Invitro Anthelmintic Efficacy of Aqueous Extract of *Parkiabiglobosa* Fruit Husk on Albino Rats

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ABSTRACT

The toxicity and anthelmintic efficacy of the aqueous extract of Parkiabiglobosa fruit husk were studied in albino rats. The oral acute toxicity study was conducted using the standard method. The anthelmintic efficacy of the extract was conducted using the fecal and larval recovery test tube paper technique and the egg hatch assay. The result of the studies showed that the LD_{50} of the oral administration of the extract was 1120mg/kg b.w indicating that the extract was moderately toxic. The extract did not significantly reduce egg hatch as compared to levamisol and albendazole used in this study, hence does not have anthelmintic effect. In conclusion the aqueous extract of Parkiabiglobosa fruit husk can be said to be safe but does not possess anthelmintic activity.

Keywords: *Parkiabiglobosa fruit husk, albino rats, aqueous extract, herbs or plant products*

INRODUCTION

The use of herbs or plant products by man as medicine has been from time immemorial. Majority of our population particularly those living in villages depend largely on herbal remedies. Some herbal remedies have stood the test of time; however, not much scientific data regarding their identity and effectiveness are available (Sofowora, 1984). Traditional medicine and medicinal plants have been used widely for maintenance of good health in developing countries (UNESCO, 1996). An increasing reliance on the use of medicinal plants in the industrialized societies have been traced to the extraction and development of several drugs and chemotherapeutics from these plants, as well as from traditionally used rural herbal remedies (UNESCO, 1998). *Parkiabiglobosa* popularly known as African locust bean tree is also known as "*Dorawa*" in Hausa, "*Irugba*" in Yoruba, and "*Origili"* in Ibo (Ajaiyeoba, 2002). *Parkiabiglobosa* is a large perennial deciduous tree of up to 7-20m in height but can reach 30m of height in exceptional cases with a dense spreading

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crown (Sabiti and Cobina, 1992). Its leaves alternate and are dark green in colour, bipinnate up to 30cm long (Orwa, Mutua, Kindt, Jamnadass and Anthony, 2009). The Pinnae are up to 17 pairs with 13-60 leaflets. The leaflets are oblong to linear 0.8-3.0cm x 0.2-0.8cm, its flowers are red or orange in colour, clustered in heads up to 7cm in diameter and borne on hanging peduncle up to 10-35cm long (Oruwa, Mutua, Kindt, Jamnadass and Anthony, 2009). Pods are linear, can be 12-30cm x 15-25cm glabrous brown. It has a very slow growth and begins fruiting in 8 years. At 15-20 years, it produces 25-100kg of pods per tree. The bark, leaves, flowers and pods have innumerable medicinal and food utilizations (Sabiti and Cobbina, 1992). The pods in particular (husk and pulp) are suitable food for humans and are stored in households. The foliage contains saponins, but it is considered palatable to cattle.

Flowers are rich in nectar and bee-hieves are often placed on the branches. The locust bean seed are fermented to make a black, strong smelling, tasty food ingredient, high in protein normally use by women in most African communities as cooking ingredient (Sabiti and Cobbina, 1992), referred to as "*Dawa-dawa*" in Hausa land or "*iru*" in Yoruba. It is rich in protein, lipids and vitamin B_2. The fat in the bean seed nutritionally contains approximately 60% unsaturated fat. The seeds are used as coffee substitute, they contain up to 29% crude protein and up to 60% saccharine. They are rich in vitamin C and high in oil content (Alabi, Akinsulire and Sanyoaolu, 2005). The pulp is eaten raw or made into a refreshing drink and is used as sweetener; it is press into cake for storage. The young seedlings are nutritious and heavily browsed by livestock; other products such as the burnt husk are added to tobacco to increase its pungency (Ajaiyeoba, 2002). In Gambia the leaves and roots are used in preparing a lotion for sore eyes. A decoction of the bark of *Parkiabiglobosa* is used as bath for fever, as a hot mouthwash to steam and relieve toothache. The pulp bark is used along with lemon for wound and ulcers (Irvine, 1961). *Parkiabiglobosa* has been identified as a source of tannins, saponins, gums, fuel and wood seeds of various species have also been investigated for their protein and amino acid contents (Fatuga, Babatunde and Oyenuga, 1974). It also contains fatty acids with Arachidonic acid being most abundant (Ajaiyeoba, 2002).

