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Full Length Research Paper

Toxicological evaluation of aqueous extracts of *Hermannia incana* Cav. leaves in male Wistar rats

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The effects of the administration of aqueous extract of *Hermannia incana* Cav. leaves at 200, 400 and 600 mg/kg body weight for 14 days on some biochemical parameters of male rats were investigated. Extract administration did not produce any significant effect on the liver and kidney body weight ratios, red blood cell, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, neutrophils, monocytes, basophils, cholesterol, triacylglycerol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and atherogenic index. The extract did not also affect the levels of sodium, potassium, chloride, inorganic phosphorus, urea, creatinine, total protein, globulin, albumin, total and conjugated bilirubin. The activities of alkaline phosphatase, gamma glutamyl transferase and alanine aminotransferase in the serum were increased by the extract. While the large unstained cells and platelets increased at 400 and 600 mg/kg body weight only, the same dose levels also decreased the activity of aspartate aminotransferase in the serum. The levels of lymphocytes and eosinophils were significantly affected at 600 mg/kg body weight. The available evidence in this study suggests that the extract of *H. incana* leaf extract is mild, parameter and dose specific. Therefore, it may not be completely 'safe' as an oral remedy.

Key words: *Hermannia incana*, biochemical parameters, serum lipid, function indices, mild toxicity, haematological parameters, Sterculiaceae.

INTRODUCTION

Hermannia incana Cav. (Sterculiaceae), also known as Mavulakuvaliwe (Xhosa) and sweet yellow bells (English), is a sparsely, hairy prostrate herb with yellow flowers. It is found in the grassland and marshes of the Eastern Cape Province of South Africa. *H. incana* is used as an emetic and the leaf sap extracted in cold water is used to treat stomach-ache and diarrhoea. Decoctions of the whole plant are taken to soothe coughs (Appidi et al., 2008). Our recent study has also validated the anti-diarrhoeal activity of *H. incana* leaves.

The anti-diarrhoeal activities of plants are due to the presence of biologically active compounds. Unfortunately, along with these bioactive agents, plants also produce potentially toxic compounds. As a result, continued evaluations of traditional medicinal plants are required not only to establish the scientific basis for activity, but to make clarifications on the safety or toxicity risk (Palambo, 2006).

To the best of our knowledge as at the time of carrying out this study, there was no suffient information on the toxic implications of aqueous extract of *H. incana* leaves in male Wistar rats. Therefore, the aim of this study was to provide information on the safety/toxicity risk of the aqueous extract of the plant leaves using albino rats as a model.

MATERIALS AND METHODS

Plant material

The plant samples were collected in August 2007 from a natural population of *H. incana* within the premises of University of Fort Hare, Alice, South Africa. The plant was identified by Prof. D.S. Grierson of the Department of Botany, University of Fort Hare, and a voucher specimen (Jaipal Med 001) was deposited at the Giffen Herbarium of the University.

Animals

Male, albino rats (180.18 ± 4.64 g) were obtained from the Animal

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House of the Agricultural and Rural Development Research Institute, University of Fort Hare. All the animals were housed in clean metabolic cages placed in well-ventilated house conditions (temperature 23 ± 1˚C; photoperiod: 12 h natural light and 12 h dark: humidity: 45-50%). They were also allowed free access to Balanced Trusty Chunks (Pioneer Foods [Pty] Ltd, Huguenot, South Africa) and tap water. The cleaning of the cages was done daily.

Assay kits

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

Preparation of extract

The leaves were separated from the stem, washed under running tap and air-dried at room temperature for 4 days. The dried material was pulverized with an electric blender. Fifty grams of the powder was extracted in 500 ml of distilled water for 48 h on an orbital shaker (Stuart Scientific Co., Ltd., Essex, UK). The extract was filtered using a Buchner funnel and Whatman no. 1 filter paper (Maidstone, UK) and the resulting filtrate was freeze-dried (Savant Refrigerated Vapor Trap, RV T41404 California, USA) to give a yield of 6.97 g. This was reconstituted separately in distilled water to give the required doses used in this study.

Animal grouping and extract administration

Twenty, male Wistar rats were completely randomized into four groups of 5 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 200, 400 and 600 mg/kg body weight of the extract, respectively. The doses were as used in the previous study that validated the anti-diarrhoeal property of the plant.

The administration was done orally using metal oropharyngeal cannula. All the rats from each group were sacrificed 24 h after their respective 14 daily doses. The study was carried out following approval from Ethical Committee on Animal Use and Care of the University of Fort Hare, South Africa.

