Evaluation of Acute and Sub-Chronic Toxicities of the topical extract of Mangifera indica Seeds on Wistar albino Rats

Amgad A. Awad El-Gied, Ismail M. Mahmoud, Abelkareem Mohammed, Elnazeer I Hamed

ABSTRACT

Acute toxicity studies in animals are usually necessary for any pharmaceutical intended for human use. The information obtained from these studies is useful in choosing doses for repeat-dose studies, providing preliminary identification of target organs of toxicity, and, occasionally, revealing delayed toxicity. Acute toxicity studies may also aid in the selection of starting doses for Phase 1 human studies, and provide information relevant to acute over-dosing in humans. The present study was designed to investigate the acute and sub-chronic toxicities of the topical extract of Mangifera indica seed on Wistar albino rats. Toxicity study was carried out by using three different doses like 1, 2 and 4 mg/kg/day. Several parameters like aspartate transaminase, alanine transaminase, alkaline phosphatase, total protein, albumin, total bilirubin, direct bilirubin, urea, hemoglobin, red blood cell, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin concentration, white blood cell, lymphocytes, granulocytes and histopathology of liver, kidney, heart and spleen. The extract in all the doses produced significant protection and offered the therapeutic profile related to Mangifera indica extract.

Keywords: Stevioside; rebaudioside-A; photostability; diterpenoid glycosides; 1H and 13C NMR spectral data; chemical studies

INTRODUCTION

Natural products are the cornerstone of health care delivery especially in resource poor settings. Present estimates indicate that about eighty percent of the world’s population relies on traditional medicine for health care delivery (Farnsworth, et al 1985, Akah 2008, Appidi et al 2008). This should be encouraged especially in countries where access to the conventional treatment is inadequate, in as much as efficacy and safety are assured (WHO, 1980). A number of studies have reported the toxic effects of herbal medicines (Kalplovitz 1997, Calixto 2000, Jaouad et al, 2004, Taziebou et al, 2008). Studies of medicinal plants using scientific approaches showed that various biological components of medicinal plants exhibit a variety of properties and can be used to treat various ailments.

MATERIALS AND METHODS

Wistar albino rats weighing 150-200 g were obtained from Animal House facility, research centre, Khartoum and were housed at 25° ± 5° C under 12 hour light and dark cycle. Experiments were carried out according to the guidelines of the animal ethics committee of the research centre. The rats were divided into 4 groups (n=10 in each group) and fed either with the suspension of methanolic extract of Mangifera indica powder of three doses (1 mg/kg (MI 1), 2 mg/kg (MI 2) and 4 mg/kg (MI 3)) or with vehicle by oral gavage once a day for 28 days for acute toxicity studies and 7 days treatment for chronic toxicity studies, along with standard rat chow (Test Diet, USA contains protein 22.06%, fat 4.28%, fiber 3.02%, ash 7.8 %, sand [silica] 1.37% w/w) and water, ad libitum. The difference in body weight of the treated rats compared with control (vehicle), either at the beginning or at end of the study period. The treated rats did not offer any abnormal resistance to drug administration. The treatment schedule did not cause any change in food and water intake pattern. After 48 hours of the last dose, rats were heparinised [375units/200 g i.p] (Neely et al., 1972) and half an hour later rats were anaesthetized with anaesthetic ether, collection of Blood by cardiac puncture and subjected to any one of the protocols like Analysis of serum biochemical parameters, Asparate amino transferase(AST), Alanine amino transferase (ALT), Alkaline phosphatase (ALP), Total protein, Albumin, T.Bilirubin, D. Bilirubin, Urea, Analysis of Hematological parameters likeHemoglobulin (HB), Red blood cell (RBC), Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), White blood cell (WBC), Lymphocytes, Granulocytes.

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Table 1: Body weight and Body weight gain in rats orally given Mangifera indica (MI) extracts for one week

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Bodyweight (g) 0 week</th>
<th>Body weight gain (g) 4 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>85.0 ± 3.16</td>
<td>8.7 ± 4.59</td>
</tr>
<tr>
<td>MI (1mg/kg/day)</td>
<td>83.3 ± 2.79</td>
<td>10.0 ± 2.76*</td>
</tr>
<tr>
<td>MI (2mg/kg/day)</td>
<td>83.3 ± 2.79</td>
<td>9.4 ± 2.46**</td>
</tr>
<tr>
<td>MI (4mg/kg/day)</td>
<td>83.3 ± 2.79</td>
<td>8.5 ± 3.01**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E; **= not significant; *= Significant = (P< 0.05).

