Acute toxicity studies of butanol fraction of leaves of *Moringa stenopetala* in rats

Abdu Hassen Musa¹*, Prabhanjan Kumar Vata², Asfaw Debella³

¹College of Health Sciences and Medicine, Dilla University, P.O. Box 419 Dilla, Ethiopia
²College of Health Sciences and Medicine, Dilla University, P.O. Box 419 Dilla, Ethiopia
³Ethiopian Public Health Institute, P.O. Box 1242/5654, Addis Ababa, Ethiopia

ABSTRACT

The leaves of *Moringa stenopetala* are traditionally used for the treatment of various ailments such as malaria, hypertension, asthma, and diabetes. This study was carried out to evaluate the acute toxic effect of butanol fraction of the leaves of *M. stenopetala* in experimental rats. For this study the leaves were collected in Arba-Minch, Southern Ethiopia. The leaves were dried, crushed to powder and extracted with ethanol and fractionated with butanol. Fifteen female albino rats were randomly divided into four experimental groups (which received 500, 1000, 2000, and 5000 mg/kg respectively of the fraction) and one control group, which received distilled water by oral gavage. In the acute toxicity study rats treated with up to dose of 5000 mg/kg showed no toxic signs on behavior, gross pathology, and body weight, as compared with the controls. These results showed that butanol fraction of *M. stenopetala* did not produce adverse effects in treated rats after acute treatment. Although, further detailed studies should be carried out to recommend its therapeutic use.

Keywords: acute toxicity, behavioral, body weight changes, LD₅₀, Moringa stenopetala.

Introduction

*M. stenopetala* belongs to the family Moringaceae; it is a fast-growing tree with 6-12 m tall, white to pale gray smooth bark, alternate multi-pinnate leaves and long taproots with few lateral roots. It is widely distributed in the Southern region of Ethiopia and is known by different vernacular names such as Shiferaw (Amh), Halako (Gamo & Wollayita), Shelchada (Konso), and Cabbage tree (Eng).[1-3] Many primary & secondary chemical compounds have been isolated from the fresh leaves of *M. stenopetala* such as: proteins, fats, carbohydrates, minerals (K, Fe, Ca, P, and Zn, in significant concentrations), alkaloids, flavonoids, glycosides, polyphenols, saponins etc.[4-6]. In southern Ethiopia, around Arba-Minch, Konso and Wollayta the fresh leaves are eaten as vegetables and also used as herbal medicine its alcholic extract & fractions has shown significant changes on blood glucose level and body weight in experimental mice[6-11].

Despite its nutritional & medicinal values, toxicity studies of *M. stenopetala* leaves are limited. Therefore, toxicological testing is needed in *stenopetala* leaves. This study was aimed to investigate the acute toxic effects on behavior, general body weight, gross pathology of the liver and kidneys and to determine median lethal dose (LD₅₀) of butanol fraction of the leaves of *M. stenopetala* in experimental rats.

Materials and methods

Preparation of Plant Materials

The present study was conducted in the Ethiopian public health Institute (EPHI), traditional and modern drug research laboratories, in March 2011. The leaves were collected in Arba-Minch, Southern Ethiopia, about 502 kilometers south of Addis Ababa. The leaves were identified and authenticated by a taxonomist, cleansed from extraneous materials and dried under shade and powdered. The powdered leaves were extracted with 70% ethanol using percolator and the resulting ethanol extract was filtered using Whatmann no.1 filter paper and concentrated using a rotary evaporator (BUCHI Rota-vapor type R-205, Switzerland) under reduced pressure at a temperature of 40-450C. Then the residue was dried by steam bath at 40°C. The dried ethanol extract was dissolved in
warm distilled water and filtered with Whatmann no.1 filter paper. The dissolved extract was partitioned in a separatory funnel with n-hexane (3x50), dichloromethane (3x50) and n-butanol (5x50) successively until the extracting solvent became colorless in each case. After completing the separation process, the butanol fraction was collected and concentrated using a rotary evaporator. The residue was then dried by steam bath at 40°C. Finally, the dried n-butanol fraction was kept in the refrigerator for the experiments. The yield of the extract was 20% and that of the butanol fraction was 7% relative to the weight of the powdered leaves (500gm). The procedure for plant material preparation was adopted from Debella (2002), Ranjan and Reeba (2002) with some modification[13-14].

