Antipyretic, anti diarrhoeal, hypoglycaemic and hepatoprotective activities of ethyl acetate extract of *Acacia catechu* Willd. in albino rats

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**ABSTRACT**

**Objective:** To evaluate the antipyretic, anti diarrhoeal, hypoglycaemic and hepatoprotective effects of the ethyl acetate extract of *Acacia catechu* in experimental animal models.

**Materials and Methods:** Ethyl acetate extract of *Acacia catechu* was evaluated for antipyretic activity in yeast induced pyrexia and for anti diarrhoeal activity in castor oil induced diarrhoea in albino rats. Hypoglycaemic activity was studied in both normal and alloxan (120 mg/kg, s.c.) induced diabetic albino rats. The hepatoprotective potential of *Acacia catechu* was evaluated by CCl₄-induced hepatotoxicity in albino rats.

**Results:** Single administration of the ethyl acetate extract of *Acacia catechu* at doses of 250 and 500 mg/kg, p.o. showed significant antipyretic activity (*P<0.01*) in albino rats. *Acacia catechu* at a dose of 250 mg/kg, p.o., (single dose) has been found to possess highly significant anti diarrhoeal property (*P<0.001*) in respect of latent period of onset of diarrhoea, average number of stools passed and purging index. Significant reduction of blood glucose level was observed in nondiabetic albino rats following single dose treatment with the test drug at a dose of 500 mg/kg, p.o. (*P<0.01*). Significant reduction of blood glucose level was also evident in diabetic rats at doses of 250 and 500 mg/kg (*P<0.001*). Highly significant hepatoprotective activity was also observed when the extract of *Acacia catechu* (250 mg/kg) was administered prophylactically for seven days (*P<0.001*).

**Conclusion:** The present study shows that ethyl acetate extract of *Acacia catechu* (cutch/ catechu) has significant antipyretic, anti diarrhoeal, hypoglycaemic and hepatoprotective properties.

KEY WORDS: Antidiabetic, cutch, pyrexia, liver.

**Introduction**

The diverse culture of our country is a rich source of traditional medicines, many of which are of plant origin. Scientific data on such plant derivatives could be of clinical use.¹³ Catechu or cutch (Katha in Hindi and Manipuri), the extract prepared from the hard wood of *Acacia catechu*, has been used for treating fever, diarrhoea, leucorrhoea, piles and erysipelas.¹³ The juice of its fresh bark has been used in treatment of haemoptysis and gonorrhoea. Catechuic acid found in cavities of the wood of the *Acacia catechu* tree (Leguminosae) was valued for facilitating expectoration in chest infection.¹⁵ Catechu contains catechuic acid, catechuttannic acid (25%–33%), acacatechin (10%–12%), catechu red, quercetin, catechin (2%–12%), epicatechin, phlebotanin (25%–33%), gummy matter, quercitrin, quercitrin and moisture. Quercitrin is a phenolic flavonoid and catechu of acacia is a pseudotannin. Catechu and epicatechin usually accompany other flavonoids.¹³, ¹⁴

It is reported that *Acacia catechu* has hypoglycaemic activity⁶ and catechu, a product of *Acacia catechu*, has hepatoprotective, antipyretic and digestive properties.⁶,⁷ Cyanidanol, an active principle of *Acacia catechu*, is claimed to be effective in treating liver diseases.⁶ Catechu was used in the treatment of diarrhoea and throat infection⁹ because the tannin and polyphenols present in it impart astringent activity. Considering the above facts, the present study was undertaken to evaluate the antipyretic, anti diarrhoeal, hepatoprotective and hypoglycaemic properties of *Acacia catechu* in experimental animal models.

**Materials and Methods**

Collection and preparation of extract

Catechu (250 g) was collected (from the local market during the month of October), identified and differentiated from *Uncaria gambier* by gambier fluorescin test.¹⁰,¹¹
The ethyl acetate extract of *Acacia catechu* was obtained by the procedure as described by Jayasekhar et al.\[6\] and Seikel.\[12]\ The powdered catechu was defatted with a nonpolar organic solvent such as petroleum ether (40°C–60°C) to remove the phenolic and nonpolar substances from the dried material. After defatting, catechu was extracted with 95% ethanol. Then the dried ethanol extract was again extracted with ethyl acetate to concentrate the minute amounts of phenolic materials present. The yield at the end of extraction was 29.2%.

