Phytochemical screening and effect of aqueous root extract of *Raphia hookeri* (raffia palm) on metabolic clearance rate of ethanol in rabbits

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Abstract

The phytochemical screening of the aqueous root extract of *Raphia hookeri* (Raffia palm) and its effect on plasma ethanol level in male rabbits were investigated. Phytochemical screening revealed the presence of tannins, flavonoids sterol/triterpenes and saponins in high concentrations. Cardiac glycosides and alkaloids were detected in moderate amounts while cyanogenic glycosides and deoxysugars were present in trace amounts. The extract was however devoid of reducing sugars, phlobatannins, chlorogenic acid and anthraquinone glycosides. Rabbits given root extract of *Raphia hookeri* orally prior to ethanol administration were found to metabolize ethanol faster than the control. The metabolic clearance rate (MCR) of the test animals was 12% higher than the control. This suggest the effect of the extract on ethanol clearance rate in Rabbits

Key words: *Raphia hookeri*, ethanol, phytochemical screening, plasma, rabbits

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INTRODUCTION

The raffia palm (Raphia hookeri) is a member of the family palmaceae. It is commonly found in West Africa and in abundance particularly in South Eastern Nigeria and usually grows up to 12m high.

Scientific research into the cultivation, management and economic products of R hookeri has received greater attention in recent times, particularly in Nigeria. The major economic products of raffia palm are: wine, raffia fibre, and pulp for paper production. The fruit is large, cone-shaped with a single hard nut having an outer layer of rhomboid-triangular and overlapping reddish brown scales. Between this outer layer of scales and the very hard seed is a yellow, mealy, oil-bearing mesocarp or pulp. Raffia oil is very similar to palm oil in chemical composition and is used for cooking, as liniment, as lubricant, for lighting and in cosmetics; and could be used for making soap and margarine. The pulp is normally consumed with boiled, sliced cassava and may be pounded with other plant substances and also used as fish poison. It has been reported that the active ingredient that causes fish poisoning is a water-soluble saponin.

Edem et al. investigated the chemical composition of the pulp of the raffia palm fruit. It was reported to contain 6.1% protein, 11.8% fat, 61.4%, nitrogen free extract, 17.7% fibre and 3.0% ash on dry matter basis. The tannin content was 597mg/100g dry weight. It has been observed that the pulp of the full mature raffia palm fruit contained insignificant (or trace) amount of starch, but had soluble sugar content of 30.9% dry sample. The phytochemical and antimicrobial properties of the fruit pulp of raffia have been reported.

The root extract of raffia palm is used in traditional medicine for the treatment and prevention of several diseases. The cool root extract is normally given to infant with stomach pain. Again, the effect of root extract on the plasma level of ethanol has been observed in acute and chronic intoxication in human being. There are claims by traditional medicine practitioners that the root extracts of R. hookeri can be used in the treatment of alcoholic intoxication in man. This assertion lacked scientific justification as literature is scarce on this. This paper attempts in part to establish scientific basis for these claims. The authors therefore report on the effect of root extract of R. hookeri on plasma level of ethanol in male rabbits with acute alcoholic intoxication.

MATERIALS AND METHODS

Collection and treatment of samples
The fresh root of Raphia hookeri was obtained from East Itam community in Itu Local Government Area, Akwa Ibom State, Nigeria in the month of November, 2000. The sample was taken to Dr R. Ubom a taxonomist in the Department of Botany, University of Uyo, Nigeria for confirmation. The brownish red root were washed clean with distilled water, drain dried and cut into small pieces and dried in an oven at 60°C, ground and stored in an airtight container prior to use.

Experimental animals
Six (6) male rabbits weighing between 1000-1060g were purchased from a commercial rabbit farmer in Uyo and confined in a wire cage. They were fed with growers mash containing, 15% protein, 3.5% fat, 7.5% fibre, 1.0% calcium, 0.4% average phosphorus and 2400kcal/kg net energy, for a period of one month. This was approved by the Animal Unit of University of Uyo, Uyo.

