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Study of toxicity of aqueous extract of *Psorospermum febrifugum* Spach root bark on *Wistar* rats

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Abstract

Psorospermum febrifugum Spach was a very common plant in tropical regions. In Benin, its roots bark was effective in curing anemia. The aim of this work was to test the biological tolerance of the aqueous extract of the bark of these roots. On the aqueous root bark extract of the plant, were conducted *in vivo* acute and sub-chronic oral toxicity tests according to OECD guideline 423. For this purpose, *Wistar* rats were either force-fed at a dose of 2000 mg extract/kg body weight and monitored for 14 days, or at 200 mg extract/kg body weight daily for 28 days. Blood tests were performed on days 0, 14 and 28 as well as histology of the liver, kidney and spleen. In the acute and sub-chronic oral toxicity tests, the weight of the rats did not change significantly compared to controls. AST and ALT transaminases did not increase suggesting an absence of hepatic cytolysis, confirmed by the normal appearance of the hepatic parenchyma. Uremia and serum creatinine also did not significantly change, indicating no renal dysfunction, confirmed by normal histology of the kidney. The number of blood leukocytes did not significantly vary, suggesting no disturbance of immunity, confirmed by the normal histology of the spleen, a secondary immune organ. So, the aqueous extract of *Psorospermum febrifugum* root bark did not reveal toxicity. This study deserves to be continued by chronic toxicity tests and clinical trials for transformation into Traditional Improved Medicine (MTA).

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Introduction

The use of plants for therapeutic purposes is a multisecular practice (Kassel, 2003). In underdeveloped countries, access to pharmaceutical drugs is difficult for the vast majority of people because of poverty. 80% of these people mostly located in rural areas resort to medicinal plants to solve their various health problems (Schmincke, 2003; Yakubu *et al.*, 2009Pingali *et al.*, 2015). These plants may contain inherent active ingredients used to cure disease or relieve pain (Okigbo *et al.*, 2008). Unfortunately, the medicinal use of plants is accompanied by little or no knowledge about the toxicity of such plants (Oduola *et al.*, 2007). Therefore, this raises the question of the efficacy and safety of these traditional medicines.

Some finding highlighted potential toxic effects associated with some common herbal medicines marketed for different indications. Chaparral, creosote bush made with *Larrea tridentata* leaves and twigs and used for blood thinner, weight loss or as antioxidant, anticancer, anti-arthritis showed carcinogenic, nephrotoxic and hepatotoxic activities (Arteaga, 2005). Aloe drug derived from *Aloe Vera* leaves used as wound healing and laxative displayed cytogenetic toxicity (Verma, 2012).Renal damage has been associated with the use of the medicinal plants in the treatment of different disorders, including diabetes mellitus (Mapanga and Musabayane, 2010).
So toxicity of herbal medicines needs attention in order to prevent side effects (Haq, 2004; Philomena, 2011; Nasri, 2013). WHO encouraged scientific investigations in this area to select the best ones for beneficial use.

Psorospermum febrifugum Spach is widely used in traditional medicine in Africa. It grows in savannas and tropical ears and belongs the family of Hypericaceae (Arbonnier, 2000).It is employed in the treatment of varied conditions including fevers and skin problems. Phytochemical screening of the different parts of the plant revealed the presence of various medically active compounds.The root bark contained catechuic tannins. Steroids and terpenes are present in the bark and roots (Burkill, 2004).

In Benin, various plants are used in traditional medicine to cure anemia (Nwaehujor *et al.*, 2015; Senou *et al.*, 2016; Tchogou *et al.*, 2016). One of them is *Psorospermum febrifugum* Spach. The present work aimed to test the safety of the aqueous extract of this plant roots bark in experimental animal model.

Materials and methods

Animal material

Animal material consisted of *Wistar* albino rats of average body weight 200 g approximately, having free access to water and food and acclimated to farming conditions from the pet of the Biomembrane and Cell Signaling Laboratory in Faculty of Sciences and Techniques of the University of Abomey-Calavi (UAC) in Benin Republic. Breeding was done in a well ventilated room, with a day-night rhythm of 12h.

The animals were kept in wire mesh cages with metal feeders and drinking troughs. Their daily diet was made from a mixture of food in the form of croquettes and marketed by Vet Services (Benin). The enclosure was regularly cleaned to ensure optimal development of the animals avoid infection.

Identification and preparation of plant material

Psorospermum febrifugum Spach(Clusiaceae) roots bark was collected from Atlantic Department in Benin during April 2015.