**Phytochemical studies**

Tests for presence of flavonoid compounds in the catechu were done according to the methods described by Kokate\[11\] and Seikel.\[12\] However, identification and estimation of different flavonoid compounds like catechin, quercetin and cyanidol were not done.

**Animals**

Colony bred, healthy, Wistar strain, albino rats of either sex weighing 150–200 g were used for the study. They were housed in standard polypropylene cages, under room temperature (24±2°C); relative humidity (60%–70%) and exposed to 12:12 h light:dark cycle. The rats were fed with standard diet and water *ad libitum*. The ethics committee, Regional Institute of Medical Sciences, Imphal, approved the protocol of the present study.

**Drugs**

The following chemicals (analar/GR) and drugs were used: gum acacia, castor oil, tablet Lomotil (diphenoxylate 2.5 mg + atropine sulphate 0.025 mg per tablet), acetyl salicylic acid, dried yeast, carbon tetrachloride (Qualizen), olive oil, silymarin (Micro Lab), glibenclamide (Aventis) and alloxaan moonhydrate.

**Antidiarrhoal studies**

Antidiarrhoal activity was evaluated as described by Yegnanarayan and Shrotri\[13\] with slight modifications to ensure evenness of dose of castor oil in rats according to body weight. Fifty albino rats were screened initially by administering 1 ml of castor oil orally and those (32) which developed diarrhoea were selected (consistency i.e. loose stool, was the criterion for selection). The experimental set up was as follows.

Group A (Control): received 3% aqueous gum acacia suspension 1 ml/200 g, p.o., at '0' hour and castor oil 1 ml/150 g, p.o., one hour later.

Group B (Test): received ethyl acetate extract of *Acacia catechu* 250 mg/kg as 5% suspension in 3% gum acacia in DW (1 ml/200 g, p.o.) at '0' hour and castor oil 1 ml/150 g, p.o., one hour later.

Group C (Standard): received diphenoxylate 10 mg/kg (0.2%) with atropine sulphate (0.002%) suspension in 3% gum acacia in distilled water at a dose of 1 ml/200 g, p.o. and castor oil 1 ml/150 g, p.o. one hour later.

Percent respondents X Average number of stools of respondents

Purging index (PI) = __________________________________________

Average latent period of onset of diarrhoea

**Antipyretic activity**

Antipyretic activity on albino rats was studied with fever induced by 20% Brewer’s yeast as described.\[14, 15\] Albino rats (150–200 g) were fed uniformly till 24 h before giving drugs, when food was withdrawn. After measuring rectal temperature of the animals by introducing 1.5 cm of digital thermometer in rectum, pyrexia was induced by injecting, subcutaneously, 20% suspension of dried yeast in 2% gum acacia in normal saline at a dose of 20 ml/kg of body weight. After 18 h of yeast injection, rats which showed a rise in temperature of at least 1°F (0.6°C) were taken for the study. Animals in the various groups were treated as follows.

- **Group A**: 3% aqueous suspension of gum acacia (1 ml/200 g) as vehicle, orally.
- **Group B**: Aqueous suspension of ethyl acetate extract of *Acacia catechu* 250 mg/kg (1 ml/200 g) with 3% gum acacia as 5% suspension, orally.
- **Group C**: Aqueous suspension of ethyl acetate extract of *Acacia catechu* 500 mg/kg (1 ml/200 g) with 3% gum acacia as 10% suspension, orally.
- **Group D**: Aqueous acetyl salicylic acid, 300 mg/kg (1 ml/200 g with 3% gum acacia as 6% suspension, orally.

Rectal temperature was recorded every hour for four hours after administration of drugs.

**Hepatoprotective activity**

Hepatoprotective activity of *Acacia catechu* was studied using the method described by Jayasekhar et al.\[6\] Blood was collected from the orbital sinus of albino rats and allowed to coagulate for 30 min. Serum was separated by centrifugation at 2500 rpm for 20 min and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed. AST and ALT levels were estimated from blood by the widely used method of Reitman and Frankel.\[27\] The protocol for the study of hepatoprotective activity is shown in Table 1.

