 2.

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

Table:1. % Reduction of Liver weight and volume by treatment with methanol extract of *Alysicarpus monilifer* against CCl₄ induced hepatotoxicity in albino rats.

Treatment Group	Weight of liver(gm)	% Reduction in Wt.	Volume of liver(cc)	% Reduction in vol.
Positive control	4.2	--	5.0	--
CCl ₄ Treated	9.0	--	10	--
Silymarin (standard)	4.5	93.75	5.0	100
AMME 200mg/kg	6.5	52.08	6.6	68
AMME 400mg/kg	5.5	72.91	5.5	90
AMME 800 mg/kg	5.2	79.16	5.4	92

Table:2 Effects of methanodic extract(80%) of *Alysicarpus monilifer* whole plants against CCl₄ induced hepatotoxicity in albino rats in terms of serum biochemical parameters.

Treatment group	Serum biochemical parameters			
	SGOT (IU/L)	SGPT (IU/L)	ALP(IU/L)	TB(mg/dl)
Control (olive oil 1ml/kg p.o)	86.07±1.83	46.0±0.35	160.08±1.60	0.31±0.02
Toxic- CCl ₄ (1ml/kg s.c.)	550.48± 12.33	456.18± 1.38	375.66±2.46	1.86±0.01
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)*
AMME (200 mg/kg p.o)	257.33±1.25(63.12)*	237.9± 1.4 (53-21)*	265.06±1.06(51.30)*	0.67±0.02(75.31)*
AMME (400mg / kg p.o)	213.66±1.38(72.52)*	168.01±0.86(70.25)*	243.11±1.25(61.48)*	0.58±0.01(81.01)*
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 Methanolic extract(80%) of *Alysicarpus monilifer* whole plants against CCl₄ induced hepatotoxicity in albino rats

(0.31±0.06 to 1.86±0.14 mg/dl). The liver showed significant increase in its weight (Liv.Wt - 9 gms), and volume (Liv. Vol -10 cc) 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 CCl₄ 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 and 2).

Treatment with methanodic extract of *Alysicarpus monilifer* whole plant (200, 400 and 800 mg/kg p.o doses) on CCl₄ intoxicated rats revealed a significant dose dependant reduction (p<0.01) in the levels of SGOT, SGPT, ALP, TB, Liv.Wt. as well as Liv Vol. respectively (Table 1 and 2; Fig 2 and 3), compared to that of CCl₄ intoxicated group.

