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Evaluation of hepatoprotective activity of ethanolic extract of *Aquilaria agallocha* leaves (EEAA) against CCl_4 induced hepatic damage in rat

Kamala Vakati[1], Habibur Rahman*[2], M. Chinna Eswaraiah[1], A.M. Dutta[3]
[1]Department of Pharmacology, Anurag Pharmacy College, Ananthagiri(V), Kodad(M), Nalgonda(dist.), Andhra Pradesh.
[2]Research Scholars, Asaam Down Town University, Guwahati, Assam and Asst. professor, Department of Pharmacology, Anurag Pharmacy College, Ananthagiri (V), Kodad(M), Nalgonda(dist.), Andhra Pradesh, India.
[3] Professor, Asaam Down Town University, Guwahati, Assam

Corresponding author
Habibur Rahman
Email: habiburruh@gmail.com

Abstract – The present study was carried to evaluate the hepato-protective activity of ethanolic extract of *Aquilaria agallocha* leaves (EEAA) against CCl4 induced hepatic damage in rat. The hepatoprotective activity was evaluated by estimated serum hepatic enzyme levels and hisopathological study of liver tissues of rats. Ethanolic extract of *Aquilaria agallocha* leaves (EEAA) at dose 200 mg/kg and 400mg/kg body weight were administered orally for 10 days in rats and compared with standard silymarin (100 mg/kg) orally. The results showed significant decrease in serum ALT, AST and ALP levels treated groups which were increased due to CCl_4 induced liver damage are comparable with standard drug. Histopathological study of liver tissue ravels the hepatoprotective activity of EEAA

Keywords – *Aquilaria agallocha*, CCl_4induced liver damage, serum enzyme levels, Histopathology.

INTRODUCTION

Agar wood (*Aquilaria agallocha* of family Thymelaeaceae) oil is extremely rare and precious oil available in North Eastern India, Bhutan and parts of South East Asia. The different extracts of the plant has reported to possess anti nociceptive [1], anti-microbial [2], lower hypersensitivity reactions [3], laxative [4], anti oxidant activity [5], CNS activity [6], sedative effect [7] and anti-hyperglycaemic activity [8].

The liver is the main drug metabolizing organ and performs many functions and target organ for toxic drug-induced lesions. The liver transforms and excretes many drugs and toxins. These substances are frequently converted into inactive form by reactions that occur in the hepatocytes. Transformations that occur in the liver that render many drugs water soluble and they readily excreted by the kidneys [9]. The physiological response to injury results such as necrosis, cholestasis, steatosis, inflammation and fibrosis.

Hepatitis is an autoimmune disorder, produce inflammation in the liver, leads to injury or destruction. In most common hepatitis cases (viral hepatitis), specific viruses incite the immune system to fight off infections. Specific immune factors become over-produced that cause injury. Hepatitis caused by drugs, alcohols, chemicals and environmental toxins. CCl_4 is chemical which induce hepatotoxicity through lipid peroxidation by its free radical derivative (CCl_3, CCl_3O_2). Excessive production of the reactive species manifests in tissuethiol depletion, lipid peroxidation, plasma membrane damage etc., culminating into severe hepatic injuryoxic [10].

Carbontetrachloride toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures. The toxic metabolite CCl_3 radical is produced by microsomal oxidase system binds covalently to the macromolecule and causes peroxidative degradation of lipid membrane of the adipose tissue. [11] Results of this hepatotoxicity increase the serum enzyme levels such as aspartate aminotransferase, alanine aminotransferase and alkaline phosphate.

Present study was conducted to evaluate the protective effect of ethanolic extract of *Aquilaria agallocha* leaves (EEAA) against CCl4 induced hepatic damage in rat.

MATERIAL AND METHODS

Collection and extraction of drug

The leaves of *Aquilaria agallocha* were collected in the month of October- November, 2011 from Nagaon Dist, Assam and authentified by Prof. Venkaiah, Dept.of botany, taxonomist, Andhra University. A voucher specimen was kept in department for reference. The leaves and were dried in shade at room temperature then subjected to size reduction to a fine powder with the help of electric grinder. The grinded plant material was subjected to Soxhlet extraction (45^0-55^0C) employing 95% Ethanol as solvent. Appearance of colourless solvent in the siphon tube was taken as the termination of extraction. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated.The percentage

yield of the extract was 19.56% w/w. The extract was kept in air tight container in a refrigerator below 10^{o}C.

