Acute and Subacute Toxicity Studies of Zingiber Officinalis Roscoe Essential Oil on Mice (Swiss) and Rats (Wistar)

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ABSTRACT

All substances are toxic, only the dose could differentiate a poison from a medicine. The oral acute and subacute toxicity studies of the essential oil extract from the rhizomes of Zingiber officinalis Roscoe were respectively investigated in albino mice and rats. Acute toxicity studies were carried out in both female and male mice. The extract was administered at doses of 4 and 7 g/kg body weight. Subacute toxicity studies were also carried out in both female and male rats. The extract was administered at doses of 1, 1.4 and 1.8 g/kg body weight. In subacute toxicity, the parameters measured included food intake, body weight, alanine transaminase and aspartate transaminase activities (ALAT and ASAT) as well as the histological analysis, creatinine, blood cell count and total proteins. We have registered two death mice in acute toxicity at 7 g/kg body weight. In subacute toxicity, rats treated with this extract had significant (P < 0.05) decrease in body weight as compared to the control as well as the food intake. Creatinine concentration, ALAT and ASAT activities were significantly increased (P < 0.01) with dose dependent. Furthermore, the total protein concentration was significantly higher (P < 0.0001) in groups that received the extract (0.6g/kg, 1 g/kg, 1.4 g/kg and 1.8 g/kg). The histological examination of livers presented diffuse clarification of hepatocytes, congestion and necrosis at higher doses (1.4 and 1.8 g/kg). Thus this extract should be consumed at a dose less than 0.6 g/kg of body weight.

KEYWORDS: Acute toxicity, Subacute toxicity, Liver function, Renal function, Medicinal plants
INTRODUCTION

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**Zingiber officinalis** (ZO) Roscoe is an herbaceous plant belonging to the *Zingiberaceae* family. It was initially discovered in Asia (1) and is commonly known as vegetable and is usually used for spices and aroma (2). *Zingiber officinalis* relieves joint pains. It has antianaemic, antihistaminic and hypocholesterolemic properties (3). This plant contains among others extracts, an essential oil. Essential oils are volatile substances generally colourless, mainly called aromatics, obtained by hydro-distillation of plants (4). Phytochemical studies carried out on *Zingiber officinalis* have been reported. Three majority compounds were isolated: zingiberene, sesquiphellandrene and camphrene (5). Its essential oil is commonly used to treat articular pains (3). The safe use and analysis of such herbal preparation must be preceded by toxicological screening to identify and evaluate the toxicity caused by the plant products in the body at a given dose, since there is no drug without side effects (6). Due to the large consumption of *Zingiber officinalis* in Cameroon as spice and for articular pain, there is the need for a toxicological study so as to guard against food poisoning or overdose.

The present study reports on the acute and subacute toxicity effect of the essential oil from the rhizomes of *Zingiber officinalis*, a traditional spice, aroma product and medicinal plant used in Cameroon.

MATERIALS AND METHODS

**Collection and Extraction of Essential Oil of ZO**

The rhizomes of *Zingiber officinalis* was bought at the Dschang market and identified in the National Herbarium of Yaounde. After being washed, 3 kilograms were crushed and hydrodistilled with 3 litres of water for about 6 hours. The essential oil rid of water using a sodium sulphate pillar. The yield of extract was 0.2 %.

**Experimental Animals**

Male and female rats were used with the permission of the scientific committee of the University of Dschang and the Cameroon National Ethics Committee (Reg. No. FWA – IRB00001954). Animals were maintained on a 12 h light/dark cycle, at constant temperature and humidity and were given free access to food and water.

**Acute Toxicity**

Eight weeks old male and female Swiss albino mice were used in this experiment. The acute toxicity was studied by preparing two doses of the extract (2 and 7 g/kg.b.w.). A single administration was made orally and the mortality was noted after 48 h. The control group was given distilled water.