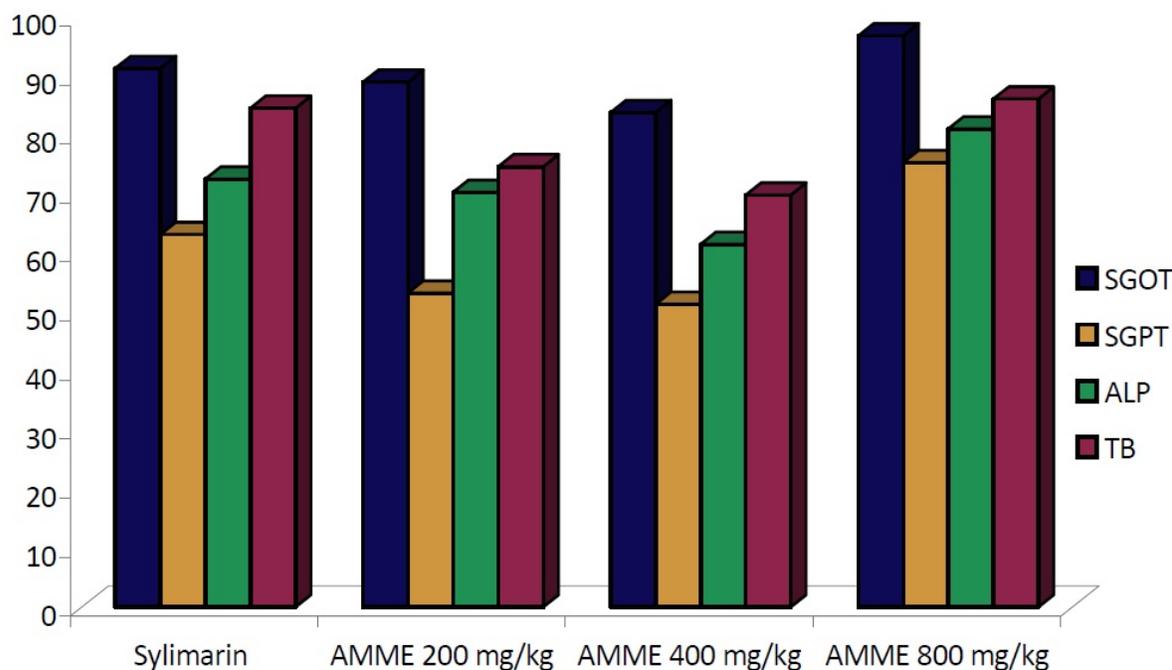


Figure 2. Hepatoprotective activity of methanodic extract (80%) of *Alysicarpus monilifer* whole plant against CCl₄ induced hepatotoxicity in albino rats showing Percentage reduction of various serum biochemical parameters.

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 (Figure 3A). The liver section of CCl₄ intoxicated group showed complete disarrangement of normal hepatic cells with intense centrilobular necrosis, vacuolization, neutrophile infiltration, fatty changes and sinusoidal hemorrhages and dilatation (Figure B). The liver sections of silymarin treated rats at 50mg/kg dose showed apparently normal liver lobule with no sign of necrosis in centrilobular area and portal vein but only a few inflammatory cells were observed in the centrilobular 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 (Figure 3C).