The collected samples were identified and certified at the National Herbarium of the University of Abomey Calavi under the number AA6625 / HNB. The samples were dried at moderate temperatures (20-25° C), protected from moisture for four weeks. They were then crushed into powder and stored in suitable containers at room temperature. 50 g of the powder was boiled in 500 ml of distilled water contained in a 1000 ml flask for 30 minutes. After cooling the filtrate collected is evaporated in a rotary evaporator between 50° C and 60° C. The extract was dried in an oven at 50° C. The dry residue obtained was powdered and kept in the refrigerator in a black bottle.

Acute toxicity test

Acute toxicity test was carried out as recommended by the guideline 423 of the Organization for Economic Cooperation and Development for the testing of chemicals (OECD, 2002).

The substance was tested in a sequential process in which three animals including multiparous females and no pregnant aged 8 to 12 weeks are used at each stage. The absence or the manifestation of substance related mortality in a group dosed at a step would determine the next step. The initial dose was selected from the following four doses: 5, 50, 300 and 2000 mg/kg body weight. We administered by gavage to animals 2000 mg, of *Psorospermum febrifugum* Spach roots bark aqueous extract/kg body weight. The animals were observed carefully during the four (4) hours and then daily for 14 days. They were weighed and blood was collected by orbital puncture at the start of the experiment and then after 14 days.

Sub-chronic toxicity test

Five *Wistar* rats received the *Psorospermum febrifugum* Spach roots bark aqueous extract at 200 mg/kg body weight, daily for 28 consecutive days by gavage (Biswas *et al.*, 2010). They were weighed and blood was collected by orbital puncture at the start of the experiment and then after 28 days.

Blood tests

Biochemical parameters such as uremia and serum creatinine measured to explore kidney function. Transaminases AST and ALT were assayed for liver function. The WBC count was performed as hematological parameter.

Histology

At the end of the experiment, the animals were dissected. The liver, the kidney and the spleen were removed, fixed in Bouin solution, and embedded in paraffin.

The specimens sections (5 µm) were mounted on glass slides, deparaffinated, and hydrated. For histological analysis, sections were stained with hematoxylin and eosin (H&E), following a standard protocol (Sènou *et al.*, 2009). The pictures were taken at 400X magnification.

Statistical analysis

The values were presented as mean plus or minus twice the standard error on the mean (mean ± 2 SEM). The means were compared using Mann-Whitney test. The significance level was set at 5%.

Results

Aqueous extract of Psorospermum febrifugum Spach root bark did not exhibit any acute toxicity

Acute oral toxicity was assessed by measuring physical parameters such as the weight of rats, liver function parameters as AST and ALT transaminases, kidney function indicators such as uremia and serum creatinine, and immune function parameter such as the number of blood leukocytes (Table 1).

Table 1. Acute oral toxicity.

Parameters	Means at D0	Means at D14	P value	Difference
Rat weight (g)	210 ± 46	214 ± 50	0.9	no significant
Uremia (g/L)	0.16 ± 0.01	0.16 ± 0.01	0.9	no significant
Creatinine (mg/L)	6.0 ± 1.0	6.0 ± 2.0	0.9	no significant
Transaminase AST (IU/L)	124 ± 10	124 ± 8	0.9	no significant
Transaminase ALT (IU/L)	54 ± 25	55 ± 25	0.9	no significant
White Blood Cells (G/L)	6.8 ± 1.2	7.3 ± 1.1	0.8	no significant

The mean weight of the rats was 210 ± 46 g at day 0 and did not significantly change at day 14, suggesting no disturbance of the physical parameters of the rats.

The mean serum urea and creatinine levels were 0.16 ± 0.01 g/L and 6 ± 1 mg/L, respectively. They did not significantly change on Day 14 suggesting an absence

of renal function impairment. Transaminases AST and ALT were respectively 116 ± 8 IU/L and 65 ± 10 IU/L on day 0. They did not significantly change on Day 14 suggesting no impairment of liver function. The mean number of blood leukocytes was 6.9 ± 1.3 G/L. It did not significantly change on day 14, suggesting no disturbance of immune function.

Table 2. Sub-chronic toxicity.

Parameters	Means at D0	Means at D28	Pvalue	Difference
Rat weight (g)	194 ± 18	202 ± 15	0.6	no significant
Uremia (g/L)	0.27 ± 0.04	0.29 ± 0.03	0.7	no significant
Creatinine (mg/L)	8.0 ± 1.4	8.2 ± 1.4	0.9	no significant
Transaminase AST (IU/L)	122 ± 13	116 ± 16	0.8	no significant
Transaminase ALT (IU/L)	55 ± 11	53 ± 13	0.7	no significant
White Blood Cells (G/L)	5.9 ± 1.4	6.5 ± 1.7	0.8	no significant

Aqueous extract of Psorospermum febrifugum Spach root bark did not show sub-chronic toxicity

Sub-chronic oral toxicity was assessed by the same parameters previously measured for acute oral toxicity, namely: rat weight, transaminases, uremia, creatinine, and white blood cell counts (Table 2).

