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Repeated toxicological study and cardiotoxicity of hydroalcoholic root extract of *Paullinia pinnata* L (Sapindaceae)

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ABSTRACT

Paullinia pinnata L. is a plant widely used in African traditional medicine especially in the treatment of erectile dysfunction. This study aims to evaluate the cardiotoxicity of 50% hydroalcoholic extract of the roots of *P. pinnata*. The result of the acute toxicity test has shown a LD50 greater than 5000 mg/kg. During the 28 days subchronic administration, *P. pinnata* has increased significantly the relative weight of kidney. *P. pinnata* has induced also a microcytosis and an isolated hypochromia. Renal injuries were observed with doses of 400 mg/kg and 800 mg/kg; and are noted by the increase in blood urea, creatinine, potassium and chlorine. Cardiac disorders characterized by the increase of creatinine phosphokinase with *P. pinnata* at 800 mg/kg has been noted; as well as cholestasis, characterized by an increase in the ALP at 200, 400 and 800 mg/kg. The study conducted on the isolated auricle of guinea pigs, has shown that *P. pinnata*, at increasing concentrations (0.5 to 2.5 mg/mL) has caused an increase in the force of contraction (positive inotropic effect) and simultaneously a decrease in heart rate (negative chronotropic effect). The positive inotropic effect observed could justify the traditional use of this plant as an aphrodisiac.

INTRODUCTION

Paullinia pinnata L is a medicinal plant which belongs to the Sapindaceae family. *P. pinnata* leaves and roots are used in traditional medicine in the treatment of erectile dysfunction, malaria and dysentery (Abbiw, 1990). *P. pinnata* leaves are also used in the treatment of pathologies such as: snake bite, rabies, mental disorders, eye disorders, blindness, abdominal pain. *S*everal studies including pharmacological and chemical studies have shown that *P. pinnata L* has an antimicrobial (Annan and Houghton, 2010; de Souza *et al.*, 1995), dermatological, antiemetics, antiparasitic, antispasmodics, (Chabra *et al.*, 1991; Dokosi, 1998; Yusuf *et al*., 2014), antipyretics (Gill, 1992; Fred-and Jaiyesimi *et al.*, 2011) and antimalarial (Maje *et al.*, 2007) properties. The anticonvulsant activity of the stem bark of *P. pinnata* has been demonstrated by Maiha *et al.*, (2009). For its aphrodisiac use, *P. pinnata* roots are often soaked in a mixture of water and alcohol. *P. pinnata* is found to contain no alkaloids but a triterpene saponin, a triterpene aglycone and a flavotannin (Kerharo and Adam, 1974). *P. pinnata* flavotannin has a cardiotonic effect on isolated frog's heart and on the heart of mammals (Broadbent, 1962). Zamble *et al.*, (2006) have demonstrated the vasodilatory activity of *P. pinnata*, and then it's mechanism on the penis erection. *Paullinia cupana*, a very close species to *P. pinnata*, also called Guarana, is used in sweetened or carbonated soft drinks and energy shots, an ingredient of herbal teas or contained in capsules (Weinberg and Bealer, 2001). It was demonstrated that *P. pinnata* leaves and roots extracts is rich in phenolic compounds, saponins, triterpene, catechols and cardiac tannins (Bowden, 1965; Zamble *et al.*, 2006; N'Guessan *et al.*, 2011; Abourashed *et al.*, 1999; Dongo *et al.*, 2009) Adinortey *et al.*, (2012) have studied the toxicity of the ethanolic roots of *P. pinnata*.

Doses up to 850 mg/kg per os, for 14 days, have induced a leukocytosis and microcytosis. Because of the extensive use of *P. pinnata* root's hydroalcoholic extract in traditional medicine, especially in erectile dysfunction and the presence of cardiac tannins in this plant, we aim in this study to evaluate the 28 days subchronic toxicity and cardiotoxicity of the 50% root hydroalcoholic extract of *P. pinnata*.

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MATERIALS AND METHODS

The study was conducted following an approved animal use protocol from the institutional Ethical Committee for Teaching and Research (ref no. CNCB- CEER 2801/2014). Animal care and handling are conducted as conformed to accepted guidelines (OECD, 1998; 2002; 2008).

Plant materials

P. pinnata roots were collected at Aképé (Togo) in August 2014. They were identified by Prof K. Akpagana from the Botany department of University of Lome (Togo) and a voucher specimen was kept in the herbarium of the Laboratory of Botany and Plant Ecology (Faculty of Science/University of Lome) under the reference number Togo 15074.

