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Histopathological Studies of Physostigma venenosum Balf. on Wistar Rats

Aihiokhai M. O.[1], Idu M.[2], Ovuakporie-Uvo O.[1]

[1] Department of Biological Sciences, Faculty of Natural and Applied Sciences, Michael and Cecilia Ibru University, Agbarha-Otor, PMB 100, Ughelli, Delta State, Nigeria.

[2] Phytomedicine Unit, Department of Plant Biology and Biotechnology, University of Benin, PMB 1154, Benin City, Nigeria.

*Corresponding author: Ovuakporie-Uvo O., E-mail: oghale.uvo@gmail.com

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ABSTRACT

Physostigma venenosum Balf. seeds which have been implicated for treating diverse ailments were investigated for possible toxicity of the liver in both sexes of Wistar rats. Sixteen Wistar were weighed and divided into four groups of three treatment groups and one control group. Animals were allowed to acclimatize in standard metal cages for 7 days thereafter, fed with graded doses of *P. venenosum* extract. Group A (control) was administered 10 mg/kg distilled water only, while groups B, C and D were respectively administered with 10, 20 and 30 mg/kg b.w of *P. venenosum* seed extract of by gastric intubation for 14 days. On day 14, animals in all groups were anaesthetized, their liver were isolated and processed for histopathological studies. Rats treated with 10 mg/kg extracts showed presence of activated kupffer cells, sinusoidal dilatation and moderate vascular congestion**,** while the groups treated with 20 mg/kg and 30 mg/kg b.w showed mild vascular congestion, mild kupffer cell activation and mild tissue separation, which were significantly different from the control. Histopathological examination suggests that doses higher than 20 mg/kg may possibly induce liver cell injury.

Keyword: *Physostigma venenosum* (Balf.), Liver, Histopathology

INTRODUCTION

Medicinal plants are extensively used traditionally in treating all forms of diseases. Usually, infusions, decoctions with unspecified quantities are often consumed without consideration of the lethal and other antagonistic effects. Results of many acute, chronic and sub-chronic toxicity tests of various plant extracts show that the liver and kidneys are the major organs usually affected because of the metabolic role both organs play in the body [1]. Patel [2] reported that the liver is the key organ for metabolism of various xenobiotics and therapeutic agents which accumulate in various tissues, while they are carried to the bile for elimination via the hepatocytes. Hepatotoxic and nephrotoxic effects are to be expected, since the liver acts as the main detoxifying organ for chemical substances; hence in a bid to detoxify these toxins, the liver could be compromised or overwhelmed when faced with excess toxins [3]. The use of herbal medicine is sometimes associated with liver injuries ranging from mild elevation of liver enzymes to liver failure often requiring a new transplant and carcinogenesis [4].

The plant *Physostigma venenosum* (Fabaceae) produces seeds commonly called Calabar beans. It is a large, herbaceous, twining climbing perennial plant, with woody stem at the base that grows up to 2 inches (5 cm) in diameter and attains a height of about 50 feet (16 m). The beans, which are thick, with deep brown chocolate colour ripens at all seasons, but are best and most abundant during the rainy season in Nigeria, June – September [5]. Ethnobotanically, *P. venenosum* is used to treat diseases of the eye, chronic constipation, epilepsy, cholera, Alzheimer and hypodermically in acute tetanus [6]. Calabar beans have a dramatic history of human use. The people in the Calabar region of Nigeria made great use of it as a type of botanical judge and an ordeal poison to know persons possessed by evil spirits. Fortunately, the practice was outlawed by the British and strict measures were enforced to put an end to the trials [6]. Although, the plant has been reported to be very poisonous and toxic, there are no literature reports on possible histological changes caused by the administration of *P. venenosum* on internal organs at low concentration [7]. Hence, the present research was aimed at investigating; the possible histopathological effects of ethanolic seed extract of *P. venenosum* on the liver of Wistar rats at graded dose levels.

MATERIALS AND METHODS

Plant Material

The seeds of *Physostigma venenosum* used for this research were obtained dried from a local market in Calabar metropolis in Cross Rivers State of Nigeria and was identified and authenticated at the Herbarium unit of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria.

Extraction of the plant

The dried seeds were further dried in an oven at 60°C for 1 hr after which they were blended into fine powder. Four hundred and thirty grams (430 g) of the powder were extracted by maceration with 95% ethanol and concentrated in a rotary evaporator to (Buchi 461, Switzerland) at 55 °C to obtain 72 g (16.7% yield) of the crude extract.