Extraction of Bioactive principles
1.0 kg of the ground root powder of Raphia hookeri was soaked overnight in a Winchester bottle containing 2.5 liters of distilled water. The soaked sample was shaken continuously. After 24hrs, the extract was filtered and the residue subjected to a second extraction for another 24 hours and filtered using a suction pump. The weight of the deposit was 0.969. The filtrates were combined, concentrated by rotary evaporation at 50°C, centrifuged at 1,500 rev min for 20min and the supernatant made
up to the required volumes through serial dilution and used for the experimental studies.

**Determination of ethanol concentration in blood plasma of male rabbits**

Standard solution for ethanol determination was prepared by diluting aliquots (2-10ml) of 4.0% (w/v) aqueous solution of ethanol to 100ml, with water. 3.0ml of K$_2$CrO$_7$ (0.071% (W/V)K$_2$CrO$_7$ in 50%(v/v) H$_2$SO$_4$ was pipetted into a 25ml volumetric flasks, 0.1ml of the standard solution was added to each, stoppered and heated in a water bath at 55-65°C for at least one hour. It was then cooled and 10ml of water was added, 3.0ml of 1% (w/v) brucine solution in H$_2$SO$_4$ was added and was diluted with water to mark. The extinction was read at 425nm after 5min against water blank.

**Experimental procedures**

Phytochemical Screening: phytochemical analysis of the root sample was done following standard methods of Trease and Evans and Iwu.

**Animals and treatment**

Two sets of experiments were performed. In each experiment a control group of 3 rabbits (n=3) were administered distilled water (0.5ml) only. The treated group (n=3) was administered root extract of *Raphia hookeri* (200mg/kg in 0.5ml of water) orally using a feeding annular immediately after the water or root extract. Before the administration of the extract, ethanol intoxication was induced by administration of ethanol (60%) diluted 1.1 with distilled water and given orally (3.2g/kg) .This concentration has been confirmed to induce intoxication in Rabbits. Blood sample (0.2-0.3ml) was drawn from a tail vein into a heparinized syringe before administration of extract and at 0.5, 1, 2, 3, and 5 hours after dosing. The heparinized blood was stored stoppered on ice until the end of the experiment. The blood samples were centrifuged for 15min at 3,000rpm, and plasma was removed and assayed immediately for content of ethanol.

**Determination of Blood plasma ethanol**

Ethanol content of blood plasma was analyzed using an assay kit from Sigma (St Louis, MO) based on the method of Bucher and Redetzki. Half –life was calculated from the terminal portion of the log concentration versus time curve for ethanol. Area under the plasma concentration time curve (AUC) was calculated using the trapezoidal rule for data points 0-5hrs and extrapolated to infinity for calculation of clearance. Clearance (MCR) was calculated as (F) (dose/AUC) where F is bio-availability of an oral dose and apparent volume of distribution (AVD) was calculated as (MCR) (T$_{1/2}$/0.693).

**RESULT AND DISCUSSION**

The summary of the phytochemical studies on the aqueous root extract of *Raphia hookeri* are shown in Table 1. The sign (+) indicates the presence of the constituent while (-) indicates the absence of bioactive agents. Some bioactive substances such as tannins, saponins, polyphenols, flavonoids, and sterol/triterpenes were present in high concentration (+++). Cardiac glycoside and alkaloids were moderately (++) present while cyanogenic and deoxy sugars were present in low concentration (+). The sample was however devoid of reducing sugar, phlobatannins and chlorogenic acid.

<table>
<thead>
<tr>
<th>Constituents Analyzed</th>
<th>Remarks</th>
</tr>
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<tbody>
<tr>
<td>Tannins</td>
<td>+++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
</tr>
<tr>
<td>Sterol and triterpenes</td>
<td>+++</td>
</tr>
<tr>
<td>Saponins</td>
<td>+++</td>
</tr>
<tr>
<td>Polyphenol</td>
<td>+++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>++</td>
</tr>
<tr>
<td>Cynogenetic glycoside</td>
<td>+</td>
</tr>
<tr>
<td>Deoxy sugar</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>-</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinone glycoside</td>
<td>-</td>
</tr>
</tbody>
</table>

+++  High concentration
++  Moderate concentration
+  Low concentration
-  Not detected
The presence of these bioactive substances in the root extract could be responsible for the pharmacological of the root extracts and may act synergistically. Saponins, tannins and flavonoids are actually reported to possess antimicrobial activities\(^{11}\).