Preparation of hydroalcoholic extract

The roots were washed in running water, then dried and ground to a powder. The powder was soaked in ethanol-water (50-50: v/v) for 72 h with manual discontinue agitation. The solution was filtered and evaporated using a rotary evaporator (yield: 11.89). Appropriate concentrations of the extract were made every day in distilled water and used in the animal experiments. The study was conducted in Animal Physiology Department, Faculty of Sciences, University of Lome, Togo.

Animals

Wistar rat of either sex (150-200 g) and guinea pigs were provided by the department of Animal Physiology of University of Lome (Togo) were used. They were housed in a standard environmental condition and fed with rodent standard diets and water ad libitum.

Acute toxicity test

The limit test dose of 5000 mg/kg was used as stipulated in Organization for Economic Cooperation Development (OECD) guidelines (2002). Three female rats, each sequentially dosed at intervals of 48 h, were used for the test. The animals were observed individually for acute toxicity signs and behavioral changes 1 h post-dosing, and at least once daily for 14 days.

Subchronic toxicity test

The repeat dose oral toxicity study was carried out according to OECD guideline 407 (1998). Male wistar rats were divided into four groups of 8 animals each. Group 1 received 10 mL/kg of distilled water and served as control. Group 2, 3 and 4 received *P. pinnata* hydroalcoholic extract at 200, 400 and 800 mg/kg body wt. respectively. Doses were chosen on the basis of therapeutic doses used in the literature (Chabra *et al.,* 1991; Dokosi OB, 1998; Yusuf *et al.,* 2014). Extract was administered daily for 28 days at similar time. Animals were observed at least twice daily for morbidity and mortality. Body weight of animals was evaluated daily. On the 29th day, after an overnight fast, rats were anaesthetized with ether and blood sample for haematological and biochemical analysis were collected into tubes with or without EDTA respectively. Haemoglobin (Hb), haematocrit (Ht), red blood cells count (RBC), white blood cells count (WBC), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and platelet count were determined using automatic counter Sysmex (K21, Tokyo, Japan).

Biochemical analysis were performed in serum obtained after centrifugation of total blood without anticoagulant, at 2500 rpm for 15 min. Standardized diagnostic kits (Labkit®) and a Biotron® spectrophotometer were used for spectrophotometrical determination of the following biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine phosphokinase (CPK), creatinine, alkaline phosphatase (ALP), glucose (Glu), total proteins, γGT and urea.

Necroscopy of all animals was carried and the organ weights (heart, liver, kidney and spleen) were recorded. Each weighed organ was then standardized for percentage body weight of each rat (relative organ weight). Histological study of organs was done after sacrificing the animals on 29th day.

Study of *P. pinnata* on the isolated heart activity

The guinea pigs were sacrificed by cervical dislocation after ether anesthesia. The atria were quickly removed and placed in Mac Ewen physiological solution (NaCl: 15 g; KCl 0.24 g; CaCl: 0.48 g; PO_4H_2Na: 0.29 g; CO_3HNa: 2 g; $MgCl_2$: 0.1 g; Glucose 4 g) at 37 ° C. The atria were then mounted into the vessel body. The right atrium was attached to the hook which is situated at the bottom of the tank and the left atrium is connected to the transducer. The body under a base tension of 1 g was bathed in the physiological Mac Ewen solution maintained at 37 °C and aerated with pure oxygen. The atria were washed several times just after installation.

After 60 minutes of equilibration, the increasing concentrations of 0.5 mg/mL; 1 mg/ mL; 1.5 mg/ mL; 2 mg/ mL and 2.5 mg/ mL of extract (prepared with distilled water) was tested on the amplitude and frequency of contraction. The contraction of the organ was recorded using Harvard isotonic transducer (Biopac system, MP 100) and displayed on a computer with Acq Knowledge (Acq Knowledge III software). The effect of each focus on the atria was recorded for 25 minutes. Between two concentrations, the organ was washed three times over a period of 15 minutes.

Concentration–response curve was obtained by the cumulative addition of *P. pinnata* at 15-min intervals. All experiments were conducted in parallel with time-matched controls using the tissue from the same animal and adding an equivalent volume of vehicle. The same operation was repeated three times with each concentration.

Statistical analysis

The results are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed by one way analyse of variance (ANOVA) with Tukey test to evaluate

significant differences between groups. Values of $p< 0.05$ were considered significant. All statistical analysis were carried out using the Instat Statistical package (Graph Pad software, Inc. USA).

