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A STUDY ON THE HEPATOPROTECTIVE EFFECTS OF ETHYL ACETATE EXTRACT OF *SPILANTHES ACMELLA* IN PARACETAMOL-INDUCED HEPATOTOXICITY IN EXPERIMENTAL ANIMALS

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ABSTRACT: *Spilanthes acmella* plant has been used for various purposes in traditional medicine since time immemorial. The present study aimed to determine the hepatoprotective activity of ethyl acetate extract of *Spilanthes acmella* in Paracetamol-induced hepatotoxicity in experimental animals. The leaves and flowers of this plant were used for this study, and the experiment was carried out in albino rats. The ethyl acetate extract at 100mg/kg and 200mg/kg body weight was evaluated by inducing hepatotoxicity with paracetamol at a dose of 2g/kg body weight and using Silymarin 100mg/kg as the standard reference drug. The hepatoprotective activity was monitored biochemically by estimating serum levels of AST, ALT, ALP, Total Protein, and Total and direct bilirubin. The extract exhibited significant ($p<0.05$) hepatoprotection in a dose-dependent manner in paracetamol-intoxicated albino rats. The hepatoprotective effects of the extract were comparable to the Standard drug, Silymarin, therefore suggesting further in-depth studies.

INTRODUCTION: The liver is the largest solid organ in the body, which performs several vital functions, including secretion of bile, vascular and hematologic functions, and also the metabolism of fats, proteins, carbohydrates, metabolic detoxification, as well as storage of minerals and vitamins [1]. In recent years, though there has been tremendous advancement in the field of hepatology, liver diseases are quite common. Only a few drugs are available for treating liver ailments, which are either inadequate or can have serious side effects [2, 3].

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Therefore, there is increasing interest in therapeutic evaluation and use of medicinal plants which may counteract the detrimental effects of various hepatotoxins [4, 5]. The present study evaluated the hepatoprotective effect of ethyl acetate extract of *Spilanthes acmella* in paracetamol-induced hepatotoxicity in albino rats. *Spilanthes acmella* is an annual or short-lived herb known throughout the world as para cress, toothache plant, Brazilian cress, sechuan button, and eyeball plant [6, 7].

Its medicinal properties are mainly due to the presence of a wide range of compounds, such as alkylamides (spilanthol), phenolics (ferulic acid and vanillic acid), coumarin (scopoletin) and triterpenoids, like â-sitostenone and stigmasterol. Of these, the most abundant principle is Spilanthol, an antiseptic alkylamide, (2E, 6Z, 8E)-deca-2,6,8-trienoic acid N-isobutyl amide [8]. Extracts from

Spilanthes acmella plant have shown different pharmacological response, which includes hepatoprotective effect, [9] anticonvulsant, analgesic, anti-inflammatory, vasodilation, diuretic, antimalarial effects [10]. They have shown local anaesthetic and antipyretic activities as well [11].

Paracetamol overdose may lead to hepatotoxicity and acute liver failure, mainly due to cellular damage by NAPQI (N-acetyl-para-benzo-quinone imine) [12]. Silymarin is a well-tolerated and effective drug for use in cases of hepatotoxicity produced by a number of hepatotoxic agents [13].

MATERIALS AND METHODS:

Chemicals and Reagents: Tablet Paracetamol and tablet Silymarin were purchased from M/S Strassenburg Pharma Ltd. and Micro Lab Ltd. Reagents. Petroleum ether, ethanol, ethyl acetate, and Sodium Carboxy methyl cellulose were brought from various commercial sources.

Experimental Animals: 30 healthy albino rats of either sex weighing 200-250 grams were recruited from the animal house of JNIMS, Porompat, Imphal, and housed in the department polypropylene cages for 10 days for acclimatization in the laboratory atmosphere. They were fed with a standard laboratory diet and water ad libitum, while maintaining 12 hours dark-light cycle.

Ethical Clearance: Ethical clearance to conduct the study was obtained from the IAEC of the Regional Institute of Medical Sciences, Manipur, India (Reg No. 1596/GO/a/12/CPCSEA).

Plant Material: Fresh whole plants of *Spilanthes acmella* were collected from the Imphal East area randomly and authenticated by Dr. Bisheshwori Thongam, Scientist E, Plant Taxonomist, Institute of Bioresources and Sustainable Development (IBSD), Takyepat, Imphal, Manipur (Acc. No. - IBSD/M-257). The leaves and flowers were separated from the plants, washed, shade dried, powdered, and stored in tight containers until the extraction was done.

