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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *ACACIA CATECHU*

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ABSTRACT

The main chemical constituents of Black catechu are flavonoids (catechin, (-) epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, rocatechin, phloroglucinol, procatechuic acid, catecutannic acid, quercetin, quercitrin), alkaloids (kaempferol, dihydrokaempferol, taxifolin, (+) -afzelchin gum), glycosides (poriferasterol, poriferasterol acylglucosides), tannins (gallic acid, phlobatannins), sugars (d-galactose, d-rhamnose and larabinose). Acacia catechu wild has been shown to possess multifarious medicinal properties such anti-bacterial, anticancer, anti-diarrheal, anti-inflammatory, antimicrobial, antioxidant, antipyretic, anti-ulcer, antisecretory, hepatoprotective, hypoglycemic, sore throat and wound healing etc. The present review article provides up-to-date information on the medicinal properties of the plant. We have also tabulated the phytochemical constituents of Acacia catechu wild. We hope this literary criticism can facilitate the scientists operating within the space of ancient medicines and medicative food in their future endeavor.

KEYWORDS

Acacia catechu wild, Phytoconstituents and Hepatoprotective activity.

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INTRODUCTON

Catechu (Katha) is a vital product from acacia (Leguminosae) that contains a combination of many catechins, catechutannic acid, quercetin etc.[1,2]. Katha is used as an astringent, digestive, antipyretic, antidiarrheal and anthelmintic in indigenous medicine[3]. Cyanidanol (+), the active principle in Acacia Catechu is reported to be effective in liver disease 4. An *in vivo* method for hepatoprotective activity study was employed using CCl4 as Hepatotoxin. CCl4, is reported to produce free radicals which affect the cellular permeability of hepatocytes, leading to elevated levels of serum biochemical parameters like serum glutamate

pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), serum alkaline phosphatase, bilirubin etc. It causes massive histopathological changes in the experimental dose like necrosis, congested vessels, multifocal area of fatty changes, nuclear disintegration, sinusoidal dilation, kupffer cell hyperplasia etc. The reverse of these phenomenon can be considered as the index of hepatoprotective activity. The ethyl acetate extract of 'Katha' has been taken up in the present study to screen hepatoprotective agents from simple plant extract.

MATERIAL AND METHODS

Animals

healthy albino rats (*Rattus norvegicusus*) 150-200gm of wister strain were given to standard diet with water ad libitium during the entire period of the experiments as per the recommendation of the central committee for the purpose of control and supervision of the experiments on animals Regd-1146/AC/07/CPCSEA for laboratory animals facilities.

Drugs

All drugs suspension were prepared for the different groups with 3 % (W/V) aqueous suspension of gum acacia as vehicles.

Test Drug

Acacia catechu alcoholic leaf extract (ASE). This was prepared as follows: One kilogram of fresh Acacia catechu heart wood, was collected and washed thoroughly with cold water, dried in the shade at room temperature and, thereafter, crushed in and electrical mixer-grinder. Hundred grams of this air-dried powder of the heart wood was soaked in 90% ethyl alcohol and was allowed to stand for 15min in a tightly covered container. The soaked powder was then transferred to a percolator, where it was firmly packed in and allowed to macerate for 24 h, at room temperature, followed by slow percolation. The procedure was repeated over the next 24 h, with sufficient amount of 90% alcohol until no further extraction was possible. Alcohol was evaporated to a soft extract and the residue was transferred to a vacuum desiccator, thus, obtaining the dried leaf alcoholic extract of Acacia catechu[103]. We got 5g of a dark greenish-black and sticky extract (5% dry weight of powdered heart wood). The ASE suspension was used in doses of 200mg/kg BW and 100mg/kg BW for the respective groups as per previous studies on other models of hepatoxicity[100,102].

Standard hepatoprotective

Slilymarin (SILY) powder (obtained from Micro Labs Ltd, Bangalore, India) used to make the suspension in doses of 100mg/kg BW and 50mg/kg BW for the respective groups following the method of Mankani *et al*, and Mansour *et al*[104,105].

