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AN *IN VIVO* ASSESSMENT OF ANTI-DIARHEAL ACTIVITY OF SOLVENT EXTRACTS OF LEAF AND STEM BARK OF GHANAIAN *PARKIA BIGLOBOSA* AGAINST CASTOR OIL-INDUCED DIARRHEA IN ALBINO RATS

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Abstract: *Parkia biglobosa* is a wild plant belonging to family Fabaceae. The plant has widely been reported for its medicinal properties and used traditionally in the northern part of Ghana in the management of diarrhea. The present study evaluated the phytochemical profile, acute toxicity and anti-diarrheal effects of aqueous and ethanolic leaf and stem bark extracts of *P. biglobosa* against castor oil-induced diarrhea in albino rats. Preliminary phytochemical screening revealed the presence of reducing sugars, tannins, cardiac glycosides, anthocyanosides and terpenes in both leaf and stem bark. Flavonoids and saponins were only found in the leaf extracts. Alkaloids and anthraquinones were only found in the stem bark. The LD_{50} of the extracts was found to be greater than 5000mg per kg body weight of the rats. The extracts induced a dose dependent anti-diarrheal effect at 200, 600 and 1000 mg per kg body weight by significantly ($p<0.05$) reducing the frequency of stooling in castor oil-induced diarrhea. The anti-diarrheal activities of the various extracts were significant ($p<0.05$) compared with the control. Diarrheal droppings were completely eliminated at dose 1000mg per kg body weight after five hours. The antidiarrheal activity of the extracts at dose 1000mg per kg body weight was comparable to that of the standard drug loperamide at 2mg per kg body weight. Results from this study show that aqueous and ethanolic extracts of *P. biglobosa* leaf and stem bark possess anti-diarrheal effects on castor oil-induced diarrhea models and this scientifically validates its traditional use in the management diarrheal conditions.

Key Words: *Parkia biglobosa*, phyotchemicals, anti-diarrheal, castor oil-induced model, albino rats

INTRODUCTION

Diarrhea is an increase in the frequency of bowel movements, an increase in the looseness of stool or both. Severe diarrhea leads to fluid loss, and may be life-threatening, particularly in young children, malnourished people and those with impaired immunity. Diarrhea is second only to pneumonia in infant mortality (UNICEF/WHO, 2009). It kills more young children than AIDS, malaria and measles combined, (WHO/UNICEF, 2009).

According to the World Health Organization (WHO, 2001), phytomedicine is defined as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes. These preparations may be produced for immediate consumption or as the basis for other herbal products. Traditional medicine is a major African socio-cultural heritage. It has been used to treat various diseases in Africa including diarrhea. Although synthetic or chemical drugs can have greater or quicker effects than do equivalent traditional medicines, they present a higher degree of side effects and risks, (Odama *et al.,* 1997). These shortcomings include their toxicity, the ability of organisms to develop resistance to the drugs previously known to be effective, and loss of potency of the drug with time. On the other hand, the merits of herbal medicine over orthodox drugs include; minimal or no side effects on the organic functioning of the body, consistent potency, and the fact that they are well absorbed and distributed in the area of infection (Cheij, 1988; Nkere, 2003; Okigbo and Omodamiro, 2006).

Different medicinal plants have been employed in the treatment of various ailments across the globe, especially in Africa. Medicinal plants also present the basis for the development of many synthetic drugs. Despite reports on traditional use of medicinal plants, only a few of these plants or their phytochemical constituents have been proven scientifically to have medicinal value.

Parkia biglobosa is common perennial plant in the nothern part of Ghana. The plant has widely been used in the Ghanaian and other West African rural communities to treat a variety of diseases including malaria, inflammatory diseases, and diarrhea. In spite of the wide use of this plant in managing diarrheal conditions, there has been little scientific data available to support these claims. The present research was aimed at screening for phytochemicals present in ethanolic and aqueous leaf and stem bark extracts of *Parkia biglobosa* and evaluating the anti-diarrheal activity of these extracts against castor oil-induced diarrhea in albino rats.

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MATERIALS AND METHODS

Plant material

Healthy leaves and stem bark of *Parkia biglobosa* were collected from Navrongo in the Upper East Region of Ghana. The leaves were air dried under shade, milled in a blender, sieved to obtain smooth powder and then stored in an airtight container.

