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## Acute and sub-acute oral toxicity assessment of hydro-alcoholic root extract of *Paullinia pinnata* on haematological and biochemical parameters

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### Abstract

The trusted efficacy of *Paullinia pinnata* (*Sapindaceae*) that has made many people to patronize it despite the absence of toxicity studies emerges from its long history of use in Ghana. The aim of this study was to evaluate the acute and sub-acute toxicity effects of *P. pinnata* roots on biochemical and haematological indices in rats. The dried *Paullinia* roots after milling was macerated in 70% ethanol and concentrated to obtain the semi-solid extract. For the acute toxicity study, rats were exposed to dose levels of 2,000, 2,500, 3,000, and 5,000 mg/kg p.o. and monitored for 48–72 hours. Dose levels of 375, 750, and 850 mg/kg were used for the sub-acute toxicity studies. The results of haematological and biochemical parameters in the present study showed that the root extract of *P. pinnata* is potentially safe for oral consumption up to dose of 850 mg/kg.

**Keywords:** *Paullinia pinnata*; sub-acute toxicity; Ghana.

### Introduction

Medicinal plants play a vital role in the maintenance of human health throughout the world notably in the Tropics. According to WHO, 70–95% of the population of developing countries rely on traditional medical systems especially plant based to meet their primary health care needs (The World Medicines Situation, 2011). Plants have been used in traditional medicine for several years. Knowledge of medicinal plants is useful for conservation of cultural, tradition, and biodiversity, community healthcare and drug development (Namsa *et al.*, 2011).

*Paullinia pinnata* is a medicinal plant belonging to the family *Sapindaceae* (Ghana Herbal Pharmacopoeia, 2007). It is a woody or sub-woody plant commonly found in secondary forests and also along streams and savannah belt. The plant is commonly called 'bread and cheese'. The leaves and the roots are

the main parts of the plant used for medicinal purposes such as treatment of erectile dysfunction, malaria, wounds, and dysentery. The plant has also been shown to have strong fibroblast stimulatory action (Annan and Houghton, 2010).

The trusted efficacy of the plant that has increased its patronage emerges from its long history of use despite the fact that its toxicity has not yet been scientifically investigated. These days there is no doubt regarding the value and potential of phytomedicines. According to WHO, plant medicine has become the building block of primary healthcare (The World Medicines Situation, 2011). Proof of safety of these medicines in animals should take precedence over establishing efficacy. Hence, this study sought to investigate the acute and sub-acute toxicity effects of *P. pinnata* roots on blood, kidney, and liver indices in Wistar albino rats.

## Materials and Methods

### (a) Plant collection, preparation, and extraction

The roots of *P. pinnata* were collected from the University of Cape Coast (U.C.C.) Botanical Garden. The plant was authenticated at the herbarium unit of the School of Biological Sciences where voucher sample # CCG3527 has been deposited. The roots were thoroughly washed with distilled water and chopped into pieces to aid drying for a period of 2 weeks. The dried roots were milled into powder, weighed and kept in an air tight plastic bag and 400 g of the powdered sample was extracted with 2.6 L of aqueous ethanol (70% v/v) by cold maceration for 48 hours with the aid of a mechanical shaker. The mixture was filtered by suction and concentrated using rotary evaporator to dryness. Extraction was repeated on the residue with 1.5 L ethanol for 48 hours. This was also filtered, concentrated, and bulked with the first crude extract. A reddish-brown crude material was obtained and was labelled *Paullinia pinnata* root extract (PPRE). This was kept dry in a desiccator at 25 degree centigrade room temperature. A yield of 40.2 g representing 10.05% was obtained.

### (b) Animal care and treatment

Healthy young adult Wistar albino rats between 150 and 270 g were obtained from the Animal house of the Department of Biochemistry, School of Biological Sciences, U.C.C. Both male and female sexes were selected for the study. All the rats were housed in groups in metal cages with soft wooden shavings as bedding and maintained under normal laboratory conditions allowing free access to food and freshwater daily. The protocol of the study was approved by the Local Ethical Committee for Animal Experimentation of the Department of Biochemistry, School of Biological Sciences, U.C.C.

### (c) Acute toxicity studies

Twenty-five healthy young Wistar albino rats of both sexes were randomly selected and put into five groups with five rats in each group. Rats were marked and kept in their respective cages for 72 hours to acclimatize to the prevailing laboratory conditions prior to administration of the extract. The dosages administered to rats were strictly based on the average weight of rats in the individual cages. The animals in groups II, III, IV, and V were exposed to doses of PPRE at levels of 2,000, 2,500, 3,000, and 5,000 mg/kg p.o., respectively with Group I serving as control which received 10 mL/kg p.o. of Tween-80 enrich from

sigma Aldrich. The rats in the various groups were observed for over 24 hours for signs of toxicity and other behavioural effect and later for 72 hours.

