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Hepatoprotective activity of methanolic extract of *Ecbolium viride* (Forssk.) alston roots against paracetamol induced hepatotoxicity

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ABSTRACT

In the present study, hepatoprotective activity of methanolic extract of Ecbolium viride (Forssk.) Alston roots in paracetamol induced hepatotoxicity were investigated. The activity was assessed against paracetamol induced hepatotoxicity by measuring the levels of serum enzymes like SGOT SGPT and ALP, total proteins, total bilirubin, and triglycerides. Further, hepatic tissues were also subjected to histopathological studies. The extract showed significant hepatoprotective activity at 200 mg and 400 mg/kg b.w by decreasing the levels of SGPT, SGOT, ALP, total bilirubin, triglycerides and increasing the level of total proteins when compared to toxicant group. The histopathological studies further supported the activity. The results of the present study proved the hepatoprotective potential of methanolic extract of Ecbolium viride (Forssk.) Alston

Key words: *Ecbolium viride* (Forssk.) Alston, Paracetamol, Hepatoprotective activity, Serum enzymes, Histopathological studies

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INTRODUCTION

Liver is one of the major organs responsible for maintenance of metabolic functions, secretion and storage including regulation of various physiological processes. It involves in synthesizing useful principles and detoxicates toxic substances. Hepatotoxicants like alcohol, toxic chemicals, infections etc induce liver diseases mainly through oxidative damage and lipid peroxidation. Thus, hepatic diseases considered as most health serious problems [1]. Its damage is always associated with increase in tissue lipid peroxidation, cellular necrosis, and depletion in the tissue GSH levels. In addition to the above, serum levels of many biochemical markers like SGPT, SGOT, ALP, triglycerides, cholesterol, bilirubin are elevated and total proteins depleted. Hepatic disorders have been recognized worldwide as an important cause of morbidity and mortality in man and animals all over the globe [2]. Herbs are known to play a major role in the treatment liver disorders and many traditional healers have claimed that numerous medicinal plants can be extensively used for the treatment of various liver disorders.

Ecbolium viride (Forssk.) Alston is a low shrub with erect branches, cylindrical, thickened above the nodes and glabrous. It is commonly known as Kappubobbili and belongs to a family Acanthaceae and the roots of the plant are reported to be used in the treatment of jaundice, menorrhagia and rheumatisms [3]. Roots were reported to contain glycoflavones such as Orientin, Vitexin, Isovitexin and Isoorientin [4]. Traditional uses and phytoconstituents of *Ecbolium viride* (Forssk.) Alston prompt us to take up this study.

MATERIALS AND METHODS

Collection of plant material

The plant material was collected from vicinity of Tirumala hills, Tirupati, identified and authenticated by Dr. Madhava chetty, Asst.Professor, Botany Dept, Sri Venkateswara University, Tirupati (Voucher specimen No : EV-1768)

Preparation of plant extracts

The roots of the plant were separated, washed and dried at room temperature. After complete drying, it was powdered in a multi mill grinder and passed through a 60 mesh sieve. Dried coarse powdered drug was subjected to successive solvent extraction using Soxhlet apparatus (petroleum ether, benzene, chloroform and methanol) and macerated with chloroform water.

Phytochemical Screening

Extracts obtained on successive solvent extraction and maceration was subjected to phytochemical screening for the detection of various phytosconstituents[5].

Animal studies

Experimental animals

The pharmacological studies were carried out on Albino Wister rats of either sex weighing 150-225 g. The animals were housed in the animal house, maintained in controlled temperature (27 ± 2^0C) and light cycle (12 hr light and 12 hr dark). They were fed with rat feed (rat pellets from VRK Nutritional solutions, Sangli, Maharashtra, India) and water ad libitum. The study protocol was approved by the institutional Animal Ethical Committee of MSRCP (IAEC certificate No: MSRCP/P-11 2010, dated 3/12/2010)

Acute toxicity studies

Acute toxicity study was performed on methanol extract following OECD-423 guidelines.[7] After fasting overnight, rats were administered with extract of *Ecbolium viride* (Forssk.) Alston in a single dose up to the highest dose of 2000 mg/kg orally. The animals were observed continuously for 1 h and then hourly for 6 h and finally after every 24 h up to 15 days for any toxicological symptoms.

Experimental design [8]

Animals were divided into 5 groups containing six animals each

Group I was served as normal control and received 2% w/v acacia suspension. Group II was maintained as toxicant group, received 2g /kg b.w paracetamol on 5^{th} day and vehicle for 7 days orally. Group III was orally administered with standard Silymarin at 100mg/kg once daily for 7 days and 2g /kg b.w paracetamol on 5^{th} day. Group IV and V were orally administered once daily for 7 days with MEEV extract at a dose of 200 & 400 mg/kg b.w respectively and 2g /kg b.w paracetamol on 5^{th} day. On the 8^{th} day all the animals were anaesthetized for the collection of blood from retro-orbital sinus.