**Subacute Toxicity Study**

Eight weeks old Wistar albino male and female rats were used. They were divided into several groups and were given 0.6, 1, 1.4 and 1.8 g/kg body weight of the essential oil of ZO respectively, every day during 4 weeks, while the control group received distilled water only. Food and water intake were monitored daily with measurement of body weight. After 30 days of
exposure, blood was collected from the animal by cardiac puncture, for blood cell count and serum biochemical assays. The animal livers were isolated for histological analysis (7). ALAT and ASAT were assayed using the method of Yushend and Zhonglin (8). Creatinine and total proteins were determined respectively by the method of Jaffe (9) and Gornall (10) while White Blood Cell (WBC) and Red Blood Cell (RBC) were counted using the method of WHO (11).

**STATISTICAL ANALYSIS**

Results are expressed as mean ± Standard Error of Mean (SEM). Significance differences between control and experimental groups and between experimental groups were assessed by ANOVA two factors’ test followed by a Student Newman Keuls. SPSS hardware for Windows 8.0 was used. P values less than 0.05 were considered to be significant.

**RESULTS**

From all mice treated with the essential oil extract of *Zingiber officinalis* in acute toxicity, 25% of mortality was registered at 7 g/kg.b.w. As shown in Table 1 below, this extract decreases significantly the food intake of the rats at the higher doses (1, 1.4 and 1.8 g/kg.b.w) compared to the control group.
Table 1

Variation in food intake of animals during subacute toxicity testing

<table>
<thead>
<tr>
<th>Dose (g/kg)</th>
<th>$M \pm SD$ (males)</th>
<th>$M \pm SD$ (females)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>89.75 ± 1.01</td>
<td>89.75 ± 1.01</td>
</tr>
<tr>
<td>0.6</td>
<td>86.14 ± 1.03</td>
<td>86.14 ± 1.03</td>
</tr>
<tr>
<td>1</td>
<td>78.25 ± 0.25**</td>
<td>78.25 ± 0.25**</td>
</tr>
<tr>
<td>1.4</td>
<td>70.53 ± 0.53***</td>
<td>70.53 ± 0.53***</td>
</tr>
<tr>
<td>1.8</td>
<td>64.50 ± 1.29***</td>
<td>64.50 ± 1.29***</td>
</tr>
</tbody>
</table>

The growth weight result is shown in Table 2.
Table 2

Growth weight variation of animals during subacute toxicity

<table>
<thead>
<tr>
<th>Doses (g/kg.b.w)</th>
<th>M ± DS (males)</th>
<th>M ± DS (females)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.27 ± 2.90</td>
<td>9.10 ± 1.21</td>
</tr>
<tr>
<td>0.6</td>
<td>0.78 ± 0.31</td>
<td>0.19 ± 1.24</td>
</tr>
<tr>
<td>1</td>
<td>-2.10 ± 1.00**</td>
<td>-2.40 ± 0.89**</td>
</tr>
<tr>
<td>1.4</td>
<td>-5.00 ± 5.28**</td>
<td>-4.97 ± 4.08**</td>
</tr>
<tr>
<td>1.8</td>
<td>-10.79 ± 4.65***</td>
<td>-10.00 ± 3.42***</td>
</tr>
</tbody>
</table>

** p<0.01 ***p<0.0001

M ± SD: mean for four weeks ± standard deviation

Table 2 demonstrates a decrease in the growth weight which was dose dependent, and which become significant at the higher doses of the extract (1, 1.4 and 1.8 g/kg.b.w).
The effects of essential oil of ZO on liver enzymes (ALAT, ASAT), creatinine and total proteins are shown respectively in Table 3.