**Table 1**

Protocol for study of hepatoprotective activity of *Acacia catechu* in albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (1-7) days</th>
<th>Toxicant on 7th day single dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Aqueous 5% gum acacia</td>
<td>Olive oil</td>
</tr>
<tr>
<td></td>
<td>1 ml / 200 g</td>
<td>0.12 ml / 100 g</td>
</tr>
<tr>
<td>Toxic control</td>
<td>Aqueous 5% gum acacia</td>
<td>CCl₄ + Olive oil (1:1)</td>
</tr>
<tr>
<td></td>
<td>1 ml / 200 g</td>
<td>0.25 ml / 100 g</td>
</tr>
<tr>
<td>Standard</td>
<td>0.5% silymarin 25 mg/kg</td>
<td>CCl₄ + Olive oil (1:1)</td>
</tr>
<tr>
<td></td>
<td>1 ml / 200 g</td>
<td>0.25 ml / 100 g</td>
</tr>
<tr>
<td>Test drug</td>
<td><em>A catechu</em> extract 250 mg/kg</td>
<td>CCl₄ + Olive oil (1:1)</td>
</tr>
<tr>
<td></td>
<td>in 5% gum acacia</td>
<td>0.25 ml / 100 g</td>
</tr>
</tbody>
</table>

Estimation of AST and ALT was done on 8th day of experiment. All the drugs were administered orally. CCl₄ was used as toxicant with olive oil. n=6 in each group.
Study of hypoglycaemic activity of Acacia catechu

Hypoglycaemic activity of Acacia catechu was studied both in normal and alloxan induced diabetic rats.

Hypoglycaemic activity in normal rats

Twenty-four albino rats weighing 150–200 g were fasted for 18 h and were divided into four groups of six animals in each. The groups included i) vehicle control (5% gum acacia in normal saline, 1 ml/200 g rat); ii) test drug (250 mg/kg, p.o. 5% w/v, 1 ml/200 g rat); iii) test drug (500 mg/kg, p.o., 10% w/v, 1 ml/200 g rat) and iv) standard control, glibenclamide (0.5 mg/kg, p.o., 1% 1 ml/200 g rat). One millilitre of blood from the orbital sinus of each rat was collected at ‘0’ hour. At two hours of treatment, blood samples were collected again from the treated animals and blood glucose was estimated by glucose oxidase method.[18]

Hypoglycaemic activity in diabetic rats

Albino rats (n = 44) were fasted for 48 h. Diabetes was induced by administering[5, 17-19, 25, 26] freshly prepared alloxan monohydrate 2.4% in normal saline subcutaneously at a dose of 120 mg/kg, body weight as single dose.[17] After 72 h of alloxan, 18 h fasting blood was collected from those that survived (n=34)[18] and blood sugar estimated by glucose oxidase method. Twenty-four diabetic rats with blood glucose level of 300-500 mg% were selected and were divided into four groups of six each. The selected groups were treated with the vehicle (5% gum acacia, 1 ml/200 g), test drug (250 mg/kg, p.o.), test drug (500 mg/kg, p.o.) and glibenclamide (0.5 mg/kg, p.o.), respectively, for seven days.[19] On the eighth day blood samples were collected after 18 h of fasting and blood glucose was estimated again.

Statistical analysis

The results were analysed for statistical significance using one-way ANOVA followed by Dunnett’s test. Nonparametric data were analysed by Kruskal–Wallis one-way ANOVA. P values <0.05 were considered significant.

Results

Antidiarrhoeal studies

The extract of Acacia catechu (250 mg/kg) markedly reduced the percentage of animals that had diarrhoea (50%).

