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Evaluation of Hepatoprotective Activity of Ethanolic Extracts of Aerial Parts of *Smilax perfoliata* and *Flemingia wightiana*

Venkatehwar Reddy Atla[1*], Rama Rao T[2], and Naidu A[3]

[1]Anwarul Uloom College of Pharmacy- Hyderabad, Andhra Pradesh, India.
[2]Mohammedia College of Pharmacy-Khammam, Andhra Pradesh, India.
[3]JNTUH- Hyderabad, Andhra Pradesh, India.

ABSTRACT

The present study was conducted to evaluate the hepatoprotective activity of alcoholic extracts of *Smilax perfoliata and Flemingia wightiana* against Carbon tetrachloride induced liver damage in rats. The alcoholic extracts of *Smilax perfoliata and Flemingia wightiana (*100mg/kg &200mg/kg*)* were administered orally to the animals with hepatotoxicity induced by Carbon tetrachloride (0.7 ml/kg). 1 ml/kg of Liv-52 perorally was given as reference standard. The plant extracts were effective in protecting the liver against the injury induced by Carbon tetrachloride in rats. This was evident from significant reduction in serum enzymes SGOT,SGPT,SALKP and bilirubin. It was concluded from the result that the alcoholic extracts of *Smilax perfoliata and Flemingia wightiana* possess hepatoprotective activity against Carbon tetrachloride induced hepatotoxicity in rats.

Keywords: *Smilax perfoliata Flemingia wightiana*, Carbon tetrachloride, hepatoprotetive and hepatotoxicity

Corresponding author

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INTRODUCTION

Hepatotoxicity means damage of liver by drugs, chemicals or by microorganisms. The liver plays vital role in renovating and clearing chemicals and is also vulnerable to toxicity from these agents. Certain drugs when taken in overdose and even when administered at therapeutic dose may cause injury to liver [1]. Other chemical agents that are used in laboratories, industries, microcystins produced by cyanobacteria and herbal remedies can also induce hepatotoxicity. The synthetic drugs may cause serious adverse effects. There are many herbal preparations available in India used in folklore for their hepatoprotective activity and there is need for the systemic study to evaluate the effectiveness of these preparations [2,3]. *Smilax perfoliata* and *Flemingia wightiana* are climbing and erect shrubs respectively seen in higher elevations of southern Andhra Pradesh used in folklore for various ailments for the past several decades [4]. The study was conducted to establish the hepatoprotective effect of these plants against Carbon tetrachloride induced hepatotoxicity.

MATERIALS AND METHODS

Animals

Wistar albino rats of either sex weighing between 200-250 gm were housed under standard environmental conditions (temperature of 22 $\pm$ 1°C with an alternating 12 hour light–dark cycle and relative humidity of 60 $\pm$ 5 %), one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethics committee. They were fed with standard laboratory diet and water was allowed *ad libitum* during the experiment [5].

Plant Material

Fresh plats were obtained from Tirumala hills of Andhra Pradesh identified and authenticated by the botany department of S V University, Tirupati. The plants were cleaned, shade dried and milled into coarse powder by mechanical grinder.

Preparation of Extract

The coarse powder of the plants was extracted using ethanol as solvent by soxhlet apparatus. The solvent was removed under pressure to a semi solid mass. Standard methods were used for screening the phytochemicals and it was found that the plants contain alkaloids, flavonoids, tannins and sterols [6].

Chemicals

All the chemicals, solvents and reagents used in this experiment were of analytical grade. Carbon tetrachloride (S.D. fine chemicals Ltd., Mumbai, India), Serum SGOT, SGPT,

Bilirubin, Alkaline phosphatase (Span Diagnostic Ltd., Surat, India). Liv – 52 (The Himalaya Drug Co., Bangalore, India) were used in this experiment.

