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Acute Toxicity Study and Hepatocurative Effect of Aqueous Stem Bark Extract of Parkia Biglobosa in Wister albino Rats

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Abstract
Parkia biglobosa plant is widely is used in folk medicinal practices to treat and/or manage various diseases including diabetes, malaria, diarrhea and pains. The current research seek to establish the toxicity profile and hepatocurative ability of aqueous stem bark extract of the plant. Twelve (12) rats were used for Oral LD_{50} determination, and were grouped into four (4) groups of three rats (3) each. The first three groups were administered with 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight of the extract respectively, while the last group was subdivided into three groups of one rat each and were administered with 2500mg/kg, 3500mg/kg and 5000mg/kg body weight of the extract respectively. For the hepatocurative studies, twenty five (25) experimental rats were divided into five groups of five (5) rats each. Group I served as normal rats, Group II served as test Control while Groups III to V were induced with liver damage and administered with 50mg/kg, 100mg/kg and 150mg/kg of the extract respectively. The LD_{50} was found to be greater than 5000mg/kg, while phytochemical screening revealed the presence of Flavanoids, Glycosides, Tanins, Saponins, Steroids and Phenols, with the absence of Anthraquinones. For the hepatocurative study, a significant ($p<0.0.5$) increase in serum albumin and liver enzymes (AST, ALT and ALP) was observed in test control compared to normal control. Upon administration of the extract, a significant ($p<0.0.5$) fall in Albumin, AST, ALT and ALP was recorded in a dose dependent pattern. No significant difference ($p>0.05$) was observed between groups in total protein, direct and total bilirubin. The research concludes that the extract is practically non-toxic and possess strong hepatocurative ability which might be due to the phytochemicals present.
Keywords: Acute toxiicity; CCl_4; Liver; P. biglobosa; Phytochemical and wistar rats.
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1.0 Introduction

Medicinal plant have continued to attract attention in the global search for effective methods of using plants' parts (e.g. seeds, stems, leaves, roots and bark etc) for the treatment of many diseases affecting humans. Many important drugs used in medicine today are directly or indirectly derived from plants due to its bioactive constituents. In recent years, secondary plant metabolites previously with unknown pharmacological activities have been extensively investigated as sources of medicinal agents (Krishnaraju *et al.,* 2005).

P. biglobosa, also called the African locust bean is a popular tree found scattered around many countries of West Africa. It is a popular food plant whose seeds are fermented to make dawadawa (Daddawa) -a strong smelling tasty food rich in protein by the Hausa people of Northern Nigeria as well as a coffee substitute while the pulp is made into a refreshing drink or eaten raw (Abbiw, 1990). Parkia species have found usage traditionally as foods, medicinal agents and are of high commercial value. The pulverized bark of P. bicolor is employed in wound healing. *P. biglobosa* is known to provide an ingredient that is used in treating leprosy, and for treating hypertension. In Gambia, the leaves and roots are used in preparing a lotion for sore eyes. A decoction of the bark of *P. biglobosa* is also used as a bath for fever, as a hot mouthwash to steam and relieve toothache. The pulped bark is used along with lemon for wound and ulcers. *P. biglobosa* is found commonly everywhere in the Savannah and it grows up to about 20m high. The pinnae of the former is about 10 - 26 pairs while that of the latter is about 6 - 11 pairs. The leaflets of P. bicolor occur in 20 – 55 pairs, those of *P. biglobosa* in 14 - 30 pairs (Abbiw, 1990).

Liver, the principal organ of metabolism and excretion is subjected to a number of diseases which may be classified as liver cirrhosis, acute chronic hepatitis (inflammatory disease) and hepatitis (non-inflammatory condition), Jaundice or disease within the tissue of the liver itself. The predominant type of liver disease varies according to country and may be influenced by local factors. Causative factors of liver disorders include virus infection, exposure to certain chemicals e.g., the excessive inhalation of chlorinated hydrocarbons or overindulgence, medication with antibiotics, chemotherapeutic agents. In the recent years in vivo and in vitro test models have been developed for evaluation of plants for their anti-hepatotoxic activities (Trease and Evans, 2002).

Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics (Navarro and senior, 2006). The chemicals that cause liver injury are called hepatotoxins or hepatotoxicants. Hepatotoxicants are exogenous compounds of clinical relevance and may include overdoses of certain medicinal drugs, industrial chemicals, natural chemicals like microcystins, herbal remedies and dietary supplements (Willett *et al.,* 2004). Certain drugs may cause liver injury when introduced even within the therapeutic ranges. Hepatotoxicity may result not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically-mediated response affecting hepatocytes, biliary epithelial cells and/or liver vasculature (Deng *et al.,* 2009). The hepatotoxic response elicited by a chemical agent depends on the concentration of the toxicant which may be either parent compound or toxic metabolite (Kedderis, 1996). Hepatotoxicity related symptoms may include a jaundice appearance causing yellowing of the skin, eyes and mucous membranes due to high level of bilirubin in the extracellular fluid, pruritus, severe abdominal pain, nausea or vomiting, weakness, severe fatigue, continuous bleeding, skin rashes, generalized itching, swelling of the feet and/or legs, abnormal and rapid weight gain in a short period of time, dark urine and light colored stool.

The current study was carried out to evaluate hepatocurative activity of aqueous extract of *P. biglobosa* against CCl_4 induced Hepatotoxicity albino rats. It also established the acute toxicity of the extract as well as the phytochemicals present.

2.0 Material and Methods

2.1 Collection and Identification of Plant Material

The plant materials were collected from Wudil Local government, Kano state, Nigeria. They were authenticated by the staff of the herbarium section of the Biological Sciences Kano University of Science and Technology, Wudil, Kano, Nigeria.

2.2 Preparation of Extract

The stem bark were washed in clean water and dried at room temperature, after which it was pulverized to coarse powder using mechanical grinder. Aqueous stem extract of *Parkia biglobosa* was prepared according to Mittal *et al* (1981) by soaking One thousand grams (1000g) of the powder in 2000cm^3distilled water, the content of the flask was mixed vigorously and allowed to stand for 48 hours. The aqueous extract was obtained by filtration using whatman No1 filter paper and concentrated using vacuum evaporator at 60^oC in water bath (OSL200 water bath and shaker Grand instrument, Cambridge). The concentration and total yield of the aqueous stem extract of *Parkia biglobosa* was determined and stored in air tied container for further analysis.

2.3 Acute Toxicity Study ($LD_{50\ (Oral,\ Rats)}$) Determination

The $LD_{50\ (Oral,\ rats)}$ was determined according to Lorke (1983) method. In the first phase, nine rats were divided into three (3) groups of 3 rats each. The rats were orally administered with 10, 100, 1000mg/Kg of the extract. The rats were monitored for general behavior and mortality for 24 hours.

In the absence of any toxicity, three rats were grouped into three groups of one rat each, and were orally administered with 2500mg/kg, 3500mg/kg and 5000mg/kg body weight of the extract. They were observed for signs of toxicity which include: paw licking, salvation, rubbing of nose on floor, change in body weight and death within 24 hours. The number of death in each group within 24 hours was recorded and LD_{50} was calculated from the relation

$LD_{50} = \sqrt{\text{min conc. full death} \times \text{max conc. no death}}$

2.4 Phytochemical Screening

Phytochemical tests were carried out by using the standard methods of Sofowora (1993), Parekh and Chanda (2007), Trease and Evans (1989), El- Olemyl *et al* (1994).

2.5 Induction of Liver Damage

Liver damage was induced using Carbon tetrachloride (CCl_4) by the method of Alhassan *et al*. (2009). A stock solution of CCl_4 was prepared in 1:1 by dissolving 25cm^3 of CCl_4 in 25cm^3 pure olive oil (which was used as a vehicle) .The liver damage was induced by single intraperitonial injection of CCl_4 (120mg/kg). The volume of CCl_4 administered was determined by the weight of the rat according to the following relationship:

$$\text{Volume to be administered } (cm^3) = \frac{\text{Weight of rats (kg)} \times 120\text{mg/kg}}{\text{Concentration of } CCl_4 \text{ solution } (0.1538\ \text{mg/cm}^3)}$$

