Evaluation of Anti-Diarrhoeal Activity of Different Bark Extracts of Faidherbia albida (Delile) A (Chav) in Albino Rats

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Abstract

Diarrhoea is a major concern in the health sector as it is the second leading cause of death and growth retardation in children under five years old, and is responsible for killing significant number of children every year. Various drug treatments available are not readily accessible, affordable and may have associated adverse or side effects, and in some cases resistance to antimicrobial agents have developed. This research focuses on the antidiarrhoeal activity of the aqueous and ethanol extracts of Faidherbia albida bark commonly used in herbal medicine in the Northern part of Nigeria to treat diarrhoeal diseases. The phytochemical screening of the bark extracts of Faidherbia albida revealed the presence of alkaloids, carbohydrates, flavonoids, saponins and tannins. However, glycoside was not found in both extracts. Both extracts had significant antidiarrhoeal effect. The ethanol extract was found to have higher antidiarrhoeal activity when compared to the aqueous extract. The extracts of Faidherbia albida bark had dose dependent activity in castor oil induced diarrhoea by significantly reducing the number of wet faecal droppings and delayed time of onset of diarrhoea. The bark extracts of Faidherbia albida was found to contain phytochemicals which may be responsible for the antidiarrhoeal activity and this justifies the traditional use of this plant in the treatment of gastrointestinal diseases including diarrhoea.

Keywords: Faidherbia albida, diarrhoea, LD_{50}, in vitro, in vivo

Introduction

Diarrhoea is defined as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual) (WHO, 2013). Since stool weight is largely determined by stool water, most cases of diarrhea result from disorders of intestinal water and electrolyte transport. Diarrhoea can be caused by an increase osmotic load within the intestine, excessive secretion of electrolytes and water into the intestinal lumen, excretion of protein and fluid from the mucosa, and altered intestinal motility resulting in rapid transit (Jafri and Pasricha, 2001). Diarrhoea can be common in under developing and developing countries (WHO 2013). However it can be distressing and unpleasant until it passes, which normally takes few days to a week

(NHS, 2014). This condition can be quiet embarrassing if it occurs at work or in any public places. The rush for the nearest convenience is always quiet eventful. Diarrhoea is the second leading cause of death in children under five years old, and is responsible for killing around 760, 000 children every year (WHO, 2013). Diarrhoea can last several days, and can leave the body without the water and salts that are necessary for survival. Most people who die from diarrhoea actually die from severe dehydration, fluids and electrolytes losses (Diurno *et al.,* 1996; WHO, 2013). Children who are malnourished or have impaired immunity as well as immune-compromised patients like people living with HIV are at most risk of life threatening diarrhoea (WHO, 2013). Diarrhoea is usually a symptom of an infection in the intestinal tract, which can be caused by a variety of bacterial, viral and parasitic organisms. Infection is spread through contaminated food or drinking-water, or from person-to-person as a result of poor hygiene (WHO, 2013). Modern medicine is yet to provide a satisfactory effective therapy to cure diarrhoea. Also, unpredictable and unwanted effect of most antidiarrhoeal medications has been a burden to the patient and health care providers (Diurno *et al.,* 1996; WHO, 2013). As a result of these challenges, there is increasing interest in research into natural products with antidiarrhoeal activity, with mild side effects and more affordable compared to orthodox medicine.

Faidherbia albida is a genus of leguminous plants belonging to Fabaceae family containing one specie. The plant is native to Africa and the Middle East. The Hausa people of Northern Nigeria call it 'Gawo', while in Fulfulde it is called 'Chayski'. Common names for it include apple-ring acacia (their circular, indehiscent seed pods resemble apple rings), ana tree, balanzan tree and winter thorn. Phytochemical studies reveal that plants in this family contain tannins (Barry and McNabbs, 1999), which account for their use in making of dyes. In addition to this, Tijani *et al* (2008) reported the presence of alkaloids and saponins in the stem bark extract of *Faidherbia albida*. Traditionally, it is used in treating fevers by the Masai people of Kenya and as antidiarrhoea in Tanganyika (Irvine, 1961; Tijani *et al.,* 2008). It is also used for colds and hemorrhage. A liniment, made by steeping the bark, is used for bathing and massage in pneumonia. The bark infusion is used by traditional birth attendant for difficult delivery, and is used as a febrifuge for cough (Irvine, 1961). Kubmarawa *et al* (2007) demostrated the antimicrobial activity of *Faidherbia albida* against *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Salmonella typhi*. In Northern Nigeria, especially among the cattle rearing nomads, a decoction of the stem bark is usually taken for the management of the sleeping sickness. This study was conducted to investigate the antidiarrhoeal activity of the aqueous and ethanol extracts of *Faidherbia albida* bark.