Aqueous extraction

About 1kg of the powdered leaf and stem bark samples were separately boiled in 5L of distilled water for 1 hour. The decoctions were decanted, centrifuged at 4500rpm for 10 minutes and freeze dried. The yields were 21.56% and 20.73% w/w of leaf and stem bark samples respectively. The freeze dried samples were stored in an airtight container and used for the study.

Ethanolic extraction

The ethanolic extracts were obtained from 75% ethanol using Soxhlet apparatus for 24 hours. The extracts was filtered through Whatman No.1 filter paper and evaporated under reduced pressure using a rotary evaporator. The filtrates were freeze-dried using lyophilizer to yield 13.34% and 13.33%w/w of leaf and stem bark samples respectively. The dried extracts were stored in an airtight container and used for the study.

Phytochemical screening

Qualitative phytochemical tests were conducted for tannins, anthraquinones, cardiac glycosides, flavonoids and anthocyanosides on the ethanolic and aqueous leaf and stem bark extracts of *Parkia biglobosa* using standard methods described by Evans and Trease, 2002. Alkaloids were screened for using the method described by Sofowora (2008) whereas saponins, terpenes and reducing sugars were screened for using the methods described by Persinos and his colleagues (Persinos, 1967).

Test animals

All the pharmacological experiments were conducted using Albino rats, weighing between 120-200g and were purchased from the Animal department of the Center for Research into Plant Medicine, Mampong in the Eastern Region of Ghana. The animals were acclimatized for three days before the commencement of the study.

Acute toxicity test

The LD_{50} (Mean lethal dose) of the extracts was determined using the method described by Lorke, 1983) with some modifications. Nine rats were divided into three groups of three rats per group; the three groups were administered orally with doses of 100, 400 and 1000 mg per kg body weight of aqueous extracts respectively. These groups were observed over a period of 6 hours for signs of toxicity and after 24 hours, they were scored for mortality. In the second phase, two groups of two rats per group were administered orally with 2500 and 5000mg per kg body weight of the aqueous extract respectively based on the findings in the first phase. The above procedure was then repeated for the ethanolic extracts.

Antidiarrheal activity (castor oil induced diarrhea model)

Forty (40) overnight fasted rats were used for this antidiarrheal test. They were randomly divided into eight (8) groups of five rats per group. Group 1 received loperamide 2mg per kg body weight (standard drug), Groups 2, 3 and 4 received 200, 600 and 1000 mg per kg body weight of *Parkia biglobosa* aqueous leaf extracts respectively. Group 5 animals received 10 ml per kg body weight distilled water. Groups 6, 7 and 8 animals received 200, 600 and 1000mg per kg body weight of the ethanolic leaf extracts respectively. After 10 minutes of administration of the extracts, 1ml of castor oil was administered to each rat in all the groups. All drugs and controls were administered orally using feeding syringe. The various groups of animals were then placed in individual cages lined with clean white paper adsorbent. The above procedure was repeated for the stem bark extracts.

The animals were observed for the presence of characteristic diarrhea droppings at one-hour intervals and recorded over a period of six hours. The total score of each group were taken and mean total number of droppings determined for each group.

Percentage inhibition of defecation in each group is calculated using the formula:

$$\% \text{ inhibition} = \frac{(\text{Mean defecation of control group} - \text{Mean defecation of treated group})}{\text{Mean defecation of control group}} \times 100$$

Statistical analysis

Data are expressed as mean ±standard error of mean (S.E.M.) and statistical analysis was carried out by One-way analysis of variance (ANOVA) at $p<0.05$ significance level using Graphpad prism 5.0 for mac.

RESULTS AND DISCUSSION

Table 1 presents result of qualitative phytochemical screening of aqueous and ethanolic extracts of leaf and stem bark of *Parkia biglobosa.* Phytochemical analysis reveals the presence of tannins, reducing sugars and cardiac glycosides in all extracts. Terpenes were only absent in the ethanolic leaf extract while anthocyanosides were absent only in the aqueous stem bark extract. Leaf extracts did not contain alkaloids and anthraquinones while saponins

and flavonoids were absent in the stem bark extracts. Our result is in agreement with the works of Tijani *et al.*, 2009 and Ezekwe *et al.*, 2013.

