

In-vivo Hepatoprotective Activity of Methanolic Extracts of *Sphaeranthus amaranthoides* and *Oldenlandia umbellata*

Somnath De^{1*}, Ramalingam Suresh², Akula Murali Sri Sudhakar Babu³, Siddabathuni Aneela¹

ABSTRACT

Objective: The present study was carried out to evaluate the *in vitro* hepatoprotective activity of unexploited plants, *Sphaeranthus amaranthoides* and *Oldenlandia umbellata* on CCl₄ induced liver injury, which are indigenous to South India. **Methods:** in the present study the methanolic extracts from *Sphaeranthus amaranthoides* and *Oldenlandia umbellata* were studied against the carbon tetrachloride hepatotoxicity. **Results:** significant hepatoprotective effect was obtained against carbon tetrachloride induced liver damage as judged from serum marker enzyme activities (SGOT, SGPT, ALT, and TB) and a normal architecture of liver compare to toxic control. **Conclusion:** the result revealed that methanolic extracts of *Sphaeranthus amaranthoides* and *Oldenlandia umbellata* could be useful in preventing CCl₄ induced liver injury. **Key words:** Hepatoprotective activity, *Sphaeranthus amaranthoides*, *Oldenlandia umbellata*, CCl₄, SGOT, SGPT, ALP, TB.

INTRODUCTION

Liver is the largest and most complex internal organ in the body. It plays an important role in maintenance of internal environment with the help of its multifarious and several functions. It is connected to intermediary metabolism of proteins, fats, and carbohydrates as well as in the synthesis of number plasma proteins such as albumin, fibrinogen and in the production of various enzymes, formation and excretion of bile.

Drug-induced liver injury is a major health problem that remonstrance not only health care profession but also pharmaceutical industry and drug regulatory agencies. According to the United States Acute Liver Failure study group, drug-induced liver injury accounts for more than 50% of acute liver failure, including hepatotoxicity caused by overdose of acetaminophen (39%) and idiosyncratic liver injury triggered by other drugs (13%).¹ Liver diseases have become one of the major causes formorbidity and mortality in man and animals all over globe and hepatotoxicity due to drug appears to be the most common contributing factors.² Among the many diseases that can affect the liver that is "Viral Hepatitis" (inflammation of liver caused by viral infection). Hepatitis can be caused by the drugs, viruses, bacteria, mushrooms, parasite like moebas or giardiasis. In India about 40 poly herbal commercial formulations reputed to have hepatoprotective action are being used. It has been reported that 160 phyto constituents from 101 plants have hepatoprotective activity.³ Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenoids, carotenoids, glycosides, and xanthines. Plant extracts of many crude drugs are also used for the treatment of liver disorders. Extracts of different plants of about 25 plants have been reported to cure liver disorders.⁴

S. amaranthoides Burm. Is a small herb decumbent herb, with stem rooting and pubescent with exploited hair leaves palmately 3- foliolate. Features of the herb: low annual with spreading branches, stem e erect, glabrous, sometimes as thick as the little finger, but short, branches e not winged and 8-12 inches, leaves of 2-4 inches, linear, oblong narrowed at the base. This plant is well known for its medicinal value for the treatment of eczema, blood disorder, stomach warms, and filarial fever and as a remover of kapha, vata and piles. It is also known to cure the skin diseases.⁵ *S. amaranthoides* belongs to Plant kingdom, Dicotyledon class, Gamopetale sub class, Inferae series, Asterales order, and *Astraceae* (*compositae*) family. It is weed of paddy field of southern India. The genus *O. umbellata* (family *Rubiaceae*) consists of different species, many of which are used in traditional medicine.⁶ *O. umbellata* is commonly known as Indian madder, known to yield a color-fast red dye from its roots and has been used in diverse applications since ancient times. The root bark, preferably of a 2 year old plant, when used with a mordant will confer red color to calico, wool, and silk fabrics.⁷

There is growing interest in herbal medicines because of their effectiveness, minimal side effects in clinical experience and relatively low cost. Herbal drugs or their biological active compounds are unknown.

Therefore, studies with plant extracts are useful to know their efficacy and mechanism of action and safety. Natural remedies from medicinal plant are considered to be effective and alternative treatment for hepatotoxicity.

