Supra-supplementation levels of Dawadawa (*Parkia biglobosa*) powder has toxicity potential in Sprague-Dawley rats

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**Abstract**
Dawadawa (*Parkia biglobosa*) seed powder is used in Ghana as flavouring agents in local dishes, as nutraceutical in the management of diabetes mellitus and cardiovascular diseases. There are concerns about its safety at supra-supplemental levels. Both acute (5000 mg/kg) and subacute (2000 mg kg⁻¹, 500 mg kg⁻¹ and 100 mg kg⁻¹) studies using Sprague–Dawley rats of both sexes (150–200 g). The doses were selected based on the LD₅₀ which was above 5000 mg/kg. Blood samples were obtained via cardiac puncture and used for haematological and clinical chemistry analysis. Gross necropsy and histopathology were performed on the kidney, heart and liver. Haematological studies showed a decrease in WBC levels (p<0.052) and platelet count (p<0.038) while histopathological studies showed narrowing and congestion of the lumen of central vein with RBCs as compared to the controls in 60% of the animals treated with both the high and median doses and 20% of photomicrographs from the high dose showed evidence of periglomerular oedema. These results show that dawadawa seed powder is a relatively safe product to be used domestically and as medicinal however, extremely high doses has the capability of causing hepatic and renal disorders.

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1. Introduction
1.1 *Parkia biglobosa*
*Parkia biglobosa* is a perennial tree of legume, belonging to the family Leguminosae (Campbell-Platt, 1980) and is used by the people of the three northern regions of Ghana as a food additive to beef up the flavour and texture of soups and stews (Irvine, 1961). Dawadawa, popularly known as Kololo or Kpalugu in the Gurune language, is the fermented seeds of *Parkia biglobosa* or the African locust bean plant prepared from the seed of the Dawadawa tree. It is reputed to protect one from acquiring various diseases especially heart related diseases and diabetes mellitus (Builders et al., 2011; Miotlogo-Kone et al., 2008; Nnemeka et al., 2009; Silva et al., 2007; TTL, 2003). The hepatoprotective effect of *Parkia biglobosa* stem bark 200 mg/kg has been proven in paracetamol induced hepatic injury in rats (Komolafe et al., 2013). Both the aqueous and methanolic extracts have a favourable lipid profile, which is probably an indication of their possible anti-arteriogenic property (hypertension and ischaemic heart diseases being common complications in diabetes mellitus) the methanolic extract has
shown possible contraindication to ischaemic heart diseases (Odetola et al., 2006).

1.2 Traditional value as a nutraceutical
The antidiabetic potentials of the methanol extract of *Parkia biglobosa* seed, its chloroform, hexane, and mother liquor fractions have been evaluated in glucose-loaded and alloxan-induced diabetic rats (Fred-Jaiyesimi and Abo, 2009). Further, the anti-inflammatory and analgesic activity of the extract of *Parkia biglobosa* have also been demonstrated in animals (Inngjerdingen et al., 2004; Meraiyebu et al., 2013) while the antifungal (Fawole and Abioye, 2002), anti-diarrhoeal (Agunu et al., 2005), anti-hypertensive (Ouedraogo et al., 2012) and antibacterial (Ajaiyeoba, 2002a; 2006b) properties have also been proven.

The growing interest in nutraceuticals for the management of non-communicable diseases of purely natural origin calls upon various studies to ascertain the safety of such products (Silva et al., 2007). With regard to *Parkia biglobosa*, its leaf extract contains cardiac and saponin glycosides (Ajaiyeoba, 2002a; 2006b) while the fruit pulp and seeds are rich in proteins and lactose (Agunu et al., 2005). Research has also shown that the seeds contain antinutritional factors like oxalate, hydrogen cyanide, tannin and phytate (Mohan and Janardhanam, 1993) and the bark of the plant have been found to contain epigallocatechin, epicatechin 3-O-gallate and epigallocatechin 3-O-gallate (Agunu et al., 2005). There have been cases during preliminary studies, where recommendations have been made to include more than 6 g/kg of “Dawadawa” in treated meat as this had improved crude protein content, indicating that Dawadawa sausages could be more nutritious than traditional sausages (Teye et al., 2013). This calls for a safety assessment of Dawadawa powder with regard to regular human consumption as reports concerning its toxicity are very rare. It is against this background that this study is being conducted.