RESULTS AND DISCUSSION

Acute toxicity

The limit dose of 5 g/kg did not cause any mortality or any sign of acute toxicity in the rats dosed for a short period (48 h) and long period (14 days). But on the 14th day, *P. pinnata* at 800 mg/kg has significantly decreased ($p<0.05$) the rats body weight. These results can be compared to those of Adinortey *et al.,* (2012). In our study, *P. pinnata* has caused a significant ($p <0.05$) decrease in rats' bodyweight on the 14th day. Several authors agree that body weight change in animals is a sign of toxicity and it's usually due to a lack of appetite (Raza *et al.,* 2002; Teo *et al.,* 2002).

Subchronic toxicity test

In this test, no behavioral changes and death were observed at the end of treatment. Similarly, no significant differences in body weight were observed between control and treated groups during this period (table 1).

Table 1: Mean body weight of rats after a single administration of *P. pinnata* hydroalcoholic root extract.

Days	Mean body weight (g, ± SEM)	
	Control	Extract 5000 mg/kg
D0	191.1 ± 10.50	198.3 ± 7.84
D7	208.2 ± 7.57	192.3 ± 13.86
D14	212.2 ± 9.54	187.7 ± 8.68*

The results are mean ± S.E.M.; (N): number of animals=3; *P<0.05 (control group versus extract).

It is also observed that *P. pinnata* at 800 mg/kg has significantly ($p<0.001$) increased the relative weight of rats' kidney (table 2). The evaluation of organ's weight such as liver, kidney, spleen, testis, heart, pancreas, brain and lung is very important in toxicological studies.

The weight of the body or even more, the relative weight is an important parameter used in physiology and toxicology (Dybing *et al.,* 2002). This increase in the kidney relative weight is not confirmed by the histological studies. Tables 3 and 4 have shown respectively the haematological and biochemical analysis results. *P. pinnata* at the three doses (200, 400 or 800 mg/kg body wt.; p.o.) has increased significantly ($p<0.01$) VGM, TCMH and platelet number. For the biochemical parameters, the three doses of *P. pinnata* has decreased significantly ALT and ALP ($p<0.01$). At 400 or 800 mg/kg body wt. *P. pinnata* has increased significantly urea, creat, K^+ and Cl^- ($p<0.001$). At 800 mg/kg *P. pinnata* has increased significantly the CPK. No clear histological damage was observed in rat liver, kidney, spleen, colon, stomach and testis tissues treated with *P. pinnata* hydroalcoholic extract when compared to control. The evaluation of the haematological parameters is very important in toxicological studies. The hematopoietic system is a favorite target of toxic substances, and consequently an important parameter of humans' or animals' physiology (Oslon *et al.,* 2000; Diallo *et al.,* 2008). In our study *P. pinnata* has decreased significantly ($p <0.05$) some haematological parameters (MCV, MHC) and has increased the number of platelets and WBC. These results are different from those of Adinortey *et al.,* (2012) who have observed an isolated microcytosis. The dosage of transaminases (ALT, AST), alkaline phosphatase and glucose is very important in the assessment of liver toxicity (Corns, 2003; Pittler and Ernst, 2003). *P. pinnata* has decreased the ALT as compared to control and has increased the level of ALP that means there were no liver cell lesions, but rather a functional impairment with biliary obstruction (cholestatic hepatitis). The results of the histological sections of the rats' liver do not clearly confirm the results of biochemicals parameters changes. Urea and creatinine were significantly ($p <0.001$) increased indicating renal damage. *P. pinnata* has also caused a hyperkalemia and a hyperchloremia. CPK levels have been significantly ($p <0.001$) increased with the dose of 800 mg/kg and it may be due to heart' cells necrosis. However the heart histology does not allow us to confirm this result.

Table 2: Mean body weight of rats after 28 days treatment with hydroalcoholic root extract of *P. pinnata*.

Days	Control	Extract dose		
		200 mg/kg	400 mg/kg	800 mg/kg
D0	180.3± 10.32	180.3± 10.22	178.1 ± 7.4	179.3 ± 9.89
D7	185.0± 11.12	175.5± 10.58	174.4 ± 8.69	184.1 ± 8.4
D14	193.3± 10.44	180.1± 11.37	173.1 ± 8.08	191.8± 10.53
D21	201.3± 10.54	191.1 ± 9.92	183.8 ± 7.77	190.9 ± 6.88
D28	201.5± 11.04	191.9± 10.04	186.8 ± 8.26	193.9 ± 5.44

The results are mean ± S.E.M.; (N): number of animals=8.

Table 3: Relative organ weight of rats after 28 days treatment with hydroalcoholic root extract of *P. pinnata*.