2.6 Hepatocurative studies

Twenty five (25) experimental rats were divided into five groups of five (5) rats each. Groups II to V were

administered with CCl_4 (120mg/kg) to induce liver damage.
Group I: Normal rats.
Group II: (Test Control) No extract administered but CCl_4 was given.
Group III: Administered with aqueous stem extract of *Parkia biglobosa* (50mg/kg) once daily.
Group IV: Administered with aqueous stem extract of *Parkia biglobosa* (100mg/kg) once daily.
Group V: Administered with aqueous stem extract of *Parkia biglobosa* (150mg/kg) once daily.

The rats in groups I and II were sacrificed 24 hours after inducement with CCl_4 and blood samples were collected to confirm inducement of liver damage in group II. The rats each from Group III, IV and V were sacrificed after four (4) weeks oral administration of aqueous stem extract of *Parkia biglobosa*.

2.7 Biochemical analysis of serum

The rats were sacrificed 24 hours after last extract administration. The blood samples collected were allowed to clot and serum was separated for determination of Liver function. Aspartate Aminotransferase (AST) and Alanine aminotransferase (ALT) were assayed using the method of Reitman and Frankel (1957). Alkaline phosphatase (ALP) was assayed by Roy, (1970), and serum bilirubin by Molley and Evolyn. (1937), Total protein concentration using the method of (Tietz, 1995).

2.8 Data Analysis

Results were expressed as mean ± standard deviation and analyzed using ANOVA, with p value <0.05 considered significant followed by Tukey´s post hoc test. GraphPad Instat3 Software version 3.05 by GraphPadInc was used to analyze the data.

3.0 Result

3.1 Phytochemical Analysis

The results of the phytochemical screening was presented in Table 1. The extract was found to contain Flavanoids, Glycosides, Tanins, Saponins, Steroids and Phenols, while Anthraquinones was absent.

Table 1. Phytochemical screening of aqueous extract of stem bark of *P. biglobosa*

S/N	**Phytochemical constituents**	**Results**
1	Flavanoids	+
2	Glycosides	+
3	Tanins	+
4	Saponins	+
5	Steroids	+
6	Phenols	+
7	Anthraquinones	-

Key: + (Present) ; - (Absent)

3.2 Acute Toxicity Studies

The result for LD_{50} determination is presented in table 2. In the initial phase of no mortality and toxic symptoms were observed in all the extract. Although some rats exhibited symptoms of weakness, rubbing of nose and mouth on the floor of the cage and restlessness in the second phase, no mortality was observed (Table 2b)

Table 2a: phase I of LD_{50} determination

Doses (mg/kg)	Aqueous extract of *Parkia biglobosa*
10	0/3
100	0/3
1000	0/3

Table 2b: Phase II of LD_{50} determination

Doses (mg/kg)	Aqueous extract of *Parkia biglobosa*
2500	0/1
3500	0/1
5000	0/1

3.3 Hepatocurative Studies

Table 3 present the effect of oral administration of the extract on CCL_4 induced hepatoxocity. Hepatotoxicity was induced by administration of CCl_4. A significant ($p<0.0.5$) increase in serum albumin and liver enzymes (AST, ALT and ALP) was observed in test control compared to normal control. Upon administration of the extract, a significant ($p<0.0.5$) fall in Albumin, AST, ALT and ALP was recorded in a dose dependent pattern. No significant difference ($p>0.05$) was observed between groups in total protein, direct and total bilirubin.

Table 3: Serum Liver Function Indices of Rats Administered with Aqueous Extract of *Parkia biglobosa* Stem Bark