In our society today, there is increasing consumption of alcohol and beverages with increasing risk of liver diseases. In attempt to find cheaper alternative means of reducing alcohol intoxication this study was put forth. There are claims by herbalist that if the root of *Raphia hookeri* is chewed after excess consumption of alcohol, its toxicity can be minimized.

Alcohol intoxication was induced prior to administration of extract by oral administration of 3.2g/kg ethanol. Although the actual toxic dose was not determined, the above concentration is established to be a toxic dose in animals such as rabbits\(^{7}\). The result of the experiment revealed that the plasma concentration of ethanol reduced with time compared to the control (Table 2) especially when the extract was administered.

Metabolism of alcohol is the main means of removing alcohol from the body, such small amount, usually less than 2% of a dose, are extracted unchanged in urine, expired air and sweat\(^{12}\). A concentration of 30g/100ml alcohol is reported to cause superficial erosion, hemorrhages, and partial paralysis of smooth muscle of the stomach\(^{13}\). The values of all data (Table 2) were considered as possible indices to ascertain the effect of the extract on the metabolism of ethanol. The mean plasma concentration time (Fig 1) for ethanol administered orally with extract of *R. hookeri* was lower than administration of ethanol without root extract. Over the years, many researches have been conducted on the effect of some drugs and other natural plant for extracts on the plasma level of ethanol in both humans and non-humans\(^{14}\). Nicorandil for example has been shown to lower the plasma level of ethanol when administered orally simultaneously with alcohol\(^{14}\).

### Table 2: Results of the effects of the root extract of *Rapia hookeri* on plasma level of ethanol.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>EXPERIMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R. hookeri</strong></td>
<td>Control</td>
</tr>
<tr>
<td>Time of peak plasma concentration (hr)</td>
<td>1.06</td>
</tr>
<tr>
<td>Area under the plasma Concentration-time curve (0-hrs) (Mg.h.ml(^{-1}))</td>
<td>6.97</td>
</tr>
<tr>
<td>Half-life (hr)</td>
<td>2.66</td>
</tr>
<tr>
<td>Metabolic clearance rate (MCR) Lh. kg(^{-1})</td>
<td>0.41</td>
</tr>
<tr>
<td>Apparent volume of Distribution (AVD) (1.kg(^{-1}))</td>
<td>1.18</td>
</tr>
</tbody>
</table>

*Values are mean + S.D. for 6 rabbits.*
The effect of ginseng extract on the deposition of ethanol was also studied in 344 male Fisher rats. Reports reveal that the preventive and therapeutic effect of ginseng on ethanol intoxication was due to enhanced ethanol metabolism. Researchers claimed that simultaneous administration of ginseng and ethanol lowered plasma level of ethanol by enhancing its plasma clearance. It has also been reported that acute or chronic treatment with ginseng enhances ethanol metabolism by increasing the activity of alcohol dehydrogenase, aldehyde dehydrogenase, and by inducing the cytodrome p450 mono oxygenase system. Treatment of Rabbits with R. hookeri resulted in a 25% decrease in area under the plasma concentration time curve (0-5h) of ethanol administered orally (Fig 1). This value compares well with a 21% decrease in area when freaked with ginseng. When the rabbits were also treated with R. hookeri extracts, there was a 32.2% increase in apparent volume of distribution, shorter half life and a shorter time peak plasma concentration. These observations are however consistent with 24.9% increase reported for Ginseng.

This research seems to justify the use of root extract of R. hooker in the treatment of alcohol intoxication. It was generally observed that
extracts of *R. hookeri* lowered plasma level of ethanol in rabbits when the two are administered together orally. This work does not support enhanced systemic clearance of ethanol as the mechanism by which this occurs but rather suggests that the lower plasma levels of ethanol result from either decreased absorption or enhanced first pass gastrointestinal mechanism. The ability of the extract to lower the plasma concentration of ethanol in rabbits may be attributed to the synergistic actions of the identified secondary metabolites. Although this work was not exhaustive, identification of the potent compounds responsible for this action is therefore necessary. These findings provide possible basis for more scientific research with a view to finding the panacea for alcohol intoxication in man.

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