Materials and Methods

Plant collection

The fresh bark of *Faidherbia albida* was collected from Faculty of pharmacy, University of Maiduguri, Borno State, Nigeria. The plant was identified by a plant taxonomist by Prof. S.S Sanusi, Department of Biological Sciences, University of Maiduguri, Borno State.

Preparation of extracts

The fresh stem bark of *Faidherbia albida* was collected, air-dried under the shade for one week and then grounded into powdered form using a clean pestle and mortar. Grounded sample was extracted using ethanol (95%) and water as solvents for the ethanol and aqueous extracts respectively for 24 hrs. The mixtures were filtered and the residue was macerated. Ethanol was recovered using soxhlet rotary evaporator, while the aqueous extract was evaporated using water bath. The percentage yields (w/w) of extracts were calculated. The extracts obtained were stored in a desicator at -4°C until required for use.

Drugs Used

Loperamide (Imodium, RPG Life Science Ltd.), castor oil (BELL, SONS and Co. (DRUGGISTS) LTD, SOUTHPORT PR9 9AL, ENGLAND. Normal Saline solution (0.9% NaCl), and vehicle (distilled water) were used. **Animals Used**

Normal healthy Wister albino rats of both sexes were housed and acclimatized in the animal house facility of the Faculty of Pharmacy, University of Maiduguri, Borno States, Nigeria. The rats were fed with standard pellet diet and water *ad libitum*. The research was carried out at the Pharmacology laboratory, Faculty of Pharmacy, University of Maiduguri, in accordance with the rules governing the use of laboratories animals as accepted internationally.

Preliminary Phytochemical screening

The bark extracts of *Faidherbia albida* were screened for the presence of different chemical constituents using standard procedures (Trease and Evans, 1989; Sofowora, 1993). Secondary metabolites evaluated includes; alkaloid, saponins, tannins, glycosides, carbohydrates and flavonoid.

Acute toxicity tests

The method previously described by Lorke (1983) was modified and adopted using rats for the study. In the first phase, four doses of the aqueous extract (10, 100, 100 and 2000 mg/kg) were administered intraperitoneally (IP) to four groups, each containing two rats. In the second phase, which was based on the results of first phase, usually consist of three geometric dose levels at which the median lethal dose (LD_{50}) was determined as the square root of the geometric mean of the highest non-lethal dose and lowest lethal dose. LD_{50} was calculated as such; square root ($\surd$) of the highest effective dose for which animal survived x minimal lethal dose for which the animal died. The geometrical mean of the smallest dose that killed the rat and the highest dose that did not kill the rat was taken as the mean lethal dose (LD_{50}) of the extract.

Effect of castor oil-induced diarrhoea

The rats were starved for 12 hours prior to the commencement of the experiment, they were randomly divided into five groups of five rats each weighing between 120-200 g. The rats in the first group received 2ml/kg distilled water orally, while the rats in the second group received 3mg/kg Loperamide as a standard positive control. The third, fourth, and fifth groups were administered doses of 250mg/kg, 500mg/kg and 1000 mg/kg of the aqueous and ethanol extracts respectively. After 60 minutes of administration of the extracts, castor oil 3ml/kg was administered orally to animals in all groups. The animals were placed on individual special cages over white clean Whatman filter paper. The number of both wet and dry diarrhoeal droppings was counted every hour for a period of 4 hours (Awouters *et al.*, 1978). A numeric score based on the stool consistency was assigned as follows: normal stool = 1, semisolid stool = 2 and watery stool = 3. The total score of diarrhoeal faeces of negative control group was considered as 100%. Results were expressed in terms of percentage of inhibition (Zaval *et al.*, 1998). The absence of stool was recorded as a protection from diarrhoea (Diurno *et al.*, 1996) and the percentage protections were calculated using the formula as follows (Offiah and Akah, 1999); *Percentage protection or inhibition* = **[(**mean defecation of control – mean defecation of treated group) / (Mean defecation of control group)] ×100.