Experimental Protocol

In this study, Animals were divided into seven groups of six rats in each [7]. The entire groups of animals were fasted over night and administered with respective drugs [9]. The rats of group 1 was administered with 10 ml/kg of 2% v/v aq. Tween 80 solution per orally. Animals of group 2 (Toxic control) were administered with 0.7 ml/kg of Ccl_4 in olive oil (1:1) i.p. once daily for 7 days. Animals of group 3 administered simultaneously with EESP 100 mg/kg perorally and 0.7 ml/kg of CCl_4 in coconut oil (1:1) i.p. once daily for 7 days. Group 4 animals administered simultaneously with EESP 200 mg/kg perorally and 0.7 ml/kg of CCl_4 in coconut oil (1:1) i.p. once daily for 7 days. Group 5 Treated animals administered simultaneously with EEFW 100 mg/kg perorally and 0.7 ml/kg of CCl_4 in coconut oil (1:1) i.p. once daily for 7 days. Group 6 Treated animals administered simultaneously with EEFW 200 mg/kg perorally and 0.7 ml/kg of CCl_4 in coconut oil (1:1) i.p. once daily for 7 days. Group-7: Drug control animals administered simultaneously with 1 ml/kg of Liv-52 perorally and 0.7 ml/kg of CCl_4 in coconut oil (1:1) i.p. once daily for 7 days. On the 8^{th} day, blood was collected from the retro-orbital and allowed to clot for 45 minutes at room temperature. Serum was separated by centrifugation and subjected to various biochemical estimations of glutamic oxaloacetic transaminase (SGOT), glutamate pyruvate transaminase (SGPT), alkaline phosphatase (SALKP) and serum total bilirubin were assayed [8]. The percentage of inhibition of various biochemical parameters was calculated by

Percentage of inhibition = 100 X (value of toxic control – value of test sample)/ (value of toxic control – value of control).

Groups	SGOT (IU/L)	SGPT (IU/L)	SALKP (IU/L)	Total serum Bilirubin (mg/dl)
Group 1	52.6 ± 1.4	38.4 ± 1.32	56.9 ± 0.98	1.28 ± 0.14
Group 2	158.9 ± 1.9	124.3 ± 1.4	152.8 ± 1.46	2.86 ± 0.03
Group 3	62.8 ±1.6*** [90.40]	51.4 ± 1.5*** [84.87]	71.3 ± 1.9*** [84.98]	1.51 ± 0.04** [85.44]
Group 4	72.40± 1.7** [81.37]	56.4 ± 1.2** [79.05]	78.5 ± 1.3** [77.48]	1.74 ± 1.07** [70.89]
Group 5	65.9 ± 1.3*** [87.49]	53.2 ± 1.0*** [82.77]	74.5 ± 1.1*** [81.65]	1.65 ± 0.9*** [76.58]
Group 6	98.6 ± 2.9** [56.73]	70.1 ± 2.7** [63.10]	103.6 ± 2.9** [51.30]	1.94 ±0.07** [58.23]
Group 7	84.2 ± 2.3*** [70.27]	62.8 ± 1.8*** [71.59]	87.5 ± 2.1*** [68.09]	1.71 ± 0.06** [72.78]

Each value represents the mean ± SEM. n = 6 number of animals in each group. **P<0.01, ***P<0.001 Compared to respective control CCl_4 group-2

