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Original article

Safety evaluation of aqueous root extract of *Hermannia geniculata* EckL. & Zeyh. (Streculiaceae) in Wistar rats

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Abstract

Introduction: *Hermannia geniculata* is a medicinal plant used by the Basotho tribe (South Africa) for the management of diarrhoea and sugar-related disorders either individually or in synergy with orthodox antidiabetic drugs.

Methods: Aqueous root extract of *Hermannia geniculata* was evaluated for its safety and or toxicity in Wistar rats. A preliminary evaluation in rats observed signs of toxicity over a 14 day period after a single oral dose of 5000 mg/kg body weight of *H. geniculata* extract. Detailed experiments were then conducted by orally administering graded doses (75, 150 and 300 mg/kg) of *H. geniculata* extract to rats daily for 28 days. Behavioural changes as well as haematological, biochemical and histological parameters were then evaluated.

Results: The extract significantly reduced ($p < 0.05$) white blood cells and other haematological parameters. The levels of conjugated bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase and calcium ion were also significantly affected ($p < 0.05$) by the administration of *H. geniculata* extracts. Histopathological study on the lungs, liver, kidney and heart of the animals after treatment with *H. geniculata* extract, showed that the organs were not structurally different compared to that of the control.

Conclusions: Results suggest that at these doses the administration of aqueous root extract of *H. geniculata* to Wistar rats did not produce any deleterious effect on the livers, kidneys, lungs or hearts of the animals. However, caution is needed as continuous usage could lead to reduction of systemic immunity.

Keywords: *Hermannia geniculata*; Toxicity testing; Safety; Pharmacology; White blood cells

Introduction

The use of medicinal plants in the treatment of disease conditions has been used for many years [1]. Indigenous knowledge of medicinal plants has made them a valuable tool in the treatment of several ailments. The knowledge about the various functions of plants is communicated from generation to generation through frequent usage and by oral tradition [2]. Natural products are known to be a major source of chemical substances with possible therapeutic activity. World Health Organization (WHO) reported that 80% of the world's population use medicinal plants as their main primary health care source in the treatment of diseases [3].

Herbs and herbal formulations for the treatment of ailments have continued to receive prominent attention because of the strong belief that these products are safe [4,5]. This assumption to a large extent may have influenced the indiscriminate use of these formulations by many, particularly amongst the rural populace. However, general acceptability of herbal medicines has been limited by lack of defined chemical characterization, dose regimen and adequate toxicity data to evaluate their safety [6]. The incidence of adverse effects and sometimes life-threatening conditions emanating from these herbal medicines has been reported in previous studies [7,8]. Several warnings have also been issued regarding the potential adverse effects of herbal remedies [9,10].

Hermannia geniculata is in the Streculiaceae family and commonly known as Seletjane among the Basotho tribe of the Eastern Free State Province of South Africa [11]. There are about

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six to eight species of Hermannia but only *H. geniculata* is often used in the traditional Basotho medicine after *H. depressa*. The dry root material is chopped, boiled in water and taken three times daily to ameliorate blood sugar disorders. It is also used in the management of diarrhoea, heartburn, stomach disorder and flatulency called "leletha" in pregnant Sotho women [11]. Despite its usage as source of medicine among the Basotho tribe, there is dearth of scientific information on the efficacy as well as safety and or toxicity of this plant. To the best of our knowledge, there is no previous report on the safety or toxicological evaluation of this important plant in the literature. Hence, the objective of this study was to assess the *in-vivo* toxicological potential of the aqueous root extract of this plant in Wistar rats.

Materials and methods

Plant collection

The plant material (*Hermannia geniculata*) was collected from the vegetation along Wetsi cafe at Monontsha in Qwaqwa, eastern Free State Province. The species identity was confirmed with the herbarium specimen (collected by Prof. Moffett in 1993 with voucher specimen file number 5056.000-10700) at the University of the Free State, Qwaqwa campus herbarium and further authenticated at the Bew's herbarium, University of KwaZulu Natal Pietermarisburg campus. Voucher specimen of the new collection (AshMed/05/2013/QwHB) was prepared and deposited at the Qwaqwa herbarium.

Preparation of extract

The root was separated, washed under running tap to remove all debris and chop into small pieces before being dried in the oven for 9 days at 45 °C to a constant weight. The dried root material was pulverized into fine powder using Waring laboratory blender (Labon, Durban, South Africa). 85 g dry powdered material was extracted in 1.0 L distilled water for 72 h on an orbital shaker at room temperature. This was centrifuged at 1500 × *g* for 5 min and the filtrate further filtered with Watman No 1 filter paper and freeze dried using Virtis BenchTop (SP Scientific Series, USA) freeze dryer and the yield was 13.80 g. This was reconstituted in distilled water to various concentrations before administration to experimental animals.

Animals

Male albino rats (160–200 g) of Wistar strain were obtained from the animal facility of the University of the Free State, Bloemfontein campus. The animals were kept in clean metallic cages placed in a well ventilated house with optimum condition (temperature: 23 ± 1 °C; photoperiod: 12 h natural light and 12 h dark; humidity: 45–50%). They were allowed free access to commercial pelleted rat chow (Opiol Mice Cubes, Durban, South Africa) and water *ad libitum*. The floors of the cages were filled with sterilized (autoclaved) saw dusts and the cleaning was done on every other day basis. The study was carried out after obtaining approval from the University of the Free State Interfaculty Ethics Committee on the use and care of animals with approval number NR-02-2013.

Preliminary evaluation

Preliminary evaluation was performed in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines [12]. Ten (10) animals weighing 180–200 g were divided into two groups consisting of five 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 body weight (b/w) of the extract. All animals were observed for clinical signs including mortality and morbidity, 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 macroscopically.

Experimental design

Forty male Wistar rats (215–235 g) were randomized into four groups of ten animals each. Group 1 (control) were orally administered with 1 mL distilled water. Groups 2 to 4 were orally treated with 1 mL of 75, 150 and 300 mg/kg body weight/day of *H. geniculata* root extract. The treatment continued for 28 days at approximately 9 o'clock in the morning daily and the administration was done using metal oropharyngeal cannula.

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 × *g* 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, heart and lungs 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 Haematologic Analyzer (Sysmex, KX-21, Japan) was used to analyse 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, triacyglycerol, 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 [13–16]. The levels of other parameters were determined as described for