As it is with most African plants, this plant is used in traditional medical treatment of diseases to relieve diarrhoea; the bark is boiled to make tea, for infectious wounds and fever. The bark when macerated has been used in the treatment of haemorrhoids and burns. Its flowers, when gulled and macerated have been used in the treatment of hypertension and in the prevention of leprosy in some communities. Some use the decoction of the seed made by concentrating its extract through boiling to bring about emotional stability. It is also used to treat mouth sores and insect bites (Alabi, Akinsulire and Sanyaolu, 2005). Helminthosis is a disease that is highly prevalent in societies of low socio-economic status. It is common in Africa, the middle and Far East, Central and South America and other tropical region of the world, but less common in industrialized countries of the West. Tropical diseases are mostly caused by helminths (Aliu, 2007). Helminth infections constitute a hazard to both human and animal health with great economic loss in terms of morbidity and loss of domestic animals. One difference between helminth and microbial infections is

that most helminth parasites do not multiply in the host as do protozoa or bacteria. Consequently the severity of the helminth infections often depends on the number of larvae entering the host. Hence, the inhibition of growth commonly practiced in bacteriae chemotherapy is not a useful approach in helminthe chemotherapy, rather the aim is to weaken the worm, expel it or kill it out rightly (Aliu, 2007). Anthelmintics introduced into infected person or domestic animal are substances (drug) that are toxic to the helminthes parasite but not to the host. The drug should be selective in its effect interfering with physiology or biochemical processes essential for the functional integrity of the worm. Selectively toxicity can also be achieved in the case of helminthes residing in the lumen, by using orally active, non-absorbable drugs which affect parasites function by direct contact in the gut. The prevalence of helminthe diseases in Nigeria is as high as 100% in animals especially during the wet season (Suleiman, Maman, Aliu and Ajanusi, 2005). The economic loss due to mortality, decrease growth rate and reduced wool production constitute a major problem in livestock production. In Nigeria the major control strategy for helminth adopted is the use of antihelmintics (Ibrahim, Nwude, Aliu and Ogunsusi, 1983). However the high cost of modern antihelmintics has led to the limited effective control of these parasites (Monteiro et al., 1998).

The development of resistance strains of pathogenic helminthe has prompt the search for other chemotherapeutic agents which may allow efficient control of helminth parasites (Hammond, Fielding and Bishop, 1997). The solution to this is the use of less expensive and available resources within our environment which includes the study of indigenous plants to serve as a remedy. Herbal treatment in Nigeria is practised by nomadic Fulani and human herbalists (Nwude and Ibrahim, 1980; Nwosu, 1998). This study is designed to provide information which will either invalidate or support the folkloric usage of *Parkiabiglobosa* fruit husk as an anthelmintic agent and will also serve as a guide for potential users of the plant. The objective of the study is to evaluate the *in-vitro* anthelmintic efficacy of the crude aqueous extract of *Parkiabiglobosa* fruit husk.

MATERIALS AND METHOD

Plant Collection and Identification: The leaves, stems and fruits of *Parkiabiglobosa* were collected from Chibok town of Borno State and submitted for identification and authentication by a botanist with the Department of Biological Sciences, University of Maiduguri. A Voucher specimen; *P.biglobosa:* 001 was deposited at the department of Veterinary Physiology, Pharmacology and Biochemistry herbarium.

Extract Preparation: The fruit husk was completely dried at room temperature ($37^{O}C$) and then ground into fine powder using pestle and mortar, and 200gm of the powder was weighed and mixed with 1 liter of distilled water in a conical flask. The mixture was shaken and heated at $65^{0}C$ for 30 minutes. It was allowed to cool and filtered using Whatman No.1 filter paper. The filtrate was concentrated in a rotary evaporator and stored at $4^{o}C$ until needed.

Experimental Animals: White albino rats of both sexes were used for this study. They were kept in a plastic rat cages and allowed to acclimatize to laboratory environment for the period of two weeks before the commencement of the experiment. They were fed with growers' mash (Vital feeds Nig. Ltd) and water *at libitum*. The experiments were conducted with the international guiding principles for biochemical research involving animals (C.I.O.M.S. 1985).