MATERIALS AND METHODS

Plant Materials and Extraction:

The plants *S. amaranthoides* (entire plant) and *O. umbellata* (entire plant) were col-

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Somnath De^{1*}, Ramalingam Suresh², Akula Murali Sri Sudhakar Babu³, Siddabathuni Aneela¹

¹Samuel George Institute of Pharmaceutical Sciences, Markapur- 523316, Andhra Pradesh, India

²Annammalai University, Department of Pharmacy, Annammalai Nagar-600 802, Tamil Nadu, India

³A.M. Reddy Memorial Colleges of Pharmacy, Narasaraopet, Guntur-522601, Andhra Pradesh

Correspondence

Somnath De

Dr. Samuel George Institute of Pharmaceutical Sciences, Markapur-523316, Tarlapadu road, Andhra Pradesh, India.

Phone: +91-8596-224045, 9618347303

E-mail: somnath.bankura@gmail.com

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lected from the Thoothukudi district, Tamilnadu, India, voucher. No. SGIP, Ref.no: 007 and 010 in the month of December 2011 and authenticated by Dr. V. Chelladurai, retired research officer – Botany, central council for research in Ayurveda and siddha (CCRAS), government of India; Tirunelveli. Herbarium of the plants was prepared and preserved in the department of pharmacognasy, Dr. Samuel George Institute of Pharmaceutical Sciences, Markapur, Prakasam district, Andhra Pradesh. Plant materials were air dried in the laboratory for 5 days at room temperature then grinded to powder form using an electrical mill. The powder sample was kept in an air tight container until required. About 100 g of each plant sample was extracted with 500 mL methanol solvent by using soxhlet extractor. After extraction the sample was kept in dark for 72 hours with intermittent shaking. Then both the solvents were evaporated under reduced pressure using Rota-Vapor and to obtain viscous semisolid masses.

Phytochemical screening

The preliminary phytochemical screening was carried out by methanolic extract of both the plants for the presence of phyto constituents. The methanolic extract of both the plants were tested for steroids, alkaloids, phenolic compounds, flavonoids, saponins, tannins, anthraquinone and amino acids. Phytochemical screening of both the extracts was carried out according to the standard methods.⁸

Acute Toxicity Studies

The acute toxicity studies for methanolic extracts of both the plants were determined on albino mice (20-30g) the plants were determined on albino rat, maintained under standard condition. The animals were fasted overnight prior to the experiment. Fixed dose method as per OECD Guideline No. 425 method, given by CPCSEA was adopted for toxicity studies.⁹ the study was conducted by prior permission of institutional animal ethical committee (IAEC registration no. 1048/a/07/CPCSEA, approval no. 22/IAEC/CIPS/2014-15). The mice were divided in to control and test group each containing 6 animals. The test groups of mice were administrated with the dose of 25, 200, 500, 2000 and 5000 mg/kg of extracts. Carefully observe all the mice and any sign of toxicity in the

first four hours, after the administration of extracts and daily following that for the period of 14 days.

Hepatoprotective activity

Selection of animals, caring and handling

The albino rats (wistar strain 150-200 g) of either sex were used. After randomization in to various groups, animals were accustomed for a period of 10 days under standard husbandry condition.

Room temperature: $23 \pm 3^\circ\text{C}$

Relative humidity: $50 \pm 20\%$

12 hrs dark and light cycle.

All the animals were feed with rodent pellet diet and water was allowed ad-libitum under strict hygienic condition. Ethical clearances for performing experiments on animals are obtained from Institutional Animal Ethical Committee (IAEC registration no. 1218/a/08/CPCSEA, approval no. 17/IAEC/SGIP/2014-15). The animals were divided in to vehicle control, toxic standard and test extract of *S. amaranthoides* 50mg/kg, *S. amaranthoides* 100mg/kg, and *O. umbellata* 50mg/kg, *O. umbellata* 100mg/kg groups. Each consisting of 6 animals in all sets of experiments respectively. The drug treatment was given once daily per oral.

