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Acute and sub-acute toxicity of the methanolic extract of *Pteleopsis hylodendron* stem bark

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ABSTRACT

Ethnopharmacological relevance: Pteleopsis hylodendron is one of the most popular medicinal plants in Cameroon where it is used to treat measles, chickenpox, sexually transmitted diseases, female sterility, liver and kidney disorders as well as dropsy. To date there is no documented evidence corroborating its safety. This study thus aimed to determine the toxicity profile of the methanolic extract of *Pteleopsis hylodendron*.

Materials and Methods: The acute and sub-chronic toxicity of the methanolic extract of Pteleopsis hylodendron were investigated by employing established methods. The acute toxicity study was done by administering single doses (2–8 g/kg body weight) of plant extract to adult mice. For the sub chronic toxicity study, doses (85–680 mg/kg bw) of plant extract were administered daily to adult rats during 28 days after which the effect on organs, the hematological and biochemical parameters was assessed.

Results: In mice, single oral administrations of the methanolic extract of Pteleopsis hylodendron caused dose-dependent general behaviour adverse effects and mortality. The LD50 values were 3.00 and 3.60 g/kg bw for males and females respectively. In rats, daily single oral doses of the methanolic extract of Pteleopsis hylodendron provoked significant ($p < 0.05$) growth retardation in rats at all tested doses after 28 days of dosing. Haematological parameters showed a significant decrease in white blood cells count and significant increases red blood cells count; irrespective of the sex, all biochemical parameters studied, except triglycerides significantly ($p < 0.001$) increased with dose. However, a dose-dependent significant ($p < 0.007$) increase in HDL was observed only in male rats. Increases in liver enzymes (ALT and AST), proteins and creatinine levels correlate the observed histopathological damages (i.e. inflammation and vascular congestions) in the liver and kidneys.

Conclusions: The overall results of this study indicate that the methanolic extract of Pteleopsis hylodendron stem bark possesses hepatotoxic and nephrotoxic effects at doses ≥85 mg/kg bw, suggesting that this plant should be used with caution.

1. Introduction

Plants and plant-based derivatives constitute an important part of the human health care system since ancient civilization. Studies carried out by Raskin et al. (2002) have shown that 30% of the commercialized drugs contain active principles that were isolated for the first time from medicinal plants. These plants can bring about a solution to certain diseases, mostly in developing countries where synthetic drugs are relatively expensive and not very accessible to the underprivileged social strata (Kuiate et al., 2005). *Pteleopsis hylodendron* Mildbr. is a tree belonging to the family Combretaceae, commonly found in the forest regions of West and Central Africa (Irvin, 1961). Ten species of the genus *Pteleopsis* are found in Africa but only *Pteleopsis hylodendron* is present in Cameroon (Ngounou et al., 1999). This plant is highly used in Cameroonian traditional medicine. The aqueous decoction of the stem bark of *Pteleopsis hylodendron* is used to treat measles, chickenpox, sexually transmitted diseases, female sterility, liver and kidney disorders as well as dropsy (Motso, 2007). It has been reported that ethyl acetate and methanolic extracts of the stem bark of this plant possess antioxidant and antimicrobial activities against several pathogenic microorganisms namely: *Bacillus cereus*, *Corynebacterium diphtheriae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Strepto-*

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coccus pyogenes (Atta-Ur-Rahman et al., 2001; Mokale, 2009). However, to the best of our knowledge, no report is available on the safety profile of *Pteleopsis hylodendron*. It was on this basis that we carried out the toxicity studies of the methanolic extract of its stem bark.

2. Materials and methods

2.1. Collection of plant material

The stem bark of *Pteleopsis hylodendron* was collected from the Central Region of Cameroon in February 2009. A specimen was identified at the National Herbarium (Yaoundé, Cameroon) using the one registered under the reference number 44913/HNC.

2.2. Preparation of crude extract

The coarse powder of *Pteleopsis hylodendron* (370 g) was macerated at room temperature in methanol (4 L) for 48 h once, stirring at intervals. The resulting mixture was filtered and the filtrate was concentrated by evaporating the methanol at 40 °C under reduced pressure using a rotary evaporator (Büchi R200). The extract was further concentrated by allowing it to stand overnight in an oven at 30 °C. The yield of the extract (15.96%, w/w) was calculated with respect to the initial weight of the dry plant powder.