Acute Toxicity Study: A pilot test was conducted to ascertain the level of toxicity of the plant extract after which the acute toxicity was determined. Twenty five albino rats were used for the acute toxicity study; they were divided into five groups A-E of five rats each. Group E served as the control group. The extract was administered orally to the groups (A-D) using a cannula No. 20 at the dose rate of 200mg/kg, 400mg/kg, 800mg/kg and 1600mg/kg respectively while group E was administered distilled water. This was done to establish the range of doses of the extract that would produce toxic effect. The rats were observed for 24 hours post treatment for behavioural signs such as excitement, nervousness, dullness, alertness, ataxia and even death. The median lethal dose (LD_{50}) was then calculated using the arithmetic method of Karber (1931) as modified by Aliu and Nwude, (1982).

Assessment of Anthelmintic Efficacy of the Extract

Fecal Sample Collection: Fecal samples were collected from the rectum of sheep and goats at Maiduguri Abattoir. Samples were collected in a polythene bags and labelled with identification and dates of collections, brought to the laboratory one hour after collection for analysis.

Egg Counting Technique: The modified McMaster technique was used to determine the fecal egg count using saturated sodium chloride solution as the floating medium as described by Soulsby (1982) and ANON (1997). Two grams of the fecal sample was treated in 28ml of the saturated sodium chloride solution and macerated using a laboratory pestle and mortar. The resultant solution was strained using tea strainer. A further 30ml of the sodium chloride solution was added to the filtrate and thoroughly mixed. The two chambers of the McMaster slide was carefully filled with the filtrate using Pasteur pipette. The slide was then mounted on the microscope and allowed to stand for 2 minutes. Eggs within the ruled areas of the chambers and those touching the right and bottom edges of the ruling were identified and counted. For positive samples, the eggs in the two chambers of the slide were counted and added then, multiplied by 50 to get the numbers of the eggs per gram of the faeces. Only positive samples with at least 500 eggs per gram were used for the anthelmintic efficacy study. Precautions observed include: faeces were moist and crumbly, in case of dry faeces, water was added, and in case of wet faeces charcoal (or sterile bovine feaces) was added until correct consistency was obtained.

Faecal Culture and Larval Recovery: The test tube paper technique as described by Harada and Mori (1995) was used for the faecal culture and larval recovery. 0.5g of the positive fecal sample was smeared on strips of filter paper and the lower end was dipped into test tubes containing 2mls of various concentration of the extract at 25mg/ml, 50mg/ml

and 100mg/ml respectively. 2mls of albendazole was measured in test tubes at different concentrations of 6.25mg/ml, 12.5mg/ml and 25mg/ml respectively and 2mls of levamisole was also measured in test tubes at the concentrations of 15mg/ml, 30mg/ml and 60mg/ml respectively. A control test tube with 2mls of distilled water was also used in each case. The test tubes were covered with a clean cotton wool to stop evaporation and the set up was allowed to stabilize for 10 days at room temperature. After 10 days, the cotton wool covering the test tubes were removed and the filter paper carefully pulled out of the culture medium individually. The culture medium was then poured on a Petri dish. The larva was immobilized by the addition of Lugol's iodine and examined under light microscope at the magnification of x40. The infective larval stage of the parasite were identified and counted in each case based on a standard description (Soulsby, 1982; Hansen and Perry, 1990).

Egg Hatch Assay: The egg hatch assay of Kelly, Webster and Griffin (1981) was used to determine the anthelmintic efficacy. The number of the hatched egg were determined in each of the samples and compared with that recorded for the water cultures of the same fecal sample to determine the percentage reduction in egg hatch using either the test sample or the standard anthelmintic. In each case, the proportion of the hatched eggs was calculated by relating it to the total number of eggs cultured (Cheijina, 1984).

Statistical Analysis: Data collected were expressed as mean ± standard deviation (S.D.). Analysis of variance (ANOVA) was used to analyze the extent of variation among groups and Pd" 0.05 were considered significant (Mead and Curnow, 1983). The computer statistical software Graph Pad InStat® (2003) was used to analyze the data.

RESULTS AND DISCUSSION

Extraction: The extract of *Parkiabiglobosa* fruit husk was dark brown in colour and was soluble in water, the extract yield was 25% w/w.

Acute Toxicity Study: The administration of aqueous extract of *Parkiabiglobosa* fruit husk orally at various doses resulted in the mortality of some of the treated albino rats as shown on table 1. The oral lethal dose (LD_{50}) was calculated to be 1120mg/kg b.w. using the arithmetic method of Karber (1931) as modified by Aliu and Nwude (1982). Clinical signs of depression, difficulty in breathing and huddling together were observed between 10 and 17 hours after administration of the extract to the rats.