Experimental Animals

Sixteen (16) Wistar albino rats of both sexes weighing between (200 – 250) g were used for this study for a period of two weeks. The animals were housed in separate cages, kept in a clean environment and provided with standard animal chow and water *ad-libitum* at the Animal House of the Pharmacology Department, University of Benin, Benin City. The animals were allowed to acclimatize to their new environment for 7 days before experimenting with them. Care and handling of the animals was conducted in compliance with International Animal Welfare Guidelines. The 16 rats of both sexes were randomly distributed into four groups (n=4). The treatment groups (B, C and D) were orally administered with ethanol extract of *P. venenosum* at 10, 20 and 30 mg/kg/day, respectively for two weeks, while the control group (A) received distilled water (10 ml/kg) with no add-on substance for 14 days. A pilot test was first carried out to determine the dose regimen of the plant

extract. At the end of the experimental period, all rats were sacrificed and liver tissues were immediately collected for histopathological analysis.

Histopathological studies

The liver of the dissected rats were taken and fixed in 10 % v/v formalin solution for 24 hours. After fixation, the method described by Aliyu *et al.* [8] with slight modifications, was adopted for histopathological analysis. Tissue slides were stained in Hematoxylin & Eosin (H & E) and examined under a light microscope then photomicrographs of the tissues were taken using a 14 mega pixel sony camera.

RESULTS

Photomicrographs of liver sections derived from rats in the control and treatment groups are shown on Plates 1. In the control group, the liver exhibited a normal architecture of hepatocytes separated by vascular sinusoids, with no observed pathological abnormalities. The treatment groups administered different concentrations of *P. venenosum* extract showed mild kupffer cell activation, tissue separation, moderate vascular congestion and dilatation.

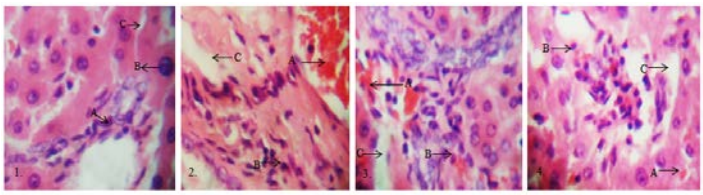

Plate1: Rat liver

1. Control: Rat liver composed portal triad 'A', hepatocytes, 'B' separated by sinusoid 'C' (H&E x 40).
2. Group treated with 10 mg/kg of body weight *P. venenosum* for 7 days showing moderate vascular congestion and dilatation 'A', mild kupffer cell activation 'B' and mild tissue separation 'C' (H&E x 40).
3. Group treated with 20 mg/kg of body weight *P. venenosum* for 7 days showing mild vascular congestion 'A', mild kupffer cell activation 'B' and mild tissue separation 'C' (H&E x 40).
4: Rat liver treated with 30 mg/kg of body weight *P. venenosum* for 7 days showing mild vascular congestion 'A', moderate kupffer cell activation 'B' and mild tissue separation 'C'(H&E x 40).

DISCUSSION

Herbal medicines are widely erroneously assumed to be free from side effects because they are natural. Hence, medicinal herbs are usually self-prescribed by the consumers with no proper control and review in terms of dose, mode and frequency of administration [9]. This ought not to be because, the chemicals in herbal drugs may be natural to the plant, but they are not natural to the human body. Any compound with therapeutic effect has the potential to be incorrectly prescribed or overdosed and may exhibit some adverse results [10]. This is not different with *P. venenosum*. Previous study as reported by Aihiokhai *et al.* [11] showed that the ethanolic seed extract of *P. venenosum* has immune-system inducing activity with obvious toxicity symptoms at high doses on rats based on their hematological and biochemical parameters. According to Olson *et al.* [12], the safety of drugs and plant products for human use can be determined using toxicological evaluation which is usually carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a safe dose in humans. From this present study, mild activation of kupffer cells, tissue separation, sinusoidal dilatation and moderate vascular congestion of rat liver treated with 10, 20 and 30 mg/kg body weight of *P. venenosum* extract which are significantly different from the control group are notable caution signals for the use of *P. venenosum* seeds in humans. These responses to treatment suggest that whenever *P. venenosum* may be considered as a medicine, doses within or even below 10 mg/kg should be considered as any dose higher than 20 mg/kg may be injurious to the liver when taken over a long period of time.

In conclusion, although, the plant extract at a low dose level produces mild stimulation of the kuffer cells, it may still be considered in the manufacture of useful drugs subjected to monitoring to determine its mechanism of action on the liver and the kidney.

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