Drugs and Chemicals

Silymarin (Allied FabriChem Private limited, Hyderabad) used as the standard hepatoprotective drug, Hexane and Carbon tetra chloride (Sd. Fine Chemicals, Mumbai) were obtained from from the institute store and are analytical grade. SGOT, SGPT and ALP enzyme kit (Span diagnostics limited, Surat) were purchased.

Animals

Rats of either sex weighing 150-200 g were used in experiment. Animals were obtained from Anurag Pharmacy College, Kodad. Animals were kept under standard conditions at 23-25^{0}C 12 hr light/dark cycle and given standard pellet diet and water. Before performing the experiment the ethical clearance was obtained from institutional animal ethics committee (IEAC). IAEC No.-177/99/CPCSEA.

Preliminary phytochemical tests

The ethanolic extract of *Aquilaria agallocha* leaves (EEAA) were tested for different phytoconsituents like alkaloids, glycosides, saponinins , tannins, protein, carbohydrates using standard procedures [14-15].

Acute oral toxicity studies

Acute oral toxicity study was carried out for ethanolic extract of *Aquilaria agallocha* leaves (EEAA) using Acute Toxic Class Method as described in OECD (Organization of Economic Co-operation and Development) Guidelines No. 423 in Female Wister rats.

Evaluation of Hepatoprotective activity

For evaluation of hepatoprotective activity of the first day, all animals were randomly divided into five groups of six animals each. Each group of animals were treated with respective vehicles or drugs for 10 days, after 30minutes post dose administration all groups(except group-1 normal) were received CCl_4 at the dose of 1.5ml/kg(1:1 v/v of CCl_4 in olive oil)orally to induced liver damage[16-17].

Group-I: Normal (2% Tween80,P.O) for 10days
Group-II: Control (2%Tween80, P.O for 10days) with CCl4 on 10thday
Group-III: EEAA (200 mg/Kg, P.O for 10days) with CCl4 on 10thday
Group-IV: EEAA (400mg/Kg, P.O for 10days) with CCl4 on 10thday
Group-V: Silymarin (100mg/kg, P.O for 10days) with CCl4 on 10thday

On 11th day (after 24 hr of CCl_4 administration), the blood samples were collected by retro-orbital puncture from each animal for estimation of hepatic enzyme levels. Blood samples were centrifuged for 15 mins at 3000rpm to separate the serum. Alkaline phosphates (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST) were estimated using standard kits.

Histopathological studies

On the 11th Day, after sacrification of rats by cervical dislocation, liver samples were dissected out and washed immediately with ice-cold saline to remove as much blood as possible. A portion of liver tissue in each group was preserved in 10% formaldehyde solution for histopathological studies. Haematoxylin and eosin were used for staining and later the microscopic slides of the liver tissue were photographed at magnification 40X.

Statistical Analysis

The statistical analysis was carried by one way ANOVA followed by Dunnet's multiple "t" test. P values < 0.05 (95% confidence limit) was considered statistically significant, using software Graph Pad Prism5.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Ethanolic extract of *Aquilaria agallocha* (EEAA) leaves were subjected for phytochemical screening and found EEAA of leaves to contain carbohydrates, flavonoids, glycosides, saponins, tannins and triterpines and Phenolic compounds.

Acute oral toxicity studies

Ethanolic extract of *Aquilaria agallocha leaves* (EEAA) was screened for toxicity by oral toxicity studies according to OECD guidelines 423 taking three female Wister rats with starting dose of 2000mg/kg body weight and found to be non-toxic i.e- Category 5 or Unclassified and two test dose level as low 200 mg/kg, and high 400 mg/kg selected for experiment.

Evaluation of Hepatoprotective activity

Hepatoprotective activity of Ethanolic extract of *Aquilaria agallocha leaves* (EEAA) were evaluated on carbon tetrachloride induced hepatotoxicity in rats by estimated serum hepatic enzyme levels. The results are given in Table-1.

Hepatic cells appear to participate in a variety of enzymatic metabolic activities and administration of carbon tetrachloride (CCl_4) damages hepatic cells and elevate serum level of AST, ALT, ALP and bilirubin significantly. There was significant ($p<0.001$) increase in hepatic enzyme levels were observed in control group and the drug treated animals with EEAA (200mg/kg) and EEAA(400mg/kg) showed reduction in serum enzyme levels and are comparable with standard silymarin.