Organ relative weight	Control	Extract		
		200 mg/kg	400 mg/kg	800 mg/kg
Liver	2.88 ± 0.08	2.82 ± 0.12	2.79 ± 0.07	3.07 ± 0.08
Kidney	0.52 ± 0.02	0.55 ± 0.01	0.55 ± 0.01	0.62 ± 0.01***
Heart	0.33 ± 0.01	0.37 ± 0.02	0.35 ± 0.02	0.39 ± 0.02
Testis	1.99 ± 0.04	1.27 ± 0.04	1.23 ± 0.06	0.99 ± 0.13
Epididyme	0.23 ± 0.02	0.23 ± 0.01	0.21 ± 0.02	0.26 ± 0.03
Spleen	0.18 ± 0.02	0.19 ± 0.03	0.15 ± 0.01	0.21 ± 0.03

The results are mean ± S.E.M.; N (number of animals) =8; ***P<0.0001 (control group versus extract).

Table 4: Haematological parameters for rats after 28 days treatment with hydroalcoholic extract of *P. pinnata* roots.

Parameters	Control	Extract		
		200 mg/kg	400 mg/kg	800 mg/kg
RBC (10^6/µl)	7.85 ± 0.11	8.02 ± 0.19	7.1 ±0.74	8.14 ± 0.06
WBC (10^3/µl)	7.04 ± 0.49	7.06 ± 0.38	7.7 ± 0.59	10.37± 0.47 ***
Haemoglobin(g/dL)	14.03 ± 0.16	13.58 ± 0.34	13.90 ± 0.20	14.28 ± 0.17
Haematocrit (%)	43.36 ± 0.38	42.65 ± 1.06	42.07 ± 1.14	44.14 ± 0.51
MCV (fl.)	55.28 ± 0.41	53.20 ± 0.39***	53.99 ± 0.52***	54.46 ± 0.51**
MCH (pg)	17.88 ± 0.18	16.91 ± 0.13***	17.22 ± 0.27***	17.61 ± 0.17**
MCHC (%)	32.31 ± 0.19	31.85 ± 0.28	32.89 ± 1.09	32.35 ± 0.37
Platelet (10^3/µl)	839.1 ± 26.60	1072± 36.45***	1054± 42.57***	1004 ±54.52***

The results are mean ± S.E.M.; N (number of animals) = 8; **P<0.01 (control group versus extract); ***P<0.0001 (control group versus extract).

Table 5: Biochemical parameters for rats after 28 days treatment with hydroalcoholic extract of *P. pinnata* roots.

Parameters	Control	Extract		
		200 mg/kg	400 mg/kg	800 mg/kg
CPK (U/L)	83.0 ± 20.29	81.4 ± 23.24	209.7 ± 36.63	416.3± 1.43***
Urea (g/L)	0.39 ± 0.04	0.49 ± 0.03	0.69 ± 0.05***	0.53 ± 0.02***
Gluose (g/L)	1.05 ± 0.19	0.82 ± 0.05	0.85 ± 0.07	1.24 ± 0.14
Creatinine (mg/L)	4.93 ± 0.07	4.92 ± 0.11	5.61 ± 0.31***	5.51 ± 0.2***
ALP (U/L)	221.6 ± 21.38	290.5 ± 26.17**	283.6± 3.32***	270.4± 0.87***
ALT(U/L)	75.00 ± 6.82	53.00 ± 2.85***	50.83 ± 1.47***	55.50 ± 6.29***
AST(U/L)	118.5 ± 9.55	129.9 ± 6.6	121.0 ± 5.78	129.5 ± 5.18
Ca^{2+}(mg/L)	95.00 ± 1.53	91.25 ± 3.68	91.75 ± 3.15	100.3 ± 1.15
Na^+(mEq/L)	141.8 ± 4.17	144.0 ± 1.69	144.1 ± 1.79	133.5 ± 4.98
K^+(mEq/L)	4.45 ± 0.1	4.56 ± 0.16	4.62 ± 0.13**	5.10 ± 0.10***
Cl^-(mEq/L)	108.0 ± 0.35	107.3 ± 0.57	110.3 ± 1.34***	112.1 ± 1.37***

The results are mean ± S.E.M.; N (number of animals) = 8, **P<0.01 (control group versus extract); ***P<0.0001 (control group versus extract).

Effect of *P. pinnata* on the heart

The effect of the extract of *P. pinnata* on the contractility (inotropy) and on the contraction frequency (chronotropy) on isolated guinea pig atria is shown in Figure 1. *P. pinnata* at increasing concentrations of 0.5 mg/mL; 1 mg/mL; 1.5 mg/mL; 2 mg/mL and 2.5 mg/mL, has increased significantly the force of contraction (positive inotropic) from the 5th minute after the *P. pinnata* administration (Figure 1).