Table 1: Phytochemical screening of leaf and stem bark extracts of *P. biglobosa*

Phytochemicals	Leaf		Stem bark	
	Ethanolic	Aqueous	Ethanolic	Aqueous
Flavonoids	+	+	-	-
Alkaloids	-	-	+	+
Saponins	+	+	-	-
Tannins	+	+	+	+
Terpenes	+	-	+	+
Reducing Sugars	+	+	+	+
Cardiac Glycosides	+	+	+	+
Anthraquinones	-	-	+	+
Anthocyanosides	+	+	+	-

(+) = phytochemical present, (-) = phytochemical absent

Oral administration of aqueous and ethanolic extracts of *P. biglobosa* leaf and stem bark to rats up to 5000mg per kg caused no death in the two phases of the test after 72 hours. There was no mortality in animals at all doses of the extracts up to 5000mg per kg. The absence of death at doses up to 5000mg per kg implies that LD_{50} of the extracts of *P. biglobosa* is greater than 5000mg per kg. This suggests that the extracts of *P. biglobosa* have low toxicity. The low toxicity obtained may be responsible for its widespread use in different therapeutic interventions.

Table 2a: Anti-diarrheal activity of leaf extracts of *P. biglobosa*

Treatment	Average number of diarrheal stool within six hours (n=5)					
	1hour	2hour	3hour	4hour	5hour	6hour
Control (distilled water)	4.6	4.2	4	3.8	3.6	3.4
Loperamide (2mg/kg)	1.8	1.2	0.4	0.4	0.0	0.0
Aqueous extract (200mg/kg)	4.2	3.4	2.4	2.0	1.0	0.0
Aqueous extract (600mg/kg)	3.8	3.2	2.4	1.8	0.8	0.0
Aqueous extract (1000mg/kg)	3.6	3.0	1.8	1.2	0.6	0.0
Ethanolic extract (200mg/kg)	3.6	2.6	2.0	1.2	0.4	0.0
Ethanolic extract (600mg/kg)	3.2	2.6	1.8	1.0	0.2	0.0
Ethanolic extract (1000mg/kg)	2.6	2	0.8	0.4	0.0	0.0

One hour after administration of the castor oil, diarrhea was clinically apparent in most animals of the control group (distilled water treated animals), which continued for the next 6hours. A marked dose-dependent reduction in the number of defecations over the six-hour period was achieved with leaf extracts of *P. biglobosa* at doses of 200, 600 and 1000 mg per kg (Table 2a). A similar trend was observed for the stem bark extracts Table 2b).

Table 2b: Anti-diarrheal activity of stem bark extracts of *P. biglobosa*

Treatment	Average number of diarrheal stool within six hours (n=5)					
	1hour	2hour	3hour	4hour	5hour	6hour
Control (distilled water)	4.8	4.6	4.2	3.8	3.8	3.6
Loperamide (2mg/Kg)	1.8	1.0	0.6	0.4	0.0	0.0
Aqueous extract (200mg/Kg)	4.0	3.6	2.2	1.8	0.8	0.0
Aqueous extract (600mg/Kg)	3.6	3.0	2.2	1.6	0.8	0.0
Aqueous extract (1000mg/Kg)	3.4	2.8	1.6	0.8	0.4	0.0
Ethanolic extract (200mg/Kg)	3.4	1.8	1.6	1.2	0.4	0.0
Ethanolic extract (600mg/Kg)	3.0	1.8	1.4	1.0	0.2	0.0
Ethanolic extract (1000mg/Kg)	2.0	1.4	0.6	0.2	0.0	0.0

Table 3a: Percentage inhibition of diarrhea by leaf extracts of *P. boglibosa* in 6 hours

Treatment	Mean ± SEM (in 6hours)	Inhibition (%)
Control(distilled water)	3.9333±0.1764	
Loperamide (2mg /kg)	0.6333±0.2940	83.91
Aqueous extract (200mg /kg)	2.1667±0.6270	44.90
Aqueous extract (600mg /kg)	2.000±0.5865	49.15
Aqueous extract (1000mg /kg)	1.700±0.5675	56.78
Ethanolic extract (200mg/kg)	1.6333±0.5572	58.47
Ethanolic extract (600mg/kg)	1.4667±0.5283	62.70
Ethanolic extract (1000mg/kg)	0.9667±0.4455	75.41

Values are presented as Mean ± SEM

Table 3b: Percentage inhibition of diarrhea by stem bark extracts of *P. boglibosa* after 6 hours

