African Journal of Pharmacy and Pharmacology Vol. 5(16), pp. 1920-1924, 29 October, 2011
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP11.537
ISSN 1996-0816 ©2011 Academic Journals

Full Length Research Paper

Hepatoprotective effect of methanolic extract of the leaves of *Kydia calycina* on carbon tetrachloride induced hepatotoxicity in albino rats

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Accepted 30 September, 2011

The hepatoprotective activity of methanolic extract of *Kydia calycina* (Malvaceae) (KCME) at doses of 250 and 500 mg/kg were evaluated by carbon tetrachloride (CCl_4) intoxication in rats. The toxic group which received 1 ml/kg (50% CCl_4 in olive oil) per oral, alone exhibited significant increase in serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphate (ALP) and total bilirubin (TB) levels. It also exhibited significant ($P < 0.001$) decrease in serum total protein (TP) and albumin (ALB) levels. The groups that received pretreatment of *K. calycina* leaves extract at a dose of 250 and 500 mg/kg per oral showed reduced levels of the ALT, AST, ALP and TB effects were compared with standard drug (Silymarin 50 mg/kg). The KCME 500 showed a better hepatoprotective activity ($P < 0.001$) than KCME 250 comparable to the reference standard drug (Silymarin). The histopathological studies indicated that the hepatic damage induced by CCl_4 were remarkably reduced by the standard silymarin, KCME 250 and KCME 500, and showed a reduced fatty changes, necrosis and broad infiltration of lymphocytes produced by CCl_4.

Key words: *Kydia calycina*, carbon tetrachloride, silymarin.

INTRODUCTION

Liver plays a very important role in the metabolism of foreign compounds entering the body. The exposure to the foreign compounds may be through consumption of alien/contaminated foods from exposure to chemical substances in the occupation environment or through synthetic drugs consumed for various pathological conditions; these compounds have many toxic effects on the human liver (Rajesh, 2004). The liver gets injured also by viruses, chemicals, alcohol and autoimmune diseases. Liver diseases remained one of the serious health problems, so medicinal plants and herbs have been use for treating such problems as in the Indian traditional systems of medicine, especially Ayurveda. Recently, a scientific basis was proved to justify the various medicinal uses of herbs (Pohocha and Grampurohit, 2001). India is well-known for a plethora of medicinal plants. The medicinal use of many plants (as hepatoprotectants) like *Andrographis paniculata, Azardiracta indica, Cassia fustula, Elephantopus scaber, Hibiscus rosasinensis, Phyllanthus debilis, Picrorrhiza kurroa* and *Glycyrrhiza glabra* linn has been reported in the literature (Rajesh, 2004; Rajesh and Latha, 2001; Anandan et al., 1997).

Kydia calycina Roxb (Malvaceae) synonyms *Kydia fraterna* Roxb, *Kydia roxburghiana* Wight are distributed in tropical Himalayas from the Indus eastwards to Myanmar (Burma) and in peninsular India from Northern Maharastra and Madhya Pradesh southwards, chiefly in mixed, moist and deciduous forests. The leaves of *K. calycina* were 7.5 to 15 cm long and wide, usually 3 to 7 lobed, apex angled or rounded, base cordate, palmately 7-nerved and hoary-tomentose beneath; petioles 2.5 to 5 cm (Parrot, 2001). Among the Santalis, a paste of the grounded leaves is appled to relieve body pains, arthritis and lumbago; a poultice of the leaves is traditionally used to treat skin diseases (Parrot, 2001; Ramarao and Henry, 1996). In the present investigation, we have evaluated the hepatoprotective effect of methanolic extract of the

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Table 1. Effect of methanolic extract of the leaves of *K. calycina* (pre-treatment) on different biochemical parameters in CCl_4 induced liver damage in rats.