Hepatotoxin

Paracetamol (PCM) powder (I.P) (obtained from Bharat chemicals. Tarapur, Gujarat, India) was used to make the suspension in dose of 2g/kg BW for the respective groups.

Methods

The experiment was carried out on 30 healthy albino for 24 days, before starting the experiment, the animals were allowed to acclimatize to the laboratory environment for 1 week.

Grouping and treatment schedule

The rats were randomly divided into five groups of six animals each after weighing, recording and numbering. Each group received treatment as follows.

DOSING AND ADMINISTRATION OF DRUGS

The drug suspension and the vehicle were administered per orally by an intragastric feeding tube at a uniform volume of 5ml/kg BW.

INDUCTION OF HEPATIC INJURY

A single dose of 2g/kg BW/day was given to groups B, C, D and E on the eight day of the experiment. It was administered after overnight fasting of the animals, i.e. the diet was restricted 12 h prior to the administration of paracetamol. However, free access to water was permitted[106].

LABORATORY ASSESSMENTS

On the 24th day, blood was collected from the hearts of the animals under light ether anesthesia, the blood was kept undisturbed for 30 min and the clot was dispersed with a glass rod. The samples were centrifuged for 15 to 20 min at 200 rpm to separate the serum and then sent for liver function tests (LFT), namely total serum protein, albumin

globulin ratio, alkaline phoshates (ALP), asparate aminotransferase (AST) and alanine aminotransferase (ALT)[107-109].

STATISTICAL ANALYSIS

The results, obtained from the LFT were presented as mean and standard error of mean (SEM) for each (mean+-SEM). All groups were subjected to one way analysis of variance (ANOVA), which was followed by Bonferoni's test to determine the intergroup variability. A comparison was made with the experimental control (paracetamol) group and with the standard (silymarin). We took a P value of <0.01(highly significant) as our desired level of significance.

RESULTS AND DISCUSSION

The LFT results are summarized (Table No.1) and expressed as mean =- SEM (n=6). The histopathological examination (HPE) of group A livers showed a normal arrangement of the hepatocytes, with clearly visible nuclei, central vein and portal triad. We observed areas of congestion of sinusoids, cloudy welling, and congestion of central vein, centrilobular fatty changes and necrosis of hepatocytes in all animals of group B (Figure No.1) b. In group C, D and E, there was marked reduction in sinusoidal congestion, cloudy swelling and fatty changes, with areas of regeneration as well. (Table No.2) Effect of the alcoholic leaf extract of *Acacia catechu* aminotransferase and alanine aminotransferase in paracetamol induced hepatoxicity in albino rats (24^{th} day of the experiment).

Result are expressed mean +_ SEM (n=6) compared with the PCM group $p^{\#}<0.01$ compared with silymarine group; P<0.01 obtained is highly significant AST, separate Aminotransferase; ALT, aline aminotransferase; ALP; alkaline phosphate, PCM, paracetamol; ASE, osmium sanctum extract, SILLY, silymarine.

Figure No.2. Photomicrographs of rat liver (hematoxylin and eosin) under low power (x100). (A) Shows normal hepatic architecture; (B) shows hepatic necrosis; (C, D and E) shows varying degree of hepatic regeneration.