Treatment	Mean ± SEM (in 6hours)	Inhibition (%)
Control (distilled water)	4.133±0.1978	-
Loperamide (2mg/Kg)	0.6333±0.2801	84.67
Aqueous extract (200mg/Kg)	2.067±0.63386	49.99
Aqueous extract (600mg/Kg)	1.867±0.55056	54.83
Aqueous extract (1000mg/Kg)	1.500±0.55558	63.71
Ethanolic extract (200mg/Kg)	1.400±0.48990	66.13
Ethanolic extract (600mg/Kg)	1.233±0.45142	70.17
Ethanolic extract (1000mg/Kg)	0.7000±0.33764	83.06

Previous studies showed that anti-dysenteric and antidiarrheal properties of medicinal plants were mostly due to tannins, alkaloids, flavonoids, saponins, terpenes and sterols (Longanga-Otshud *et al.*, 2000; Veiga *et al.*, 2001; Al-rehaily *et al.*, 2001). Flavonoids

have also been shown to possess antidiarrheal activity attributable to their ability to inhibit intestinal motility and hydro-electrolytic secretion (Rao *et al.,* 1997). Flavonoids present in the plant extracts are reported to inhibit release of autacoids and prostaglandins, thereby inhibiting motility and secretion induced by castor oil. The antidiarrheal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretions which are altered in this intestinal condition. Tannins may also evoke an anti-diarrheal effect since these substances can precipitate proteins of the electrolytes and reduce peristaltic movement and intestinal secretions. The presence of one or more of these phytochemicals in the extracts may be responsible for the observed anti-diarrheal activities shown by the extracts. The anti-diarrheal activity of the leaf extracts was slightly higher than those of the stem bark extracts and this may partly be attributed to the presence of flavonoids in the leaf extracts.

Generally, diarrheal inhibition by the stem bark extracts was slightly higher than those of the leaf extract. This observation may partly be attributed to the abundance of terpenes in the stem bark extract. The inhibition of diarrhea caused by extracts at dose 1000mg per kg weight was comparable to that of the standard drug loperamide (at dose 2 mg per kg body weight).

Although the exact mechanism of the anti-diarrheal activity of *P. biglobosa* extracts is uncertain in this study, one of the most likely mechanism of anti-diarrheal activity of the extracts may be their ability to enhance fluid and electrolyte absorption through the gastro intestinal tract since castor oil produces diarrhea by preventing fluid and electrolyte absorption and thus resulting in an increase in intestinal peristalsis. The anti-diarrhoeal activity of the extract may also be due to the presence of denatured proteins forming protein tannates (a salt of tannin); protein tannates make the intestinal mucosa more resistant and reduce secretion (Tripathi, 1994).

Furthermore, castor oil is made up of 90% ricinoleate (Mekeon *et al.,* 1999) which is metabolized to ricinoleic acid. Liberation of this ricinoleic acid results in irritation and inflammation of the intestinal mucosa thus leading to release of prostaglandins which results in stimulation of secretion (Pierce *et al.,* 1971) thereby preventing the reabsorption of NaCl and H_2O (Galvez *et al.,* 1993). The activity of *P. biglobosa* can thus be attributed to the inhibition of gastrointestinal hyper-secretion and hypermobility by enhancing electrolytes (reabsorption of NaCl), solutes and water absorption from the intestinal lumen by decreasing intestinal motility.

Castor oil also activates chlorine channels that enhance the secretion of water into the intestinal lumen and creating massive watery diarrhea (Ammon and Soergel, 1985). The extracts activity could also be attributed to inactivation of the Cl^- channels thereby reverting this mechanism.

CONCLUSION

The use of medicinal plants in traditional healing is widespread in the developing world. Following the potential role of plants in drug discovery, the search for new drug leads from medicinal plants has been on the increase in recent times. Crude extracts of *P. biglobosa* showed significant ($p<0.05$) anti-diarrheal activity *in vivo* in our study. Our result suggests that aqueous and ethanolic extracts of *P. biglobosa* contain bioactive compounds capable of significantly reducing the severity and frequency of diarrhea induced by castor oil in albino rats. Furthermore, our work demonstrates that aqueous and ethanolic leaf and stem bark extracts of *P. biglobosa* has anti-diarrhea activity comparable to that of loperamide. Our findings therefore corroborate the traditional usage of *P. biglobosa* in the treatment and management of diarrheal conditions.

We therefore suggest further studies be conducted to identify and isolate phytochemicals responsible for the anti-diarrheal activity of *P. biglobosa*. It is also worthwhile to study the possible mode of action of fractions of the leaf and stem bark extracts.

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