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose and route</th>
<th>Mean latent period (h)</th>
<th>Mean total no. of stools passed</th>
<th>Purging index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1 cc/200 g, p.o.</td>
<td>2±0.36</td>
<td>3.2±0.53</td>
<td>160</td>
</tr>
<tr>
<td>3% Gum acacia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test A. catechu</td>
<td>250 mg/kg, p.o.</td>
<td>5.25±0.31**</td>
<td>0.7±0.26**</td>
<td>16.28</td>
</tr>
<tr>
<td>Standard Diphenoxylate</td>
<td>10 mg/kg, p.o.</td>
<td>5.95±0.05**</td>
<td>0.2±0.13**</td>
<td>3.48</td>
</tr>
<tr>
<td>One-way ANOVA</td>
<td></td>
<td>F 38.29</td>
<td>20.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &lt;0.001</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=10 in each group. df=2,27. **P<0.001 as compared to control. Latent period of the animals which did not pass stool within 6 h was considered as 6 h.
Studies on A. catechu

Antipyretic activity of Acacia catechu in albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug and dose</th>
<th>Temperature (°F) at 1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3% gum acacia in DW, 1 ml/200 g</td>
<td>99.07±0.34</td>
<td>101.7±0.42*</td>
<td>101.75±0.29</td>
<td>101.88±0.28</td>
</tr>
<tr>
<td>Test</td>
<td>A. catechu 250 mg/kg</td>
<td>99.11±0.18</td>
<td>100.9±0.25**</td>
<td>101.16±0.45</td>
<td>100.73±0.33*</td>
</tr>
<tr>
<td></td>
<td>500 mg/kg</td>
<td>99.20±0.13</td>
<td>100.91±0.41*</td>
<td>100.9±0.38</td>
<td>100.6±0.39*</td>
</tr>
<tr>
<td>Standard</td>
<td>Aspirin 300 mg/kg</td>
<td>98.98±0.26</td>
<td>100.75±0.27*</td>
<td>100.08±0.37</td>
<td>99.23±0.29**</td>
</tr>
</tbody>
</table>

One-way ANOVA: F 0.39, P 0.84, 0.039 < 0.05

Table 4

Hepatoprotective activity of Acacia catechu in albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>AST on 8th day (i.u./l)</th>
<th>ALT on 8th day (i.u./l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 5% Gum acacia in DW 1 cc/200 g, p.o.</td>
<td>14.7±2.25</td>
<td>36.2±2.69</td>
</tr>
<tr>
<td>Toxicant (CCl₄) 2.5 mg/kg, p.o.</td>
<td>954.7±19.23*</td>
<td>838.3±30.23*</td>
</tr>
<tr>
<td>Test A. Catechu 250 mg/kg, p.o.</td>
<td>330.7±31.76**</td>
<td>343.5±33.4**</td>
</tr>
<tr>
<td>Standard Sylimarin, (25 mg/kg)</td>
<td>242.3±37.8</td>
<td>263.5±36.77**</td>
</tr>
</tbody>
</table>

One-way ANOVA: F 0.99, P <0.0001

Table 3

Antipyretic activity of Acacia catechu in albino rats

Discussion

In the present study, the mean initial basal rectal temperature of rats before yeast injection corresponds with findings of other workers. In our study, the initial rise of temperature after 18 h of subcutaneous yeast injection was 1.7°F–2.1°F (0.95°C to 1.67°C), which corresponds with the findings of Hajare et al. There was no significant difference between the initial mean basal temperature of the different groups and the mean temperature between the groups of pyrexia rats, after 18 h of yeast injection. Body temperatures of pyrexia rats were lowered significantly with the test drug. The antipyretic effect of the test drug may be due to presence of flavonoid compounds, as some flavonoids are predominant inhibitors of cyclooxygenase or lipoxygenase. Castor oil induced model of diarrhoea incorporates both secretory and motility diarrhoea as a result of prostataglandin release from the intestinal mucosa. Acacia catechu significantly decreased the number of stool passed (P<0.001). The percentage of respondents and purging index were also significantly decreased. Highly significant (P<0.001) increase in the latent period of onset of diarrhea, as compared to the control group, was also produced. Parameters in the control group of animals agree with the findings of previous workers. The result shows that the ethyl acetate extract of Acacia catechu (test drug) is as effective as the standard drug, glibenclamide. The antidiarrhoeal property of the ethyl acetate extract of Acacia catechu appears to be due to its tannin and flavonoid content, which has astringent property. Results of the present study shows that ethyl acetate extract of Acacia catechu (500 mg/kg) significantly decreases fasting glucose levels of normal rats (P<0.001). However, the reduction was found to be less effective than that of glibenclamide. The test drug at doses of 250 and 500 mg/kg/day for seven days reduced the blood glucose level of diabetic rats significantly (P<0.001). Effect of the test drug at doses of 250 and 500 mg/kg, orally, on blood glucose level was comparable with that of the standard drug, glibenclamide. The hypoglycaemic effect of A. catechu may be due to presence of flavonoids which acts as insulin secretagogues. Epicatechin, a flavonoid compound, is reported to promote regeneration of B cells of the Islets of Langerhans. Carbon tetrachloride is a hepatotoxin commonly used for the production of experimental liver toxicity. The serum transaminase level is most widely used as a measure of hepatic injury, due to its ease of measurement and high degree of sensitivity. It is useful for the detection of early damage of hepatic tissue and requires less effort than that required for a
histologic analysis, moreover without sacrifice of the animals. In the present study, AST and ALT levels in normal control group were in conformity with the findings of Kapoor et al.\(^26\) Seven days pretreatment with the test drug (250 mg/kg) protected the animals significantly (\(P<0.001\)) from CCl\(_4\)-induced hepatotoxicity as compared to toxic control, and reflects the hepatoprotective activity of ethyl acetate extract of \textit{A. catechu}. There was no significant difference between the test drug group and standard drug group. The hepatoprotective activity of \textit{A. catechu} could be due to the presence of bioflavonoids which have hepatoprotective and antioxidant properties.\(^{11, 12, 28}\)