### (d) Sub-acute toxicity studies

Twenty healthy rats of both sexes were randomly selected and put into four groups with five rats in each group. Food was withheld overnight but water made available. The animals were weighed prior to administration of the extract and those in Group I served as control receiving Tween-80 of 10 mL/kg p.o. Groups II, III, and IV were administered with PPRE at dose levels of 375, 750, and 850 mg/kg p.o., respectively. The administration of the extract was done on daily basis for 14 days. The animals were observed continuously for behavioural responses, mortality and other signs of toxicity during the 14 day period.

### (i) Haematological analysis

A volume of 1.5 mL of blood sample drawn through the sublingual vein was collected in vacutainers containing K<sub>2</sub>EDTA as an anticoagulant. The samples were then refrigerated prior to analysis for the following haematological indices; white blood cell count (WBC), lymphocyte count (LYM), mid cell count (MID, which may include less frequently occurring cells and rare cells correlating to monocytes, eosinophils, basophils, blasts, and other precursor white cells), granulocyte count (GRAN), red blood cell count (RBC), haemoglobin concentration (HBC), haematocrit (HCT) or packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width (RDW), and platelet concentration (PLT).

### (ii) Biochemical parameters

Alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total bilirubin, direct bilirubin, indirect bilirubin, total protein, albumin, globulin, blood urea nitrogen (BUN), and creatinine (CRE) were the parameters measured. A 2 mL of blood sample drawn through the sublingual vein. This method has been found to be suitable for most laboratory animals' well-being (Heimann *et al.*, 2009). The blood samples were placed in dry vacutainers and centrifuged after they had clotted in order to avoid fibrin clot. The sera obtained were kept in sterile tubes and stored at -20°C for later analysis. The sera were later assayed for the various parameters using the automated Elan ATAC 8000 Random Access Chemistry analyzer (WS-ATAC8000).

*(e) Statistical analysis*

All values were expressed as mean  $\pm$  SEM and the statistical significance between treatment and control groups were analysed using student t-test with the aid of SPSS version 16 software at 5% level of significance.

**Results and Discussion**

In the determination of safety of drugs and herbal products for human consumption, toxicological evaluation is carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a 'safe' dose in humans.

In the present acute toxicity study carried out in rats, a maximum dose of 5,000 mg/kg p.o. of PPRE did not cause any toxicity-no food refusal, changes in posture, lacrimation, convulsion, and mucous secretions or death even up to 72 hours, thus the LD<sub>50</sub> was indetermined. This suggests that the PPRE is relatively non-toxic since in acute toxicity studies, a product is considered non-toxic if no deaths are registered and no clinical signs of toxicity are observed at doses of or below 5,000 mg/kg.

According to (Oslon *et al.*, 2000), there is a correlation of toxicity in haematological, gastrointestinal and cardiovascular adverse effects between animals and humans. Haematological indices in animals are important to determine the toxicity risk since the changes in the blood

system have a higher predictive value for human toxicity. In this study, haematological and biochemical indices were used to assess sub-acute toxicity of PPRE in Wistar albino rats.

The haematological parameters namely RBC, HBC, and MCV showed significant changes ( $p < 0.05$ ) between treatment (375 and 750 mg/kg) groups and the control group. Furthermore, there were significant changes ( $p < 0.05$ ) in HCT, MCHC, PLT, and MID between the 375 mg/kg group and the control. Again, there was significant change ( $p < 0.05$ ) in LYM and RDW for the 750 mg/kg group when compared with the control. The remaining haematological parameters for the 375 mg/kg and the 750 mg/kg groups showed no significant changes ( $p > 0.05$ ) between them and the control. There were relative increases ( $p > 0.05$ ) in values for RBC, HBC, HCT, MCV, WBC, and LYM with relative decreases, for MCH, MCHC, PLT, MID, GRAN, and RDW in the group with a dose of 850 mg/kg (Table 1). The significant increase in RBC and HBC levels for the 375 and 750 mg/kg groups coupled with the relative increase for the 850 mg/kg group are indications that the root extract might have caused an increase in RBC and HBC production. The marked increase in HCT values for 375 mg/kg group coupled to the relative increases in other groups rule out the possibility of anaemia or disturbances in the erythrocyte or haemoglobin production. This therefore means that the root extract enhances the oxygen-transport capacity of the blood. MCV,