Preparation of serum

The procedure described by Yakubu et al. (2005) was employed in the preparation of serum. Briefly, under ether anaesthesia, rats were made to bleed through their cut jugular veins which were slightly displaced (to prevent blood contamination by interstitial fluid) into clean, dry centrifuge tubes. An aliquot (2 ml) of the blood was collected into sample bottles containing EDTA (BD Diagnostics, Preanalytical Systems, Midrand, USA) for the hematological analysis. Another 5 ml of the blood was allowed to clot for 10 min at room temperature and then centrifuged at 1282 g x 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 thereafter quickly dissected in the cold; the liver and kidney were excised and transferred into ice-cold 0.25 M sucrose solution. The organs were freed of fat, blotted with clean tissue paper and then weighed.

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, gamma glutamyl transferase, alanine and aspartate aminotransferase, cholesterol, triacylglycerol, HDL-C and LDL-C were determined in the serum using assay kits from Roche Diagnostics on Roche modular (model P800) Mannhein, Germany. The Horiba ABX 80 Diagnostics (ABX pentra Montpellier, France) was used for the determination of haematological parameters: red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), large unstained cell (LUC), red cell distribution width (RCDW), white blood cell (WBC), neutrophils, monocytes, lymphocytes, eosinophils, basophils and platelets. The organ-body weight ratio was computed using the expression of Yakubu et al (2008a).

Statistical analysis

Data were expressed as means of five replicates ± SD. Data were subjected to one way Analysis of Variance (ANOVA) and complemented with paired student's t-test. Significant levels were tested at $P < 0.05$.

RESULTS

When compared with the control, administration of the aqueous extract of *H. incana* leaves at 200, 400 and 600 mg/kg body weight did not produce any significant change ($P>0.05$) in the liver and kidney body weight ratios of the animals (Table 1).

The extract produced dose specific effects on some haematological indices. While the values of RBC, Hb, PCV, MCV, MCH, MCHC, RCDW, WBC, neutrophils, monocytes and basophils at all the doses compared favourably with the control values, the LUC and platelets significantly increased at 400 and 600 mg/kg body weight of the extract. The highest dose (600 mg/kg body weight) also increased the level of lymphocytes whereas the same dose decreased the level of eosinophils in the animals (Table 2).

Administration of the extract at the doses investigated did not alter the serum concentrations of cholesterol, triacylglycerol, high and low-density lipoprotein cholesterols and the computed atherogenic index (Table 3).

Whereas the extract did not significantly alter the levels of sodium, potassium, chloride, inorganic phosphorus, urea, creatinine, total protein, globulin, albumin, total and conjugated bilirubin; the serum alkaline phosphatase, gamma glutamyl transferase and alanine aminotransferase activities increased. The activity of aspartate aminotransferase was decreased by the doses of 400 and 600 mg/kg body weight of the extract (Table 4).

DISCUSSION

Although, poisonous plants abound everywhere, herbal medicine is still used by up to 80% of the population of

Table 1. Effect of the *Hermannia incana* leaf extract on the organ- body weight ratios of Wistar rats.

Test samples	Dose (mg/kg body weight)	Liver-body weight ratio (%)	Kidney-body weight ratio (%)
Control	0.5 ml distilled water	3.621 ± 0.04-[a]	0.826 ± 0.03[a]
Hermannia incana leaf extract	200	3.713 ± 0.06[a]	0.815 ± 0.02 [a]
	400	3.670 ± 0.08[a]	0.822 ± 0.04 [a]
	600	3.832 ± 0.05[a]	0.828 ± 0.03 [a]

n = 5, x ± SD. Test values are not significantly different (P< 0.05) from their controls.

Table 2. Effect of *Hermannia incana* leaf extract on haematological parameters of rats.