Preparation of experimental animals and treatment

For this study fifteen healthy adult female rats (weighing 172 - 212 gm, with age of 10 - 12 weeks old) were obtained from animal house of EPHI. They were kept under standard conditions (at a temperature of 22+2°C, with 12 hours light/12 hours dark cycle) and provided with free access to standard pellet laboratory diets and drink tap water ad-libitum. Before the experiment they were grouped into 5 groups (3 female rats per group, one control and four test groups) and then kept in their cages for 5 days to allow acclimatization to the laboratory conditions. After acclimatization all groups were fasted overnight. Following the fasting period, they were weighed and the doses and the vehicle were calculated based on their body weight (2 ml/100g body weight). The butanol fraction was then administered orally, using oral gavage, at a single dose of 500, 1000, 2000, and 5000mg/kg body weight of rats in the test groups II, III, IV, & V respectively. The control group (I) received distilled water. This acute toxicity study was conducted based on the Organization for Economic Co-Operation and Development (OECD 423) guideline[15].

Statistical analysis

The toxicity results were expressed as mean + standard error of mean (SEM). The data obtained was analyzed by a one-way analysis of variance (ANOVA) using the SPSS version 20 program followed by a Dunnet’s t-test. P-values < 0.05 were considered statistically significant.

Results

Acute toxic effects of fraction of M.stenopetala

In the present toxicity study the fraction of M.stenopetala leaves up to the highest dose of 5000mg/kg did not show any significant changes on behavior (such as (alertness, aggressiveness, irritability), gross physical appearance (condition of fur, general cleanliness) compared with the controls. No death was observed, at any of the doses employed, including the dose 5000mg/kg. The gross pathological examination on the liver and kidneys of treated rats showed no significant changes in color, size, shape, and texture compared with the rats in the control group. Moreover, as summarized in table-1, the mean absolute weights of the liver & kidneys increased in a dose - dependent manner, though, were not statistically (p>0.05) significant as compared with the control groups.

Table: 1. Comparison of organ weights of rats treated with different doses of the butanol fraction

<table>
<thead>
<tr>
<th>Doses</th>
<th>Liver weight (g)</th>
<th>Kidney weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (DW)</td>
<td>6.84 ± 1.02</td>
<td>0.71 ± 1.07</td>
</tr>
<tr>
<td>500mg/kg</td>
<td>7.08 ± 2.03</td>
<td>0.74 ± 0.12</td>
</tr>
<tr>
<td>1000mg/kg</td>
<td>7.26 ± 2.09</td>
<td>0.80 ± 0.01</td>
</tr>
<tr>
<td>2000mg/kg</td>
<td>7.32 ± 2.74</td>
<td>0.82 ± 0.02</td>
</tr>
<tr>
<td>5000mg/kg</td>
<td>7.46 ± 0.15</td>
<td>0.83 ± 1.04</td>
</tr>
</tbody>
</table>

*Values are expressed as Mean ± SEM, n= 3/group

There was a gradual increase in the body weight of both the treated and control rats. At the end of the experiment (after 14 days) the mean body weight gain for rats treated with 500 mg/kg, 1000 mg/kg,
2000mg/kg, and 5000mg/kg, were 14.03 g (6.9%), 14.74 g (7.19%), 23.66 g (11.68%), and 22.52 g (10.93%) respectively, while the control rats was 9.34 g (4.4%). As summarized in table-2 and graph-1, the mean body weight gains for rats treated at different doses of the fraction were not statistically (p>0.05) significant as compared to that of the control groups.