Extraction: The powdered material (70 grams) was extracted by P. Jayasekhar *et al*. [14] with slight modification. They were defatted with petroleum ether (60-80°C) in the Soxhlet apparatus and then extracted successively with 99.9% ethanol and ethyl acetate. Then, the yield (30 grams) was stored in a porcelain jar at 4°C for future use.

Acute Toxicity Study: The acute toxicity study for *Spilanthes acmella* leaves and flowers test extracted was carried out using OECD/OCED guidelines 425. Five healthy young albino rats (200-250 grams) were used for this study. Animals were fasted overnight with free access to water, and proper care was taken to prevent coprophagy. The drug was administered orally to one animal at a 2000mg/kg dose, and changes were observed. When it survived, the other four animals were administered the drug at the same dose, sequentially, and eventually, all of them survived. Animals were observed individually at least once during the first 30 minutes after administering the drug, periodically during the first 24 hours (with special attention during the first 4 hours) and daily thereafter, for a total of 14 days.

Study of Hepatoprotective Activity: The method of P. Madhu Kiran *et al.* [15], Rajasekaran and Periyasamy [16] were followed. 30 healthy albino rats (200-250 grams) were randomly divided into 5 groups, each consisting of 6 animals, and treated orally using feeding tube:

Group I (Normal Control): Received normal saline, 5ml/kg body weight, daily for 7 days.

Group II (Toxic Control): Treated like Group 1.

Group III (Test Dose I): Received ethyl acetate extract of *Spilanthes acmella* leaves and flowers 100 mg/ kg body weight per day suspended in 0.5% CMC for 7 days.

Group IV (Test Dose II): Received ethyl acetate extract of *Spilanthes acmella* leaves and flowers 200 mg/kg body weight per day suspended in 0.5% CMC for 7 days.

Group V (Standard): received the standard drug, Silymarin 100 mg/kg body weight daily for 7 days.

On the 7th day, Paracetamol suspension in 0.5% CMC was given orally, 2g/kg body weight, to all the Groups except Group 1, which was given CMC.

Biochemical Studies: On 8th day of the experiment, *i.e.*, 24 hours after administration of paracetamol, all rats were anesthetized with ether,

and blood samples were collected from the orbital sinus. Then the blood was kept for 30 minutes without disturbing. The clots were dispersed with a glass rod and then centrifuged for 20 minutes at 2000 rpm to separate the serum. The serum from each animal was investigated for different biochemical parameters, namely AST, ALT, ALP, Total and direct bilirubin, Total protein using specific kit for each parameter in the Ortho Clinical Vitros 250 Chemistry System.

Statistical Analysis: Mean, standard deviations and standard error were used for descriptive statistics. For analytical statistics, ANOVA (analysis of variance) was applied, and Post-hoc Tukey-Kramer, multiple comparisons test, was used whenever statistical significance was found. The F-ratio expressed the significance in the test, and p-values of 0.05 or less were considered significant.

RESULTS AND DISCUSSION:

Acute Toxicity Studies: The ethyl acetate extract of *Spilanthes acmella* did not show any sign or symptom of toxicity and mortality up to 2000 mg /kg dose.

Effects of Ethyl Acetate Extract on AST, ALT, ALP, Total and Direct Bilirubin, Total Protein: The results of the hepatoprotective effect of the test extract on paracetamol-induced hepatotoxicity in rats have been summarised in **Table 1** and **Fig. 1** and **2**.

TABLE 1: SHOWING MEAN ± SD VALUES OF SIX OBSERVATIONS OF SERUM LEVEL OF AST, ALT, TOTAL PROTEIN, TOTAL BILIRUBIN, DIRECT BILIRUBIN, AND ALP IN EACH GROUP

Groups	AST	ALT	Total protein	Total bilirubin	Direct bilirubin	ALP
Group-I	71.67±12.66	97.50±4.68	6.17±0.75	0.98±0.31	0.58±0.304	77.68±5.42
Group-II	174.33±24.74$^{@}$	159.83±22.21$^{@}$	2.83±0.71$^{@}$	3.29±0.54$^{@}$	1.44±0.43$^{@}$	130.38±12.32$^{@}$
Group-III	90.83±9.52*$^{@}$	100.67±6.80*$^{@}$	4.52±0.51*$^{@}$	2.08±0.23*$^{@}$	0.90±0.11*$^{@}$	80.17±4.67$^{*@}$
Group-IV	97.50±9.63$^{*@}$	108.17±18.23*$^{@}$	5.43±1.24*$^{@}$	1.98±0.5*$^{@}$	0.52±0.12*$^{@}$	76.54±4.45*$^{@}$
Group-V	77.00±15.15*$^{@}$	86.67±9.69*$^{@}$	6.00±0.39*$^{@}$	0.99±0.38*$^{@}$	1.07±0.49*$^{@}$	86.13±3.44*$^{@}$

@ p< 0.05 compared to corresponding values of group I. *p<0.001 compared to corresponding values of group II.