2.3. Experimental animals

Adult healthy *Swiss* albino mice (10–12 weeks old; 20–30 g) and *Wistar* rats (8–10 weeks old; 160–200 g) of both sexes were used respectively for the acute and sub-acute toxicity experiments. They were obtained from the Animal House of the Department of Biochemistry (University of Dschang) and housed in plastic cages under normal laboratory conditions (12 h light/dark cycle: 25 ± 2 °C) for an acclimatization period of 7 days prior to the experiments. All the animals were given food and water *ad libitum*. The bioassay was conducted in accordance with the internationally acceptable guidelines for evaluating the safety and efficacy of herbal medicines (WHO, 2000; OECD, 2008).

2.4. Acute oral toxicity study

In order to study any possible toxic effect or changes in normal behavior, 5 groups of 10 mice (5 males and 5 females) each were used in this experiment. The control group received the vehicle (1% DMSO in distilled water), and test groups received arithmetic doses of 2, 4, 6, and 8 g/kg body weight of the extract. Those doses were chosen after several screenings on mice. The experimental animals were deprived of food for 18 h prior to extract administration. They were monitored continuously for 3 h thereafter for any signs of toxicity such as reduction in locomotion, aggressiveness, reaction to stimuli (tail pinch, noise), social interactions, aspect of feces and mortality. After this period, the animals were supplied food and water *ad libitum*. Dead animals in each group were counted within 48 h following the administration of the extract and DL_{50} values were determined in both sexes using the Behrens and Karber's formula (1983). The surviving animals were monitored daily for 14 days for changes in body weight, food and water consumptions.

2.5. Sub-acute oral toxicity study

In a repeated dose experiment, fifty albino rats of both sexes were used. They were kept under the same conditions as described above. The animals were randomly divided into five groups of 10 animals (5 males and 5 females) each. Animals of group 1 served as control and received the vehicle only (1% DMSO in distilled water) while those of groups 2, 3, 4 and 5 were treated daily with methanolic extract of *Pteleopsis hylodendron* at 85, 170, 340, and 680 mg/kg body weight respectively for 28 days. These doses are fractions of the LD_{50} values obtained in acute toxicity, the highest dose being the fifth of the average LD_{50} value. These animals were observed for morbidity and mortality. Body weights were recorded at the beginning, then once after every two days during the study. Water and food consumptions were noted daily and individually. This work was carried out following the welfare of animals as recommended by WHO (2000).

2.5.1. Preparation of serum samples

At the end of the sub-acute experiment, the animals were subjected to a 24 h fast, anaesthetized by intraperitoneal injection of thiopental and then dissected. The blood samples were collected by cardiac puncture then introduced into two sets of plastic tubes with one containing EDTA and the other nothing. The blood collected without EDTA was allowed to stand for complete clotting. The clotted blood samples were centrifuged at 3000 rpm for 15 min and serum samples were aspired off and frozen.

2.5.2. Preparation of tissue homogenate

The homogenate of each organ (i.e. liver and kidneys) was prepared in 0.9% NaCl solution at 15% (i.e. 15 g organ in 100 ml solution).

2.5.3. Hematological and biochemical analysis

A Malassez's chamber was used to quantify the total red blood cells (RBCs) and white blood cells (WBCs) counts on the EDTA-collected blood. Serum biochemical parameters such as creatinine, ALT, AST, total cholesterol, triglycerides, HDL and LDL cholesterol were estimated using commercial kits (Hospitex Diagnostics std, Roma, Italia). Urine, serum and organ proteins were determined by the Biuret method as described by Gornall et al. (1949).

2.6. Histological analysis

Slices of the liver and kidney tissues of animals of each group were fixed in 10% formalin for one month and then, embedded in paraffin. Sections of 5–6 mm were routinely stained with haematoxylin and eosin (H & E) and examined under a light microscope (Olympus CH02). Any alterations compared to the normal structure were registered.

2.7. Statistical analysis

The values were expressed as mean ± standard deviation. For each parameter, the One-Way ANOVA was used to detect significant differences between the groups. When significant differences existed, the Waller–Duncan test ($p < 0.05$) was used to compare the means.

3. Results

3.1. Acute toxicity

3.1.1. Behavioral observations and mortality patterns

Mice treated with the methanolic extract of the stem bark of *Pteleopsis hylodendron* showed behavioral changes like prostration, motionlessness, slow response to external stimuli and rapid breathing at all the tested doses starting from 15 to 45 min after extract administration. This happened for a period of 5 days. After that, everything was restored to survivors.