Histological Examinations

The hearts were removed, washed immediately with saline and then fixed in 10% buffered formalin. The hearts stored in 10% buffered formalin were embedded in paraffin, sections cut at 5 µm and stained with hematoxylin and eosin. These sections were then examined under a light microscope for histological changes.

Statistical Analysis

Values are expressed as Mean ± SEM; significance is set at P ≤ 0.05. One Way Analysis of variance (ANOVA) was carried out to test the significance of the serum biochemical and Hematological data of the different groups. Chi-square test was used to determine survival /mortality significance of different groups.

RESULTS AND DISCUSSION

Body weight/body weight gain ratio

The effect on the body weight and body weight gain of rats given daily oral doses of Mangifera indica seeds aqueous extract is presented in Table 1.

Acute toxicity group

There is a significant (p<0.05) body weight gain in the rats treated with MI 2 treated groups when compared to control.

Chronic toxicity group

There is a significant (p<0.05) body weight gain in the rats treated with MI 1 treated groups when compared to control. There is no change observed in the other treatment groups.

Haematological changes

Hemoglobin

The HB level is significantly (P<0.005) increased in the MI 1, MI 2 and MI 3 treated groups (13.7± 1.34 g/dl, 14.23±1.28 g/dl & 14.43±0.63 g/dl) respectively, when compared to the group C (9.66± 0.53g/dl).

Red blood cell (RBC)

The RBC level is significantly (P<0.001) increased in the

Table 3: Haematological analysis of rats given Mangifera indica (MI) aqueous extract orally for one week

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One week</td>
</tr>
<tr>
<td></td>
<td>1.Control (normal diet)</td>
</tr>
<tr>
<td></td>
<td>2. MI 1 (1mg/kg/day)</td>
</tr>
<tr>
<td></td>
<td>2. MI 2 (2mg/kg/day)</td>
</tr>
<tr>
<td></td>
<td>4. MI 3 (4mg/kg/day)</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>9.66 ± 0.53</td>
</tr>
<tr>
<td>RBC (X10^6/m³)</td>
<td>5.78 ± 0.42</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>32.47 ± 2.67</td>
</tr>
<tr>
<td>MCV (m³)</td>
<td>56.34 ± 1.36</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>16.73 ± 0.46</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>29.73 ± 0.46</td>
</tr>
<tr>
<td>WBC (X10^3/m³)</td>
<td>4.25 ± 0.46</td>
</tr>
<tr>
<td>Lymphocytes(%)</td>
<td>53.46 ± 9.52</td>
</tr>
<tr>
<td>Granulocytes (%)</td>
<td>40.89 ± 9.95</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E; ** = not significant; *= Significant = (P< 0.05).
MI 1, MI 2 and MI 3 treated groups (8.52 ± 0.80 X10^6 m^3, 8.97 ± 0.95 X10^6 m^3 & 9.19 ± 0.41 X10^6 m^3) respectively, when compare to the group C (5.78 ± 0.42 X10^6 m^3).

Packed cell volume (PCV)

The PCV level is significantly (P<0.001) increased in the MI 1, MI 2 and MI 3 treated groups (51.41 ± 5.08%, 53.01 ± 5.47% & 56.55 ± 2.45%) respectively, when compare to the group C (32.47 ± 2.67%).

Mean corpuscular volume (MCV)

The MCV level is significantly (P<0.001) increased in the MI 1, MI 2 and MI 3 treated groups (60.34 ± 1.75 m^3, 59 ± 1.26 m^3 & 61.83 ± 2.45 m^3) respectively, when compare to the group C (56.34 ± 1.36 m^3).

Mean corpuscular hemoglobin (MCH)

The MCH level is significantly (P<0.001) increased in the MI 1, MI 2 and MI 3 treated groups (16.05 ± 0.30 pg, 15.86 ± 0.59 pg & 15.7 ± 0.71 pg) respectively, when compare to the group C (16.73 ± 0.46 pg).

Mean corpuscular hemoglobin concentration (MCHC)

The MCHC level is significantly (P<0.001) increased in the MI 1, MI 2 and MI 3 treated groups (26.5 ± 0.70 %, 25.5 ± 0.62 % & 30.38 ± 0.85 %) respectively, when compare to the group C (29.73 ± 0.46 %).

White blood cell (WBC)

The WBC level is significantly (P<0.001) increased in the MI 1, MI 2 and MI 3 treated groups (9.11 ± 2.29 X10^3 m^3, 7.16 ± 3.92 X10^3 m^3 & 9.35 ± 7.42 X10^3 m^3) respectively, when compare to the group C (4.25 ± 0.46 X10^3 m^3).