Group	ALT(IU/L)	AST(IU/L)	ALP(IU/L)	DB(mg/dl)	TB(mg/dl)	TP(g/dl)	ALB(g/dl)
Normal Control	29.43 ± 2.90[a]	23.897 ± 2.02[a]	56.96 ± 1.85[a]	0.34 ± 0.06	1.85 ± 0.16	7.01 ± 1.11	5.97 ± 0.16[a]
Test Control	159.67 ± 12.9[a]	119.76 ± 9.23 [a,b,c]	178.55 ± 16.0[a,b,c]	0.56 ± 0.16	2.05 ± 0.33	7.43 ± 1.78	9.56 ± 2.34[a,b,c]
50mg/kg	26.01 ± 3.72[b]	19.26 ± 4.08[b]	44.91 ± 3.04[b]	0.47 ± 0.03	1.31 ± 0.34	7.16 ± 1.24	5.70 ± 0.82[b]
100mg/kg	20.65 ± 6.73[c]	15.50 ± 3.00[c]	44.46 ± 4.04[c]	0.38 ± 0.03	2.05 ± 0.38	7.86 ± 1.32	5.46 ± 0.33[c]
150mg/kg	20.05 ± 2.63[d]	14.7 ± 3.8[d]	36.23 ± 2.05[d]	0.40 ± 0.28	2.77 ± 0.10	6.63 ± 2.03	5.19 ± 0.95[d]

Results are expressed as mean ±SD, n=5. Values in the same row bearing the same superscript are significantly different at $P<0.05$.

4.0 Discussion

Medicinal plants were the major sources of products used to sustain health until the nineteenth century. In 1828 the German chemist Friedrich Wohler, in an attempt to prepare ammonium cyanide from silver cyanide and ammonium chloride, accidentally synthesized urea. This will be the first organic synthesis in history and it heralded the era of synthetic compounds (Mendonça-Filho, 2006).

The liver is the largest and heaviest internal organ of the body with a weight of 1 to 1.5 kg and representing 1.5% to 2.5% of the lean body mass. It is a reddish wedge shaped and covered by a network of connective tissue called Glisson capsule. It is located in the right upper quadrant of the abdominal cavity, resting just below the diaphragm and lies to the upper right side of the stomach and overlies the gallbladder (Guyton and Hall, 1996).

The liver regulate the flow of nutrients to the rest of the body as it controls the release of absorbed materials in to the systemic circulation and has a central role in carbohydrate, proteins and fat metabolism. The liver store substances, such as minerals, blood and vitamins, which can be released when required (Finlayson *et al.*, 1995).

Acute toxicity studies does not show any sign of toxicity or mortality up to a higher dose of 5000mg/kg. The result of the acute toxicity study indicated that the plant is practically non-toxic and can be used for many therapeutic purposes. This agrees with the findings of many studies that reported LD50 of P. biglobosa to be more than up to 5g/kg body weight (Tijani et al., 2009; Udobi and Onaolapo, 2009).

Liver damage was successfully induced using 12omg/kg body weight of CCl_4. This was proven by the high level of liver enzymes (ALT, AST and ALP) in test control compared to normal control. The enzymes were reported to reach higher than normal levels in the blood when there is necrosis of the parenchymal cells of the liver as in viral or toxic hepatitis (Sule, 2004). ALP is also a non-plasma specific enzyme involved in the hydrolysis of a variety of phosphate esters at alkaline pH. These enzymes were reported to reach higher than normal level in the blood in events of impaired liver function (Price and Stevens, 2003).

Administration of aqueous extract of *Parkia biglobosa* stem bark led to a significant decrease ($p<0.05$) in the mean serum levels of liver enzymes and albumin. This finding is in accordance with the findings of Muhammad *et al.* (2015) who reported a lower activities of transaminases in the liver of CCl_4 induced rats administered aqueous with *Khaya senegalensis* stem bark extract. One possible mechanism for the hepatocuration may be due to antioxidant properties of the extrcat, which could counteract the toxic effect of CCl_4. Natural antioxidants present in plants are responsible for inhibition or prevention of the deleterious consequences of oxidative stress. Herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. In the present study, phytochemical screening shows the presence of phenols, flavonoids and glycoside which may bind to the trichloro methyl-free radical, preventing its covalent binding to microsomal lipid and protein and thereby preventing lipid peroxidation which is thought to be the cause of liver damage by CCl_4 (Maiti *et al*., 2008)

5.0 Conclusion

This study has shown the chemical constituent of aqueous stem bark extract of *P. biglobosa* as well the acute toxicity of the extract. The study also established the hepatocurative potential of the extract which might be due

to the phytoconstituents.

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