2. Objective of Research
The frequent and widespread use of Dawadawa is likely to continue due to prevailing socio-cultural/economic conditions, healthcare services that are not readily accessible, especially to rural populations and the general perception that plant medicines are efficacious and free from side effects. The growing interest in nutraceuticals of purely natural origin calls upon various studies to ascertain the safety of such products. This study looks at the possible toxic effects likely to be associated with high consumption of Dawadawa powder in Sprague–Dawley rats.

3. Materials and Methods
Packaged *Parkia biglobosa* powder (PBP) was purchased from the Ghanaian market in August 2012 and *Parkia biglobosa* aqueous extract (PBEE) prepared by dissolving the powder in distilled water (100 g/100 ml). The eight-week old Sprague-Dawley rats acquired from Noguchi Memorial Institute for Medical Research (NMIMR) were housed in cages in regulated room temperature of 26°C, and humidity, 40 and 60%. The animals were allowed to acclimatize for 1 week with 12 hour light/dark cycle. Animal feed (AIN-93G Formulation obtained from GAFCO-Ghana) and water were given ad libitum, but feed was withdrawn 8 h prior to treatment to ensure effective absorption from the gastrointestinal tract after oral administration. Feed was, however, reintroduced 30 min after treatment. Food was withheld for a further 3-4 hours after administration of PBEE.

3.1 Animals and experimental design
Forty (40) Sprague-Dawley (S-D) rats of both sexes (weighing 150–200 g) were obtained from the NMIMR. During the acclimatization period, clinical observations as well as body weight measurements of the animals were conducted and they were found healthy. The rats were assigned into groups including a control group by the stratified random method according to their body weight. S-D rats were fed standard chow diet (AIN-93G formulation, obtained from GAFCO – Ghana) ad libitum.

3.2 Housing conditions
S-D rats were housed in metal cages with stainless steel tops in the animal care facility of NMIMR, where room temperature, humidity and ventilation were controlled according to international standards. The rats were maintained in a 12-h light-cycle and were studied for 14 days. Prior to sacrifice, they were anesthetized with diethyl ether and later euthanized. All visible organs and tissues were macroscopically examined and harvested. Blood collection was by cardiac puncture.

3.3 Route of administration
The route of administration was by oral gavage in accordance with the main route of intake of *P. biglobosa* powder by humans for food and medicinal purposes.
3.4 Acute toxicity
PBEE was administered in a single dose by gavage via a stomach tube in accordance with the expected route of administration. Various doses were administered in order to yield a 50% death of the animals. The animals were observed for a period up to 7 days individually twice every 30mins, then periodically during the first 24 hours with special attention given to the first four hours.

3.5 Sub acute toxicity studies
This was performed at doses of 2000 mg kg\(^{-1}\), 500 mg kg\(^{-1}\) and 100 mg kg\(^{-1}\) over a 14-day period and the animals once again observed for signs of toxicity. The doses were selected based on the LD\(_{50}\) which was above 5000 mg/kg. Blood samples were obtained via cardiac puncture into EDTA and plain gel test-tubes, processed and used for haematological and clinical chemistry analysis. Autopsy was performed on all the animals and the heart, liver and kidneys were harvested and weighed. The organs were isolated and fixed in 10% buffered formalin as soon as necropsy was performed. Gross necropsy and histopathology were performed on the kidney, heart and liver. The protocol was approved by the institutional ethical committee and conforms to the approved guidelines (OECD, 2001, WHO, 2000).

3.6 Statistical analysis
All data are expressed as means ± SEM. Data was analysed using analysis of variance (ONE-WAY ANOVA). Level of significance was set at p<0.05. Bonferroni multiple comparison post hoc test was performed when p<0.05 and entire data processed using graphpad prism 5.

4. Results

4.1 Mortality
No death was recorded at any of the doses administered during the acute and sub-acute toxicity studies. There was however decrease in alertness and staggering of gait in the first few hours at the high doses.

4.2 Clinical signs
Lacrimation and repetitive circling was noticed in the high dose animals during the first 10 minutes.

4.3 Body weight changes
There were no changes in the body weight of the animals.

4.4 Gross pathology
There were no visible pathological changes in the heart, kidney, liver when different doses of PBEE were administered to the animals.