Group	ALT (IU/L)	AST (IU/L)	ALP (KA/dL)	TB (mg/dL)	TP (gm %)	ALB (gm %)
Control	14.10 ± 0.06	11.10 ± 1.20	34.20 ± 3.36	3.15 ± 0.08	10.20 ± 0.12	7.50 ± 0.13
Toxic	155.01 ± 2.50	144.5 ± 2.03	130.18 ± 5.00	9.09 ± 0.40	5.10 ± 0.10	1.54 ± 0.20
Standard (Silymarin-50)	40.50 ± 3.20***	24.40 ± 1.06***	42.50 ± 2.30***	4.01 ± 0.08***	9.10 ± 0.11***	6.94 ± 0.10***
KCME 250	81.11 ± 7.03**	113.16 ± 12.10	125.5 ± 2.10	8.90 ± 0.01	6.40 ± 0.22**	4.51 ± 0.16**
KCME 500	52.21 ± 3.20***	48.18 ± 1.21**	64.30 ± 6.03***	5.62 ± 0.06***	7.42 ± 0.50***	5.15 ± 0.13***

n = 6, values expressed as mean ± SD. Significant *P < 0.05, **P < 0.01, *** P < 0.001 compared with standard and toxic group.

leaves of *K. calycina* on carbon tetrachloride induced hepatotoxicity in albino rats.

MATERIALS AND METHODS

Collection of plant material

The leaves of *K. calycina* were collected in the month of August from Thirumala hills, Thirupathi, Andhra Pradesh, India, and authenticated by Dr. Raju S. Vastavaya, Professor, Department of Botany, Kakatiya University, Warangal, Andhra Pradesh, India. Voucher specimens are being maintained in the herbarium of University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Andhra Pradesh, India.

Preparation of extracts

Leaves of *K. calycina* (3 kg) were cleaned from any adherent foreign material and air-dried, macerated with methanol in a round-bottom flask for 7 days separately. The content of the flask was stirred intermittently to ensure the efficiency of the extraction. After a week, it was filtered and concentrated under reduced pressure (Rotavapour, Buchi) to yield corresponding extract; the obtained extract was kept in a desiccator to remove moisture and stored properly until used.

Animals

Female albino Wistar rats weighing 100 to 150 g were purchased from Mahaveera Agencies, (Regn. No. 146/1999/CPCSEA), Hyderabad and maintained in the animal house of University College of Pharmaceutical Sciences, Warangal. Animals were provided with standard rodent pellet diet and the food was reserved 18 to 24 h before the experiment, while water was allowed *ad libutum*. They were maintained at (27 ± 2°C) 12 h light and dark cycle throughout the period of acclimatization and experimentation. All the animal experimental protocols were duly approved by the Institutional Animal Ethics Committee (Reg No.169/1999/UCPSc, KU).

Phytochemical analysis

Phytochemical tests were carried out to identify the phytoconstituents, such as carbohydrates, glycosides, alkaloids, steroids, flavonoids and phenols (Paech and Tracy, 1979).

Experimental design

Acute toxicity study of methanolic extract

Wistar albino mice of 25 to 30 g were divided into ten groups of six animals each. Acute toxicity study was carried out according to the method described (Palanichamy and Nagarajan, 1990). The methanolic extract of *K. calycina* (KCME) were suspended in 5% gum acacia in doses of 100, 200, 400, 600, 800, 1000, 1200, 1400, 1800 and 2000 mg/kg and were given orally to albino mice. The animals were observed continuously for any change in autonomic or behavioral responses for first few hours and later at 24 h intervals for a period of 48 h. At the end of this period, the mortality rates in all groups were noted.

Hepatoprotective activity

In the present study, the animals were pretreated with test extract before inducing liver damage with CCl_4. Seven days after acclimatization, the rats were divided into five groups (I to V) each group consisting of six animals. All animals were kept on same diet for 7 days.

Group I: Served as a normal group and received 1 ml/kg of 2% w/v gum acacia in water for seven days.
Group II: Treated with vehicle (1 ml/kg of 2% w/v gum acacia in water) daily for seven days followed by CCl_4 on the seventh day.
Group III (standard-Silymarin): Animals received 50 mg/kg of Silymarin for seven days orally followed by CCl_4.
Group IV-V: These test groups were treated in the similar way using methanolic extract of *K. calycina* (KCME) of 250 and 500 mg/kg, respectively followed by CCl_4 administered orally on the seventh day.

