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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF ACONITUM HETEROPHYLLUM ROOT IN PARACETAMOL INDUCED LIVER TOXICITY

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ABSTRACT

The goal of the study was to evaluate the hepatoprotective activity of ethanolic extract of Aconitum heterophyllum root in paracetamol induced hepatic damage in Wistar albino rats. Thirty six albino rats weighing 150-200gr were selected and divided into six groups with 6 in each group. Paracetamol (3gr/kg) was given to induce hepatotoxicity in the animals group II, III IV & V. N-acetyl cysteine (100mg/kg) was given as standard drug in group III. Group I given with normal saline. Group II given with paracetamol (3gm/kg) to induce hepatotoxicity. Group III was given paracetamol and standard drug N-Acetylcysteine (100mg/kg). Group IV and group V were given paracetamol and Aconitum heterophyllum (225mg/kg) and 450mg/kg respectively. Group VI was given plant extract root alone 450mg/kg. The hepatoprotective activity of ethanolic extract of Aconitum heterophyllum root was evaluated by the assessment of biochemical parameters such as serum glutamic oxaloacetic transaminases (SGOT), serum glutamic pyruvic transaminases (SGPT), alkaline phosphatise (ALP), total bilirubin, serum protein, and histopathological studies of the liver. Ethanolic extract of Aconitum heterophyllum root significantly reduced the liver damage and all biochemical parameters. The 450mg/kg extract showed greater response than 225mg/kg. This confirms the activity of ethanolic extract of Aconitum heterophyllum root has hepatoprotective activity.

KEY WORDS: - Hepatoprotective, Paracetamol, Aconitum heterophyllum root, N-Acetylcysteine

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INTRODUCTION

Liver disease is still a worldwide health problem. Liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds, any injury to it or impairment to its functions may lead to many implications on one's health. Hepatic dysfunction due to inhalation of hepatotoxin is increasing worldwide [1, 2] among the various mechanisms involved in the hepatotoxicity by hepatotoxin, one is oxidative damage through free radical generation [3, 4]. Management of liver disease is still a challenge to the modern medicine. Conventional medicine is now pursuing the use of natural products such as herbs to provide the support that the liver needs on a daily basis [5]. The physiological activity of the liver results in the generation of highly reactive free radicals which covalently bonds with membrane lipids causing lipid peroxidation. Lipid peroxidation alters the membrane permeability and causes tissue damage. Since the liver is involved in various biochemical reactions, the liver cells are prone to attack and necrosis by the free radicals [6]. In addition, these radicals have been implicated as important pathological mediators in many clinical disorders such as heart disease, diabetes, gout and cancer [7]. However, inbuilt antioxidant systems such as superoxide dismutase (SOD), tissue glutathione (GSH) etc. protect the tissues from free radical attack. Excessive release of reactive oxygen species overcome this system resulting in organ damage. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects [8]. In the absence of a reliable liver protective drug in modern medicine there are number of medicinal plant preparations in recommended for the treatment of liver disorders in Ayurveda [9].

These limitations create a need for alternative treatment for hepatotoxicity such as medicinal plants and plant based formulations. Among the medicinal plants Aconitum heterophyllum root is the most important one which is widely distributed in Asian countries has more medicinal values. The genus Aconitum, belonging to the family Rannunculaceae, is widely distributed in the alpine and subalpine regions. The plants are usually perennial or biennial herbs, often with stout leafy stems, bulbs or creeping rhizomes. Leaves are mostly cauline, lobed, rarely divided and dentate. Flowers are simple or branched recemes. It comprises of over 300 species, including some ornamental and medicinal plants[10]. The roots of the Indian plant Aconitum heterophyllum, or atis, have long been known to contain an alkaloid, atisine which belongs in the category of simpler aconite alkaloids or alkamines of low toxicity. The possibility of a structural relationship between this group of alkaloids and the more complicated highly toxic aconitine group makes a study of their chemistry of particular importance[11].

MATERIALS AND METHODS

Animals

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Chettinad Hospitals and Research Institute in its meeting dated on 24.01.13. Adult male Swiss albino mice weighing 25-35 g from our breeding stock were used in this study. The animals were housed at 24±2°C with 12:12 h light and dark cycle. They had free access to food and water. The animals were acclimatized for a period of 7 days before the study. The animals were used according to the CPCSEA guidelines for the use and care of experimental animals.

Chemicals

Paracetamol 500mg tablet, N-Acetyl cysteine 100mg tablet were purchased from our chettinad pharmacy.