The mean weight of the rats was 194 ± 18 g on day 0. There was a slight increase on day 28, which however is not statistically significant suggesting no physical involvement of the rats.

The mean serum urea and creatinine levels were 0.27 ± 0.04 g/L and 8.0 ± 1.4 mg/L, respectively. They did not significantly change on day 28, suggesting a lack of renal function impairment. The mean values of the AST and ALT transaminases were 122 ± 13 IU/L and 55 ± 11 IU/L, respectively, on D0. They slightly decreased at day 28, suggesting a liver function protection. However, this decline was not statistically significant. The mean number of blood leukocytes was 5.9 ± 1.4 G/L at day 0. It did not significantly change at day 28, suggesting no disturbance of immune function.

Aqueous extract of Psorospermum febrifugum Spach root bark did not alter the hepatic, renal and splenic parenchyma in the acute or sub-chronic state

In acute (Fig. 1B) or sub-chronic (Fig. 1C) toxicity tests, liver parenchyma was typical as that of untreated rats (Fig. 1A). Hepatocytes (arrows) showed no visible atypia and were well ranged in rays around centrilobular veins (V). Between these rays, the sinusoids (S) were clearly visible.

Similarly, in acute (Fig. 1E) or sub-chronic (Fig. 1F) toxicity test, the kidney displayed the typical parenchyma of untreated rats (Fig. 1D). The glomeruli (G), proximal tubules (PT), distal tubules (DT) and collecting ducts (CD) underwent the characteristic appearance without visible abnormalities.

Splenic parenchyma was also typical in the acute (Fig. 1H) or sub-chronic (Fig. 1I) oral toxicity test as in untreated rats (Fig. 1G).Peri-arteriolar sheaths (AS) of lymphocytes around central arteries (CA) and germinal centers (GC) of white pulp were typical.It was the same for the vein sinusoids (S) and the Billroth cords (BC) of the red pulp.

Discussion

The aqueous extract of *Psorospermum febrifugum* root bark did not alter either the behavior or the weight of the rats in the acute or sub-chronic oral toxicity tests. Similar results were obtained with aqueous suspensions of *Jatropha tanjorensis* leaves and also with the roots or shell extracts of *Cocos nucifera* which also exhibit antianemic property (Costa *et al.*, 2011; Idu, 2014; Tchogou *et al.*, 2017). In the acute or sub-chronic oral toxicity tests, root bark extract did not affect liver function, since AST and ALT transaminases did not increase and hepatic

parenchyma did not display atypia. Similar results are obtained with the methanoic or aqueous extract of the leaf sheath of *Sorghum bicolor*, which also had hematopoietic effects (Nwinyi, 2009; Sènou *et al.*, 2017). A hepatoprotective effect was also observed with the leaves and fruits of *Solanum macrocarpon*, a hypocholesterolemic plant (Dougnon *et al.*, 2013). In contrast, administration for 14 days of *Sorghum bicolor* leaf sheath extract to *Sprague* rats showed a slight increase in ALT Transaminase with no effect on other liver parameters (Akande *et al.*, 2010).