RESULTS

The preliminary phytochemical investigation of crude methanolic extract of the leaves of *K. calycina* revealed the presence of phytoconstituents, that is, carbohydrates, glycosides, flavonoids and phenols. In acute toxicity study, no mortality and symptoms of toxicity were observed up to a dose level of 2000 mg/kg body weight. The activities of various biochemical parameters in control, toxic, standard and treated groups were represented in Table 1 and Figure 1. The activities of serum AST, ALT, ALP and TB levels were significantly ($P < 0.001$) increased with a significant decrease in total

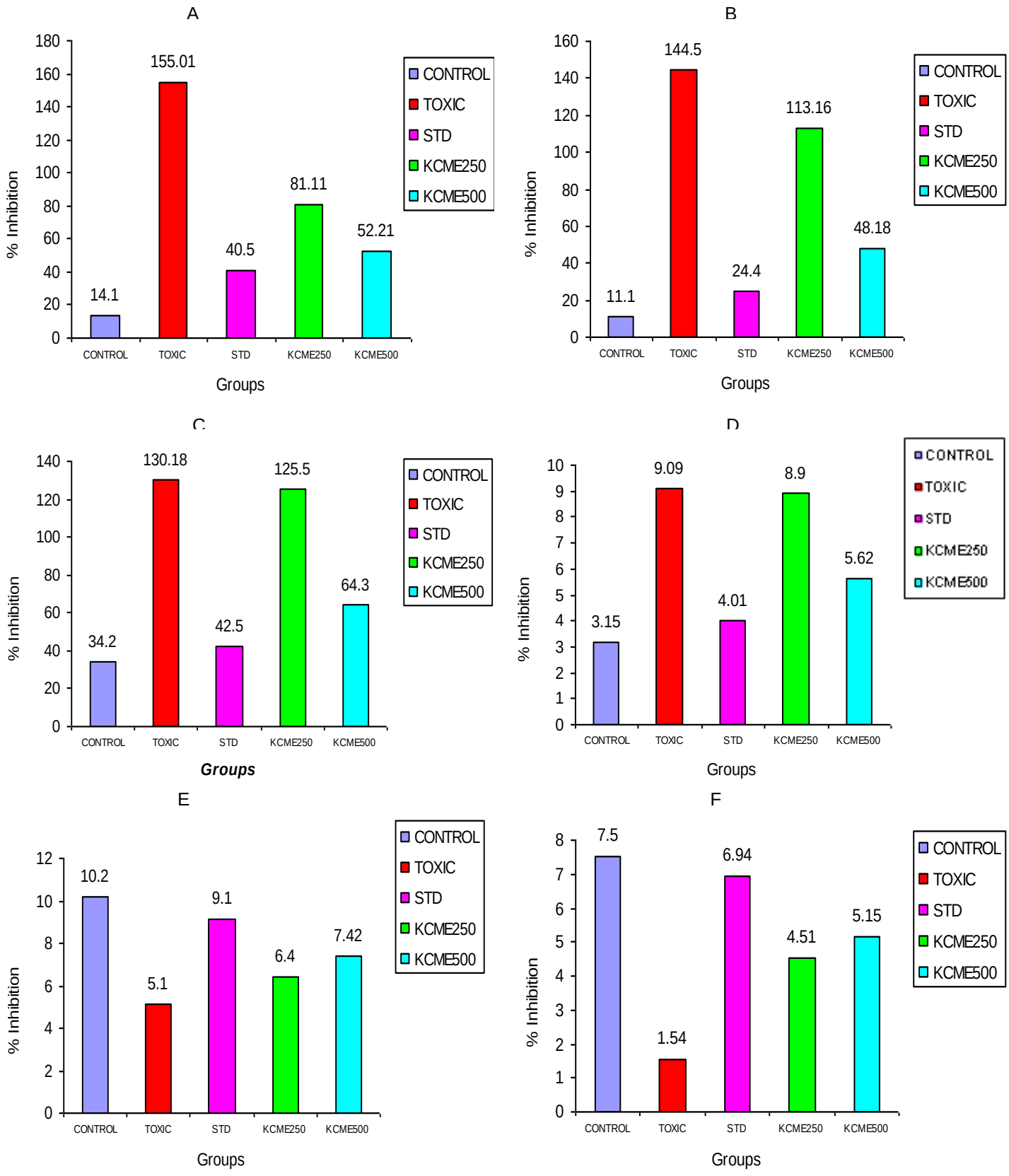

Figure 1. Effect of KCME (pre-treatment) on different biochemical parameters in CCl_4 induced liver damage in rats. (A) Effect of KCME on serum ALT levels (B) Effect of KCME on serum AST levels, (C) Effect of KCM on serum ALP levels, (D) Effect of KCME on serum TB levels (E) Effect of KCME on serum TP levels (F) Effect of KCME on serum ALB levels.