In the present study, preliminary phytochemical screening of \textit{Acacia catechu} found the presence of flavonoids and reports substantiate the presence of catechutannic acid, quercetin,\(^26\) catechin,\(^4, 22\) pseudotannin,\(^4\) phlobatannin,\(^4\) polypeholic,\(^9\) epicatechin\(^22\) and cyanidanol\(^6\) compounds in \textit{A. catechu}. The antiarrheoidal and hepatoprotective properties of \textit{A. catechu} could be attributed to the presence of tannins\(^9\) and cyanidanol\(^6\) and quercetin,\(^23\) respectively. The antipyretic and hypoglycaemic properties of \textit{Acacia catechu} may be ascribed to the presence of flavonoids, which have been shown to inhibit cyclooxygenases\(^{23}\) and promote \(\beta\)-cell regeneration besides having insulin secretagogues and antioxidant properties.\(^{11, 5, 18, 19, 21}\)

The results of the present study suggest that ethyl acetate extract of \textit{Acacia catechu} in doses of 250 and 500 mg/kg, significantly reduced the temperature of pyretic rats, and also illustrate significant hypoglycaemic activity. The extract of \textit{A. catechu}, 250 mg/kg, p.o., has also shown to possess significant antiarrheoidal and hepatoprotective property.

### References

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<table>
<thead>
<tr>
<th>Table 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypoglycaemic activity of \textit{Acacia catechu} in normal and diabetic albino rats</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose - in normal rats</th>
<th>Blood glucose - in diabetic rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fasting</td>
<td>2 h after treatment</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% Gum acacia</td>
<td>78.42 ± 9.23</td>
<td>73.05 ± 5.73</td>
</tr>
<tr>
<td>Test I \textit{A. catechu} (250 mg/kg)</td>
<td>78.23 ± 6.43</td>
<td>67.73 ± 4.93</td>
</tr>
<tr>
<td>Test II \textit{A. catechu} (500 mg/kg)</td>
<td>77.15 ± 3.36</td>
<td>50.67 ± 3.75*</td>
</tr>
<tr>
<td>Standard</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gibenclamide (0.5 mg / kg)</td>
<td>75.0 ± 5.06</td>
<td>43.00 ± 2.24**</td>
</tr>
</tbody>
</table>

| | | |
| --- | --- |
| F | 0.03 | 0.0002 | 0.03 | 290.82 |
| ANOVA | 0.99 | 0.0002 | 0.99 | <0.0001 |

Values are (mg%) mean±SEM. n=6 in each group, df=3.23. *\(P<0.01\); **\(P<0.001\) as compared to respective control.
Studies on A. catechu

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CAL DVD

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IJP would like to thank the following for allowing us to include their software in the DVD:

Prof. Henk van Wilgenburg, The Netherlands - Microlabs 2006
Prof. M. Saghaei, Iran - Random Allocation Software

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