Full Length Research paper

Safety evaluation of the extract from the roots of *Pelargonium reniforme* Curtis in male wistar rats

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**Pelargonium reniforme** Curtis is an herb used for the treatment of various human and animal diseases especially in the Eastern Cape of South Africa. The effects of the oral administration of aqueous extract of the plant roots at 100, 200 and 400 mg/kg body weight for 21 days on some haematological and biochemical parameters in male Wistar rats were investigated. Oral treatments with this extract did not cause any significant change in the white blood cell count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, neutrophils, monocytes, large unsustained cells, basophils, total and conjugated bilirubin. Also, the extract did not affect the level of albumin, gamma glutamyl transferase, alanine aminotransaminase, aspartate aminotransaminase, cholesterol, high density lipoprotein cholesterol and the organ body-weight ratio of the animals. The levels of potassium, urea, calcium and magnesium were also not affected by the extract. However, the red blood cell count, haemoglobin, platelets, lymphocytes, total proteins, globulin and sodium levels were increased significantly while the levels of alkaline phosphatase, chloride and uric acid were reduced significantly by the extract. In addition, the levels of packed cell volume, red cell distribution width, eosinophils, triglycerides, creatinine and inorganic phosphorus were altered at specific doses. The available results of this study suggest that the aqueous root extract of *P. reniforme* is not toxic at the doses used in this study and may be safe for medicinal uses.

**Key words:** *Pelargonium reniforme*, haematological parameters, biochemical parameters.

INTRODUCTION

*Pelargonium reniforme* Curtis (Geraniaceae) is an attractive erect shrublet of up to 100 cm height with kidney-shaped leaves and pink flowers. It is indigenous to the Eastern Cape Province of South Africa and occurs mainly in the coastal regions (Latté and Kolodziej, 2004; Kolodziej, 2007).

Previous phytochemical screenings have shown that the aerial parts of *P. reniforme* contain benzoic and cinnamic acid derivatives and also flavonoids and tannins which are its principal phenolic contents. The occurrence of tannins may explain the traditional use of the aerial parts as wound healing agent (Kolodziej, 2007), which may be attributed, at least in part, to their astringent action. A similar rationale explanation based on the presence of tannins may be provided for its use in traditional medicine for the treatment of gastrointestinal disorders such as diarrhea. Decoctions and infusions of the roots enjoy a wide reputation by traditional healers and are highly valued by the southern African native population for its curative and palliative effects in the treatment of respiratory tract infections, gastrointestinal disorders, hepatic disorders and menstrual complaints (Watt and Breyer-Brandwyk, 1962; Hutchings, 1996). Proanthocyanidins present in the roots of this plant may explain its traditional use in the treatment of several ailments (Scholz, 1994; Hör et al., 1995). The claimed curative effects related to hepatic disorders may be explicable on the basis of the radical scavenging activities of the broad range of phenolic compounds which are present in the root (Latté and Kolodziej, 2004; Kolodziej, 2007). The coumarins and phenolic constituents also display moderate antibacterial and fairly high immunomodulatory properties (Kayser and Kolodziej, 1997). The root extracts have been shown to have antibacterial, antifungal and antitubercular activity and this may justify its use by the people of South Africa.
in the treatment of coughs and tuberculosis (M ativandilela et al., 2006). The boiled leaves of this herb are used to protect wounds against maggots (Smith, 1895). In animals, the plant is used to prevent purging in horses and also to treat liver complaints in sheep and calves (Batten and Bokelman, 1966).

Despite the usage of plants in folk medicine over ages, there seems to be paucity of information on its possible toxicity. This study therefore seeks to assess \textit{P. reniforme} for its toxic effects by using haematology, serum chemistry and organ-body weight ratios as indices of toxicity.

\section*{METHODS}

\subsection*{Collection and identification of plant material}

The plant samples were collected in December 2008 from a natural population of \textit{P. reniforme} from Grahamstown in the Eastern Cape Province of South Africa. The plant was identified by Prof. D.S. Grierson of the Department of Botany, University of Fort Hare, and a voucher specimen (GER 3928) was deposited at the Giffen Herbarium of the University.