Lymphocytes

The Lymphocytes level is significantly (P<0.001) increased in the MI 1, MI 2 and MI 3 treated groups (48.85 ± 29.24 %, 36.13 ± 6.81 % & 52.67 ± 21.20 %) respectively, when compare to the group C (53.46 ± 9.52 %).

Granulocytes

The Granulocytes level is significantly (P<0.001) increased in the MI 3 treated groups (46.5 ± 31.16 %, 63.06 ± 6.78% & 43.5 ± 22.73 %) respectively, when compare to the group C (40.89 ± 9.52 %).

Serobiochemical changes

AST

In the MI 1, MI 2 and MI 3 treated groups (16.05 ± 0.30 pg, 15.86 ± 0.59 pg & 15.7 ± 0.71 pg) respectively, when compare to the group C (16.73 ± 0.46 pg).

Mean corpuscular hemoglobin concentration (MCHC)

The MCHC level is significantly (P<0.001) increased in the MI 1, MI 2 and MI 3 treated groups (26.5 ± 0.70 %, 25.5 ± 0.62 % & 30.38 ± 0.85 %) respectively, when compare to the group C (29.73 ± 0.46 %).

White blood cell (WBC)

The WBC level is significantly (P<0.001) increased in the MI 1, MI 2 and MI 3 treated groups (9.11 ± 2.29 X10^3 m^3, 7.16 ± 3.92 X10^3 m^3 & 9.35 ± 7.42 X10^3 m^3) respectively, when compare to the group C (4.25 ± 0.46 X10^3 m^3).

Lymphocytes

The Lymphocytes level is significantly (P<0.001) increased in the MI 1, MI 2 and MI 3 treated groups (48.85 ± 29.24 %, 36.13 ± 6.81 % & 52.67 ± 21.20 %) respectively, when compare to the group C (53.46 ± 9.52 %).

Granulocytes

The Granulocytes level is significantly (P<0.001) increased in the MI3 treated groups (46.5 ± 31.16 %, 63.06 ± 6.78% & 43.5 ± 22.73 %) respectively, when compare to the group C (40.89 ± 9.52 %).

Serobiochemical changes

AST

In the MI 1 treatment group the AST levels is not significantly (p<0.005) changed (11.53± 0.54) when compare to Group C (11.22+0.45 IU). But there is a significant (p<0.001) change in the M2 and M3 treatment groups (15.18+1.49 IU and 12.16+0.15 IU).

ALT

In the MI 1 treatment group the ALT levels is not significantly (p<0.005) changed (31.33 ± 1.50) when compare to Group C (32.5 ± 1.04 IU). But there is a significant (p<0.001) change in the M2 and M3 treatment groups (49.34 ± 4.36 IU and 38.4 ± 2.25 IU).

Table 4: Serobiochemical analysis of rats given Mangifera indica (MI) aqueous extract orally for one week

<table>
<thead>
<tr>
<th>Parameters</th>
<th>1. Control (normal diet)</th>
<th>2. MI 1 (1mg/kg/day)</th>
<th>2. MI 2 (2mg/kg/day)</th>
<th>4. MI 3 (4mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (iu)</td>
<td>11.22 ± 0.45</td>
<td>11.53 ± 0.54</td>
<td>15.18 ± 1.49</td>
<td>12.16 ± 0.15</td>
</tr>
<tr>
<td>ALT (iu)</td>
<td>32.5 ± 1.04</td>
<td>31.33 ± 1.50</td>
<td>49.34 ± 4.36</td>
<td>38.4 ± 2.25</td>
</tr>
<tr>
<td>ALP (iu)</td>
<td>201.7 ± 20.9</td>
<td>206 ± 13.7</td>
<td>219 ± 2</td>
<td>209 ± 10.09</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>6.1 ± 0.14</td>
<td>6.1 ± 0.19</td>
<td>5.96 ± 0.22</td>
<td>5.95 ± 0.32</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.45 ± 0.08</td>
<td>3.55 ± 0.19</td>
<td>3.55 ± 0.10</td>
<td>3.5 ± 0.16</td>
</tr>
<tr>
<td>T.Bilirubin (mg/dl)</td>
<td>0.15 ± 0.08</td>
<td>0.18 ± 0.09</td>
<td>0.16 ± 0.08</td>
<td>0.18 ± 0.10</td>
</tr>
<tr>
<td>D.Bilirubin (mg/dl)</td>
<td>0.04 ± 0.01</td>
<td>0.03 ± 0.02</td>
<td>0.04 ± 0.01</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>Urea(mg/dl)</td>
<td>20.67 ± 2.42</td>
<td>21.5 ± 3.27</td>
<td>36.5 ± 4.08</td>
<td>27.16 ± 2.48</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E; ** = not significant; *= Significant = (P< 0.05).