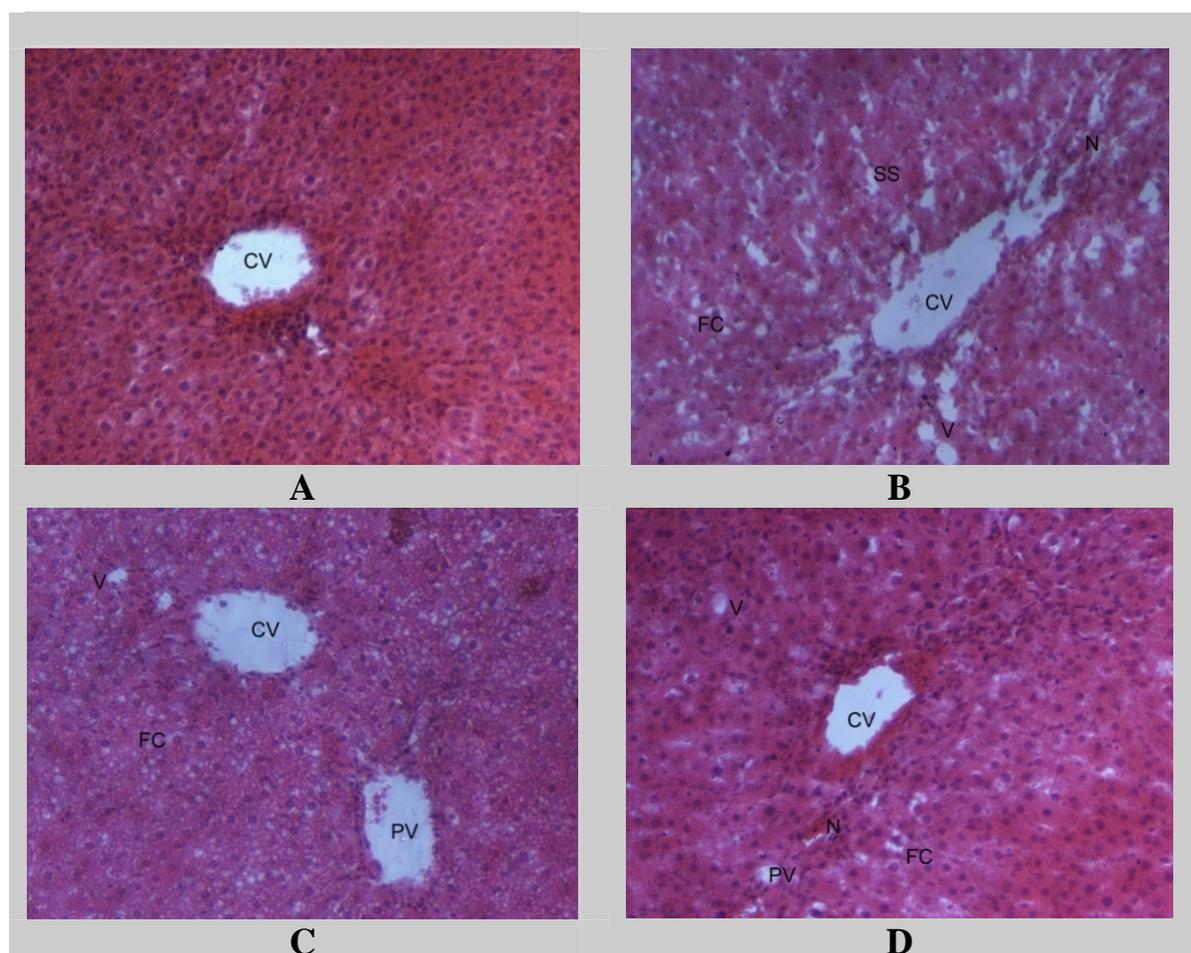


Figure 3. Photographs of liver sections stained with haematoxylin and eosin, taken using Nikon Trinocular microscope with image analyzer CV- central vein, PV – portal vein, N - necrosis, SS – sinusoidal spaces, FC – fatty changes (A) Normal control provided with olive oil showing normal liver architecture (B) Negative control- treated with CCl₄+ vehicle (1:1)1ml/kg b.w.,s.c.. showing complete disarrangement of normal hepatic cells with intense centrilobular necrosis, vacuolization, neutrophile infiltration, fatty changes and sinusoidal haemorrhages and dilatation (C) Positive control- liver tissue treated with Standard drug Silymarin (50mg/kg) and CCl₄ showing normal hepatic architecture with less vacuole formation and absence of necrosis (D) Liver tissue treated with methanol extract of *Alysicarpus monilifer* whole plant(AMME- 400mg/kg b.w.,p.o.) and CCl₄ (1ml/kg b.w., s.c.) showing absence of necrosis and less fatty accumulation preserving cellular architecture of liver indicating a marked protective activity

The Histopathological examination of rats administered with methanolic extract of *Alysicarpus monilifer* whole plant (200,400 and 800mg/kg p.o doses) intoxicated with CCl₄ showed fatty changes with sinusoidal dilatation and absence of necrosis and with higher dose 800 mg/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 (Figure 3D)

Discussion

The present study indicates the potential hepatoprotective activity of *Alysicarpus monilifer* whole plant through its bioactive constituents.. As there was no report on the hepatoprotective activity of this plant, the dose range of 200mg/kg to 800mg/kg of the methanolic extract dose fixed from the preliminary testing and the results demonstrated hepatoprotective activity.

Liver damage was assessed by biochemical studies (SGOT, SGPT, ALP and total bilirubin) and by histopathological examinations. CCl₄ produces damage that histologically resembles viral hepatitis (James and Pickering, 1976). Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures (Recknagel,1983). The hepatotoxicity of CCl₄ has been reported to be due to the formation of the highly reactive trichloro free radical, which attacks polyunsaturated fatty acids. It produces hepatotoxicity by altering liver microsomal membranes in experimental animals (Ashok et al., 2001). It has been suggested that the trichloromethyl radical (CCl₃) is the toxic intermediate responsible for maximum damage to liver (Recknagel et al., 1989; Koop, 1992). The free radicals can react with sulfhydryl groups, such as glutathione (GSH) and protein thiols. The covalent bonding of trichloromethyl free radicals to cell protein was considered the initial step in a chain of events, which eventually lead to membrane lipid peroxidation and finally cell necrosis (Brattin et al., 1985; Recknagel et al., 1989, 1991)