Collection of serum

The blood was collected from retro-orbital sinus of the animals by anaesthizing under light ether anesthesia using a heparinized capillary tube. Then it was allowed to clot and serum was separated from clotted blood by centrifugation at 8000 rpm for 10 min. The separated serum was used for the estimation of SGPT (ALT), SGOT (AST), alkaline phosphatase (ALP), total proteins, total bilirubin and triglycerides.

Isolation of liver

Liver was carefully excised and washed in ice cold normal saline solution and pressed between filter paper pads and weighed. A portion of liver (one animal of each group) was preserved in 10% neutral formalin for histopathology studies.

Biochemical estimation

Blood was allowed to clot and centrifuged at 12000 rpm for 10 min to separate the serum. The serum thus obtained was used for the estimation of Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxalloacetate transaminase (SGOT) [9], alkaline phosphatase (ALP)[10], tri glycerides[11], total proteins [12] and total bilirubin [13]. All these estimations were performed following International Federation of Clinical chemistry and Laboratory

medicine (IFCC) standard procedures. All the determinations were carried out using standard kits (Agappe diagnostics, Span Diagnostics) by using Semi-automatic B4B Diagnostic Division Chemistry Analyzer CA-2005 Ranbaxy diagnostic division

Histopathology studies

Paraffin sections were prepared from formalin fixed liver samples and stained with haematoxylin and eosin. Histological samples were categorized based on the extent of hepatic injury [14].

Statistical analysis

All values are expressed as Mean± SEM and tested with One Way Analysis of Variance (ANOVA) followed by Tukey- Kramer multiple comparison test.

RESULTS

Phytochemical screening

Phytochemical screening of different extracts reveal methanolic extract was a good source of flavonoids, tannins, alkaloids and other phenolic compounds. Hence, the methanolic extract was selected for the present study.

Acute toxicity studies

Methanolic extract at the dose of 300mg/kg and 2000mg/kg showed no toxic symptoms or death in any of the animals upto one week and till the end of the study. 1/10 and 1/5th of highest dose as selected for the studies.

Paracetamol induced hepatotoxicity

The level of marker enzymes, total proteins, total bilirubin, and triglycerides is shown in Table 1. The liver weight, serum levels of SGPT, SGOT, ALP, triglycerides and bilirubin were increased significantly, while the level of total proteins decreased in toxicant group. The treatment with the extract altered serum parameters significantly.

The serum levels of SGPT were significantly (P<0.001 for 200mg/kg and 400 mg/kg) reduced in the extract treated group. The serum levels of SGOT and ALP were also significantly (P<0.001 for 200mg/kg and 400 mg/kg) reduced in the extract treated group. Tri glyceride levels significantly reduced for (P<0.001 for 200mg and 400 mg/kg). Total protein levels were significantly increased (P<0.001 for 400 mg/kg) and bilirubin levels were significantly reduced (P<0.001 for 200mg/kg and 400 mg/kg) in the extract treated group. The extract also brought down the liver weight significantly.

Table 1 : Effect of MEEV on serum parameters in Paracetamol induced hepatotoxicity

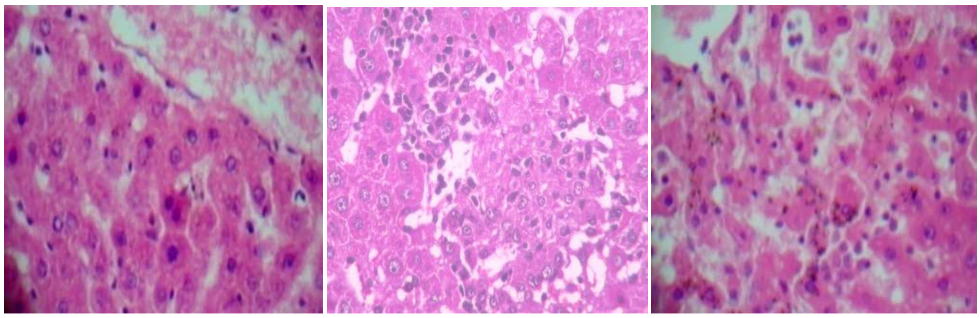
Groups	SGPT (U/I)	SGOT (U/I)	ALP (U/I)	Total proteins (g/dl)	Total bilirubin (mg/dl)	Triglycerides (mg/dl)
Normal control	51.61 ±4.241	99.33 ±7.09	151.71 ±6.07	9.137 ±0.321	0.483 ±0.127	83.84 ±5.386
Toxicant group	196.06 ±5.585	290.14 ±7.22	314.05 ±6.7	4.895 ±0.279	2.468 ±0.1502	182.18 ±4.20
Standard Silymarin	102.52 ±4.66***	136.9 ±4.44***	110.95 ±9.0***	7.210 ±0.26***	0.9617 ±0.033***	94.09 ±4.1***
MEEV (200mg)	154.38 ±4.14***	214.04 ±6.8***	222.17 ±6.8***	5.515 ±0.140	1.473 ±0.204***	146.22 ±4.9***
MEEV (400mg)	122.06 ±4.93***	151.03 ±5.6***	157.68 ±4.4***	6.942 ±0.11***	1.072 ±0.042***	104.65 ±3.4***