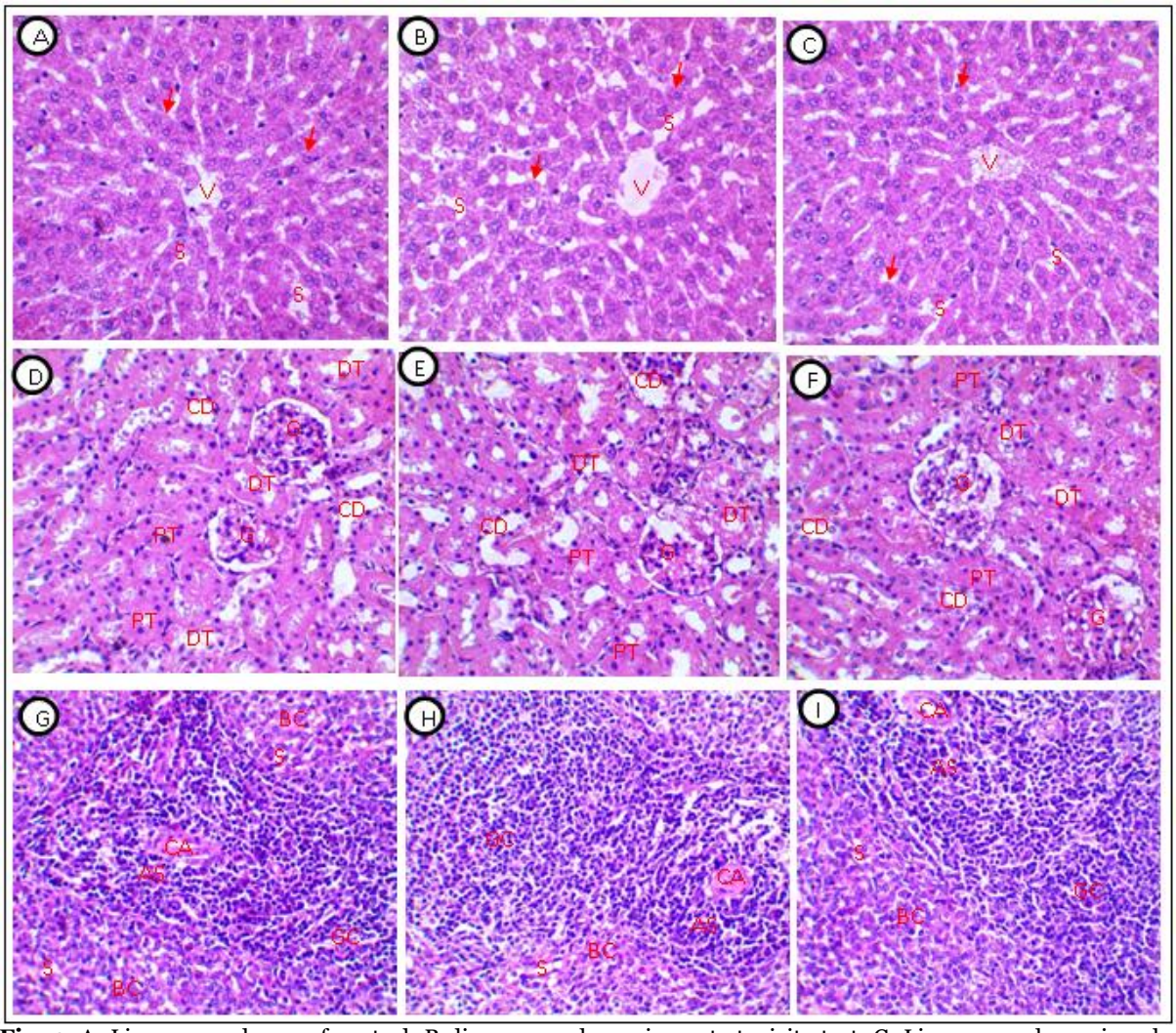

Fig. 1. A: Liver parenchyma of control; B: liver parenchyma in acute toxicity test; C: Liver parenchyma in sub-chronic toxicity test. Arrow: hepatocytes; S: sinusoid; V: centrilobular veins; D: Kidney parenchyma of control; E: kidney parenchyma in acute toxicity test; F: kidney parenchyma in sub-chronic toxicity test. G: glomérules; PT: proximal tubule; DT: distal tubule; CD: collecting duct. G: Splenic parenchyma of control; H: splenic parenchyma in acute toxicity test; I: splenic parenchyma in sub-chronic toxicity test. AS: Peri-arteriolar sheaths (AS) of lymphocytes; CA: central artery; GC: germinal center; S: vein sinusoid; BC: Billroth cords.

The renal balance assessed by of serum urea and creatinine did not change in the acute or sub-chronic oral toxicity tests, suggesting that *Psorospermum febrifugum* root bark extract was not nephrotoxic. The observation was confirmed in histology showing a renal parenchyma with glomeruli, proximal and distal tubules, and collecting ducts with normal architecture. The same observation was made with the root aqueous extract of *Cocos nucifera* (Tchogou *et al.*, 2017). A protective effect of renal function was also observed with *Cocos nucifera* nut water, which lowered uremia and serum creatinine in a model of

ethylene glycol-induced nephropathy (Gandhi *et al.*, 2013).

The immune balance assessed by blood leukocytes count cells was not affected at acute or sub-chronic oral toxicity tests. That was the same for histology of the spleen, peripheral immune organ which was not modified by the tests, suggesting an absence of disturbance of immunity by the extract. The same observations were previously made with the aqueous extracts of the leaf sheath of *Sorghum bicolor* and the roots of *Cocos nucifera* which did not alter the immune function (Sènou *et al.*, 2016; Tchogou *et al.*, 2017). Morever, an anti-inflammatory effect was noted with the ethyl acetate extract of *Cocos nucifera* fiber following a xylene local induction of inflammation in animal experiments (Silva *et al.*, 2009). The extract phytochemical screening revealed the presence of many compounds, some of which were antioxidants (Burkill, 2004).

The lack of cytotoxic effect observed in the various organs may be partly due to the antioxidant protection of these chemical groups.

Conclusion

Psorospermum febrifugum root bark extract seemed to protect the main vital functions of the body, as shown acute and sub-chronic toxicity tests. However, the investigations on its biological tolerance deserve to be continued by the chronic toxicity tests and the clinical trials before considering a possible transformation in Traditional Improved Medicine (TIM).

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