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HEPATOPROTECTIVE EFFECT OF *SOLENA AMPLEXICAULIS* (TUBER*)* ON ACUTE CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY

Parameshwar H, Narsimha Reddy Y, Ravi Kumar B and Krishna Mohan G*
Department of Pharmacognosy and Centre for Ethnopharmacological Studies,
University College of Pharmaceutical Sciences, Kakatiya University, Warangal 506009, India.
E. Mail: *drgkrishnamohan@yahoo.co.in*

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ABSTRACT

The hepatoprotective activity of methanolic extract of *Solena amplexicaulis* (SAME) (Lam.) Gandhi (Cucurbitaceae) at doses of 250 mg/kg and 500 mg/kg were evaluated by carbon tetrachloride (CCl4) intoxication in rats. The toxic group which received CCl_4 (0.3 ml/kg of CCl_4 dissolved in 1:1 ratio in olive oil by subcutaneous (s.c) alone exhibited significant increase in serum alanine amino transferase(ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and total bilirubin (TB) levels. It also caused significant ($P<0.001$) decrease in protein levels. The groups received pretreatment of SAME at a dose of 500 mg/kg b.w.p.o. had controlled the AST, ALT, ALP and total bilirubin levels and the effects were comparable with standard drug (silymarin 100 mg/kg b.w.p.o). The total protein (TP) and albumin (ALB) levels were significantly increased in the animals received pretreatment of the extract at the higher dose level. The animals received pretreatment of the extract shown decreased necrotic zones and hepatocellular degeneration when compared to the liver exposed to CCl_4 intoxication alone. Thus the histopathalogical studies also supported the protective effect of the extract.

Keywords: CCl_4 induced liver injury, *Solena amplexicaulis*, hepatoprotective activity, biochemical parameters and hepatocellular degeneration

INTRODUCTION

Liver is the most important glandular organ, which plays a pivotal role in regulating, synthesizing, and restoring the various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles. Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages**.** In general, liver is the organ, which has the capability to regenerate itself. Inspite of tremendous advances in modern medicine, there are no effective drugs available that stimulate liver function, offer protection to the liver from damage or help to regenerate hepatic cells. In absence of reliable liver protecting drugs in modern medicine, there are a number of medicinal preparation in Ayurveda recommended for the treatment of liver disorders and their usage is in vogue since centuries and are quite often claimed to offer significant relief [1]. The plant *Solena amplexicaulis* (Lam.) Gandhi (syn: melotheria heterophylla) belonging to Cucurbitaceae family are widely distributed Indian, Srilanka, China, Taiwan. The tubers, leaves and seeds of the plant are extensively used in traditional system for various ailments like hepatosplenomegaly, spermatorrhoea, thermogenic, appetizer, cardiotonic, diuretics, haemorrhoids and invigorating [2, 3].

The literature survey revealed that there are no scientific studies carried out regarding hepatoprotective activity of the tubers of *S. amplexicaulis*. Hence the present study is focused to evaluate the hepatoprotective potentials of the tubers against carbon tetrachloride induced liver injury

in albino rats and the analyzed parameters included ALT, AST, ALP, TPL and ALB activity and histopathology of liver damage [4].

2. MATERIALS AND METHODS

2.1. Collection of Plant Material

Fresh tubers of *S. amplexicaulis* were collected from Indervelly hills of Adilabad District, Andhra Pradesh (India). The plant was authenticated by Prof. Raju S. Vastavaya Department of Botany, Kakatiya University, Warangal, Andhra Pradesh (India) and a specimen voucher (C.No.1028/Param and V.S. Raju) was deposited for future reference.

2.2. Preparation of plant extract

The air-dried tubers of the *S. amplexicaulis* were made into a coarse powder and extracted with methanol by maceration. The crude extract was evaporated by using Rotavapour (BUCHI, Germany) under reduced pressure.

2.3. Phytochemical Screening

The extract was subjected to preliminary phytochemical screening to identify the presence of various phytoconstituents i.e. Alkaloids, Carbohydrates,Glycosides and Steriods present in the extract [5].

Precoated TLC plate of Silica gel 60F254 (MERCK, India) of 0.2mm thickness was used. TLC pattern of SAME was developed using Toluene: Ethyl acetate: Diethylenamine(70:20:10), Chloroform: Methanol(90:10), Chloroform:Water(90:10) and Ethylacetate: Methanol: Water (81:11:8) as solvent system and visualized using Vanillin-Sulphuric acid reagent. Then the plates were scanned in CAMAG TLC scanner (UV-Spctrophotometer) UV-Chamber and the spot were recorded and the Rf values were determined.

2.4. Animals

Female wistar albino rats (100-150 g) procured from M/S Mahaveera Enterprises, Hyderabad (India) and were used for the studies. The animals were housed in large polypropylene cages in a temperature controlled room ($37^0C \pm 2^0C$) and provided with standardized pellet feed and clean drinking water *ad libitum*. The study protocol was duly approved by the Institutional Animal Ethical Committee.