Table 5: Haematological analysis of rats given Mangifera indica (MI) aqueous extract orally for 4 week

<table>
<thead>
<tr>
<th>Parameters</th>
<th>1. Control (normal diet)</th>
<th>2. MI 1 (1mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>9.66 ± 0.53</td>
<td>9.6 ± 1.64</td>
</tr>
<tr>
<td>RBC (X10^6 m^3)</td>
<td>5.78 ± 0.42</td>
<td>5.70 ± 1.22</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>32.47 ± 2.67</td>
<td>31.51 ± 5.32</td>
</tr>
<tr>
<td>MCV (m^3)</td>
<td>56.34 ± 1.36</td>
<td>56 ± 2.82</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>16.73 ± 0.46</td>
<td>15.38 ± 1.07</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>29.73 ± 0.46</td>
<td>26.6 ± 0.90</td>
</tr>
<tr>
<td>WBC (X10^3 m^3)</td>
<td>4.25 ± 0.46</td>
<td>4.07 ± 1.52</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>53.46 ± 9.52</td>
<td>55.4 ± 10.36</td>
</tr>
<tr>
<td>Granulocytes (%)</td>
<td>40.89 ± 9.95</td>
<td>40.5 ± 11.05</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E; ** = not significant; *= Significant = (P<0.05).
to Group C (32.5 ± 1.04 IU). But there is a significant (p<0.001) change in the M2 and M3 treatment groups (49.34 ± 4.36 IU and 38.4 ± 2.25 IU).

Alkaline phosphatase (ALP)
The Alkaline phosphatase level is significantly (P<0.005) increased in the MI 1, MI 2 and MI 3 treated groups (206 ± 13.7 IU, 219 ± 2 IU & 209 ± 10.09 IU) respectively, when compare to the group C (201.7 ± 20.9 IU).

Total protein
There is no significantly changes in the total protein level in any of the treatment groups (6.1 ± 0.14 g/l, 5.96 ± 0.22 g/l & 5.95 ± 0.32 g/l) respectively, when compare to the group C (6.1 ± 0.14 g/l).

Albumin
There is no significantly changes in the Albumin level in any of the treatment groups (3.55 ± 0.19 mg/dl, 3.55 ± 0.10 mg/dl & 3.5 ± 0.16 mg/dl) respectively, when compare to the group C (3.45 ± 0.08 mg/dl).

T.Bilirubin
There is no significantly changes in the T.Bilirubin level in any of the treatment groups (0.18 ± 0.09 mg/dl, 0.16 ± 0.08 mg/dl & 0.18 ± 0.10 mg/dl) respectively, when compare to the group C (0.15 ± 0.08 mg/dl).

D. Bilirubin
There is no significantly changes in the D. Bilirubin level in any of the treatment groups (0.03 ± 0.02 mg/dl, 0.04 ± 0.01 mg/dl & 0.03 ± 0.02 mg/dl) respectively, when compare to the group C (0.04 ± 0.01 mg/dl).

Urea

Table 6: Serobiochemical analysis of rats given Mangiferaindica (MI) aqueous extract orally for 4 week

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1. Control (normal diet)</th>
<th>2. MI 1 (1mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (iu)</td>
<td>11.22 ± 0.45</td>
<td>12.68 ± 0.56</td>
</tr>
<tr>
<td>ALT (iu)</td>
<td>32.5 ± 1.04</td>
<td>41 ± 1.67</td>
</tr>
<tr>
<td>ALP (iu)</td>
<td>201.7 ± 20.9</td>
<td>212.16 ± 5.07</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>6.1 ± 0.14</td>
<td>6 ± 0.24</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.45 ± 0.08</td>
<td>3.53 ± 0.08</td>
</tr>
<tr>
<td>T.Bilirubin (mg/dl)</td>
<td>0.15 ± 0.08</td>
<td>0.18 ± 0.07</td>
</tr>
<tr>
<td>D.Bilirubin (mg/dl)</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.009</td>
</tr>
<tr>
<td>Urea(mg/dl)</td>
<td>20.67 ± 2.42</td>
<td>32.5 ± 4.84</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E; ** = not significant; *= Significant (P< 0.05).