Statistical analysis

Results were expressed as mean ± S.E.M and analyzed using Computer software GraphPad InStat ® @ USA, 2003. The significant difference between mean was determined using student T-test. Differences between means were considered significant at 5% level of significance.

Results Analysis Preliminary phytochemical screening

The preliminary phytochemical screening of the aqueous and ethanol bark extracts of *Faidherbia albida* revealed the presence of alkaloids, carbohydrates, flavonoids, saponins and tannins. However, glycoside was found to be absent in both extracts. Carbohydrates was found to be present in the aqueous bark extract, while alkaloids, flavonoids, saponins and tannins were found to be present in the ethanol stem bark extract of *Faidherbia albida* (Table 1).

Table 1: Phytochemical screening of the aqueous and ethanol stem Bark extracts of *Faidherbia albida*

Phytochemical constituents	Results	
	Aqueous extract	Ethanol extract
Alkaloids		
• Dragendoff's test	-	+
• Mayer's test	-	+
• Wagner's test	-	-
Carbohydrates		
• Barfoed's test	+	-
• Combined reducing sugar	+	-
• Fehling's test	-	-
• Molish test	+	-
Flavonoids		
• $FeCl_3$ test	-	+
Glycoside	-	-
Saponins		
• Fehling's test	-	+
• Haemolysis test	-	-
Tannins	-	+

- = Absent, + = Present

Acute toxicity

The acute toxicity of the ethanol bark extract of *Faidherbia albida* in rats was found to be greater than 5000 mg/kg orally and 2154 mg/kg intraperitoneally, while that of the aqueous bark extract of *Faidherbia albida* was found to be greater than 5000 mg/kg and greater than 2900 mg/kg for oral and intraperitoneal routes respectively (Table 2).

Effect of bark extracts of *Faidherbia albida* on castor oil induced diarrhoea

The bark extracts of *Faidherbia albida* showed significant activity against castor oil induced diarrhoea in rats. The ethanol extract of the plant had higher antidiarrhoeal activity when compared to the aqueous extract, while the aqueous extract was dose independent. The activity of the ethanol extract on the castor oil induced diarrhoea rats was dose dependent and significantly higher than the control ($p<0.001$). The antidiarrhoeal activity of ethanol extract at 1000mg/kg was significantly higher than the activity of loperamide group ($p<0.05$). The activity of extract at 1000mg/kg was able to inhibit the formation of semi-solid stool significantly higher than the control ($p<0.05$). However, loperamide and varying doses of the ethanol extract inhibited the formation of the semi-solid stool (Table 3 and Figures 1, 2 and 3).

Discussion

The presence of some phytochemical components detected in the present study agrees with the report of Tijani and his colleagues (2008), in which similar constituents were detected. However, the absence of glycosides in the present study that was detected by Tijani *et al* (2008) might be due the differences in the geographical location or climatic changes of the plant. This is in agreement with the report of Sampaio and his colleagues (2016) in their article they published which confirmed that specimens of same plant species growing under different environmental conditions, shows significant differences in the production and accumulation of secondary and primary metabolites. The phytochemicals detected in this plant may be responsible for its antidiarrhoeal activity (Brijesh *et al.*, 2009; Tangpu and Yadav, 2004), anti-inflammatory, anti-pyretic activity (Tijani *et al*., 2008) and as an antiemetic (Wickens, 1969). The absence of animal death due to the highest tested doses of the extracts in the present study suggests that, the plant extract is relatively less toxic and is safe in rats practically. This is in agreement with the findings of Matsumura (1975) and Tijani *et al* (2008) in which less toxicity was observed. The present study has shown that both aqueous and ethanol extracts of the plant possess significant antidiarrhoeal effect, with the ethanol extract exhibiting more potency when compared to the aqueous extract. Therefore, ethanol is recommended as an effective solvent for the preparation of *Faidherbia albida* extract to manage gastrointestinal issues. The observed differences may be due to the different degree of chemical constituent extracted by the solvent used because according to the findings of Gunner (1991) different solvent extracts of some plants may exhibit different pharmacological properties. This also agrees with the work of Mahmood *et al* (2013) who demostrated that the methanol extracts of the *Faidherbia albida* had *in vivo* antidiarrhoeal activity.