**Table 3**

Biochemical values of animals after four weeks of study

<table>
<thead>
<tr>
<th>Dose (g/kg)</th>
<th>Sex</th>
<th>ALAT (UI)</th>
<th>ASAT (UI)</th>
<th>Creatinine (mg/l)</th>
<th>Total Proteins (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Male</td>
<td>74.60±11.64</td>
<td>40.83±0.18</td>
<td>10.00±1.58</td>
<td>9.24±0.50</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>74.50±10.00</td>
<td>40.00±4.21</td>
<td>10.25±2.04</td>
<td>8.90±1.02</td>
</tr>
<tr>
<td>0.6</td>
<td>Male</td>
<td>101.66±2.92*</td>
<td>108.66±2.37**</td>
<td>27.50±5.27*</td>
<td>10.24±1.13</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>99.50±2.15*</td>
<td>100.28±1.95**</td>
<td>27.27±4.52*</td>
<td>9.98±0.45</td>
</tr>
<tr>
<td>1</td>
<td>Male</td>
<td>159.66±5.48*</td>
<td>113.00±1.82***</td>
<td>36.25±3.44***</td>
<td>18.73±1.75***</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>158.20±4.21*</td>
<td>113.12±1.51***</td>
<td>36.70±4.21***</td>
<td>17.01±1.41***</td>
</tr>
<tr>
<td>1.4</td>
<td>Male</td>
<td>221.00±1.73***</td>
<td>160.50±1.82***</td>
<td>45.00±4.33***</td>
<td>20.58±0.58***</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>220±3.10***</td>
<td>160±2.31***</td>
<td>44.95±3.12***</td>
<td>20.12±1.24***</td>
</tr>
<tr>
<td>1.8</td>
<td>Male</td>
<td>204.33±6.21***</td>
<td>184.33±12.14***</td>
<td>46.25±2.85***</td>
<td>21.16±0.73***</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>204.01±3.57***</td>
<td>183.98±8.24***</td>
<td>46.29±5.41***</td>
<td>20.89±2.45***</td>
</tr>
</tbody>
</table>

* p < 0.05  ** p < 0.01  *** p < 0.0001
These parameters increased significantly with the dose of ZO. Furthermore, animals that received higher doses of the extract (1.4 and 1.8 g/kg.b.w) presented some alterations such as the vacuum, karyorrhexie congestion and some necrosis as illustrated in Figures 2 and 3 compared to the control (Figure 1). WBC and RBC of treated rats were not different from the control group.

![Histological cross section of Group 1 liver (control received distilled water – 100 X)](image)

**Figure 1:** Histological cross section of Group 1 liver (control received distilled water – 100 X)
Figure 2: Histological cross section of Group 3 liver (received 1.4 g/kg.b.w - 100 X)

Necrosis  Congestion  karyorrhexie

Figure 3: Histological cross section of Group 4 liver (received 1.8 g/kg - 100 X)
**DISCUSSION**

The percentage of mortality obtained in acute toxicity at 7 g/kg.b.w, suggested that the LD$_{50}$ was higher than 7 g/kg body weight. Similar results were obtained by Skim et al. [12]. In subacute toxicity testing, the decrease of the growth weight after the administration of the essential oil of *Zingiber officinalis* could be as a result of decreased food intake by these animals. ALAT and ASAT are synthesized in the liver. ALAT is localised in the cytoplasm while ASAT and PAL are found in mitochondria and cytoplasm. After synthesis, these enzymes could be found in the blood. The significant elevated level of ALAT and ASAT (Table 3) in the serum of animals could explain the damage of the liver (13, 14, and 15). This could be confirmed by the elevated level of total proteins and furthermore by the histological analysis result which shows in animals that received higher doses of the extract. Thus, the damage of the liver caused the passage of the enzymatic compounds from the liver towards the blood current. A similar result was obtained by Udozen and Ojong (16) who showed in their work that the extract of the bark of *Sacoglottis gabonensis* was hepatotoxic and the histological examination obtained presented some alterations sign in the liver of the treated animals as congestion explaining the disruption of blood vessels, and vaccuum which is the result of mitochondria lysis. Karyorrhexie is noticeable by the disruption of the cell nucleus while necrosis is a process of cell death. The creatinine serum level is a parameter which gives some ideas on renal function. The result showed its increased level in the serum. This could result to the renal dysfunction (13, 14). We observed a particular toxic effect with each dose of the extract used, we can say in conclusion that the extract of essential oil of *Zingiber officinalis*, administered at the doses less than 0.6 g/kg.b.w could probably enhance its therapeutic benefits.

**ACKNOWLEDGEMENT**

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