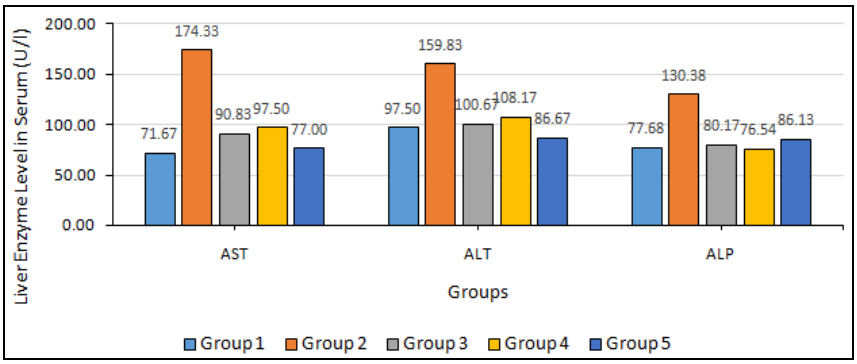

FIG. 1: BAR DIAGRAM SHOWING LIVER ENZYME LEVELS IN SERUM IN DIFFERENT GROUPS OF PARACETAMOL-INDUCED HEPATOTOXICITY IN ALBINO RATS. Each value is expressed as Mean±SD of six observations

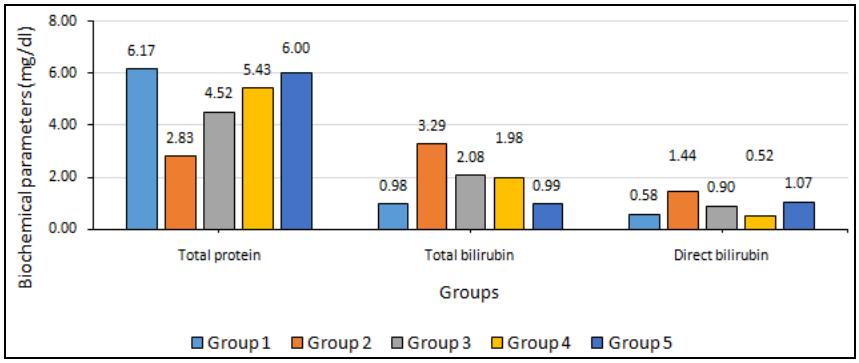

FIG. 2: BAR DIAGRAM SHOWING SERUM LEVELS OF VARIOUS BIOCHEMICAL PARAMETERS IN DIFFERENT GROUPS OF PARACETAMOL INDUCED HEPATOTOXICITY IN ALBINO RATS. Each value is expressed as Mean ± SD of six observations

The administration of paracetamol led to significant hepatocellular damage as evident from the increase in serum activities of AST, ALT, Alkaline Phosphatase (ALP), Total and Direct bilirubin and a decrease in the level of total protein, in comparison with the normal control group. Treatment of rats with the test dose, *i.e*., ethyl acetate extract of *Spilanthes acmella* leaves and flowers at a dose of 100 mg/kg and 200mg/kg given orally exhibited a significant reduction ($p<0.05$) in Paracetamol-induced elevation of serum AST, ALT, ALP, total and direct bilirubin; and increased the level of total protein. Treatment with Silymarin also significantly reversed the hepatotoxicity. Since, time immemorial, plants have been a constant source of new drugs and various chemical leads [17].

The antioxidant defence (superoxide dismutase, catalase, and glutathione peroxidase activity), reduced peroxidation, reversed hepatic fibrosis *via* enhancement of the expression of matrix metalloproteinase and removal of collagen deposits, with attenuation of hepatic stellate cells activation, their anti-inflammatory activity, and attenuation of many inflammatory processes, antifibrotic properties of plants and stimulation of extracellular matrix degradation are responsible for the hepatoprotective activity of medicinal plants [18]. Drug-induced liver injury is most commonly due to Paracetamol or Acetaminophen due to its easy access over-the-counter and also its high bioavailability [19]. Excessive formation of highly reactive intermediate metabolite N-acetyl p-benzoquinone imine (NAPQI), which depletes glutathione stores due to an overdose of paracetamol may lead to hepatotoxicity [20].

CONCLUSION: This study proved *Spilanthes acmella* to be a potential candidate in the list of hepatoprotective agents. The possible mechanism of hepatoprotective activity may be due to the reduction of oxidative stress and its ability to reduce elevated levels of serum marker enzymes [21, 22]. Further studies, including clinical trials with more purified extract, may harvest more valuable information shortly.

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