\subsection*{Animals}

Male Wistar rats (158.6 ± 33.18 g) were used for the study. They were obtained from the animal house of the Agricultural and Rural Development Research Institute, University of Fort Hare. The animals were kept in rat cages and fed on commercial rat pellets (EPOL Feeds, South Africa Ltd.) and allowed free access to fresh water \textit{ad libitum}. The project was approved by the Ethics Committee of the University of Fort Hare.

\subsection*{Assay kits}

The assay kits for creatinine, urea, calcium, sodium, potassium, chloride, phosphorus, albumin, bilirubin, cholesterol, triglycerides, high-density lipoprotein cholesterol, alkaline phosphatase, gamma glutamyl transferase, alanine and aspartate aminotransferases were obtained from Roche Diagnostic GmbH, Mannheim, Germany. All other reagents used were of analytical grade and were supplied by Merck Chemicals (Pty) Ltd., Bellville, South Africa.

\subsection*{Preparation of aqueous extract}

The roots of \textit{P. reniforme} were air-dried at room temperature for seven days. The dried material was then comminuted into coarse powder using the Waring commercial laboratory blender. Hundred grams of the powder was extracted in 1000 ml of distilled water for 48 h on an orbital shaker (Stuart Scientific Orbital Shaker, UK). The extract was filtered using a Buchner funnel and Whatman no. 1 filter paper. The resulting filtrate was freeze-dried (Savant Refrigerated Vapour Trap, RV T41404, USA) to give a yield of 6.15%. This was reconstituted separately in distilled water to give the required doses used in this study.

\subsection*{Animal grouping and extract administration}

Twenty-four male rats were completely randomized into four groups of six and were orally administered as follows: Group A (control) was administered with 0.5 ml of distilled water while groups B, C and D were given 100, 200 and 400 mg/kg body weight of the extract respectively. The administration was done repeatedly on daily basis for three weeks using metal oropharyngeal cannula. Body weights were recorded on days 1 and 21 of the experiment. All rats from each group were sacrificed 24 h after their respective 21 daily doses. The study was carried out following the approval from the Ethical Committee on Animal Use and Care of the University of Fort Hare, South Africa.

\subsection*{Preparation of serum}

The procedure described by Yakubu et al. (2006) was used in the preparation of the serum. Briefly, under ether anaesthesia, the neck area of the rats was quickly shaved to expose the jugular veins. The veins after being slightly displaced (to prevent blood contamination by interstitial fluid) were sharply cut with sterile scalpel blade and an aliquot (2 ml) of the blood was collected into EDTA sample bottles (BD Diagnostics, preanalytical systems, Midrand, USA) for the haematological analysis. Another 5 ml of the blood was allowed to clot for 10 min at room temperature and then centrifuged at 1282 g × 5 min using Hermle Bench Top Centrifuge (Model Hermle, Z300, Hamburg, Germany). The sera were later aspirated with Pasteur pipettes into sample bottles and used within 12 h of preparation for the assay. The rats were quickly dissected in the cold and the organs (liver, kidney, lungs, spleen, heart and testes) removed and freed of fat, blotted with clean tissue paper and then weighed for the determination of organ body weight ratio using the expression of Yakubu et al., (2008).

\subsection*{Determination of biochemical parameters}

Adopting the method of Tietz et al., (1994), the levels of sodium, potassium, chloride, inorganic phosphorus, urea, creatinine, total and conjugated bilirubin, albumin, globulin, total protein, alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), alanine transaminase (ALT), aspartate transaminase (AST), cholesterol, triglycerides and high density lipoprotein cholesterol were determined in the serum using assay kits from Roche Diagnostics on Roche modular (model P800) Mannheim, Germany. The Horiba ABX 80 Diagnostics (ABX pentra Montpellier, France) was used for the determination of haematological parameters which include red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), large unstained cell (LUC), red cell distribution width (RCDW), white blood cell (WBC), neutrophils, monocytes, lymphocytes, eosinophils, basophils and platelet.

