Evaluation of Haematological, Biochemical and Histopathological Effects of Aerva Javanica (Ras Elshaieb) Plant Extract on Albino Rats

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Abstract- This study was carried out to detect toxicological effects (haematological, biochemical and histopathological) of ethanolic extract of Aerva javanica plant on albino rats, which was performed as specific weight of the plant sample (500 gram) soaked in 2500 ml of 80% ethanol for about 3 days with daily filtration and evaporation of the solvent under reduced pressure using rotary evaporator apparatus. Final extract residues allowed to air in petri-dishes till complete dryness. 250 and 500 mg/kg/day of ethanolic extract of Aerva javanica whole plant administered to rats for 14 days (2 weeks) then continued for 28 days (4 weeks) as daily oral dosing. The effect on the body weight and body weight gain of rats given daily oral doses of Aerva javanica whole plant ethanolic extract at 250 and 500 mg/kg for 2 and 4 weeks showed no significant changes in body weight and body weight gain. There were no apparent clinical signs observed and no mortalities recorded. The effect on the haematological and plasma biochemical parameters of rats given daily oral doses of Aerva javanica whole plant ethanolic extract at 250 and 500 mg/kg for 2 and 4 weeks showed no significant changes in haematological and plasma biochemical findings, while 500 mg/kg for 4 weeks of the extract had caused hepatocytic necrosis, dilatation of the renal tubules and desquamation of the intestinal epithelium.

Index Items: Aerva javanica, Toxicity, Albino Rats, Ethanolic extract

I. INTRODUCTION

Medicinal plants occupy an important position in the socio-cultural, spiritual and medicinal arena of rural people in many parts of the world[1]. The World Health Organization estimated that 80% of the population of developing countries rely on traditional medicine mostly plant drugs, for their primary health care[2], and showed demand for medicinal plants is increasing in both developing and developed countries, but 90% of these materials are harvested from wild sources without applying scientific management[2]. Hence, many species are under threat to become extinct. Their sustainable management and harvesting can conserve biodiversity, sustain human and environmental health, generate employment and enhance export earnings[2]. Although, the Aerva javanica plant used in traditional medicine of several countries in Africa and Asia for treatment of various disease, little research had been done to investigate the safety of Aerva javanica plant on rodents and other species of livestock Aerva javanica is a perinennial herb belongs to the Genus Aerva from the Family Amaranthaceae. It is distributed in various parts of the world. It is native to Africa and some Asian countries. The plant is used in traditional medicine to treat wounds and inflammation of joints[3], chest pain, ascars, and diarrhoea with blood[4]. Aerva javanica shows antimicrobial[5], anti-diabetic[6], anti-plasmodial[7 and 8], antiviral[9] and anti-oxidant[10] activities. It is widely used for its therapeutic effects in relieving the swelling and pain due to kidney stones.

The objective of the present study was to evaluate the toxicological effect of the ethanolic extract of Aerva javanica (Ras Elshieb) whole Plant on haematological, plasma biochemical parameters and histopathological effects on the tissues of the heart, spleen, liver, kidneys and intestines in Albino Rats.

II. MATERIALS AND METHODS

Plant material

Aerva javanica plant was obtained from Nile river banks (November, 2012) in Khartoum state, Sudan, the research area. Plant was dried under sun-rays. The plant
was authenticated by Taxonomist Dr. Haider Abdel Gader and sample was kept in the Department of Pharmacology and Toxicology at the Medicinal and Aromatic Plants Research Institute (MAPRI).

**Preparation of the plant extract**
Extraction of the *Aerva javanica* whole plant was studied as an ethanolic extract which is performed as specific weight of the plant sample 500 gram soaked in 2500 ml of 80% ethanol for about 3 days with daily filtration and evaporation of the solvent under reduced pressure using rotary evaporator apparatus. Final extract residues were allowed to air in petri-dishes till complete dryness [1].

