Research Article
Toxicological Evaluation of Aqueous Extract of *Lauridia tetragona* (L.F.) R.H. Archer Leaf in Wistar Rats

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Abstract
Background and Objective: Medicinal plants are used in the treatment and management of various diseases. The usage of herbs and their formulations have witnessed progressive increase among the populace especially in the rural areas because of the notion that these products are safe and free from undesir able side effects. This study was aimed at evaluating the toxicological potential of aqueous extract of *Lauridia tetragona* leaf in Wistar rats. Methodology: The acute toxicity test was performed with single oral administration of 5000 mg kg\(^{-1}\) b.wt., of *L. tetragona* extract to rats and the animals were observed for 14 days for signs of toxicity. This was followed by subacute toxicity experiment conducted by oral administration of graded doses (50, 100 and 200 mg kg\(^{-1}\)) of *L. tetragona* extract daily for 28 days. Behavioural changes as well as haematological, biochemical and histological parameters were then evaluated. Data were analysed using two-way ANOVA with the aid of GraphPad Prism software. Results: Results revealed significant reduction (p<0.05) in the feed intake, serum glucose, lymphocytes and platelet levels of extract treated rats compared to the control. There were also some alterations in the liver and kidney function parameters. However, there were no treatment related differences in the histopathological evaluations. Conclusion: It can be concluded that administration of aqueous extract of *L. tetragona* may be safe at the dosages tested in this study but its continuous usage can cause anorexia.

Key words: *Lauridia tetragona*, anorexia, haematological parameters platelet, histological parameters

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.
INTRODUCTION

According to the World Health Organization (WHO), 80% of people in Africa make use of medicinal plants in the treatment and management of various diseases. This may be due to the fact that traditional medicine is readily available, affordable and reliable method of maintaining physiological well-being among the inhabitants. The usage of herbs and their formulations have witnessed progressive increase among the populace especially in the rural areas because of the notion that these products are safe and free from undesirable side effects. However, the chemical composition of herbal medicines are not known and effective dosages remain unclear. In addition, incidence of adverse effects emanating from the usage of herbal medicines have also been reported. Therefore, safety and or toxicity of medicinal plants should be evaluated.

*Lauridia tetragona* belongs to the family Celastraceae and is commonly referred to as bob-cherry or climbing saffron. Though not endemic to South Africa, the plant is distributed across five provinces of the country namely Eastern Cape, KwaZulu-Natal, Limpopo, Mpumalanga and Western Cape. The plant is an evergreen climber not more than 2.5 m high, with grey and flexible branches. The leaves are simple and opposite, leathery, 1.8-8 cm long and 0.6-5 cm wide, while they have small yellowish flowers and fleshy fruits of about 8 mm in diameter and occasionally 2 seeded. The fruits are eaten by children and by birds, while the foliage is sometimes eaten by sheep.

Despite its usage in traditional medical practice in South Africa, there is dearth of information on the efficacy and safety profile of this plant. To the best of our knowledge, there is no previous report on the safety and/or toxicological implication of this plant in literatures. Hence, this study was aimed at determining possible toxicological effects of this plant in Wistar rats.

MATERIALS AND METHODS

**Plant collection:** Matured leaf material of *Lauridia tetragona* was collected between May and June 2016, from Woody Cape backpackers, Port Alfred Area of the Eastern Cape Province. The plant was identified and authenticated by Prof. Maroyi, in the University of Fort Hare, Alice, South Africa. Voucher specimen of the new collection (Win 2016/01) was prepared and deposited at the Giffen’s herbarium. The study was performed in the in the Department of Botany, University of Fort Hare, Alice, South Africa.

**Preparation of extract:** The leaves of *Lauridia tetragona* were washed with copious amount of distilled water to remove dirt and oven dried at 40 °C for a day. The dried leaves were ground to a homogeneous powder, milled and extracted in distilled water on a shaker (Orbital Incubator Shaker, Gallenkamp) at 140 rpm for 24 h. The extract was filtered using a Buchner funnel and Whatman no. 1 filter paper. The filtrate obtained was frozen at -40 °C and dried for 24 h using a freeze dryer (VirTis benchtop, SP Scientific Series, USA). The resulting crude dried extract was stored in the refrigerator prior to use.