\subsection*{Statistical analysis}

Data were expressed as mean of six replicates and were subjected to one way analysis of variance (ANOVA). Means were separated by the Duncan multiple test using SAS. Values were considered statistically significant at p < 0.05.

\section*{RESULTS}

The effects of the graded doses of the aqueous extract of \textit{P. reniforme} on haematological parameters of Wistar rats are shown on Table 1. While there was no significant difference in the effects of all the doses on the WBC,
MCV, MCH, MCHC, neutrophils, monocytes, LUC, and basophils, some significant differences were observed in the levels of the other haematological parameters. The levels of the RBC, Hb, platelets and lymphocytes increased significantly in comparison with the control at all the three doses of the extract. However, dose specific effects were observed on the PCV, RCDW and eosinophils. There was significant increase in the level of the PCV at 100 mg/kg while there was no significant change observed at the other two doses in when compared with the control. Also, the RCDW was observed to increase significantly at 200 mg/kg, decreased significantly at 400 mg/kg but there was no significant change at 100 mg/kg. In addition, the level of the eosinophils was observed to increase significantly at the dose of 400 mg/kg and decrease at 100 mg/kg while no significant change was observed at 200 mg/kg.

The extract showed varied effects on the level of the liver and kidney function indices as shown in Table 2. The levels of total and conjugated bilirubin, albumin, GGT, ALT, AST, potassium, urea, calcium and magnesium remained unaltered in the serum. In contrast, the levels of total protein, globulin, sodium were significantly increased while ALP, chloride and uric acid levels were reduced significantly at all the three doses of the extract in comparison with the control. The serum creatinine was observed to increase significantly at 100 and 200 mg/kg but remained unaltered at 400 mg/kg. Also, the level of the inorganic phosphorus was reduced significantly at 100 and 400 mg/kg while no significant change was observed at 200 mg/kg. No deaths and clinical signs were observed in any of the groups throughout the duration of the experiment. The extract also, did not affect the organ-body weight ratio of the different organs studied.

Administration of the extracts at the different doses did not alter the levels of serum cholesterol and high density lipoprotein cholesterol except for the triglycerides where dose-dependent significant changes were observed in comparison with the control (Table 4).

### DISCUSSION

The assessment of haematological parameters in rats can be used to determine the extent of deleterious effect of a plant extract on animal blood (Yakubu et al., 2007). It is inferred from this study that the aqueous extract of *P. reniforme* may have no toxic effect on the haematological parameters which include red blood cell count, haemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin and red cell distribution width. This showed that the oxygen-carrying capacity of the red blood cells was not compromised thus; the plant may be safe as used in traditional medicine. Some plants have been reported to cause destruction of the red blood cells which leads to anaemia and ultimately cell death (Blood and Radostits, 1989; Adedapo, 2002; Adedapo et al., 2004; 2007a).