4.5 Organ weights
There were no significant changes in the weights of the kidney, liver, lungs, and heart.

4.6 Blood chemistry
These were analysed at dose levels of 2000, 500 and 100 mg kg\(^{-1}\) designated as high, medium and low doses respectively.

4.6.1 Haematology

Figure 1: Changes in haematological parameters in treated subjects (administered with high, median and low dose levels) of PBEE and controls

(a) White blood cells
(b) Red blood cell count
(c) Haemoglobin

(d) Haematocrit

(e) Mean Corpuscular Volume

(f) Mean Corpuscular Haemoglobin

(g) Mean Corpuscular Haemoglobin Concentration

(h) Platelet count

(i) Lymphocytes

(j) Lymphocyte number
4.6.2 Renal function Test

Figure 2: Renal function Tests during administration of PBEE

(a) Urea levels
(b) Creatinine

(c) Total cholesterol
(d) Triglyceride

(e) High Density Lipoproteins
(f) Low Density Lipoproteins

(g) Very Low Density Lipoproteins
(h) Total Proteins
3.6.3. Liver Function Test

Figure 3: Liver function test during PBEE administration in Sprague-Dawley rats

(a) Albumin

(b) Serum Globulin

(c) Indirect bilirubin

(d) Direct bilirubin

(e) Total bilirubin

(f) Aspartate Aminotransferase

(g) Alanine aminotransferase

(h) Alkaline Phosphatase
4.7 Histopathological Results

All animals were euthanized and various photomicrographs prepared and examined with the sole aim of identifying any with peculiar pathology or abnormalities. Those presented represents photomicrographs with abnormalities if any.

**Figure 4:** Photomicrographs of kidney from male Spraque-Dawley rats

Key: A (control males), B (5000 mg/kg bwt treated males), C (2000 mg/kg bwt treated males) and D (500 mg/kg bwt treated males).

Comments: No observable histological lesions in the glomerulus and tubules of A, C and D. However, 2 out of 10 (20%) micrographs from B showed evidence of periglomerular oedema (arrow).

**Figure 5:** Photomicrographs of cardiac muscle from Male Sprague-Dawley rats

Comments: No observable histological lesions in the A, C and D. However, 2 out of 10 (20%) micrographs from B showed evidence of periglomerular oedema (arrow).
Comments: No noticeable pathological changes in the treated and untreated groups.

Figure 6: Micrographs of liver from Male Sprague - Dawley Rats.

Comments: No histological lesion was observed in the control (A) and low dose (D) groups. Nevertheless, 60% of the lumen of central vein for B and C were narrowed and congested with RBCs compared to the controls.

5. Discussion

This study has revealed the potential of toxic effect of PBEE in Sprague-Dawley rats of both sexes though the LD$_{50}$ was estimated to be above 5000 mg/kg. Other studies have proved that acute toxicity studies on the fermented seed of Parkia biglobosa was estimated to be 1800 mg/kg in laboratory mice (Ouedraogo et al., 2012) while others however indicated the absence of renal and hepatotoxicity of PBE at a dosage of 75 mg/kg (Olaleye et al., 2013). There is also evidence of the absence of cardiotoxicity when the aqueous-methanolic leaf extract of Parkia biglobosa (PBE) was investigated for its effects on doxorubicin-induced cardiotoxicity and phytochemical constituents using adult albino rats (Komolafe et al., 2013). We proceeded to evaluate in sub-acute studies apart from the lethal dose determination, to help ascertain other investigatory parameters (Anadon et al., 2013). Haematological studies (fig. 1) portrayed reduced WBC levels ($p=0.052$) which may imply an overwhelming infections, drugs or some chemical poisoning while lymphocytes remained relatively unchanged. There was a decrease in platelet count ($P<0.038$) which normally occurs in bone marrow depression, autoimmune haemolytic anaemia, systemic lupus, severe haemorrhage or intravascular coagulation (TTL, 2003).
this case, normally individuals with liver disease develop a large spleen and as this process continues platelets are trapped within the sinusoids of the spleen. While the trapping of platelets is a normal function of the spleen, in liver disease it becomes exaggerated because of splenomegaly and so subsequently, the platelet count may reduce. The increase in lymphocyte number (p<0.0019) agrees with the findings of other works carried out where PBEE appears to increase total lymphocyte counts in rabbits at a dosage of 75 mg/kg more than at a dosage of 100 mg/kg and further possessing immunomodulatory properties (Adou et al., 2010; Janeway et al., 2005).