**Animals:** Female Wistar rats (*Rattus norvegicus*) at 6 weeks of age were obtained from the Central Animal Unit of the University of Fort Hare. The rats were housed in clean polycarbonate cages in a well-ventilated house. They were fed *ad libitum* with rat chow (Opiol Mice Cubes, Durban, South Africa) and tap water free of contaminants. The animals were allowed to acclimatize for 7 days before the start of the experiment. They were maintained at 22 ± 2 °C on a light/dark cycle for 12 h and 40-45% relative humidity. The cages were cleaned on a daily basis and treatments were in accordance with the guidelines of Ethics Committee on the use and care of Experimental Animals of the University of Fort Hare, Alice, South Africa. The study was approved (REC-270710-028-RA WIN001) prior to commencement.

**Acute toxicity:** Aqueous extract of *Lauridia tetragona* leaves was studied for acute oral toxicity according to the Organization for Economic Cooperation and Development (OECD) guidelines number 420. Twelve rats were divided into two groups consisting of six animals each. The animals were fasted overnight before the commencement of the study. Group A served as control and orally received 1 mL distilled water while group B also received 1 mL 5000 mg kg⁻¹ b.wt., of the extract. All animals were observed for clinical signs including mortality and moribundity, immediately after dosing and at 1, 2, 4, 8 and 12 h, then twice daily for 14 days. On the 14th day, all animals were sacrificed and all organs and tissues were observed microscopically.

**Subacute toxicity:** Twenty four female Wistar rats were randomized into four groups of six animals each. Group 1 (control) were orally administered with 1 mL distilled water. Groups 2-4 were orally treated with 1 mL of 50, 100 and 200 mg kg⁻¹ b.wt./day of *L. tetragona* leaf extract respectively. The extracts were freshly prepared on weekly basis and administrations were done once daily via oral intubation for 28 days. Toxic manifestations and mortality were monitored daily throughout the experimental period.
Preparation of serum and isolation of organs: After 28 days of extract administration, the rats were humanely sacrificed by halothane anaesthetization and blood collected through cardiac puncture. An aliquot (2 mL) of blood was collected into ethylene diamine tetra acetic acid (EDTA) embedded sample bottles for haematological analysis. Another 5 mL of the blood was collected and centrifuged at 1282 rpm for 5 min and the serum was carefully aspirated with a Pasteur pipette into sample bottles for the various biochemical assays. The rats were further dissected and the liver, kidney and heart excised, freed of fat, blotted with clean tissue paper and then weighed. The organ-to-body weight ratios were calculated in percentages.

Determination of haematological parameters: The automated haematology analyzer (Sysmex, KX-21, Japan) was used to analyze the haematological parameters including red blood count (RBC), haemoglobin (Hb), haematocrit, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell width (RDW), white blood count (WBC), lymphocytes and platelets.

Determination of biochemical parameters: The levels of total cholesterol, triacylglycerol, high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were determined in the serum of the animals respectively using standard procedures. The levels of other parameters were determined as described for bilirubin (total and conjugated), total protein, albumin, sodium, calcium, magnesium, potassium and chloride. The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT) and alkaline phosphatase (ALP), urea, uric acid and creatinine were determined using BS-200 automatic biochemistry analyzer (Mibady Co., Ltd.).

Histopathological examination: The organs (liver, kidney and heart) were fixed in 10% (v/v) formaldehyde, dehydrated through ascending grades of ethanol (70, 90 and 95% v/v), cleaned in xylene and embedded in paraffin wax. Tissue sections were prepared and stained with haematoxylin/eosin. The photomicrographs were taken at ×400 using the Leitz, DIALUX research microscope.

Statistical analysis: Statistical analysis was performed using GraphPad Prism 5 statistical package (GraphPad Software, San Diego MA, USA). Data were expressed as means of six replicates±SEM and were subjected to two way analysis of variance (ANOVA) followed by Duncan multiple range test. Statistical significance was considered at p<0.05.

RESULTS

Acute toxicity: The oral administration of the aqueous extract of fresh leaves of Lauridia tetragona did not cause mortality of any rat during the 14 days observation. There was also no sign of toxicity, behavioural or physiological changes. Therefore, the LD_{50} value for the oral administration of aqueous extract of Lauridia tetragona leaf in Wistar rat is greater than 5000 mg kg^{-1}. Therefore, this extract is safe at this dose and at all other dosages below it.

Subacute toxicity

Effect on body and organ weights: Table 1 showed the effects of oral administration of aqueous leaf extract of L. tetragona on the body and organ weights of Wistar rats. There was no significant difference in the body weights, organ weights as well as the relative organ weights of the treated animals compared to control. Though administration of 50 mg kg^{-1} of the extract caused significant elevation of the body weight of the animals were compared to the control.