The extract of *P. reniforme* caused an increase in the level of the total WBC count and its differentials (neutrophils, monocytes, lymphocytes, eosinophils and basophils). This is an indication that the principal function...
Table 2. Effect of the aqueous extract of *P. reniforme* on the liver and kidney function indices of rats. *n* = 6, Values are mean ± S.D. Extract concentration (mg/kg).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>100</th>
<th>200</th>
<th>400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mmol/L)</td>
<td>142.3 ± 2.39</td>
<td>146.0 ± 1.73</td>
<td>142.6 ± 0.58</td>
<td>142.6 ± 0.58</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>5.9 ± 0.17</td>
<td>5.27 ± 0.47</td>
<td>5.93 ± 0.29</td>
<td>5.93 ± 0.29</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>101.3 ± 2.89</td>
<td>100.3 ± 1.15</td>
<td>96.6 ± 1.15</td>
<td>98.8 ± 2.08</td>
</tr>
<tr>
<td>Inorganic phosphorus (mmol/ L)</td>
<td>3.8 ± 0.29</td>
<td>3.30 ± 0.17</td>
<td>3.77 ± 0.32</td>
<td>3.17 ± 0.12</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>6.77 ± 0.58</td>
<td>7.23 ± 1.46</td>
<td>6.77 ± 0.47</td>
<td>6.70 ± 0.1</td>
</tr>
<tr>
<td>Creatinine (mmol/L)</td>
<td>49.3 ± 0.58</td>
<td>50.3 ± 1.15</td>
<td>57.3 ± 1.15</td>
<td>49.0 ± 2.65</td>
</tr>
<tr>
<td>Total bilirubin (µmol/L)</td>
<td>8.33 ± 0.58</td>
<td>7.66 ± 2.08</td>
<td>9.0 ± 0</td>
<td>11.3 ± 4.04</td>
</tr>
<tr>
<td>Conjugated bilirubin (µmol/L)</td>
<td>2.67 ± 0.58</td>
<td>2.0 ± 0</td>
<td>1.67 ± 0.58</td>
<td>3.67 ± 2.08</td>
</tr>
<tr>
<td>Albumin (mmol/L)</td>
<td>17.3 ± 0.58</td>
<td>17 ± 2</td>
<td>17.6 ± 0.58</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>Globulin (mmol/ L)</td>
<td>48.0 ± 1.73</td>
<td>60.3 ± 1.15</td>
<td>52.0 ± 4.36</td>
<td>54.0 ± 1b</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>65.3 ± 1.15</td>
<td>77.3 ± 2.30</td>
<td>69.6 ± 3.79</td>
<td>73.0 ± 0a</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>472.3 ± 10.97</td>
<td>254.6 ± 43.19</td>
<td>335 ± 54.08</td>
<td>357.3 ± 44.6</td>
</tr>
<tr>
<td>Gamma glutamyltransferase (U/L)</td>
<td>8.0 ± 0a</td>
<td>6.3 ± 1.53a</td>
<td>7.0 ± 2a</td>
<td>7.0 ± 1.53a</td>
</tr>
<tr>
<td>Alanine aminotransaminase (U/L)</td>
<td>46.7 ± 2.89</td>
<td>43.6 ± 8.14</td>
<td>56.0 ± 0</td>
<td>56.3 ± 10.21</td>
</tr>
</tbody>
</table>

Means along a row with the same superscript (a, b or c) as control are not significantly different (p < 0.05).

Table 3. Effect of the aqueous extract of *P. reniforme* on organ-body weight ratios of rats *n* = 6, Values are mean ± S.D.

<table>
<thead>
<tr>
<th>Pelargonium reniforme (mg/kg body weight)</th>
<th>Control</th>
<th>100</th>
<th>200</th>
<th>400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>4.12 ± 0.81</td>
<td>3.39 ± 0.40</td>
<td>3.69 ± 0.57</td>
<td>3.46 ± 0.34</td>
</tr>
<tr>
<td>Lungs</td>
<td>1.35 ± 0.04</td>
<td>1.32 ± 0.33</td>
<td>0.93 ± 0.16</td>
<td>0.98 ± 0.19</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.89 ± 0.09</td>
<td>0.77 ± 0.08</td>
<td>0.79 ± 0.04</td>
<td>0.73 ± 0.07</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.31 ± 0.04</td>
<td>0.39 ± 0.03</td>
<td>0.37 ± 0.12</td>
<td>0.31 ± 0.09</td>
</tr>
<tr>
<td>Heart</td>
<td>0.39 ± 0.01</td>
<td>0.36 ± 0.02</td>
<td>0.33 ± 0.03</td>
<td>0.31 ± 0.02</td>
</tr>
<tr>
<td>Testes</td>
<td>1.29 ± 0.28</td>
<td>0.98 ± 0.22</td>
<td>1.24 ± 0.27</td>
<td>0.89 ± 0.17</td>
</tr>
</tbody>
</table>

Means along a row with the same superscript (a, b or c) as control are not significantly different (p < 0.05).