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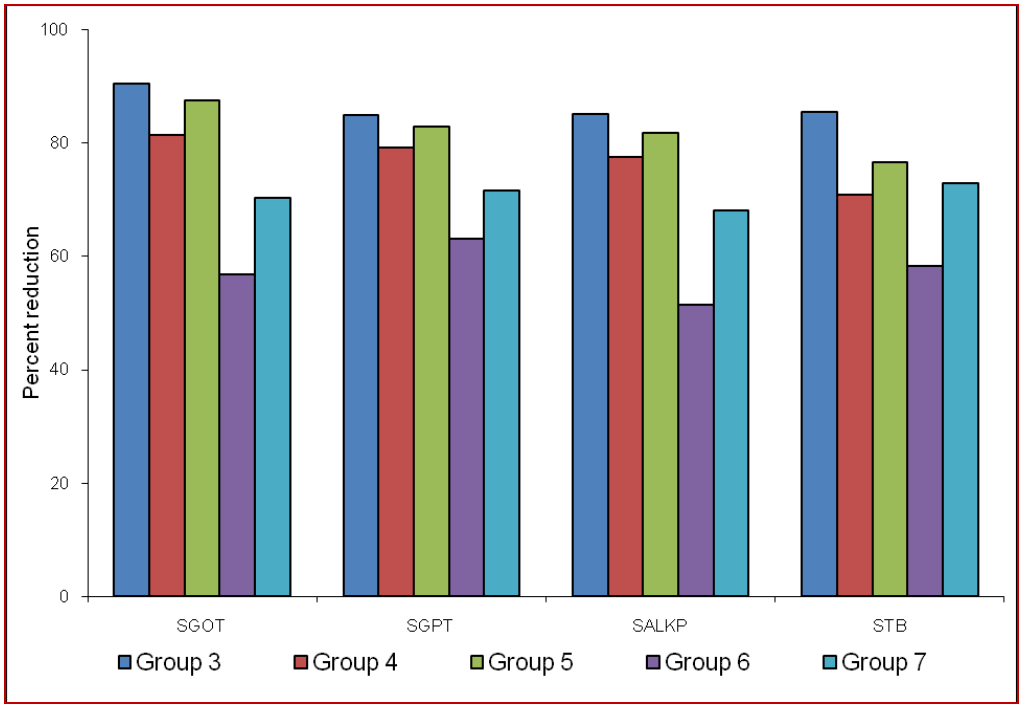
The comparative efficacy of the extracts tested for their hepato protective activity, the relationship between dose and percentage reduction in each case were depicted in the form of a bar diagram as shown



Effect of EESP and EEFW on SGOT, SGPT, SALKP and Serum Total Bilirubin levels.

Group 3: 1 ml/kg Liv – 52 + 0.7 ml/kg CCl_4 in coconut oil p.o.
Group 4: 100 mg/kg EESP + 0.7 ml/kg CCl_4 in coconut oil p.o.
Group 5: 200 mg/kg EESP + 0.7 ml/kg CCl_4 in coconut oil p.o.
Group 6: 100 mg/kg EEFW + 0.7 ml/kg CCl_4 in coconut oil p.o.
Group 7: 200 mg/kg EEFW + 0.7 ml/kg CCl_4 in coconut oil p.o.

RESULTS AND DICUSSION

The efficacy of any hepatoprotective drug essentially depends on its capability of either reducing the harmful effects or in maintaining the normal hepatic physiological mechanism, which have been imbalanced by a hepatotoxin [9].

Hepatoprotective activity of EESP and EEFW was studied in CCl_4 induced liver injury in rats. Results of the present study showed the oral administration of EESP to CCl_4 treated rats significantly decreased elevated level of hepatic microsomal enzymes (SGOT, SGPT, SALKP) and Total Bilirubin. The results were comparable to standard hepatoprotective formulation Liv 52.

CCl_4 is the most common and widely used liver intoxicator today for laboratory experiments [10]. Its action is based on membrane lipid peroxidation and induction of trichloromethyl free radical (CCl_3) Liv-52 aids quicker regeneration of hepatic parenchyma and

improved function of liver [11]. On the basis of changes in the activity of hepatic enzymes it seems that Liv-52 and both the extracts have similar mode of action. Antioxidants represent a new perspective in liver injury and fibrosis prevention [12]. Previous studies have shown that antioxidants isolated from natural source like Acacetin, Naringenin, N-acetyl cystein, Syilmarin, Vit-E, Quercetin and Rutin decreased lipid peroxidation and ameliorate liver injury [13]. The EESP extract contain myriad number of compounds like tannins, phenolic compounds, terpenoids and flavanoids like Quercetin and Rutin [14] may be responsible hepatoprotective activity.

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