Haematological parameter	*Hermannia incana* leaf extract (mg/kg body weight)			
	Control	200	400	600
RBC (x 10^{12}/l)	8.66 ± 0.27[a]	8.67 ± 0.14[a]	8.79 ± 0.51[a]	8.71 ± 0.19[a]
Hb (g/dl)	15.46 ± 0.35[a]	15.10 ± 0.25[a]	14.52 ± 0.27[a]	15.32 ± 0.83[a]
PCV (l/l)	0.42 ± 0.04[a]	0.46 ± 0.04[a]	0.48 ± 0.02[a]	0.49 ± 0.05[a]
MCV (fl)	58.91 ± 1.65[a]	58.80 ± 1.39[a]	56.85 ± 1.63[a]	59.07 ± 1.69[a]
MCH (pg)	18.26 ± 0.43[a]	18.30 ± 0.24[a]	18.96 ± 0.38[a]	18.12 ± 0.26[a]
MCHC (g/dL)	31.46 ± 0.48[a]	31.12 ± 0.5[a]	30.07 ± 0.55[a]	30.62 ± 0.15[a]
RCDW (%)	13.12 ± 0.85[a]	13.16 ± 1.03[a]	13.08 ± 1.01[a]	12.83 ± 0.42[a]
LUC (%)	8.20 ± 0.09[a]	7.94 ± 0.76[a]	10.45 ± 0.2[b]	10.88 ± 0.82[b]
WBC (x 10^9/l)	14.86 ± 0.81[a]	14.62 ± 1.19[a]	14.38 ± 1.02[a]	15.24 ± 1.12[a]
Neutrophils (%)	8.76 ± 1.28[a]	8.69 ± 0.87[a]	9.06 ± 0.97[a]	9.78 ± 0.31[a]
Monocytes (%)	29.94 ± 1.61[a]	30.55 ± 1.44[a]	29.98 ± 2.46[a]	30.77 ± 1.49[a]
Lymphocytes (%)	54.46 ± 1.84[a]	52.57 ± 2.31[a]	54.37 ± 2.76[a]	58.29 ± 2.09[b]
Eosinophils (%)	2.48 ± 0.09[a]	2.22 ± 0.07[a]	2.62 ± 0.07[a]	1.97 ± 0.07[b]
Basophils (%)	0.46 ± 0.09[a]	0.50 ± 0.02[a]	0.48 ± 0.03[a]	0.47 ± 0.05[a]
Platelet (x 10^9/l)	896.20± 9.60[a]	889.50 ± 9.47[a]	921.60 ± 6.65[b]	987.91 ± 7.23[c]

n = 5, x ± SD.
[a–c]Test values carrying superscripts different from the control across each parameter are significantly different (p < 0.05). WBC, white blood cell; RBC, red blood cell; Hb, Haemoglobin; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; LUC, large unstained cell; RCDW, red cell distribution width.

Table 3. Effect of *Hermannia incana* leaf extract on serum lipid profile of rats.

Test sample	Dose (mg/kg body weight)	Cholesterol (mmol/l)	Triacylglycerol (mmol/l)	High-density lipoprotein (mmol/l)	Low-density lipoprotein (mmol/l)	Atherogenic index (LDL-C / HDL-C)
Control	0.5 ml distilled water	1.48 ± 0.08[a]	1.21 ± 0.07[a]	1.14 ± 0.05[a]	0.72 ± 0.01[a]	0.63
H. incana leaf extract	200	1.46 ± 0.06[a]	1.24 ± 0.03[a]	1.16 ± 0.03[a]	0.70 ± 0.02[a]	0.60
	400	1.46 ± 0.02[a]	1.20 ± 0.06[a]	1.16 ± 0.05[a]	0.71 ± 0.02[a]	0.61
	600	1.40 ± 0.06[a]	1.22 ± 0.09[a]	1.22 ± 0.09[a]	0.72 ± 0.02[a]	0.59

n = 5, x ± SD. Test values are not significantly different (P< 0.05) from their controls.

developing countries. Despite widespread use, few scientific studies have been undertaken to ascertain the safety or toxicity risks of many herbal remedies (Klaassen and Eaton, 1991).

The liver and kidney body weight ratio of male Wistar rats compared favourably with those of the controls at all the doses of the extracts investigated. This may imply that the extract did not affect the secretory ability of the organs. It is also possible that the extract did not cause any cellular constriction and/or inflammation of the organs. Therefore, the extract at all the doses was not toxicologically significant on this parameter (Schmidt

Table 4. Effect of the of *Hermannia incana* leaf extract on the liver and kidney function indices of rats.