Table 4. Effect of the aqueous extract of *P. reniforme* on serum lipid profile of rats. *n* = 6, Values are mean ± S.D.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>100</th>
<th>200</th>
<th>400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>1.47 ± 0.29</td>
<td>1.73 ± 0.15</td>
<td>1.63 ± 0.35</td>
<td>1.47 ± 0.15</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.20 ± 0.0a</td>
<td>0.87 ± 0.25</td>
<td>0.77 ± 0.25</td>
<td>1.47 ± 0.15</td>
</tr>
<tr>
<td>HDL</td>
<td>1.23 ± 0.23</td>
<td>1.43 ± 0.06</td>
<td>1.40 ± 0.3a</td>
<td>1.30 ± 0.17</td>
</tr>
</tbody>
</table>

Means along a row with the same superscript (a, b or c) as control are not significantly different (p < 0.05).

of phagocytes, (which is to defend against invading microorganisms by ingesting and destroying them, thus contributing to cellular inflammatory processes), will be enhanced (Paul, 1993; Swenson and Reece, 1993; Jimoh et al., 2008). The observed changes in the levels of the WBC count and its differentials may provide a basis for the antibacterial, antifungal and antitubercular properties of *P. reniforme* (Mativandela et al., 2006).
The extract caused a significant increase in the level of platelets. Platelets, also referred to as thrombocytes, help to mediate blood clotting, which is a meshwork of fibrin fibres. The fibres also adhere to damaged blood vessels, therefore, the blood clot becomes adherent to any vascular opening and thus prevents further blood clot (Wu et al., 1996; Andrews et al., 1997; Nelson et al. 2000). The extract could thus precipitate blood coagulation or clotting, which is desirable especially in cases of severe bleeding or haemorrhage.

The aqueous extract of the plant also caused a significant increase in the levels of total protein, globulin and a dose-dependent increase in the level of albumin. This suggests that there was increased protein synthesis or mobilization. The increase in globulin levels may indicate the potential of the plant to stimulate immune response by increasing antibody production (Puri et al., 1993) and this could also account for its use as a medicinal plant. Albumin is the protein with the highest concentration in the plasma. It transports many small molecules in the blood (example, bilirubin, calcium, progesterone and drugs). It also prevents the fluid in the blood from leaking out into the tissues (Duncan et al., 1994). Since albumin is produced in the liver, increased serum albumin may indicate that the extract promotes the good functioning of the liver.

There were insignificant changes in the levels of total and conjugated bilirubin, gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) while there was a significant decrease in the level of alkaline phosphatase (ALP). Serum ALT is known to increase when there is liver disease and it has been used as a tool for measuring hepatic necrosis (Bush, 1991; Duncan et al., 1994). AST, however, is not a liver specific enzyme as high levels of the enzyme can also be found in skeletal and cardiac muscles as well as red blood cells (Adedapo et al., 2007b). Increase in serum ALP may be considered as an indicator of cholestatis in early stages or mild circumstances preceeding other indicators e.g. hyperbilirubinemia (Adedapo et al., 2007a). GGT has its highest concentration in the kidney and the liver though can be found also in the small intestine and pancreas. These observations of no significant changes in the levels of the liver enzymes, together with total and conjugated bilirubin, in comparison with the control, may indicate that the extract of *P. reniforme* has hepatoprotective effects. The observed insignificant changes in the level of urea in this study also suggest that the kidneys and liver were not adversely affected.

The absence of any significant increase in the organ-body weight ratios following the administration of the extracts suggests that the extract did not cause swelling, atrophy or hypertrophy of the organs (Amresh et al., 2008).

Alterations in the concentration of major lipids like cholesterol, high-density lipoprotein cholesterol and triglycerides can give useful information on the lipid metabolism as well as predisposition of the animals to atherosclerosis and its associated coronary heart diseases (Yakubu et al., 2008). The only significant change observed in the level of the lipids studied was on the triglycerides where significant decrease was observed at 100 and 200 mg/kg and a significant increase at 400 mg/kg. The increase in the serum triglyceride levels at the 400 mg/kg dose might be due to accelerated lipolysis. This may imply depletion in the store of fatty acids at this dose (Yakubu et al., 2008).

The administration of graded doses of the extract to the animals did not result in mortality, neither was there any behavioral change in the animals. All these observations may suggest that the plant is safe for medicinal uses.

### ACKNOWLEDGEMENT

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