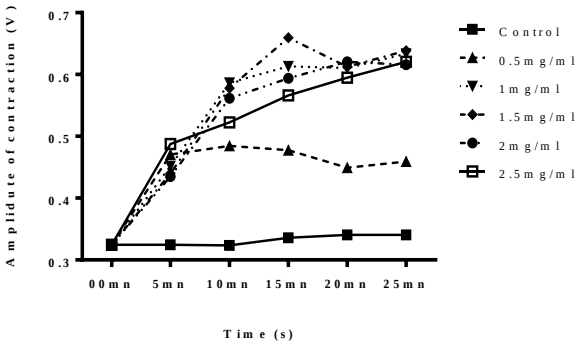

Fig. 1: Effect of *P. pinnata* on the amplitude of contraction of guinea pigs heart. The guinea pigs were sacrificed and the atria were placed in physiological solution Mac Ewen (NaCl: 15 g; KCl 0.24 g; CaCl: 0.48 g; PO_4H_2Na: 0.29 g; CO_3HNa: 2 g; $MgCl_2$: 0.1 g; Glucose 4 g) at 37 ° C. After 60 minutes of equilibration, the increasing concentrations of 0.5 mg/mL; 1 mg/mL; 1.5 mg/mL; 2 mg/mL and 2.5 mg/mL of extract was tested on the amplitude and frequency of contraction. The contraction of the organ was recorded using Harvard isotonic transducer (Biopac system, MP 100) and displayed on a computer with Acq Knowledge (Acq Knowledge III software)

Concentrations of 1 mg/mL; 1.5 mg/mL; 2 mg/mL; 2.5 mg/mL have caused a significant (p <0.001) decrease of the heart's rate (negative chronotropism), but the dose of 0.5 mg/mL, did not cause a significant change (p> 0.05) on the contraction's frequency (Figure 2). The positive inotropic effect and negative chronotropic on the heart may be due to the presence of cardiotonics tannins and high calcium content in *P. pinnata*.

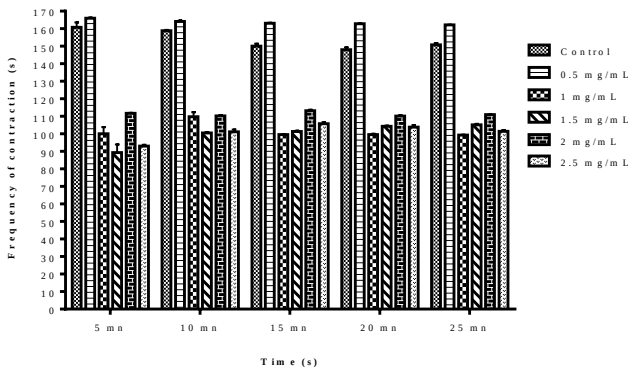

Fig. 2: Effect of *Paullinia pinnata* on the frequency of contraction. The guinea pigs were sacrificed and the atria were placed in physiological solution Mac Ewen (NaCl: 15 g; KCl 0.24 g; CaCl: 0.48 g; PO_4H_2Na: 0.29 g; CO_3HNa: 2 g; $MgCl_2$: 0.1 g; Glucose 4 g) at 37 ° C. After 60 minutes of equilibration, the increasing concentrations of 0.5 mg/mL; 1 mg/mL; 1.5 mg/mL; 2 mg/mL and 2.5 mg/mL of extract was tested on the amplitude and frequency of contraction. The contraction of the organ was recorded using Harvard isotonic transducer (Biopac system, MP 100) and displayed on a computer with Acq Knowledge (Acq Knowledge III software).

The effect of *P. pinnata* on cardiac activity is similar to the effect of cardiac glycosides that cause an increase in the strength of contraction of the cardiac muscle cells and a decrease in the cardiac frequency. Indeed, cardiotonic glycosides increase myocardial contraction force by changing ion membrane permeability of myocardial cells. Na pump, controlled by the Na^+/K^+ ATPase is inhibited by cardiac glycosides; this result in

cardiac fiber level decreased potassium and increased sodium. The increase in the Na^+ into the cells leads to a rise in ionized calcium thus strengthening myocardial contractions.

CONCLUSION

In this study, *P. pinnata* has increased and has induced a significantly increase in the relative weight of kidney, a microcytosis and an isolated hypochromia. Renal injuries are also noted by the increase in blood urea, creatinine, potassium and chlorine. Cardiac disorders characterized by the increase of creatinine phosphokinase, an increase in the force of contraction (positive inotropic effect) and a decrease in heart rate (negative chronotropic effect) have been noted. This positive inotropic effect observed could justify the traditional use of this plant as an aphrodisiac.

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