Study designing and experimental protocol

Albino rats of westar strain weighing 150-200 g were selected and divided in to 7 groups of each containing 6 animals. 5% gum acacia was used as a vehicle control for suspending the standard drugs and the extracts. Vehicle control (group-I) received vehicle aqueous 5% gum acacia. Group-II to Group-VII is administrated with CCl_4 for 7 days at a dose of 0.25ml/100 g. Whereas the Group-II received only CCl_4 , Group-III receive the standard drug silymarin at a dose of 25 mg/kg b.w and Group-IV received MESA (Methanolic extract of *S. amaranthoides*) at a dose of 50 mg/kg b.w., Group- V received MESA (Methanolic extract of *S. amaranthoides*) at a dose of 100 mg/kg b.w., Group-VI received MEOU (Methanolic extract of *O. umbellata*) at a dose of 50 mg/kg b.w., Group-VII received MEOU (Methanolic extract of *O. umbellata*) at a dose of 100 mg/kg b.w. The vehicle control and all test extract administered orally for seven days at different dosage from level and enhance the liver damage in animals. On the 7th day after 6 hrs of the administration

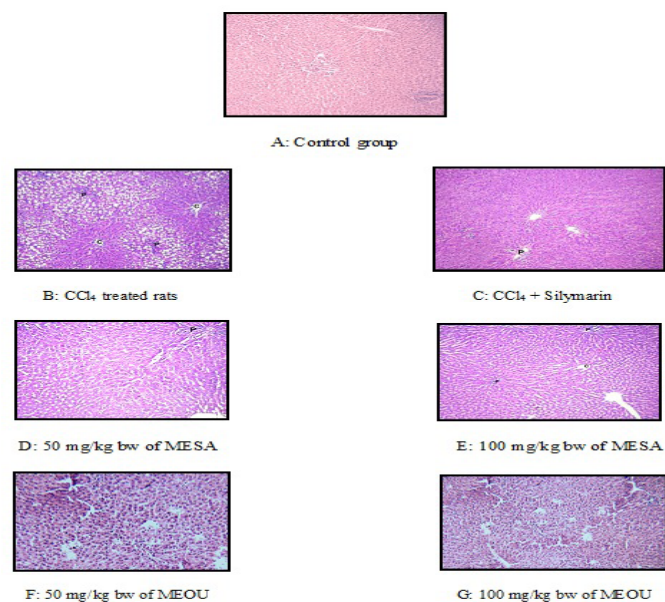


Figure 1. Dose dependent effect of methanolic extract of *S. amaranthoides* and *O. umbellata* on CCl_4 induced hepatotoxicity in albino rats.

Table: 1 Effect of methanolic extract of *S. amaranthoides* and *O. umbellata* on CCl₄ induced hepatotoxicity (Serum biological parameters) in albino rats.

SL.No	Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total bilirubin (mg/dl)
1	Control (group-I)	119±2.82	101.0±2.20	187.97±1.6	1.58±0.14
2	Toxic control (group-II)	353.16±8.79**	394.82±3.14**	444.36±7.88**	4.61±0.23**
3	Standard group-III (25mg/kg)	124.86±3.39*	123.66±1.83*	184.81±2.05*	2.05±0.03*
4	M.E.S.A. group-IV (50 mg/kg)	352.81±2.10*	328.28±1.99*	417.94±1.97*	3.28±0.028*
5	M.E.S.A. group-V (100 mg/kg)	319.14±4.25*	338.14±2.64*	394.12±3.36*	3.07±0.18*
6	M.E.O.U. group-VI (50 mg/kg)	217.48±1.84*	216.45±3.98*	226.32±2.09*	2.64±0.3*
7	M.E.O.U. group-VII (100 mg/kg)	193.22±3.30*	211.43±2.42*	204.40±3.29	2.33±0.02*

Values are mean±SEM, n=6. **p<0.01, when compared with control group, *p<0.01, when compared with toxic group

of the drug by giving a single dose of CCl₄ with liquid paraffin (1: 1 ratio). After 24 hrs blood samples were drawn from all animals by puncturing retro-orbital plexus on the day of the treatment. The blood samples were centrifuged immediately to get clear serum and subjected for estimation of various biochemical parameters namely SGPT (serum glutamic pyruvic transaminase), SGOT (serum glutamic oxaloacetic transaminase), ALP (alkaline phosphate), and TB (total bilirubin).¹⁰⁻¹⁵ Later the animals were sacrificed and liver tissues were collected from all groups for histopathological studies.¹⁶

Statistical Analysis

Results were expressed as mean <SEM, n=6. Statistical analysis was performed with one way analysis of variance (ANOVA) by Dunnett's multiple comparisons test. P value < 0.01 was considered to be statistically with control and toxicant group as possible.