**Experimental design**

**Animal, management and housing**
Eighteen Albino rats of both sexes (3 months old) with an average weight 150 ± 5 g were housed within the premises of the Medicinal and Aromatic Plant Research Institute, National Center for Research, Khartoum, were fed with standard food pellets and water provided ad libitum. The rats were allotted into three groups each of 6 rats. Group 1 fed normal diet and served as control group. Groups 2 and 3 were given the ethanolic extract of the *Aerva javanica* whole plant at doses of 250 (A) and 500 (B) mg/kg/day as water suspension which was shaken before use and gavaged daily to animals using rat gastric tube for both groups, respectively for 2 and 4 weeks duration time. Body weight measurements were done using the normal balance at day zero, after 2 weeks and after 4 weeks. By the end of week 2 half of the rats were slaughtered and the remaining rats continued daily dosing and were slaughtered at week 4.

**Blood samples**
Blood samples were taken in EDTA coated vacutainer tubes, from cervical blood vessels (as 10 ml for each) for haematological examinations, Haemoglobin (Hb) concentration, red Blood Cells (RBCs) counts, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and White Blood Cells (WBC) counts were determined.

**Preparation of the plasma**
Table 1. Effects of different doses of *Aerva javanica* ethanolic extract on B.W of Albino rats

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Body weight /g</th>
<th>Experimental periods / week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero</td>
<td>2</td>
</tr>
<tr>
<td>Control</td>
<td>106</td>
<td>116</td>
</tr>
<tr>
<td>Gr. A</td>
<td>111.2 ± 0.453</td>
<td>120.5 ± 0.463</td>
</tr>
<tr>
<td>Gr. B</td>
<td>116.7 ± 0.459</td>
<td>120.40 ± 0.518</td>
</tr>
</tbody>
</table>

Results were expressed as mean ± SD

**Haematological findings**
Haematological changes for treated rats, Gr.A and Gr.B were presented in Table 2. The values of Hb, RBC, PCV, MCV, MCH, MCHC and WBC in both treated groups were not significantly changed compared to the control group.

Samples were collected in EDTA coated vacutainer tubes (10 ml for each), and after mild shaking were centrifuged at 3000 revolutions/minute (rpm) for 15 minutes. The fluid part (plasma) was separated from the cellular part using a dropper and the plasma was placed in a new plastic sample container for each rat labelled according to the study group, time and date of collection and stored at -20°C for the analysis of the activity of aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) and for concentrations of total proteins (TP), albumin (ALB), globulins (GL), total bilirubins (BIL), cholesterol (CHOL), urea, sodium (Na) and potassium (K).

**Histopathology**
Specimens of the liver, intestines, kidneys, spleen and heart were immediately fixed in 10% neutral buffered formalin and processed for histopathology, stained with Haematoxylin and Eosin (H and E).

**Blood analysis**
Haemoglobin (Hb) concentration, Red Blood Cells (RBCs) counts, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and White Blood Cell (WBCs) counts are determined by standard methods [11]. Plasma samples were analyzed for the activity of Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) and for concentrations of (TP), (ALB), (GL), (BIL), (CHOL), (urea, K) and (Na), using standard methods [11].

**Statistical analysis**
All values are expressed as mean ± S.E.D. The significance of differences between means was compared at each time point using Duncan's multiple range test after ANOVA for one-way classified data [12].

**III. RESULTS AND DISCUSSION**

**Clinical signs, body weight and body weight gain findings**
No apparent clinical signs (the animals were observed continuously for behavioural changes and for mortality) and no change in body weight findings were observed at the two dose level, Table 1.
Table 2. Effects of different doses of *Aerva javanica* ethanolic extract on haematological parameters of Albino rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>2 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>13.33 ± 0.67</td>
<td>13.10 ± 0.20</td>
<td>13.00 ± 0.10</td>
</tr>
<tr>
<td>RBC (10^6/μl)</td>
<td>8.06 ± 0.58</td>
<td>7.12 ± 0.28</td>
<td>7.97 ± 0.16</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>38.53 ± 2.80</td>
<td>38.47 ± 1.12</td>
<td>38.30 ± 0.53</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>47.80 ± 0.65</td>
<td>54.03 ± 0.45</td>
<td>48.05 ± 0.46</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>38.53 ± 2.80</td>
<td>38.47 ± 1.12</td>
<td>38.30 ± 0.53</td>
</tr>
<tr>
<td>WBC (10^3/μl)</td>
<td>11.03 ± 2.45</td>
<td>9.07 ± 1.21</td>
<td>9.27 ± 1.20</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD.