Preparation of the extract

Aconitum heterophyllum root authenticated by Dr. Narasimhan Professor of Botany, Madras Christian College, Chennai The ethanolic extract of the roots of Aconitum heterophyllum was prepared in accordance to previously described standard extraction procedure[12]. The collected roots were air dried under shade

at room temperature and milled to a coarse powder. The obtained dried powder was subjected to continuous extraction with 80% ethanol in a Soxhlet apparatus. The powdered root material was packed in a tumble made of Whatmann's filter paper. It was extracted with ethanol for 20 cycles. The extract thus obtained was concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature. The yield of ethanolic extract of roots of A. heterophyllum was 15%. The obtained residue was green colour thick and sticky paste. The extract was stored in the refrigerator and reconstituted in gum acacia before administration to animals.

Study design

In this study six groups of rats, each group with six rats (n = 6) were selected. Hepatotoxicity was induced by administration of Paracetamol at the dose of 3gm/kg for 3 days [13]. The experimental groups were as follows Group - I (control) was given with normal saline 10ml/kg. Group - II (hepatotoxic drug control) was given with paracetamol once daily. Group - III were given with standard hepatoprotective drug N-acetyl cysteine (100mg/kg) and paracetamol. Group IV and group V were given paracetamol and Aconitum heterophyllum (225mg/kg) and 450mg/kg respectively. Group VI was given plant extract root alone 450mg/kg. Paracetamol and N-acetyl cysteine given by intra peritoneal. Normal saline and plant extract given by oral route. All dosages given once daily for two days. And the third day we collected blood for biochemical analysis and sacrificed all animals for histopathological study.

Assessment of liver function

Blood was collected from all the groups by puncturing the retro-orbital plexus and was allowed to clot at room temperature and serum was separated by centrifugation at 2500rpm for 10 min. The serum was used for estimation of biochemical parameters to determine the functional state of the liver. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were estimated by a UV kinetic method based on the reference method of International federation of Clinical Chemistry in which both SGOT and SGPT were assayed based on enzyme-coupled system; where keto acid formed by the aminotransferase reacts in a system using NADH. The coenzyme is oxidized to NAD and the decrease in absorbance at 340 nm is measured. For SGOT malated dehydrogenase is used to reduce oxaloacetate to malate where as for SGPT the pyruvated formed in the reaction is converted to lactate by lactate dehydrogenase. Alkaline phosphatase (ALP) was estimated by method described by involving hydrolysis of p-nitrophenol which gives strong yellow colour in alkaline solution [14]. The increase in absorbance due to its formation is directly proportional to ALP activity; while total bilirubin (TBL) by which ivolves the reaction of bilirubin with diazotized sulphanilic acid to form an azo ompound, the colour of which is measured at 546 nm [15].

Histopathological studies

The animals were sacrificed and the abdomen was cut open to remove the liver. The liver was fixed in Boucin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12h, then embeded in paraffin using conventional methods.10 and cut into 5µm thick sections and stained using haematoxylin-eosin dye and finally mounted in di-phenyl xylene. The sections were then observed under microscope for histopathological study.

Statistical analysis

The data was represented as mean ± SEM. Results were analysed by one-way ANOVA followed by Dunnett's multiple comparison tests using SPSS software. The minimum level of significance was set at $p < 0.05$.

RESULTS

The results of paracetamol induced hepatotoxicity were shown in below the table. In the toxic dose of paracetamol group, the significant acute hepato cellular damage, and biliary obstruction was indicated by the elevated level of SGPT, SGOT, ALP, TBL and serum protein. But the group which received the test drug of ethanolic extract at the dose of

450mg/kg body weight per oral showed a significant decrease in the elevated levels of these biochemical parameters are comparable with the standard N-acetyl cysteine hepatoprotective drug. Therefore, the N-acetyl cysteine and the ethanolic extract restored the altered level of enzymes significantly. Histopathological liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and central vein shown in figure 1, 2, 3, 4, 5& 6. Disarrangement of normal hepatic cells with necrosis and vacuolization are observed in toxic dose of paracetamol intoxicated liver.