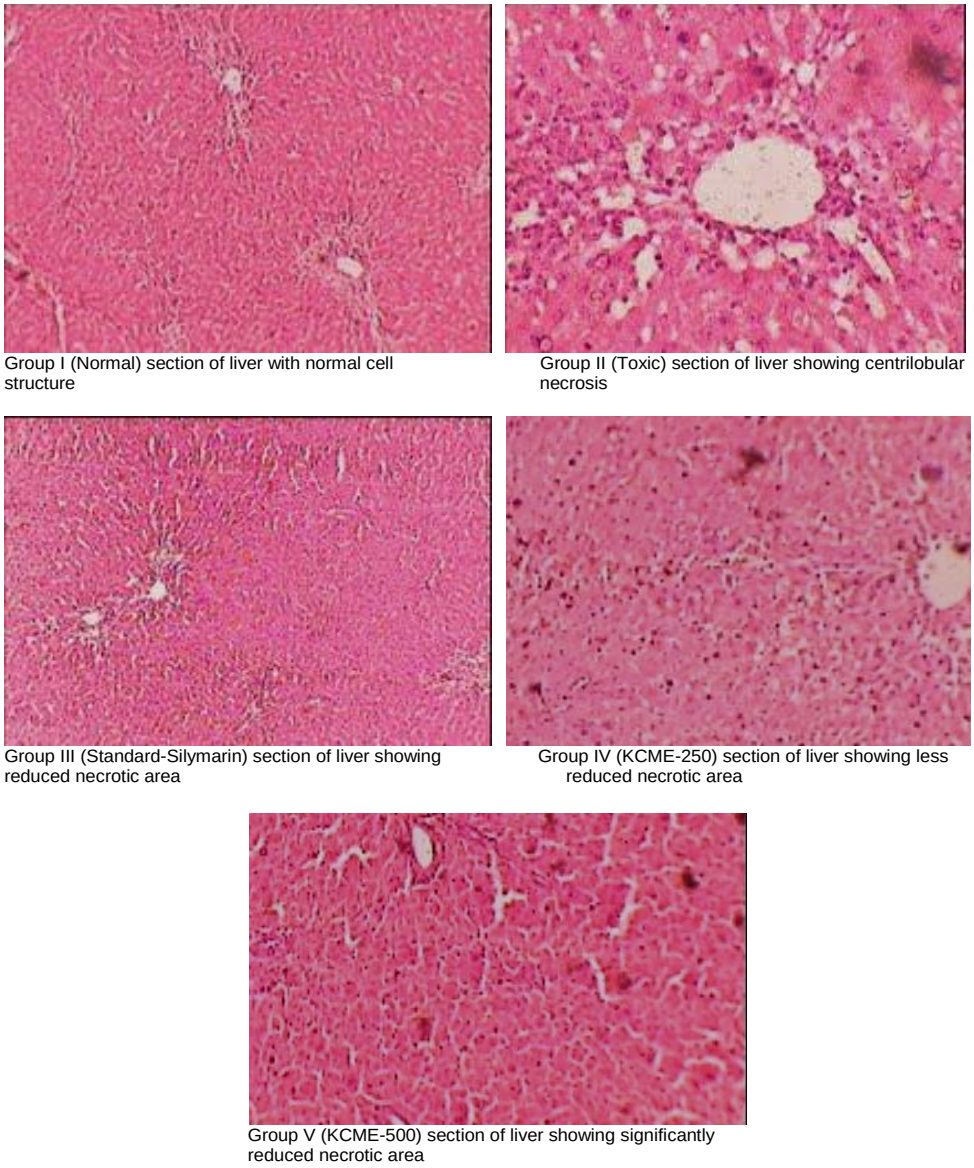

Figure 2. Effect of pre-treatment of KCME on histopathalogical changes in CCl_4 induced liver damage in rats.

protein and albumin levels in toxic group as compared to the control and standard group. The levels of the aforementioned enzymes were significantly reversed on treatment with KCME in a dose-dependent manner. The degree of protection was observed maximally with the test group KCME 500 mg/kg. The KCME 500 showed a better hepatoprotective activity ($P < 0.001$) than KCME 250 (Table 1 and Figure 1).