Although several isoforms of cytochrome P450 may metabolize CCl₄, attention has been focused largely on the cytochrome P450 2E1 (CYP2E1) isoform, which is ethanol-inducible (Koop, 1992; Raucy et al., 1993; Zangar et al., 2000). Alternation in the activity of CYP2E1 affect the susceptibility to hepatic injury from CCl₄ (Kim et al., 1997; Xiong et al., 1998 “a”, “ b”). The CCl₄ induced elevation of this enzymatic activity in the serum is in line with high level of serum bilirubin content. The methanolic extract induced suppression of the increased SALP activity with the concurrent depletion of raised bilirubin suggests the possibility of the extracts to have ability to stabilize biliary dysfunction in rat liver during hepatic injury by CCl₄. Thus, administration of methanolic extract of *Alysicarpus monilifer* whole plant revealed hepatoprotective activity against the toxic effect of CCl₄, which was also supported by histopathological studies. The preliminary phytochemical analysis of the extract has shown the presence of phytosterols, saponins, flavonoids and phenolic compounds, which have been known for their antioxidant and hepatoprotective activities (Di Carlo et al., 1999). Therefore, it can be concluded that the possible mechanism of hepatoprotective activity of *Alysicarpus monilifer* may be due to its antioxidant activity, which further may be due to the presence of flavonoids and phenolic compounds in particular flavanoid glycosides like isovitexin and vitexin in the extract.

The effects of CCl₄ are generally observed after 24h of its administration. Hence the withdrawal of the blood for biochemical parameters should be carried out only after 24h of CCl₄ intoxication. From table- 2 it is evident that the methanolic extract was able to reduce all the elevated biochemical parameters and thereby reducing the hepatotoxic intoxication as well as the levels of total proteins and albumin. The reduction is attributed to the damage which is generally localized in the endoplasmic reticulum. This results in the loss of P₄₅₀, its functional failure with a decrease in protein synthesis and accumulation of triglycerides. Intoxication with CCl₄ also resulted in inhibition of synthesis of the bile acids from cholesterol which is synthesized in vivo or derived from plasma lipids, leading to increase in cholesterol levels. Suppression of cholesterol levels suggests inhibition of the synthesis of bile acids from cholesterol is reversed by the extract. 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 CCl₄. Reduction of ALKP levels with concurrent depletion of rise in bilirubin level suggests the stability of the biliary function during injury with CCl₄. The rise 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.

Histological examination of the liver sections reveals that the normal liver architecture was disturbed by hepatotoxic intoxication. In the sections obtained from the rats treated with methanolic extract and intoxicated with hepatotoxin, the normal cellular architecture was retained compared to that of silymarin, thereby confirming the protective effect of the extract. The decrease in the necrosed area demonstrated by both of the extracts as well as decrease in the infiltration of the inflammatory cells in the liver lobules is indicative of therapeutic efficacy of the plant extracts.

Silymarin is a standard seed extract of *silybum marianum*, which contains flavonolignans. Silymarin at doses up to 100mg/kg has been used as a standard hepatoprotective agent by numerous investigators. In the present investigation 50mg/kg of silymarin showed significant difference compared to other extracts. Boigk et al., 1997 and Bhadauria et al., 2007 observed similar cases of effectiveness at 50 mg/kg of silymarin respectively.

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 CCl₄ caused hepatotoxicity. The histopathological observations also showed that in the plant extract treated liver sections against CCl₄ induced hepatotoxicity the absence of necrosis and well preserved cellular architecture. This is an indication that the cellular damage caused by hepatotoxin (CCl₄) was either prevented or repaired by the bioactive phytoconstituents of the plant, predominantly isovitexin and vitexin indicating their protective effect. This view can be further strengthened by the similar findings by other researchers as mentioned below.