Table 1
Effect of ethanolic extract of aconitum heterophyllum Root on paracetamol-induced hepatotoxicity

S. No	Received	SGOT (IU/L)	SGPT (IU/L)	ALP (mg/dl)	Total Bilirubin (mg/dl)	Serum Protein(mg /ml)
Group-I	Control (Normal saline 10ml/kg)	39.61±0.59	45.16 ± 1.25	160 ± 3.79	0.70 ± 0.03	6.31±0.04
Group-II	Paracetamol(3gr/kg)	341 ± 3.8	217.30 ± 4.5	191.50 ±7.5	0.87 ± 0.07	5.60±0.70
Group-III	Paracetamol+ N-Acetyl cysteine	43.24 ± 0.30	42.64 ± 0.33*	181.60± 0.52*	0.50± 0.01*	4.67±.27
Group -IV	Paracetamol + Plant Extract(225mg/kg)	151.35 ± 0.40*	131.95± 0.46*	187.30± 0.31*	0.83± 0.01*	4.81±0.30
Group-V	Paracetamol + Plant Extract(450mg/kg)	127.36 ± 0.42*	115.30± 1.16*	185.20± 0.30*	0.51± 0.10*	5.90±0.30
Group-VI	Plant extract alone(450mg/kg)	39.56±0.57	45.12±1.23	160±3.65	0.72±0.04	6.23±0.05

Values are expressed as Mean ± SEM (n = 6). *P < 0.05.

Histopathology study

Figure-1 Control
The control group animals showed the typical architecture of liver tissue with a central vein (CV)

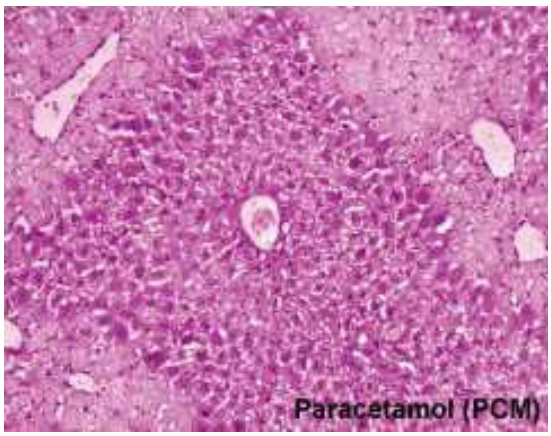

Figure -2 Paracetamo (PCM)l
The dose of Paracetamol (3gr/kg) treatment produced extensive necrosis of hepatocytes which was more pronounced in the centrizonal area.

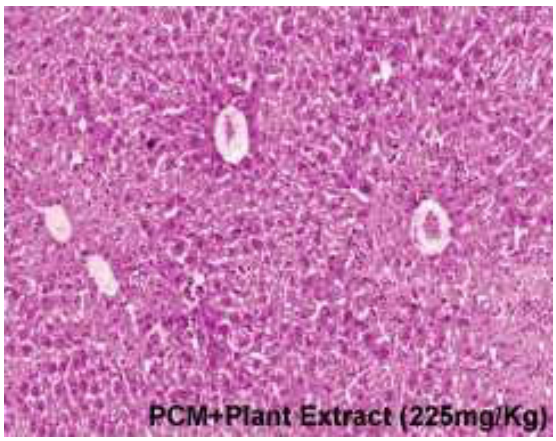

Figure – 3 PCM+ Plant Extract (225mg/kg)
The dose of 225mg/kg of extract showed partial hepatic protection with reduction in the extent of hepatic necrotic areas, fatty infiltration, and mild portal inflammation.

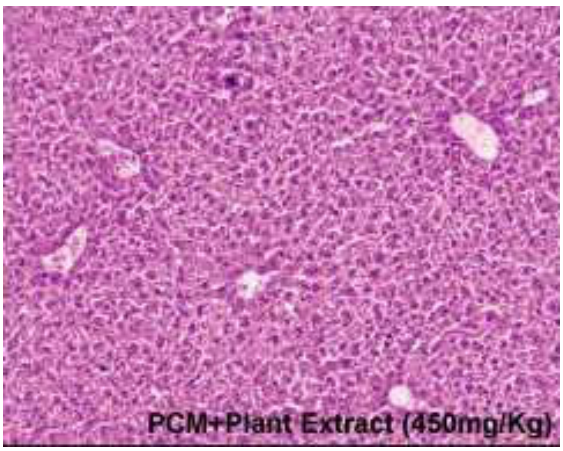

Figure – 4 PCM + Plant Extract (450 mg/kg)
The dose of 450 mg/kg of plant extract protected the liver as evidenced by determination of a normal histoarchitecture of the liver similar