2.4. Hepatoprotective Studies [6]

The animals were divided into 5 groups of six animals in each group. Group-I(Normal) received 2% gum acacia (1ml/kg per orally) daily for 7days.Group-II(Toxic) received 25% CCl_4 in olive oil (1ml/kg.p.o) daily for 7 days. Group-III (standard) received (100 mg/kg.p.o) daily for 7 days and on the 7^{th} day 25% CCl_4 in olive oil was administered 30minutes after the administration of the extract/ standard drug. Group-IV and Group-V were treated with methanolic extract of *Solena amplexicaulis* (SAME) at 250 and 500mg /kg.p.o respectively for 7 days on the 7^{th} day received 25% CCl_4 in olive oil 30min after administration of the extract or silymarin.

2.5. Biochemical estimation

All the animals were anaesthetized with thiopentone sodium (60mg/kg.i.p) and sacrificed on 7^{th} day, 36 h after administration of CCl_4 and blood was collected from the common carotid artery by carefully opening the neck region of the rat. The blood samples were allowed to coagulate at room temperature and the serum was separated by centrifugation. The serum ALT, AST, ALP, TPL and ALB were estimated by their specific methods.

2.6. Histopathological examination

The liver tissues were carefully dissected out and washed with 0.9% normal± saline solution and fixed in formalin (10% Formaldehyde), dehydrated in gradual ethanol (50-100%), cleared in xylene and embedded in paraffin, sections (4-5mm thick) were prepared and stained with hemotoxylin

and Eosin dye for photomicorscopic observation including cell-necrosis, fatty changes, hyaline degeneration, ballooning degeneration, infiltration of Kupffer cells and lymphocytes.

2.7. Statical analysis

The data were expressed as mean ± S.E.M. and statistically assessed by one-way analysis of variance (ANOVA) and subjected to Student's t-test

3. RESULTS

The preliminary phytochemical analysis of the crude extract of SAME indicated the presence of alkaloids, glycosides, sterols and terpenoids**.** The TLC studies carried out also exhibited the Rf values, which coincides with the standards. The results of the hepatoprotective studies are given in Table-1. The administration of CCl_4 induced acute liver damage, which was well indicated by increased ALT, AST, ALP and TBL when compared with the control group. The group received the toxicant alone also caused a decrease in the total protein and albumin levels. The pre-treatment of SAME at a dose of 500 mg/kg exhibited no increase in the serum levels of ALT ($P<0.001$), AST ($P<0.01$), ALP ($P<0.001$) and TBL. The TP ($P<0.001$) and ALB ($P<0.01$) levels were also prevented form a sharp decrease and were statistically significant when compared with the toxic group. The effect exhibited by SAME 500 group was comparable with the standard group treated with silymarin (100mg/kg b. w) The pre-treatment of SAME at dose of 250 mg/kg although controlled the rise of ALP ($P<0.01$) and decrease in the ALB ($P<0.05$), it was not effective in controlling the rise of other hepatospecific enzymes as well as the TB and TP levels. The increase in dose levels of SAME had exhibited an increase in efficacy which was reflected in the values of biochemical parameters.

Table 1: Effect of the SAME on the Concentrations of Serum Total Bilirubin, Hepatospecific Enzymes, Total Proteins and Albumin.

Groups	ALT (IU/L)	AST (IU/L)	ALP (KA units/dl)	TBL (mg%)	TPL (g%)	ALB (g %)
NORMAL	18.22±0.09	10.38±3.25	36.34±4.37	0.17±0.04	7.28±0.16	3.89±0.14
TOXIC (CCl_4)	162.02±5.87	151.5±45.3	126.78±6.86	0.98±0.53	5.59±0.25	1.47±0.25
STANDARD (Silymarin)	32.52±8.24**	26.69±11.6**	38.60±2.16**	0.33±0.03***	7.04±0.11***	3.84±0.12***
SAME 250	110.55±12.87	133.62±17.92	118.17±11.01**	0.79±0.08	5.73±0.24	1.81±0.16*
SAME 500	93.89±9.58**	111.66±17.1**	69.79±9.08**	0.62±0.07*	6.63±0.69***	1.96±0.12**

n = 6, Data expressed as Mean ± S.D, **P* value < 0.05, ***P* value < 0.01,

**** P* value < 0.001 compared with toxic group.

TBL =Total Bilirubin, ALT= Alanine Transaminase, AST = Aspartate transaminase, ALP= Alkaline Phosphatase, TPL= Total Protein, ALB= Albumin serum levels.