Feed and water intake: Administration of aqueous extract of L. tetragona leaf caused significant reduction (p<0.05) in feed intake at all dosages tested in this study (Fig. 1a). However,

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (mg kg^{-1})</th>
<th>50</th>
<th>100</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>82.05±2.55</td>
<td>77.56±1.12</td>
<td>83.38±2.90</td>
<td>85.20±1.74</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>182.03±4.53</td>
<td>173.30±6.65*</td>
<td>179.37±4.44</td>
<td>176.92±9.39</td>
</tr>
<tr>
<td>Weight of liver (g)</td>
<td>6.04±1.28</td>
<td>5.94±0.32</td>
<td>6.03±0.27</td>
<td>5.58±0.24</td>
</tr>
<tr>
<td>Weight of kidney (g)</td>
<td>1.33±0.13</td>
<td>1.44±0.10</td>
<td>1.47±0.05</td>
<td>1.39±0.10</td>
</tr>
<tr>
<td>Weight of heart (g)</td>
<td>0.61±0.02</td>
<td>0.67±0.02</td>
<td>0.73±0.02</td>
<td>1.04±0.07</td>
</tr>
<tr>
<td>Liver-body weight (%)</td>
<td>3.23±0.22</td>
<td>3.50±0.13</td>
<td>3.35±0.07</td>
<td>3.04±0.07</td>
</tr>
<tr>
<td>Kidney-body weight (%)</td>
<td>0.71±0.07</td>
<td>0.82±0.05</td>
<td>0.81±0.01</td>
<td>0.78±0.01</td>
</tr>
<tr>
<td>Heart-body weight (%)</td>
<td>0.40±0.02</td>
<td>0.46±0.02</td>
<td>0.42±0.02</td>
<td>0.39±0.02</td>
</tr>
</tbody>
</table>

Values are presented as Mean±SEM (n = 6), *significantly different compared to control at p<0.05.
administration of the extracts does not cause any significant alteration in the water intake of the animals except at the highest concentration (200 mg kg$^{-1}$) where it witnessed significant increase (p<0.05) compared to the control (Fig. 1b).

**Effect on haematological parameters:** Table 2 showed the effect of oral administration of aqueous leaf extract of *L. tetragona* on haematological parameters of Wistar rats. The administration of the extract, at all doses tested led to a significant reduction (p<0.05) in the lymphocytes and platelets. Though there are fluctuations in the other haematological parameters, they are not significantly affected compared to the control.

**Effect on glucose and lipid profile:** The effect of administration of aqueous extract of *L. tetragona* leaf on the serum glucose concentration and lipid profile of the rats is shown in Table 3. Administration of the extract at all dosages tested caused significant decrease (p<0.05) in the glucose concentration of the animals compared to the control. The serum triglyceride concentration also witnessed significant elevation (p<0.05) in the group treated with 200 mg kg$^{-1}$ b.wt., only, while the total cholesterol and HDL-cholesterol were not affected.

**Effect on liver function parameters:** Table 4 showed the effect of oral administration of aqueous leaf extract of *L. tetragona* on liver function parameters of the Wistar rats. There was significant increase (p<0.05) in the activities of aspartate aminotransferase (AST) in all the groups of animals tested compared to the control. Total bilirubin and alanine aminotransferase (ALT) concentration were significantly reduced (p<0.05) in the animals treated with 100 mg kg$^{-1}$ b.wt., of the extract, while alkaline phosphatase
Table 3: Effect of oral administration of the aqueous extract of *Lauridia tetragona* on glucose and lipid profile of Wistar rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>50</th>
<th>100</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol L⁻¹)</td>
<td>5.58±0.75</td>
<td>3.50±0.75*</td>
<td>4.20±0.40*</td>
<td>4.37±0.55*</td>
</tr>
<tr>
<td>Cholesterol (mmol L⁻¹)</td>
<td>0.91±0.16</td>
<td>1.25±0.15</td>
<td>1.15±0.05</td>
<td>1.19±0.03</td>
</tr>
<tr>
<td>Triglyceride (mmol L⁻¹)</td>
<td>1.60±0.67</td>
<td>1.87±0.12</td>
<td>1.46±0.08</td>
<td>3.78±0.77*</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol L⁻¹)</td>
<td>1.10±0.01</td>
<td>0.81±0.01</td>
<td>0.81±0.02</td>
<td>0.76±0.02</td>
</tr>
<tr>
<td>Log (TG/HDL-C)</td>
<td>0.16±0.01</td>
<td>0.36±0.01</td>
<td>0.26±0.01</td>
<td>0.69±0.02</td>
</tr>
</tbody>
</table>

Values are presented as Mean±SEM (n = 6), *significantly different compared to control at p<0.05.