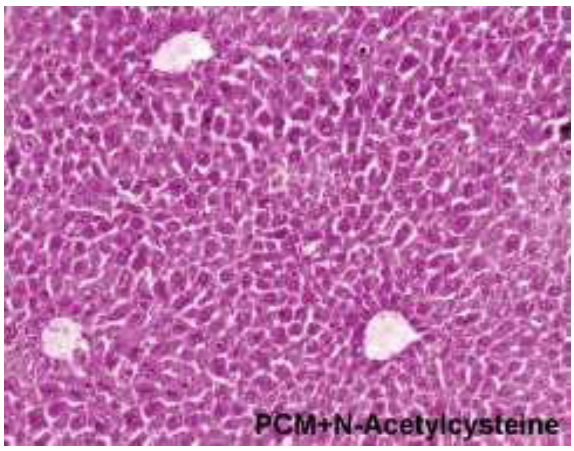

Figure -5 PCM + N-Acetylcysteine
The doses of paracetamol 3gr/kg and N-acetyl cysteine(100mg/kg) Showed normal histoarchitecture of the liver

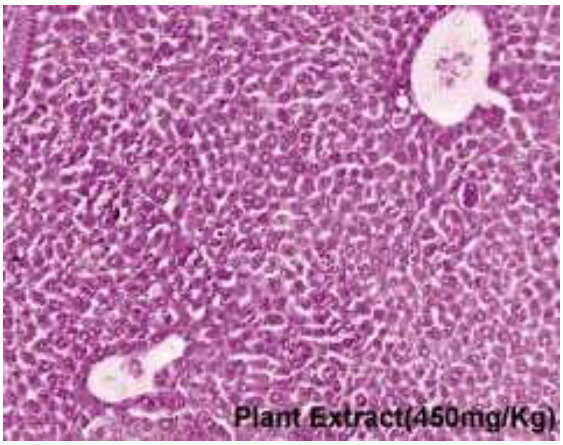

Figure – 6 Plant extract Alone (450mg/kg)
The dose of 450mg/kg of the plant extract alone showed prominent Central vein (CV) and chords of hepatocytes radiating

DISCUSSION

Hepatocytes are the main component that regulates various metabolic activities of liver. Distortion of this organ will result in disorder of body metabolism [16, 17]. Pracetamol induced liver toxicity model is a universally established model to study the potential hepatoprotective activity of extracts/compounds [18]. It is a very good antipyretic and analgesic, it is available over-the-counter. An accidental, suicidal, homicidal over dosage administration of paracetamol can result in hepatic damage [19, 20]. N-acetyl-p-benzoquinone imine (NAPQI), which is one of the metabolites of paracetamol after the latter undergoes metabolism in the liver via the action of cytochrome P450 (cyP450) monooxygenase, Several CYP450 enzymes have been known to participate in the bioactivation of NAPQI [15, 17.18]. NAPQI is normally conjugated with glutathione (GSH) and excreted in urine. NAPQI is normally conjugated with glutathione (GSH) and excreted in urine[20, 21, 22]. GSH has been highlighted to be responsible in the antioxidant defense of our body by scavenging the free radicals produced through the metabolism processes within the liver in order to prevent any subsequent cell damage [23] . Damage induced in the liver is accompanied by increase in the activity of some serum enzymes in the hepatoprotective action of

plant extract 450mg/kg was substantiated by significant attenuation of the increased level of serum enzymes in rats intoxicated with the paracetamol. Aconitum heterophyllum has several medicinal properties it is used for the treatment of diseases of nervous system, digestive system, fever and rheumatism [24]. It is found throughout the world and cultivated in tropic fields. Being rich in substances having potential biological significance, such as benzoylmesaconine, mesaconitine, aconitine, hypaconitine, heteratisine, heterophyllisine, heterophylline, heterophyllidine, atidine, isotisine, hetidine, hetisinone and benzoylheteratisine and other nutrients [25]. The plant has been reported to possess antifungal activity, cytotoxic [26], antiviral [27], immune-stimulant properties [28] and other compounds isolated from A. Heterophyllum include flavonoids, tannins, saponins and sugars [29]. Considering the traditional uses and demonstrated potential medicinal properties of Aconitum heterophyllum, present study was undertaken to investigate its hepatoprotective activity potential in paracetamol induced liver toxicity in rats.

CONCLUSION

In conclusion our present study confirmed that the ethanolic extract of Aconitum heterophyllum root has the hepatoprotective activity and this activity may be due to the antioxidants and other components.

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