Figure 1: Group I (Normal control) Section of liver with normal cell structure

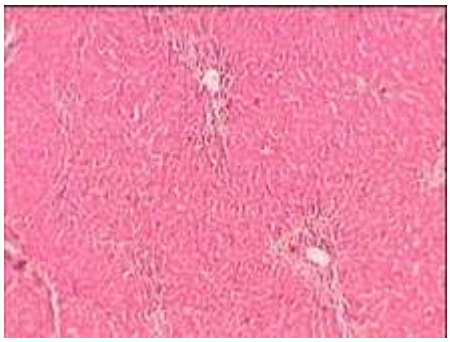


Figure 2: Group II (Toxic-CCL4) Section of liver showing centriolobular necrosis

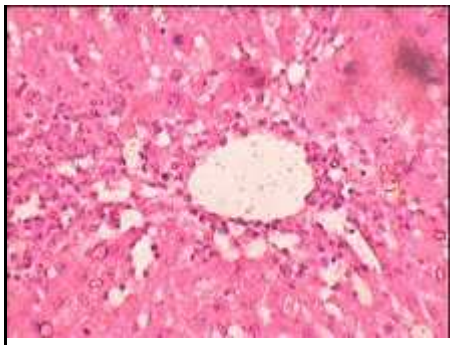


Figure 3: Group III (Standard-Silymarin) Section of liver showing reduced necrotic area

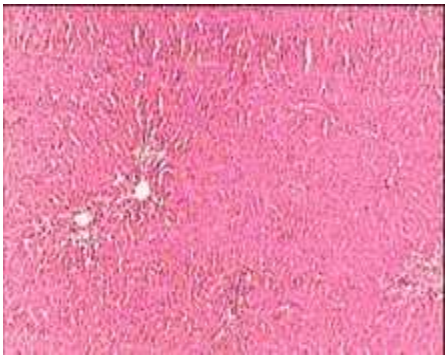


Figure 4: Group IV (SAME-250) Section of liver showing lesser area of necrosis

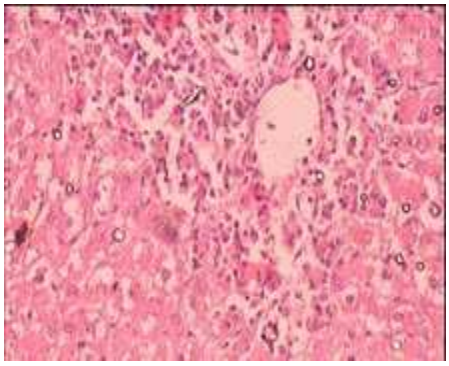
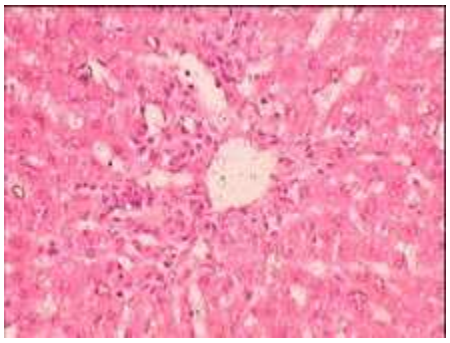


Figure 5: Group V (SAME-500) Section of liver showing significantly reduced necrotic area



4. DISCUSSION

The hepatotoxic agent CCl_4 induces selective toxicity to the liver cells due to metabolic activation and this maintains them with seminormal metabolic functions. CCl_4 is one of the most hepatotoxin experimental studies of liver diseases [7]. The hepatotoxic effects of CCl_4 are largely due to its active metabolites, trichloromethyl radical.[8]. Due to the damage caused to hepatic cells, the leakage of plasma causing an increased levels of hepatospecific enzymes in serum. The elevated serum enzyme levels like AST and ALT are indicative of cellular leakage and functional integrity of cell

membrane in liver [9]. The hepatprotective index of a drug can be evaluated by its capability to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been induced by a hepatotoxin. The measurement of serum AST, ALT and ALP levels serves as a means for the indirect assessment of condition of liver. The pretreatment of the animals with SAME (500 mg/kg per orally) with respect to intoxication with CCl_4 controlled the AST, ALT and ALP levels when compared with the toxic group.

A high concentration of bilirubin in serum is an indication for increased erythrocyte degeneration rate. It also reflects the necrotic conditions of hepatocytes [10]. The oral administration of SAME at 500mg/kg p.o reduced the serum TB levels.

The TP levels including albumin levels will be depressed in hepatotoxic conditions due to defective protein biosynthesis in liver [11]. The CCl_4 intoxication causes disruption and disassociation of polyribosomes on endoplasmic reticulum and thereby reducing the biosynthesis of protein. The pre-treatment of SAME might have reduced the polyribosomal damage and this mechanism might have aided the protective effect

The histopathological studies are direct means for assessing the protective effect of the drug from liver injuries. The groups received CCl_4 alone, the damage of cells around the central vein were well evident. Whereas, the intensity of damage was found lesser in the studies involved pretreatment of SAME. The results of the histopathological studies supported and well correlated with data obtained from evaluation of the biochemical parameters.

5. CONCLUSION

The methanolic extract of *Solena amplexicaulis* could effectively controlled the AST, ALT, ALP and TB levels and increased the protein levels in the protective studies. The histopathological studies also substantiate the activity of the drug. Therefore the study scientifically supports the

traditional use of this drug for the treatment of liver disorders. Further studies can be carried out on this plant by isolating and characterizing pure compounds, which can yield potent phytotherapeutic agent.

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***Corresponding author**

Prof. G.Krishna mohan*,
Department of Pharmacognosy and Ethnopharmacology,
University college of Pharmaceutical Sciences,
Kakatiya University, Warangal
Andhra Pradesh-506 009.
E. Mail: drgkrishnamohan@yahoo.co.in