Taking into account the high content of the polar vitexin and isovitexin in the whole leaf methanol extract of *Croton tonkinensis*, Euphorbiaceae, the correlation between the biological activities of the flavonoid glycosides and the medicinal properties of *C.tonkinensis* was established based on the results that vitexin and isovitexin displayed good antioxidant and antimicrobial activity (Phan Minh Giang (2004) The antioxidant activity of the aqueous ethanolic extract of down palm *Hyphaene thebaica* L.(*Palmae*) leaves and fruits showed inhi-

bition of reactive oxygen species attack on salicylic acid in a dose dependant manner. This is due to the substantial amount of their phenolic contents(Cook et al.,1998) specially the flavone glycosides which included vitexin and isovitexin(Omayma et al.,2009). Adamska and Lutomski (1971) isolated the C-Glycosyl flavones vitexin and vitaxin-7-glucoside from fenugreek seeds which have been used to treat stomach disorders.(Kim et al 1999)The hepatoprotective activity of the compounds vitexin and isovitexin from the aerial parts of *Beta vulgaris var. cicla* was assessed by measuring their effects on the release of glutamic pyruvic transaminase (GPT) from the primary cultures of rat hepatocytes injured by CCl₄. The compounds exhibited hepatoprotective activity almost similar to standard silymarin at the concentration of 100Mm (Inkyum Kum et al., 2004). In addition, the flvone C-glycoside,vitexin is known to possess an inhibitory activity on TNF- α induced cell death in primary cultured mouse hepatocytes (Banskota et al.,2000) .

Phytochemical analysis of *Alysicarpus monilifer* Linn. revealed C-glycosyl flavones such as vitexin and isovitexin. Therefore the bioassays with the methanolic extract of the whole plant recorded significant hepatoprotective activity. Therefore the scientific rationale of its traditional use against jaundice and other common ailments can be substantiated. Based on the above findings which are closely in agreement with our present study results, the possible mechanism of action of bioactive flavonoid glycosides vitexin and isovitexin isolated from *Alysicarpus monilifer* could be to block the active sites of reactive oxygen species to which they have greater affinity and cause cellular damage, there by offering protection to the targeted tissue acting as antioxidant.

The trichloromethyl free radicals released by CCl₄ were responsible for cell protein degradation, membrane lipid peroxidation, finally cell necrosis(Brattin et al., 1985; Recknagel et al., 1989, 1991), using Cytochrome P4502E1 (CYP2E1) isoform due to is susceptibility to hepatic injury. This mechanism of CCl₄ induced hepatotoxicity in rat liver might probably have been combated by isovitexin and vitexin and offered protection to the liver tissue from undesirable effects of trichloromethyl free radicals.

Bioflavonoids known for their antioxidant and anti inflammatory activities, are implicated to the maintenance of health. They also inhibit LDL (Low Density Lypoproteins) oxidation and impart cardioprotective effects(Kondo et al.,1996). These compounds were also known to reduce inflammation, trimorogenesis and cell damage caused by oxidation (Dempke et al., 2001).

Phytochemical analysis of *Alysicarpus monilifer* Linn. revealed C-glycosyl flavones such as vitexin and isovitexin. Therefore the bioassays with the methanolic extract of the whole plant recorded significant hepatoprotective activity. Therefore the scientific rationale of its traditional use against jaundice and other common ailments can be substantiated.

The experimental results demonstrated the potential effect of methanolic extract of *Alysicarpus monilifer* and its bioactive molecules vitexin and isovitexin in protecting liver function, reducing oxidative stress, and improving histopathological structures in the rat model of CCl₄-induced liver damage. 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

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

Acknowledgement

The author acknowledges University Grants Commission (UGC), New Delhi, India for allowing to carry out this work under Faculty Development Programme (FDP), Prof. P. Rajeswara Rao, Dept. of pharmacology, College of Pharmaceutical Science, Andhra University, Visakhapatnam, Andhra Pradesh, India for providing equipment and laboratory facilities for carrying out bioassays, Prof. B. Kishore, Dept. of Zoology, Andhra University for providing immense support in doing histopathological study and Prof. A.I. Gray, Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, Scotland, UK. for his untiring efforts in identification and structural elucidation of the isolated phytoconstituents.

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