The Alkaline phosphatase level is significantly (P<0.005) increased in the MI 1, MI 2 and MI 3 treated groups (21.5 ± 3.27 mg/dl, 36.5 ± 4.08 mg/dl & 27.16 ± 2.48 mg/dl) respectively, when compare to the group C (20.67 ± 2.42 mg/dl).

Histological changes
After one week of treatment of the topical doses of Mangiferaindica seeds extract. In rats in Group 3 and 4 there were fatty cytoplasmic vaculation of the centrilobular hepatocytes and isolated cell necrosis (Fig.3), segmentation, and packing of the glomerular tubules dilatation of the renal tubules, vaculation or desquamation of the intestinal epithelium (Fig.4) and lymphocytic accumulation. No significant lesions were observed in the heart and spleen of the test rats. The tissues of the control rats (Group 1) showed no lesion throughout the one week in vital organs.

Protocol II Chronic toxicity group (Table No 5, 6)

Haematological changes

Hemoglobin
The HB level is not significantly (P>0.005) changed in the MI 1 treated group (9.6 ± 1.64 g/dl) when compare to the group C (9.66± 0.53g/dl).

Red blood cell (RBC)
The RBC level is not significantly (P>0.001) changed in the MI 1 treated group (5.7 ± 1.22) when compare to the group C (5.78 ± 0.42).

Packed cell volume (PCV)
The PCV level is not significantly (P>0.001) changed in the MI 1 treated group (31.51 ± 5.32%) when compare to the group C (32.47 ± 2.67%).

Mean corpuscular volume (MCV)
The MCV level is not significantly (P>0.001) changed in the MI 1 treated group (56 ± 2.82m3) when compare to the group C (56.34 ± 1.36m3).

Figure 1: Kidney: Mangiferinindica MI treated groups showed the no abnormality in cortical lesion, no dilatation of distal tubules and no compression of proximal tubules (HE X10).
Figure 2: Liver: Mangiferinindica MI treated groups showed the normal hepatocytes of lobular architecture, with hepatic cords of the liver (HE X10).

Mean corpuscular hemoglobin (MCH)
The MCH level is not significantly (P<0.001) changed in the MI 1, treated group (15.38 ± 1.07 pg) when compared to the group C (16.73 ± 0.46 pg).

Mean corpuscular hemoglobin concentration (MCHC)
The MCHC level is not significantly (P<0.001) changed in the MI 1 treated groups (26.6 ± 0.90 %) when compared to the group C (29.73 ± 0.46 %).

White blood cell (WBC)
The WBC level is not significantly (P<0.001) changed in the MI treated group (4.07 ± 1.52 X10^3 m^-3) when compared to the group C (4.25 ± 0.46 X10^3 m^-3).

Lymphocytes
The Lymphocytes level is not significantly (P<0.001) increased in the MI 1 treated group (55.4 ± 10.36 %) when compared to the group C (53.46 ± 9.52 %).

Granulocytes

The Granulocytes level is not significantly (P<0.001) increased in the MI 1 treated group (40.5 ± 11.05 %) when compared to the group C (40.89 ± 9.95 %).

Serobiochemical changes

AST
In the M1 treatment group the AST levels is not significantly (p<0.005) changed (12.68 ± 0.56) when compared to Group C (11.22+0.45 IU).

ALT
In the M1 treatment group the ALT levels is significantly (p<0.005) changed (41 ± 1.67) when compared to Group C (32.5 ± 1.04 IU).

Alkaline phosphatase (ALP)
The Alkaline phosphatase level is not significantly (P<0.005) changed in the MI 1 treated group (212.16 ± 5.07 IU) when compared to the group C (201.7 ± 20.9 IU).

Total protein
There is no significantly changes in the MI 1 treated group total protein level in any of the treatment groups (6.0 ± 0.24 g/l) when compared to the group C (6.1 ± 0.14 g/l).

Albumin
There is no significantly changes in the MI 1 treated group Albumin level in any of the treatment groups (3.53 ± 0.08 mg/dl) when compared to the group C (3.45 ± 0.08 mg/dl).

T.Bilirubin
T.Bilirubin level in the MI 1 treated group there is no significantly changed (0.18 ± 0.07 mg/dl) when compared to the group C (0.15 ± 0.08 mg/dl).

D. Bilirubin
D. Bilirubin level in the MI 1 treatment group is no significantly changes (0.04 ± 0.009 mg/dl) when compared to the group C (0.04 ± 0.01 mg/dl).
Urea

The Alkaline phosphatase level is significantly (P<0.005) changed in MI 1 treated group (32.5 ± 4.84 mg/dl) when compare to the group C (20.67 ± 2.42 mg/dl).

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