***In vitro* Anthelmintic Efficacy Trial of Aqueous Extract of *P. biglobosa* Fruit Husk:** The result of the egg hatch assay of various dilutions of the extract, albendazole and levamisole is shown on table 2. All the 18 test samples cultured in plane water (control samples) show egg hatch with a mean of 417±17 being recovered. The samples cultured in 100, 50 and 25mg/ml of the extract show reduction in egg hatch by 1.43, 1.43 and 1.19% respectively, hence the extract did not significantly (P>0.05) reduce egg hatch when compared to levamisole (an imidiazazole) or albendazole (benzimidazole). In general, the experiment reveals that the reduction in egg hatch was concentration dependant as the highest concentration of the extract produced the highest inhibition in egg hatch and vice versa.

The aqueous extract of *P. biglobosa* fruit husk administered *par os* to albino rats at various doses produced a median lethal dose (LD_{50}) value of 1120 mg/kg b.w. According to the toxicity range by Clarke and Clarke any substance whose oral lethal dose (LD_{50}) in rats is between 500-5000mg/kg, is considered to be moderately toxic (Clarke, E. and Clarke, M., 1979) and could be administered with some degree of safety especially through the oral route where the absorption might not be complete but gradual due to inherent factors limiting absorption in the GIT (Dennis, 1994). The result obtained from the *in vitro* anthelmintic study of the extract reveals that the extract did not significantly ($P>0.05$) inhibit egg hatch. This is an indication that the extract does have anthelmintic property against parasites in sheep and goat. Although the result presents a negative egg hatch effect, it did not completely ruled out the folkloric believe that it has anthelmintic effect since it has not been tested on adult parasites.

CONCLUSION

This study was designed to provide information which will either invalidate or support the folkloric usage of Parkiabiglobosa fruit husk as an anthelmintic agent and will also serve as a guide for potential users of the plant. The objective of the study is to evaluate the *in-vitro* anthelmintic efficacy of the crude aqueous extract of *Parkiabiglobosa* fruit husk. White albino rats of both sexes were used for this study. They were kept in a plastic rat cages and allowed to acclimatize to laboratory environment for the period of two weeks before the commencement of the experiment. The aqueous extract of *P. biglobosa* fruit husk administered *par os* to albino rats at various doses produced a median lethal dose (LD_{50}) value of 1120 mg/kg b.w. The result obtained from the *in vitro* anthelmintic study of the extract revealed that the extract did not significantly inhibit egg hatch. This is an indication that the extract does have anthelmintic property against parasites in sheep and goat. Although the result presents a negative egg hatch effect, it did not completely ruled out the folkloric believe that it has anthelmintic effect since it has not been tested on adult parasites.

Table 1: Oral acute toxicity test of aqueous extract of *Parkiabiglobosa* fruit husk in albino rats

Dose(mg/kg BW)	**Dose difference** (DD)	**No. of mortality**	**Mean Mortality** (MM)	**(DDxMM)**
0	0	0	0	0
200	200	0	0	0
400	200	0	0	0
800	400	2	1	400
1600	800	3	2.5	2000
N=5				2400

LD_{50} = Highest dosage that caused death - $\frac{\text{Dose difference (DD)X Mean mortality (MM)}}{\text{Number of groups (n)}}$

LD_{50} = 1120mg/kg BW

$LD_{50} = (1600 - \frac{2400}{5})$

$LD_{50} = 1600 - 480$

Table 2: *Invitro* Strongyline nematode egg hatch, at various dilutions of water, extract of *P.biglobosa*, levamisole and albendazole

Extract/Drug Dilution	No. of samples showing egg hatch*	Larval Recovery** Mean±SD	Range	% reduction egg hatch
Water control	18	417±176	210-760	0***
P. biglobosa				
25mg/ml	18	412±162	200-580	1.19
50mg/ml	18	411±159	190-580	1.43[b]
100mg/ml	18	411±154	180-510	1.43[b]
Albendazole				
6.25mg/ml	10	9.50±9.0	14-21	97.7[a]
12.5mg/ml	1	1.11±4.7 ***	1	99.7[a]
25mg/ml	0	0	0	100[a]
Levamisole				
15mg/ml	1	16.6±70.7	1	96[a]
30mg/ml	0	0	0	100[a]
60mg/ml	0	0	0	100[a]

***Total number of samples tested = 18

****Mean number of eggs of feaces cultured = 417

*****Larval recovery for water control cultures was use as a standard (0% reduction in egg hatch)

[a,b]Mean in the same column with different superscripts are significantly different ($p<0.05$

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