The results of Histopathological studies of control rat liver treated with carbon tetrachloride exhibited severe necrosis with disappearance of hepatocytes, areas of inflammation and increased sinusoidal spaces. Liver

section of the rat treated with 200mg/kg of EEAA and carbon tetrachloride exhibited mild degree of necrosis, normalization of cells and reduced sinusoidal dilation. Liver section of the rat treated with 400mg/kg of EEAA and carbontetrachloride exhibited normalization of cells and reduced sinusoidal dilation along with mild inflammogens. Liver section of the rat treated with Silymarin and CCl_4 exhibited normal hepatocytes. The results are given in Fig-1(a), Fig-1(b), Fig-1(c) and Fig-1(d) respectably.

Table-1: Effect of Ethanolic extract of *Aquilaria agallocha leaves* (EEAA) on carbon tetrachloride induced hepatotoxicity in rats

Treated groups	Hepatic enzyme levels		
	AST (U/L)	ALT (U/L)	ALP (U/L)
Normal	223.2± 5.212	75.67± 3.556	65.33± 5.506
Control(CCl4)	300.3±5.783[a]***	252.0±5.842 [a] ***	111.5± 4.217 [a] ***
EEAA 200mg	250.77±4.00 [b] ***	91.34±3.14 [b] ***	95.12±3.20 [b]**
EEAA 400 mg	234.2±5.718 [b] ***	215.3±4.394 [b] ***	82.67± 6.761 [b] ***
Silymarin 100mg	235.5±4.836 [b] ***	86.7±4.807 [b] ***	75.33± 5.481 [b] ***

Values are in Mean ± S.E.M (n=6), [ns] -Non Significant, *p<0.05, **p<0.01, ***p<0.001 [a] Control compared with Normal, [b] All test groups compared with Control using One way ANOVA followed by Dunnet's "t" test.

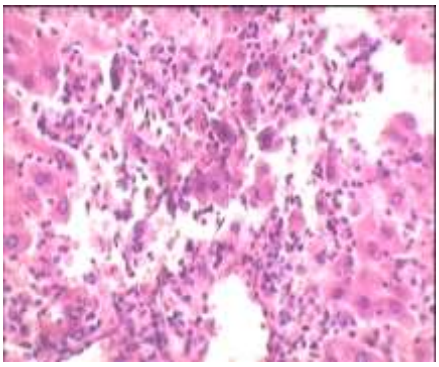

Fig-1 (a): Control group-treated with CCl_4 exhibited severe necrosis with disappearance of hepatocytes, areas of inflammation and increased sinusoidal spaces.

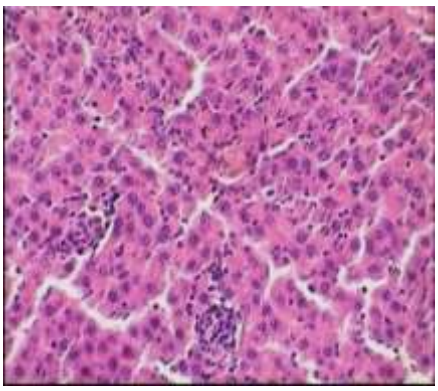

Fig-1 (b): EEAA 200 mg/kg- exhibited mild degree of necrosis, normalization of cells and reduced sinusoidal dilation.

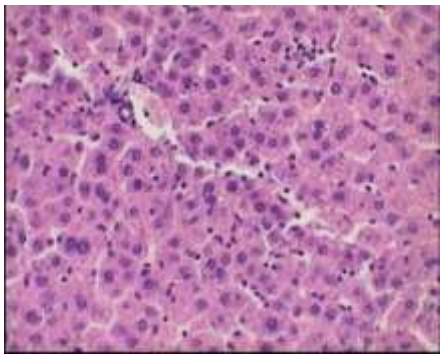

Fig-1 (c): EEAA 4000mg/kg- exhibited normalization of cells and reduced sinusoidal dilation along with mild inflammogens.

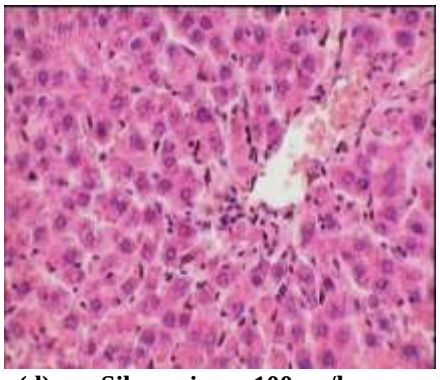

Fig-1 (d): Silymarin 100mg/kg- exhibited normalization of cells and almost exhibited normal hepatocytes.

CONCLUSION

Present study conclude that Ethanolic extract of *Aquilaria agallocha leaves* (EEAA) posses hepatoprotective activity and presence of phytoconstituents like, flavanoids, terpernoids and phenolic compounds.

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