Values arse expressed as Mean ±SEM; Data is compared against positive control group.
One way analysis of variance (ANOVA) Tukey-Kramer multiple comparison tests.
**** P< 0.001, ** P< 0.01, * P< 0.05*

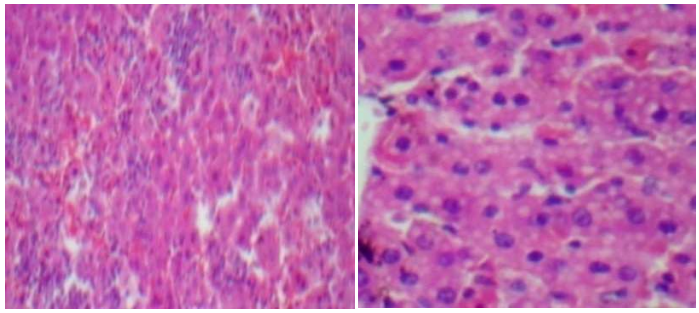
Histopathological studies

Liver of normal control showed normal hepatic architecture with central veins, portal tracts, hepatocytes and sinusoids. The perivenular and periportal region are within normal limits. Paracetamol treated group (Toxicant) showed partially effaced architecture of liver. Few hepatocytes showed epithelioid granulomas, degenerative changes, and aggregates of mononuclear inflammatory cells with congested sinusoids. The liver architecture of MEEV at 200 mg/kg was intact. The perivenular hepatocytes show hepatocyte ballooning degeneration and a moderate lymphocytic infiltration in periportal region. MEEV of 400 mg/kg treated group revealed mild

lymphocytic infiltration in periportal region and amongst the hepatic parenchyma, along with evidence of regeneration (Fig. 1).



Normal[x100] **Toxicant [x40]** **Silymarin [x100]**



MEEV (200mg)[x100] **MEEV (400mg)[x100]**

Fig 1: Histopathology of liver samples

DISCUSSION

Preliminary phytochemical investigation of different extracts was conducted to obtain the information about presence of various phytoconstituents and methanolic extract found to contain Carbohydrates, alkaloids, flavonoids, phenolic compounds and tannins. Alkaloids, flavonoids and saponins known to posses hepatoprotective activity [15] and hence, the methanolic extract was selected for the study.

The liver is major organ involved in various metabolic functions and detoxification of hazardous substances. Liver diseases remain as one of the major health problems and no satisfactory allopathic drug for the treatment is available so for. Herbal drugs play a major role in the management of various liver disorders in addition to other healing processes of the liver [16]. Paracetamol is one of the well known analgesic and antipyretic drug, but overdose of the same known to produce centrilobular hepatic necrosis. More than 90% of the Paracetamol is excreted after undergoing glucoronidation/sulfation and a small amount undergoes metabolism by cytochrome P450 enzyme to form reactive intermediate N-acetyl-P-benzoquinone imine (NAPQI). This intermediate is readily detoxified by GSH, but saturation of glucoronidation/sulfation takes place when paracetamol is administered at higher doses. This leads to excessive production of NAPQI, which in turn deplete the GSH completely and binds to proteins to form adducts. These adduct cause impairment in the function of cellular proteins [17, 18]. SGPT and SGOT catalyze the inter conversion of amino acids and α-keto acids by the transfer of an amino group. These enzymes are very sensitive and are reliable indices for hepatoprotective or curative effects of various compounds [19]. Alkaline phosphatase (ALP) is produced by bone, liver, intestine, placenta and is also excreted in the bile. In the absence of bone disease and pregnancy, there is an elevated serum ALP levels due to increased production of ALP by hepatic parenchymal or duct cells [20]. Bilirubin, a metabolic product of the breakdown of heme rises in diseases of hepatocytes, obstruction to biliary excretion into duodenum or in hemolysis [21]. Elevated levels of SGPT, SGOT, ALP and bilirubin were observed in positive control group and were reduced significantly in all drug treated groups. Liver cells synthesize various proteins like albumin, fibrinogen, haptoglobin, transferrin and antitrypsin. The blood levels of these proteins are decreased in extensive liver damage [22]. Serum proteins levels were found to decrease in positive control group which was reversed in extract treated group. Serum enzyme levels are not a direct measure

of hepatic injury, but elevated levels are indicative of cellular leakage and loss of integrity of cell membrane. Thus lowering of enzyme content in serum is a definite indication of hepatoprotection of the drug. The results were further supported by histopathological studies substantiating the use of roots of *Ecbolium viride* (Forssk.) Alston as a potential hepatoprotective drug.

CONCLUSION

The results obtained in the present study indicated that the methanolic extract of *Ecbolium viride* (Forssk) Alston posse's significant hepatoprotective activity and histopathological studies further support the activity. The activity may be due to the presence of phytoconstituents like flavonoids, alkaloids and phenolic compounds.

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