The antidiarrhoeal activity exhibited by the extracts of *Faidherbia albida* proves that, the positive control (loperamide) group was able to prevent increased contraction of the gastrointestinal tract. The extracts showed more effect when it comes to the production of solid and semi-solid stools. This means that, the extracts exhibit a dual effect on

Table 2: Acute toxicity study of the aqueous and ethanol stem bark extracts of *Faidherbia albida*

Phases	*Dose (mg/kg)*	*Aqueous extract (mg/kg)*		*Ethanol extract (mg/kg)*	
		I.P	*Oral*	*I.P*	*Oral*
I	10	-	-	-	-
	100	-	-	-	-
	1000	-	-	-	-
II	1600	-	-	-	-
	2900	-	-	+	-
	5000		-		-
LD_{50}		*>2900*	*>5000*	*2154*	*>5000*

- = no death, + = death, > = greater than, LD50 = lethal dose that can kill 50% of the population, I.P = Intraperitoneal route

preventing contraction that leads to watery stools and stabilize the intestine to normalcy, in the production of normal solid stools. This effect was observed to be dose dependent for the ethanol extract and dose independent for the water extract. According to Tunaru *et al* (2012), castor oil being one of the oldest drug normally act as a laxative when given orally which is mediated by ricinoleic acid, a hydroxylated fatty acid released from castor oil by intestinal lipases. Although the mechanism by which ricinoleic acid acts remains unknown, there are theories of its effect on water and electrolytes as well as its toxicity on the intestinal cells that may contribute to its diarrhoeal activities. Tunaru *et al* (2012) reported that the ricinoleic acid specifically binds to a particular receptor in the intestine and uterus thereby causing the laxative effect of the castor oil. Castor oil may binds to the prostaglandin receptors (EP3) to exert its effects. Since the ricinoleic acid is a selective agonist of EP3 and EP4 receptors, activation of EP3 receptors on the intestinal cells proves the pharmacological effect of castor oil. Also, it is very evident that castor oil produces diarrhoea via causing irritation and inflammation of the intestinal mucosa, leading to the release of prostaglandin which results in the stimulation of secretion (Gaginella *et al.*, 1985). Therefore, it is possible that the extracts of *Faidherbia albida*, acts on this EP3 receptor of the intestinal cells to inhibit its activation, thus its decreased contraction and stimulation of secretion of the intestine. The bark extract might have exerted the anti-diarrhoeal action via anti-secretory mechanism or the anti-diarrhoeal property may be attributed to the presence of tannins; the phytochemical

which is known to reduce the effect through denaturing the protein by the formation of protein tannate, thereby causing the intestinal mucosal more resistant and reduces secretion (Tripathi, 1994). Hence tannins present in the extracts may be responsible for their anti-diarrhoeal activity (Yu *et al*., 2000; Devi *et al*., 2002) as well as other pharmacological properties.

Table 3: Effects of the aqueous and ethanol stem bark extracts of *Faidherbia albida* on castor oil induced diarrhoea

Treatment	Number of stool		
	Solid	Semi-solid	Loose (% inhibition)
Control (2ml/kg)	2.40±0.24	4.80±0.86	22.40±2.42 (0)
Loperamide (3mg/kg)	-	-	7.00±0.71 (69)
AE (250mg/kg)	1.80±0.80	3.00±1.10	- (100)
AE (500mg/kg)	2.40±0.81	-	5.80±0.37(74)***
AE (1000mg/kg)	11.20±2.15*	2.00±0.55*	- (100)
EE (250mg/kg)	-	-	8.00±0.71 (64)***
EE (500mg/kg)	-	-	4.00±1.70 (82)***
EE (1000mg/kg)	2.20±0.66	-	3.00±1.26 (87)***#

Castor oil = 3ml/kg, n = 5, Mean±SEM, *= $p<0.05$ (significant), **= $p<0.01$ (highly significant), ***= $p<0.001$ (extremely significant), AE = Aqueous extract, EE = Ethanol extract, # = $p<0.05$ (significant when compared with loperamide) () = percentage inhibition

Conclusion

This study has demonstrated that both extract of *Faidherbia albida* possesses antidiarrhoeal activity with the ethanol extract having more activities and potency. This may be due to the presence of its phytochemical constituents and this amply justifies the traditional use of the plant in the treatment of gastrointestinal diseases including diarrhoea. The study also revealed that there was no significant difference between the antidiarrhoeal activity of *Faidherbia albida* and the traditional use or orthodox antidiarrhoeal loperamide, with the former having better activity at high dose.

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