**Table 1:** Haematological parameters of rats treated with PPRE in sub-acute toxicity studies.

Parameters	Control	Treatment		
		375 mg/kg	750 mg/kg	850 mg/kg
RBC (M/ L)	6.25 $\pm$ 0.76	8.20 $\pm$ 0.38 <sup>b</sup>	7.91 $\pm$ 0.53 <sup>a</sup>	6.63 $\pm$ 0.32
HBC (g/dL)	12.47 $\pm$ 0.88	15.25 $\pm$ 0.10 <sup>b</sup>	15.16 $\pm$ 0.32 <sup>a</sup>	13.23 $\pm$ 0.17
HCT (%)	37.15 $\pm$ 8.52	46.82 $\pm$ 0.69 <sup>b</sup>	40.92 $\pm$ 2.27	41.23 $\pm$ 2.48
MCV (fL)	59.82 $\pm$ 0.40	57.83 $\pm$ 0.84 <sup>b</sup>	52.53 $\pm$ 0.80 <sup>a</sup>	61.10 $\pm$ 1.08
MCH (pg)	20.00 $\pm$ 0.61	19.67 $\pm$ 0.70	19.66 $\pm$ 0.50	20.65 $\pm$ 0.70
MCHC (g/dL)	35.71 $\pm$ 1.75	31.71 $\pm$ 1.00 <sup>b</sup>	38.45 $\pm$ 1.41	34.08 $\pm$ 2.00
PLT (K/ L)	610.48 $\pm$ 22.57	513.90 $\pm$ 42.85 <sup>b</sup>	598.30 $\pm$ 27.84	610.52 $\pm$ 22.20
WBC (K/ L)	21.20 $\pm$ 3.07	20.10 $\pm$ 3.10	20.17 $\pm$ 3.81	22.02 $\pm$ 0.69
LYM (%)	15.90 $\pm$ 1.70	16.10 $\pm$ 1.56	17.30 $\pm$ 3.55 <sup>a</sup>	16.40 $\pm$ 1.20
MID (%)	4.10 $\pm$ 0.30	2.42 $\pm$ 0.42 <sup>b</sup>	3.70 $\pm$ 0.47	4.00 $\pm$ 0.27
GRAN (%)	3.67 $\pm$ 0.96	2.60 $\pm$ 0.28	2.90 $\pm$ 0.73	3.11 $\pm$ 0.80
RDW (%)	18.02 $\pm$ 0.21	18.27 $\pm$ 0.20	18.75 $\pm$ 0.33 <sup>a</sup>	17.90 $\pm$ 0.31

Values are represented as mean  $\pm$  SEM,  $n = 5$ .

<sup>a</sup>significant difference between 750 mg/kg and control.

<sup>b</sup>significant difference between 375 mg/kg and control ( $p < 0.05$ ).

MCH, MCHC, and RDW are indices for the basis of morphological anaemia classification (Moreno Chulilla *et al.*, 2009). Though some of these parameters had significantly low values as well as relative increases and decreases, these cannot be used in anaemia classification since RBC and HBC levels in all treatment groups recorded significant and relative increases ruling out the incidence of anaemia. The observed decrease in PLT level for the 375 mg/kg group as well as the relative decreases for the rest of treatment groups may indicate thrombocytopenia. WBC and other indices such as LYM usually show increase in activity in response to toxic environment (Robins, 1974). This therefore means that the insignificant changes in WBC values for all treatment groups as well as the significant levels in LYM which are the main effector cells of the immune system give an indication that the extract had some effects on the immune system of such treatment groups.

For the biochemical parameters, significant increase and decrease were recorded in protein and in globulin for the 375 and 750 mg/kg groups, respectively when they were compared with the control. Apart from these, no significant changes were observed for the rest of the biochemical parameters for these groups and the 850 mg/kg group when they were compared with the control. There were also no significant changes in BUN and CRE levels in any of the treatment groups when they were compared with the control (Table 2). AST is a sensitive but

non-specific liver enzyme as it is also found in the kidney, cardiac muscle and skeletal muscle in large amounts with small amounts present in the brain, pancreas and lungs while ALT is specific for the liver (Cheesbrough, 2005). The levels of these two enzymes are known to significantly increase in a toxic environment (Crook, 2006). However, in this study, these enzymes showed no significant increase in the treated animals which implies that the PPRE dose administered has no hepatotoxic effect.