Direct, indirect and total bilirubins were all elevated in the treated animals (figures 3a, 3b, 3c). This might indicate liver irritation accompanying the administration of the plant medicine (28). The liver enzymes on the other hand remained fairly unchanged indicating the possibility of the absence of hepatic cell damage although ALT levels may or may not correlate with the degree of cell death or inflammation (Arneson and Brickell, 2007; Corns, 2003). Proteins, albumin and globulins were not negatively affected but rather a slight elevation which might indicate dehydration (figures 2h, 3a and 3b). This may imply that the synthetic capability of the liver was not negatively affected. Histopathological studies did not reveal any abnormalities in 70% of the photomicrographs examined. However, 30% of the photomicrographs revealed that the lumen of the central vein of the liver were narrowed and congested with red blood cells as compared to the controls (figure 2). This is confirmed by studies that have indicated the absence of renal and hepatotoxicity of *Parkia biglobosa* leaf extract at a dosage of 75 mg/kg (Olabiniri et al., 2013; Olaleye et al., 2013; Olson et al., 2000; Ouedraogo et al., 2012).

Regarding the renal function of the test animals, urea (p<0.002) and creatinine levels (p<0.064) increased in male treated rats compared to the controls (figures 2a and 2b). Increased levels of urea indicate any condition that reduces the kidneys ability to filter body fluids in the body or interferes with protein breakdown while creatinine is a by-product of muscle metabolism and is excreted by the kidneys. Elevated levels can indicate kidney disease or urinary obstruction, muscle disease, arthritis, hyperthyroidism and diabetes. The elevation of both urea and creatinine may indicate longstanding kidney disease at these high doses of PBEE. The histopathological findings also revealed the presence of oedema (Greaves, 2007; Tobias et al., 2013) at the periglomerular region indicating that at doses within this range PBEE could be detrimental to the renal system (figure 4).

The cardiovascular system was not negatively affected irrespective of the dosage used. VLDL, LDL and TGs slightly increased especially in the male subjects (figures 2c, 2d, 2e and 2f) but the histopathology of the heart did not reveal any anomaly in the cardiac muscle. This confirms other studies that have implicated the role PB plays in cardioprotection (Komolafe et al., 2013). The totality of these findings provide evidence of the safety of Dawadawa if used at the normal recommended doses in folklore and as household flavours at low doses but has the possibility of causing renal and hepatic dysfunction if used in high doses over a long period.

**Research Highlights**

- Dawadawa (*Parkia biglobosa*) seed powder at 5000 mg/kg has the potential of producing periglomerular oedema in Sprague-Dawley rats.
- Dawadawa (*Parkia biglobosa*) seed powder caused the narrowing of the central vein lumen which was also congested with red blood cells as compared to the controls.
- Dawadawa (*Parkia biglobosa*) seed powder did not have negative effect on the heart at the doses administered.

**Recommendations**

It is recommended that sub-chronic studies be conducted to assess the effect of this nutraceutical to identify its effect on reproductive organs. This will further clarify the effects likely to expect upon prolonged use.

**Justification of Research**

This study has provided Pharmacological evidence of the potential toxic effects likely to be exhibited by supra-supplemental doses of *Parkia biglobosa* “Dawadawa” in Sprague-Dawley rats. This is of outmost importance especially as this nutraceutical is widely used by Ghanaians and most African in their regular meals.
Conclusion

The normal dosage used in meals and for prevention of diseases is far lower than 100 mg/kg. PBEE appears to be safe when used at the dosage range tested. However at extremely high doses care must be taken as it may produce functional derangement of the kidneys at persistent high doses or cumulatively after prolonged use.

Author’s Contribution and Competing Interests

Asiedu-Gyekye Isaac and Daniel Antwi originated the idea of the project. Charles Awortwe performed the experiment and statistical Analysis. Mahmood Seidu helped with the histopathological analysis. All authors reviewed the manuscript and contributed to interpretation of the data. The authors also declare there is no competing interest.

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