The administration of PCM to the animals resulted in a significant fall in the levels of total serum proteins and albumin globulin ratio and a significant rise in serum ALP, AST and ALT. In groups C, D and E, the toxic effect of paracetamol was partly reversed in the animals. Compared with the PCM (experimental control) group, significant decrease in the serum ALP, AST and ALT levels. However, no significant difference was observed in the total protein levels in these groups. Group E in comparison with silymarin (standard) showed a significant decrease in the serum AST and ALT alone. Thus, group C showed greater hepatoprotection than group E, considering the results of the LFT alone, Histology of the control group showed normal hepatic architecture. The group B animals exhibited with PCM and ASE (group C), PCM and silymarin (group D) and PCM, ASE and silymarin (group E) c-e, respectively appreciable protection of hepatic tissue from PCM. PCM, used as a tool to induce hepatoxicity in experimental animals, leads to covalent bonding of its toxic metabolite N-acetyl P bezoquineimine to sulfhydryl groups of proteins. This causes exhaustion of reduced glutathione in the liver, resulting in cell necrosis and lipid peroxidation[110]. An increase in the level of transaminase and ALP is and indication of cellular leakage and loss of functional integrity of the hepatic cell membrane[111-113]. Administration of the alcoholic extract of acacia catechu heart wood showed significant hepatoprotective activity, as shown previously in other studies[114]. Synergistic hepatoprotective activity was seen with the ASE+SILLY group. But, the ASE and ASE+SILLY combination showed lesser efficacy than SILLY alone. Eugenol, flavonoid and ursolic acid components, present in Acacia catechu heart wood, have free radical scavenging and anti-lipoperoxidative affects. Therefore, the hepatoprotective effects of Acacia catechu heart wood may be due to the antioxidant properties of its constituents[115]. The membrane stabilizing property of *Acacia catechu* is responsible for its hepatoprotective action. Moreover, the fixed oil of *Acacia catechu* contain linoleic acid, which is responsible for its anti-inflammatory activity[117]. Hence, linoleic acid may also be responsible for reversing the inflammatory features associated with hepatic injury thus adding to the hepatoprotective effect.

TableNo.1: Liver function tests (LFT)

Group A	24 Days	Normal control	5ml vehicle/kg BW/day
Group B	24 Days	Experiment control(PCM)	5mi vehicle/kg BW/day
Group C	24 Days	Test drug	200mg ASE in 5ml vehicle/kg BW/day
Group D	24 Days	Standard drug	100mg SILLY 5ml
Group E	24 Days	Test + Standard (ASE+SILY)	100mg ASE+50 mg SILLY on 5ml vehicle/kgBW/day

Table No.2: Effect of the alcoholic leaf extract of *Acacia catechu*

S.No	Groups	Total proteins(g/d)	Albumin globulin ratio	Serum ALP (KA units)	Serum AST(IU/L)	Serum ALT(IU/L)
1	A(control)	6.7 ±0.26	1.5 ±0.02	8.0 ±1.41	38 ±0.58	34 ±0.41
2	B(PCM)	5.3 ±0.21	0.1 ±0.82	20 ±1.32	750 ±4.62	523 ±17.40
3	C(PCM + ASE 200mg)	5.6 ±0.21	1.0 ±0.014$^{*\#}$	15 ±1.34$^{\#}$	272 ±30.20$^{\#}$	82 ±9.59
4	D(PCM+SILLY100mg)	6.2 ±0.18	1.3 ±0.07	12 ±1.61	97 ±2.74	41 ±6.06
5	E(PCM+ASE100mg+SILLY50mg)	5.9 ±0.18	1.2 ±0.13	1.3 ±1.26	229 ±24.38	70 ±7.94

CONCLUSION

Thus, the heart wood of Black catechu (Acacia catechu) have highly significant ($p<0.01$) hepatoprotective activity. When concurrently administered, Acacia catechu heart wood and silymarin have a highly significant ($p<0.01$) synergistic hepatoprotective activity. The acacia catechu group showed better hepatoprotection than the acacia catechu leaf extract alone and in combination with silymarin showed lesser hepatoprotective effect than silymarin alone. Silymarin is a well-known standard hepatoprotective, whereas presence of impurities in the *Acacia catechu* extract may have caused a lower hepatoprotective effect. Moreover, we used lower doses of Acacia catechu (100mg/kg) and standard hepatoprotective silymarin (50 mg/kg) in the combination group (Acacia catechu extract and silymarin) than in the silymarin group alone.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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