**Plasma biochemical findings**

Plasma biochemical changes of both treated groups, Gr. A and Gr. B were presented in Table 3. The activity of AST, ALT, ALP and the concentration of (TP), (GL), (BLI), urea, (CHOL), (K) and (Na) in the treated groups were not significantly changed compared to the control group.

Table 3. Effects of different doses of *Aerva javanica* ethanolic extract on plasmabiochemical parameters of Albino rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>2 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/dl)</td>
<td>83.50±18.50</td>
<td>56.00±18.00</td>
<td>74.50±22.50</td>
</tr>
<tr>
<td>ALT (IU/dl)</td>
<td>67.00 ± 5.00</td>
<td>69.00 ± 1.00</td>
<td>74.00± 14.00</td>
</tr>
<tr>
<td>ALP (IU/dl)</td>
<td>271.33± 1.53</td>
<td>280.50± 1.50</td>
<td>295.00±3.00</td>
</tr>
<tr>
<td>Total proteins (g/dl)</td>
<td>7.33 ± 0.65</td>
<td>7.17 ± 0.55</td>
<td>7.17 ± 0.45</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.67 ± 0.25</td>
<td>3.63 ± 0.15</td>
<td>3.60 ± 0.17</td>
</tr>
<tr>
<td>Globulins (g/dl)</td>
<td>3.70 ± 0.40</td>
<td>3.53 ± 0.70</td>
<td>3.26 ± 0.60</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.65 ± 0.05</td>
<td>0.50 ± 0.10</td>
<td>0.40 ± 0.10</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>25.00 ± 1.00</td>
<td>19.00 ± 1.00</td>
<td>20.50 ± 1.50</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>49.50 ± 0.50</td>
<td>53.50 ± 0.50</td>
<td>46.00 ± 1.00</td>
</tr>
<tr>
<td>K (mmol/l)</td>
<td>3.60 ± 0.10</td>
<td>3.63 ± 0.15</td>
<td>3.45 ± 0.15</td>
</tr>
<tr>
<td>Na (mmol/l)</td>
<td>136.50± 1.50</td>
<td>138.50± 1.50</td>
<td>138.17±5.91</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD.

**Histopathological findings**

No significant lesions were observed in the spleen and heart tissue of the both treated groups (Gr. A and Gr. B) for 2 and 4 weeks respectively.

Group A for 2 and 4 weeks and Gr. B for 2 weeks showed no significant lesions in the tissues of the vital organs (liver, kidneys, intestines) but Group B for 4 weeks showed fatty cytoplasmic vaculation of the centrilobular hepatocytes and isolated cell necrosis (Fig. 1), segmentation and packing of the glomerular tubules, dilatation and necrosis of the renal tubules (Fig. 2). There was intestinal catarrhal enteritis with desquamation of the intestinal epithelium and lymphocytic accumulation (Fig. 3).

The tissues of the control rats showed no lesions throughout the study period in all organs.
Fig 1. Liver of rats receiving daily ethanolic extracts of *Aerva javanica* whole plant at 500mg/kg for 4 weeks showing fatty cytoplasmic vaculation of the centrilobular and isolated cell necrosis, H and E x 100.

Fig 2. Kidneys of rats recessing daily ethanolic extract of *Aerva javanica* whole plant at 500mg/kg for 4 weeks showing segmentation, packing and necrosis of the renal proximal convoluted tubules, H and E x 100.