RESULT

The present study had been attempted to demonstrate the role of hepatoprotective activity of crude methanol extracts of plant materials of *S. amaranthoides* and *O. umbellata*, belonging to the different family in CCl₄ induced hepatotoxicity at different doses. The methanolic extract of both the plants are having same phytoconstituents after prior preliminary phytochemical test but here, the difference was found to be presence of anthraquinone in *O. umbellata* when compared with *S. amaranthoides*. In wellness parameter analysis no significant changes were observed for evolution toxicity. Skin, furr, eyes, mucous membrane, behavioral pattern, salivation, sleep of the treated as well as control animals were found to be normal. Tremor, lethargy, diarrhea and coma did not occur any of the animals. There was no significant damage of the liver and no significant structural changes detected in kidney, lung, and brain from all groups of the study. Carbon tetrachloride in one of the most potent hepatotoxic (toxic to the liver), and is widely used in scientific research to evaluate hepatoprotective agents.¹⁷ The result of the present study showed that, the level of SGOT [353.16 ± 08.79] IU/L, SGPT [394.82 ± 03.14] IU/L, ALP [444.36 ± 07.88] IU/L and TB [4.61 ± 0.23] mg/dL were significantly increased in toxic control treated group (P < 0.01) (Group-2) when compared with control group. Administration of (50 and 100 mg/kg b.w.) both *S. amaranthoides* and *O. umbellata* extracts treated rats showed significant reduction level of SGOT, SGPT, ALP, TB (P < 0.01) when compared with toxic treated rats. But the maximum reduction of SGOT (319.74 ± 04.25) and (193.22 ± 03.30) IU/L., SGPT

(338.14 ± 02.64) and (211.43 ± 02.42) IU/L., ALP (394.12 ± 03.36) and (204.40 ± 03.29) IU/L., TB (3.07 ± 0.18) and (2.33 ± 0.02) mg/dL were observed in the high dose group (100 mg/kg b.w) (P < 0.01) (Table 1). Histopathological sources revealed that the maximum level of fatty changes, focal necrosis, and congestion in central vein and congestion sinusoidal spaces were found in hepatotoxin treated rats. However *S. amaranthoides* and *O. umbellata* plant extracts (50 mg/kg b.w and 100 mg/kg b.w) pretreated rats showed reduction in fatty changes and focal necrosis, hydrophine changes. No visible changes (Figure-1) were observed.

DISCUSSION

The present study was revealed hepatoprotective activity in rats against CCl₄ hepatotoxin to prove its claims in folklore practice against liver disorders. A number of reports indicate that overdose CCl₄ can produce centrilobular hemorrhagic hepatic necrosis in human and experimental animals^{18, 19} Based on the result of acute oral toxicity studies for methanolic extracts of both the plants (*S. amaranthoides* and *O. umbellata*) would be rewarded as safe. There was no mortality found out during experimental periods of the animals. CCl₄ induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of medicinal plants extracts. The extent of hepatic damage is assessed by histological evolution level of various biochemical parameters in circulation. CCl₄ undergoes hepatic metabolism to give rise tri chloro methyl radicals, which upon reacting with reactive oxygen species yields tri chloro methyl peroxide radicals. These forms covalent bond with lipids and destroy the membrane integrity. It produces hepatotoxicity by altering liver microsomal membranes in experimental animals. From the table-1 it was evident that both the extracts (MESA AND MEOU) were able to reduce all the elevated biochemical parameters in the hepatotox intoxication model. The reduction is attributed to the initial damage produced and localized in the endoplasmic reticulum which results in the loss of P450. Reduction in the levels of SGOT, SGPT, ALP and TB towards the normal value is an indication of regeneration process. The histological examination of the liver sections revealed that the normal liver architecture was disturbed by hepatotoxin intoxication. In the liver sections of the rats treated with extracted and intoxicated with CCl₄, the normal cellular architecture was retend as compared to silmyrin.²⁰ thereby confirming the protective effect of both the extracts. The methanolic extract of *S. amaranthoides* and *O. umbellata* have shown very

significant hepatoprotection against CCL₄ induced hepatotoxicity in albino rats in reducing SGOT, SGPT, ALP and TB levels. Liver section of *S.amaranthoids* and *O.umbellate* treated animal groups clearly showed normal hepatic cells thereby confirming hepatoprotective activity.

CONCLUSION

In conclusion, the precious information regarding acute oral toxicity profile of both the plants would be crucial for various pharmacological screening. On the other hand methanolic extract of *S.amaranthoids* and *O.umbellate* could be an important source of hepatoprotective compounds specifically for CCL₄ induced hepatotoxicity.

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CONFLICT OF INTEREST

Authors declare that they have no competing interest.

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