The histopathological studies of the liver showed fatty changes and necrosis with loss of hepatocytes in toxic group in comparison with normal control group. The KCME treated groups showed regeneration of hepatocytes, normalization of fatty changes and necrosis of the liver. The silymarin treated group showed almost normalization of fatty changes and necrosis. The degree of the effect was as follows: standard > KCME 500 > KCME 250 (Figure 2).

Statical analysis

The data were expressed as mean ± SEM and statistically assessed by one-way analysis of variance (ANOVA) and subjected to Dunnett's test.

DISCUSSION

Literature review revealed that various chemical and biological investigations were carried out with this plant, so that the plant *K. calycina* (Malvaceae) selected for the

present investigation are medicinally important and traditionally used to treat jaundice, skin diseases, ulcers, body pains, arthritis and lumbago (Parrott, 2001; Kirtikar and Basu, 1988). Chemical compounds, such as hibiscone C, hibiscoquinone B and 8-formyl-2, 7-dihydroxy-5-isopropyl-1-methoxy-3-methyl naphthalene were reported to be isolated from stem heartwood of *K. calycina* (Joshi et al., 1983). The seed oil of *K. calycina* was reported to contain cyclopropenoid fatty acid apart from normal fatty acids (Daulatabad et al., 2006). The stem bark and leaf paste are reported to be applied for skin diseases and ulcers by Chenchus (Ramarao and Henry, 1996).

Carbon tetrachloride used as hepatotoxicants is biotransformed by the cytochrome P-450 in the liver endoplasmic reticulum to the highly reactive trichloromethyl free radical. This free radical in turn reacts with oxygen to form a trichloromethyl peroxy radical, which may attack lipids on the membrane of endoplasmic reticulum more readily than the trichloromethyl free radical. The trichloromethyl peroxy radical leads to elicit lipid peroxidation, the disruption of Ca+2 homeostasis, elevation of hepatic enzymes and finally results in cell death (Clawson, 1989).

Hepatic cells participate in a variety of metabolic activities and contain a host of enzymes. In tissues, AST and ALT are found in higher concentrations in cytoplasm and AST in particular also exists in mitochondria (Wells, 1988). In liver injury, the transport function of the hepatocytes is disturbed, resulting in the leakage of plasma membrane (Zimmerman and Seef, 1970); thereby causing an increased enzyme level in serum. If injury involves organelles, such as mitochondria, soluble enzymes like AST that are normally located there, will also be released, similarly. The elevated activities of AST and ALT in serum are indicative of cellular leakage and loss of the functional integrity of cell membranes in liver (Drotman and Lawhorn, 1978). ALP is excreted normally via bile by the liver. In liver injury due to hepatotoxin, there is a defective excretion of bile by the liver which is reflected in their increased levels in serum (Rao, 1973). Hyperbilirubinemia is a very sensitive test to substantiate the functional integrity of the liver and severity of necrosis which increases the binding, conjugation and excretory capacity of hepatocytes that is proportional to the erythrocyte degeneration rate (Sing et al., 1998).

In hepatotoxicity, a depression in total protein is observed due to the defect in protein biosynthesis (Clawson, 1989; Dubey et al., 1994) similar to our results. This is due to the disruption and disassociation of polyribosomes from endoplasmic reticulum following carbon tetrachloride administration (Clawson, 1989). Administration of KCME at a dose of 250 and 500 mg/kg body weight prevented these changes. This may be due to the promotion of the assembly of ribosomes on endoplasmic reticulum to facilitate uninterrupted protein biosynthesis.

Conclusion

On the basis of results obtained from the present study, the methanolic extract of the leaves of *K. calycina* exhibited hepatoprotective effect against carbon tetrachloride induced liver damage in a dose-dependent manner by normalizing the elevated levels of the hepatic enzymes like AST, ALT, ALP, TB and increased the serum TP and ALB levels. Histopathalogical observations also support the hepatoprotective potential of KCME. Further investigations are going in our laboratory.

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