**Table: 2 Comparison of the effect of fraction of the leaves of *M. stenopetala* on the body weight**

<table>
<thead>
<tr>
<th>Doses</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Mean weight increment (g)</th>
<th>% of weight gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (DW)</td>
<td>212 ± 3.37</td>
<td>221.34 ± 2.74</td>
<td>9.34</td>
<td>4.4</td>
</tr>
<tr>
<td>500mg/kg</td>
<td>203.3 ± 13.42</td>
<td>217.33 ± 12.83</td>
<td>14.03</td>
<td>6.89</td>
</tr>
<tr>
<td>1000mg/kg</td>
<td>205.13 ± 7.58</td>
<td>219.87 ± 4.19</td>
<td>14.17</td>
<td>7.19</td>
</tr>
<tr>
<td>2000mg/kg</td>
<td>202.56 ± 12.3</td>
<td>226.22 ± 13.10</td>
<td>23.66</td>
<td>11.68</td>
</tr>
<tr>
<td>5000mg/kg</td>
<td>206 ± 8.72</td>
<td>228.52 ± 4.98</td>
<td>22.52</td>
<td>10.93</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM, n= 3/group

**Fig.1: Comparison of the effect of fraction of the leaves of *M. stenopetala* on the body weight**

**Discussion**

Because of the widespread use of *M. stenopetala* as a food and medicinal plant in the southern Ethiopia[3]. It was felt necessary to investigate the potential toxicity the leaves of *M. stenopetala* in experimental animals. The present study was, therefore, aimed at evaluating the effects of the butanol fraction of the leaves of *M. stenopetala* on behavior, general appearance, body weight, gross pathology of the liver and kidney in laboratory-bred rats. The acute toxicity study was conducted at the doses of 500, 1000, 2000, and 5000mg/kg body weight. Administration of the fraction of *M. stenopetala*, up to the dose of 5000 mg/kg body weight, did not produce significant changes on the general appearance (condition of fur, general cleanliness) and behavioral profile (such as alertness, aggressiveness, irritability), as compared to the controls. Moreover, no death was observed in the treated groups up to the dose of 5000mg/kg during the 14 days of the experimental period. This indicates that the LD50 of the fraction could be greater than 5000mg/kg body weight. According to Clarke and Clarke (1977), any compound or drug with the oral LD50 estimate greater than 1000 mg/kg could be considered low toxic and safe. The butanol fraction may, therefore, be considered relatively safe on acute exposure. Similar results were reported by other workers and it was suggested that, no behavioral changes and death were noted following administration of the fraction of leaves of *M. stenopetala* up to dose of

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5000mg/kg body weight in mice[11-12]. (Nardos et al., 2011; Toma et al., 2012) in both normal and diabetic mice. Liver and kidneys of rats are used by many researchers to assess the safety or toxicity of drugs or plant materials[16-17]. In the present acute toxicity study, gross pathological examination of the liver and kidneys did not show any significant difference/change in size, shape, color and texture upon treatment with the fraction. Though not significant, the absolute live r and kidney weight of treated rats appeared to increase as in size, shape, color and texture upon treatment with the kidneys did not show any significant difference/change.

Researchers to assess the safety or toxicity of drugs or mice. Liver and kidneys of rats are used by many of the leaves of plant materials[16-17]. In the present acute toxicity fraction of the leaves is safe on acute exposure in rats dose of 5000mg/kg. This suggested that the butanol In the current acute toxicity study, the butanol fraction and justifies the use of the leaves of the plant as a conclusion

In the current acute toxicity study, the butanol fraction of M. stenopetala was tolerated in rats up to an oral dose of 5000mg/kg. This suggested that the butanol fraction of the leaves is safe on acute exposure in rats and justifies the use of the leaves of the plant as a source of food by some localities in the southern parts of Ethiopia. It is recommended that, further sub-chronic and chronic toxicity studies should be carried out on blood parameters, histopathological structures of kidney, liver and other vital organs in experimental animals.

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References


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