Parameter	*Hermannia incana* leaf extract (mg/kg body weight)			
	Control	200	400	600
Sodium (mmol/L)	140.20 ± 1.30[a]	139.50 ± 1.73[a]	140.60 ± 1.71[a]	140.50 ± 1.92[a]
Potassium (mmol/L)	5.52 ± 0.25[a]	5.35 ± 0.25[a]	5.55 ± 0.07[a]	5.32 ± 0.02[a]
Chloride (mmol/L)	103.80 ± 1.30[a]	104.20 ± 1.42[a]	103.70 ± 1.62[a]	104.00 ± 1.05[a]
Inorganic phosphorus (mmol/ L)	3.06 ± 0.11[a]	2.98 ± 0.03[a]	2.94 ± 0.04[a]	3.00 ± 0.02[a]
Urea (mmol/L)	6.96 ± 0.13[a]	7.01 ± 0.04[a]	7.21 ± 0.08[a]	7.32 ± 0.06[a]
Creatinine (mmol/L)	44.10 ± 1.48[a]	42.75 ± 1.50[a]	45.25 ± 1.75[a]	45.62 ± 0.95[a]
Total bilirubin (µmol/L)	7.40 ± 0.54[a]	7.32 ± 0.07[a]	7.80 ± 0.06[a]	7.83 ± 0.02[a]
Conjugated bilirubin (µmol/L)	2.40 ± 0.04[a]	2.50 ± 0.03[a]	2.45 ± 0.02[a]	2.40 ± 0.03[a]
Albumin (mmol/L)	17.80 ± 0.40[a]	17.50 ± 0.50[a]	17.25 ± 1.02[a]	17.34 ± 1.01[a]
Globulin (mmol/ L)	50.60 ± 1.34[a]	49.70 ± 1.67[a]	50.75 ± 0.97[a]	50.50 ± 1.31[a]
Total protein (g/L)	68.40 ± 1.22[a]	67.20 ± 1.34[a]	68.00 ± 1.99[a]	67.84 ± 1.99[a]
Alkaline phosphatase (U/L)	425.00 ± 1.32[a]	439.00 ± 2.48[b]	448.00 ± 1.42[b]	432.00 ± 1.88[b]
Gamma glutamyl transferase (U/L)	25.10 ± 0.09[a]	35.12 ± 0.02[a]	48.14 ± 0.05[a]	45.15 ± 0.04[c]
Alanine aminotransaminase (U/L)	67.00 ± 1.82[a]	64.10 ± 2.22[b]	68.00 ±1.94 [b]	64.60 ± 1.55[b]
Aspartate aminotransaminase (U/L)	221.49 ±2.21[a]	224.00 ± 2.87[a]	220.69 ± 3.67[a]	219.75 ± 6.18[a]

n = 5, x ± SD. [a–b] Test values carrying superscripts different from the control across each parameter are significantly different ($p < 0.05$).

et al., 2007).

In this study, the levels of RBC, Hb, PCV, MCV, MCH, MCHC, RCDW, WBC, neutrophils, monocytes and basophils at all the doses were not altered whereas those of LUC, platelets, lymphocytes and eosinophils were affected at specific doses. This may suggest mild effect on the haematological indices as well as dose and parameter specific of the extract on the blood indices (Yakubu et al., 2007).

Alterations in the concentration of major lipids like cholesterol, high and low-density lipoprotein cholesterols, and triacylglycerol 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., 2008b). Elevated levels of all lipids except the HDL-C are associated with increased risk of atherosclerosis. The absence of any effect on all the serum lipid parameters investigated in this study suggests that lipid metabolism in the animals was not altered. This might be an indication that the extract may not likely predispose the animals to atherosclerosis and its associated coronary heart diseases.

The biochemical indices of liver and kidney damage monitored in the serum in this study are useful markers for assessing the functional capacities of the organs Yakubu et al., 2003). Biochemical indices of organ function, if altered, will impair the normal functioning of the organs (Afolayan and Yakubu, 2009). Therefore, the absence of significant effect on the liver and kidney function indices by the extract of *H. incana* leaves is an indication that the normal functioning of these organs were not affected. It further indicates that the normal functioning of the nephron at the tubular and globular levels was not altered.

There are many enzymes found in the serum that did not originally originate from the serum. During tissue damage, some of these enzymes find their way into the serum, probably by leakage (Wills, 1985). Serum enzyme measurements are therefore a valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue. Therefore, the increase in serum alkaline phosphatase and gamma glutamyl transferase activities may indicate tissue damage probably by altered cell membrane permeability leading to the leakage of the enzymes from the tissues to the serum. Loss of alkaline phosphatase from the tissues to the serum may have consequential effect on the adequate transportation of required ions or molecules across the cell membrane (Akanji and Yakubu, 2000; Akanji et al., 1993). Similarly, the leakage of gamma glutamyl transferase from the tissue may affect glutathione metabolism and resorption of amino acids in the organs (Kaplan and Pesce, 1996). Alanine and aspartate aminotransaminases are considered to be sensitive indicators of hepatocellular damage and within limit can provide a quantitative evaluation of the degree of damage to the liver (Al-Habori et al., 2002). The increase in serum alanine aminotransferase is quite understandable since the enzyme is cytosolic in origin. Any disruption in the plasma membrane of the organ will consequentially lead to raised levels of the enzyme in the serum as obtained in the present study. However the dose specific decrease in the activity of aspartate aminotransferasein the serum may further imply inhibition of the enzyme as well as selective effect of the extract on the aminotransferase.

In summary, the evidence from this study has suggested that while some haematological parameters, lipid profile, liver and kidney function indices were not altered by the extract, the same cannot be described for the serum enzyme and the remaining haematological parameters. Therefore, the effect of the extract is mild, parameter and dose selective and thus may not be completely safe for oral administration 'safe' as an oral remedy as used in folklore medicine of South Africa.

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