Fig 3. Intestines of rats recessing daily ethanolic extract of *Aerva javanica* whole plant at 500mg/kg for 4 weeks showing catarrhal enteritis with minute erosion of the intestinal epithelium with lymphocytic infiltration, H and E x 200.
The haemoglobin parameters showed no significant differences in all experimental groups. The decrease in the Hb concentration and in the RBCs count, increases in MCV and decreases in MCHC observed in the present study indicated anaemia which might be due to interference of the mechanism of the hematopoiesis, hemolysis of RBCs and/or intestinal malabsorption. Previous investigations showed macrocytic anaemia in rats which had been fed a diet containing 10 or 50% of Rhazya stricta leaves [15] or in chickens which had received a diet consisting of 10% Cassia italica seeds [14].

[15] also observed anaemia of a macrocytic hypochromic type as indicated by increases MCV and decreases in MCHC. Previous investigations showed normocytic normochromic anaemia in rats on 100 g/kg F. Crispa leaves for 8 weeks or normocytic hypochromic anaemia in rats fed a diet containing 100 g/kg of Cuminum cyminum fruits for 6 weeks [15].

The granulocytes seemed to decrease probably due to infiltration in the vital organs. The decrease in the values of the WBCs count observed in the present study might be attributed to the anti-inflammatory effects of some plant constituents [16]. It is well known that the susceptibility of animals to feeding plant material is dependent on the type of the active constituents and concentrations in the amount added to the diet as well as on the rate of their metabolic conversion in the liver to metabolites and consequent excretion [15 and 17].

Although, there were no significant changes observed in the plasma biochemical parameters measured, the changes in the liver and kidneys probably contributed to the fluctuating serum AST, ALT activities and CHOL, urea and ALB concentrations. The mechanism whereby, the plant constituents injured body tissues can not be derived from the present study. There were an increase in the values of ALP activity and also decreases in the TP which was accompanied with decreases in ALB concentration, these results concomitant with the histopathological findings in the intestines which might explain interference of the plant constituents on the process of absorption and the hepatobilary functions [18], and he also mentioned that intestinal dysfunction is accompanied by an increase in none – specific ALP, and reduction in the TP and ALP values. The same author also mentioned that ALT increased when hepatic parenchyma was involved. It has been found that the increase in ALT is associated with periportal liver injury previously described by [19] in sheep, [20] in goats and by [13] in rats and the increase in urea concentration indicates a renal malfunction.

It is clear from the results of the present investigation that the liver and kidneys are the sensitive organs to the toxic action of the active constituents of plant products studied. Damage to the liver and kidneys could explain the development of gradual loss of reflexes. The liver depicted fatty vaculation of the centrilobular hepatocytes, focal necrosis, congestion, haemorrhage and lymphocytic accumulation could explain the toxic effects.

The renal lesions consisted of congestion, haemorrhage, segmented glomeruli and degeneration or necrosis of the glomerular tufts with lymphocytic infiltration could explain toxicity. The intestinal lesions showed desquamation of the intestinal mucosal epithelium and the lumen contain RBCs with aggregated lymphocytes in the intestinal lamina propria.

It is well known that a plant or drug may interact with another plant or drug and as a consequence modification in activity and/or toxicity can be observed. For example, simultaneous feeding of Citrullus colocynthis and Cassia senna resulted in an increased toxic effect on rats [21]. On the other hand, paracetamol-induced hepatonephrotoxicity in rats was reduced by feeding the seeds of Nigella sativa [22].

IV. CONCLUSION

The present results concluded that, entero-hepatorenal changes is a sequel of the Aerva javanica ethanolic extract administered specially at the dose of 500 mg/kg for 4 weeks. The present results recommended the use of the plant as a remedy for the different ailments for short periods beyond 14 days for 500 mg/kg.

Further studies are needed to determine plant constituents responsible for the toxico or immunopharmacological effect.

REFERENCES