Table 4: Effect of oral administration of the aqueous extract of *Lauridia tetragona* on liver function parameters of Wistar rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>50</th>
<th>100</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g L⁻¹)</td>
<td>48.50±2.65</td>
<td>55.33±1.53</td>
<td>53.67±2.52</td>
<td>50.67±1.15</td>
</tr>
<tr>
<td>Albumin (g L⁻¹)</td>
<td>20.00±0.82</td>
<td>18.67±1.53</td>
<td>20.00±1.00</td>
<td>19.00±1.00</td>
</tr>
<tr>
<td>Total bilirubin (μmol L⁻¹)</td>
<td>24.30±2.64</td>
<td>29.67±2.66</td>
<td>15.30±0.58*</td>
<td>18.33±3.51</td>
</tr>
<tr>
<td>Conjugated bilirubin (μmol L⁻¹)</td>
<td>9.33±2.08</td>
<td>11.67±3.51</td>
<td>6.83±0.29</td>
<td>7.67±2.52</td>
</tr>
<tr>
<td>Alkaline phosphatase (U L⁻¹)</td>
<td>169.50±6.87</td>
<td>156.33±5.36*</td>
<td>187.00±2.00*</td>
<td>175.00±2.65</td>
</tr>
<tr>
<td>γ-glutamyltransferase (U L⁻¹)</td>
<td>&lt;5.00±0.00</td>
<td>&lt;5.00±0.00</td>
<td>&lt;5.00±0.00</td>
<td>&lt;5.00±0.00</td>
</tr>
<tr>
<td>Alanine aminotransferase (U L⁻¹)</td>
<td>72.00±3.74</td>
<td>70.33±6.51</td>
<td>50.00±2.00*</td>
<td>62.67±3.02*</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U L⁻¹)</td>
<td>245.50±7.78</td>
<td>432.67±12.65*</td>
<td>358.67±8.50*</td>
<td>407.00±9.12*</td>
</tr>
</tbody>
</table>

Values are presented as Mean±SEM (n = 6), *significantly different compared to control at p<0.05.

Table 5: Effect of oral administration of the aqueous extract of *Lauridia tetragona* on kidney function parameters of Wistar rats

<table>
<thead>
<tr>
<th>Parameters (mmol L⁻¹)</th>
<th>Control</th>
<th>50</th>
<th>100</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>5.13±0.54</td>
<td>5.27±0.40</td>
<td>5.37±0.15</td>
<td>4.90±0.36</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.28±0.07</td>
<td>0.22±0.07</td>
<td>0.20±0.02</td>
<td>0.27±0.02</td>
</tr>
<tr>
<td>Creatinine</td>
<td>37.00±4.16</td>
<td>42.30±2.58*</td>
<td>42.00±3.61*</td>
<td>30.00±3.00*</td>
</tr>
<tr>
<td>Sodium</td>
<td>139.05±1.29</td>
<td>139.67±1.53</td>
<td>138.00±1.73</td>
<td>139.67±1.53</td>
</tr>
<tr>
<td>Potassium</td>
<td>5.67±0.25</td>
<td>5.00±0.01</td>
<td>5.97±0.15</td>
<td>5.53±0.32</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.34±0.22</td>
<td>2.35±0.18</td>
<td>2.44±0.11</td>
<td>2.31±0.04</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1.10±0.07</td>
<td>1.43±0.13</td>
<td>1.27±0.06</td>
<td>1.20±0.01</td>
</tr>
<tr>
<td>Chloride</td>
<td>106.50±3.42</td>
<td>102.00±3.00*</td>
<td>100.67±2.08*</td>
<td>100.33±1.53*</td>
</tr>
</tbody>
</table>

Values are presented as Mean±SEM (n = 6), *significantly different compared to control at p<0.05.

(ALP) activities was significantly affected following administration of 50 and 100 mg kg⁻¹ of the extract.

**Effect on kidney function parameters:** The effect of administration of aqueous extract of *L. tetragona* leaf on kidney function parameters of rats is shown in Table 5. While the concentration of serum chloride ion was significantly reduced (p<0.05) in all the groups of animals tested in this study compared to the control, the serum creatinine concentration witnessed significant increase (p<0.05) following administration of 50 and 100 mg kg⁻¹ of the extract. Though there were fluctuations in other parameters (urea, uric acid, sodium, potassium, calcium and magnesium), they were not significantly different from the control.

**Effect on integrity of tissues:** No gross abnormalities related to the administration of the extract were observed in any of the euthanized animals at the conclusion of the experiment.

Figure 2a-d, 3a-d and 4a-d showed that no specific lesions were found in the kidney, liver and heart of the animals treated compared to control.