An elevated serum ALP level is often associated with various disorders such as extra-hepatic bile obstruction, intra-hepatic cholestasis, infiltrative liver disease, hepatitis, and bone diseases. Unfortunately, the elevation of ALP less than three times the normal level is considered non-specific and insufficient to provide a definite diagnosis (Pagana and Pagana, 2003; Rosalki and McIntyre, 1999). Extremely high ALP activity in serum is seen in cases of obstructive jaundice and biliary cirrhosis (Mukherjee, 2005). To show the presence of bile duct obstruction, both ALP and GGT levels are expected to be high. A normal GGT level coupled to elevated ALP level has been studied to be associated with bone diseases (Mauro *et al.*, 2006; Rosalki and McIntyre, 1999). Since GGT is sensitive but non-specific to the liver, elevated GGT levels indicate that there could be damage but not specifically from the liver. Generally, the higher the GGT level the greater the extent of liver damage.

**Table 2:** Biochemical parameters of rats treated with PPRE in sub-acute toxicity studies.

Parameters	Control	Treatment		
		375 mg/kg	750 mg/kg	850 mg/kg
AST (IU/L)	25.25 ± 0.47	24.50 ± 0.35	23.90 ± 1.72	25.56 ± 0.10
ALT (IU/L)	7.32 ± 0.33	7.55 ± 0.25	7.27 ± 0.11	7.42 ± 0.22
ALP (IU/L)	112.50 ± 3.47	115.54 ± 3.52	117.22 ± 3.87	116.14 ± 2.50
GGT (IU/L)	11.20 ± 0.50	11.75 ± 0.47	10.68 ± 0.78	11.70 ± 0.80
BIL total (mg/dL)	3.84 ± 0.78	3.00 ± 0.30	2.91 ± 0.16	3.07 ± 0.02
BIL direct (mg/dL)	1.22 ± 0.78	1.02 ± 0.36	1.10 ± 0.04	1.04 ± 0.02
BIL indirect (mg/dL)	2.62 ± 0.91	1.98 ± 0.06	1.81 ± 0.45	2.03 ± 0.08
Total protein (g/dL)	7.32 ± 0.12	8.15 ± 0.08 <sup>b</sup>	7.57 ± 0.18	7.50 ± 0.15
Albumin (g/dL)	3.15 ± 0.08	3.30 ± 0.09	3.00 ± 0.09	3.20 ± 0.09
Globulin (g/dL)	5.10 ± 0.09	5.12 ± 0.10	4.47 ± 0.12 <sup>a</sup>	5.20 ± 0.05
BUN (mg/dL)	35.03 ± 3.12	36.90 ± 4.44	36.88 ± 3.37	36.84 ± 2.12
CRE (mg/dL)	0.58 ± 0.13	0.60 ± 0.14	0.63 ± 0.14	0.65 ± 0.14

Values are represented as mean ± SEM,  $n = 5$ .

<sup>a</sup>significant difference between 750 mg/kg and control.

<sup>b</sup>significant difference between 375 mg/kg and control ( $p < 0.05$ ).

However in this work, the insignificant changes in ALP and GGT levels in treatment groups when compared with the control group rule out the occurrence of hepatobiliary diseases. Bilirubin is a major breakdown product of haemoglobin. An increase in total bilirubin in the blood results in a condition called jaundice. Differential diagnosis of the types of jaundice is done by measuring the direct (conjugated) and indirect (unconjugated) bilirubin levels which determine whether the jaundice is pre-hepatic (haemolytic), hepatic or post-hepatic (obstructive) (Thapa and Walia, 2007; Mukherjee, 2005). However, in this study, no significant changes were observed in these parameters for any of the treatment groups when compared to the control ( $p > 0.05$ ) and therefore suggests that the PPRE has no abnormal effect on haemoglobin. BUN and CRE levels are used to assess renal function. An increase in the levels of these means impairment in kidney function. However, in this study, the absence of significant differences in these parameters means that the PPRE has no harmful effect on the kidney.

### Conclusion

These results suggest that the root extract of *Paullinia pinnata* is potentially safe for oral consumption at sub-acute administration up to dose of 850 mg/kg. It is recommended that further studies into sub-chronic and chronic be carried out.

### Ethical Approval

The study was approved by the Local Ethical Committee for Animal Experimentation in the Department of Biochemistry, School of Biological Sciences, University of Cape Coast.

### Conflict of Interests

None declared.

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