**DISCUSSION**

The mean body weights of animals in all experimental groups increased with the duration of the study and were not significantly different from one another. The weight gained by the animals during the experimental period may be an indication that the extract did not hamper the growth of the animals15. Evaluation of the effect of the plant extract on body weight and relative organ weights is an important test in toxicity evaluation16. The fact that there were no changes in the relative organ weight may also signify the extract did not have any deleterious effect on the integrity of the organs.

The decrease in the food consumption of animals treated with *L. tetragona* extract may imply the extract caused loss
Fig. 2(a-d): Photomicrograph of section of the kidneys of rats following administration of (a) Distilled water, (b) 50 mg kg\(^{-1}\), (c) 100 mg kg\(^{-1}\) and (d) 200 mg kg\(^{-1}\) b.w.t., of aqueous extract of *Lauridia tetragona* leaf

Fig. 3(a-d): Photomicrograph of section of the liver of rats following administration of (a) Distilled water, (b) 50 mg kg\(^{-1}\), (c) 100 mg kg\(^{-1}\) and (d) 200 mg kg\(^{-1}\) b.w.t., of aqueous extract of *Lauridia tetragona* leaf

of appetite for the animals\(^{17}\). However, this does not correspond with the water intake which remained unchanged. (50 and 100 mg kg\(^{-1}\)) and even improved at the highest dose (200 mg kg\(^{-1}\)). Therefore, the reduced feed intake without concomitant decrease in body weight of the animals may imply the extract could serve as food supplement\(^{18}\) and so
may not be regarded as an adverse effect of the extract. It was observed that all the haematological parameters between the control and L. tetragona extract treated rats were similar, with the exception of lymphocytes and platelets. Lymphocytes are the main effector cells of the immune system and so their reduction in this study may signify reduction in the immunity of the animals to fight infection. Platelets on the other hand, when present in sufficient size, number and function are involved in the process of normal coagulation of the blood. So, reduction in platelet levels across all the groups of treated rats compared to the control may be attributed to diminished effect on thrombopoietin. It is worthy of note that administration of the extract reduced the serum glucose concentration of all groups of the extract treated animals. This indicates that the extract displayed hypoglycemic effect and could ameliorate diabetes induced hyperglycemia. Administration of the 200 mg kg⁻¹ b.wt., of the extract also elicited increase in the serum triglyceride, which may be an indication of increase in the energy store of the animals. However, this may not predispose the animals to any adverse effect or danger, as other lipid parameters cholesterol and HDL-cholesterol are not affected.

Serum enzyme measurements is a valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue. The observed elevation in the levels of aspartate aminotransferase (AST) by all the extract treated groups may suggest induction of the enzyme activity by the bioactive constituents of the extract. However, decrease in the activities of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) may signify inhibition of the enzyme activities or inactivation of the enzyme activities in situ. Though there is a significant decrease in the total bilirubin level in the animals administered 100 mg kg⁻¹ of the extract, this is not toxicologically relevant as it does not correlate with other treated groups.

Creatinine is a chemical substance that is generated from muscle metabolism and transported through the blood stream to the kidneys for excretion. The observed elevation of the creatinine concentration following treatment with the extract may be an indication of renal damage. Electrolytes play a central role in gaseous exchange and intercompartmental water balance. The reduction in the serum chloride ion concentration may signify imbalance in the acid-base composition of the body, which might contribute to kidney dysfunction. However, these results may not be enough to conclude that the extract is toxic to the kidney.

The photomicrographs of some organs isolated from the animals showed there was no treatment related microscopic changes in all the organs (Fig. 1–4). All morphological changes observed in the liver was randomly distributed and the incidences were within the range of normal background lesions. It can therefore be inferred that all histological
changes observed were mild and are not considered to be
indication of toxicity of the extract. Therefore the alterations
observed in the biochemical parameters did not reflect at the
tissue level, which may signify the safety of the extract at the
dosages tested or that the alterations will take more time
before physical manifestation.

CONCLUSION

The study showed that 28 day oral administration of
aqueous extract of L. tetragona to Wistar rats at the dosages
50-200 mg kg\(^{-1}\) b.wt. did not produce any serious side effect.
It also displayed hypoglycemic effect which makes it potent
for the treatment of diabetes mellitus. However, there is need
for caution in its consumption as long term usage could lead
to anorexia.

SIGNIFICANCE STATEMENT

This study discovered that aqueous leaf extract of
Lauridia tetragona is safe when administered at the dosages
50-200 mg kg\(^{-1}\) b.wt. It will help researchers and the populace
to know that the consumption of the plant is safe at the tested
dosages.

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