The effect of *acacia nilotica* pob ethyl acetate fraction on induced diarrhea in albino rats

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ABTRACT: Three experiment (castor oil induced diarrhea, gasterointestinal enteropooling and gasterointestinal movement of charcoal in albino rats) was conducted to ascertain the effect of ethyl acetate fraction of *Acacia nilotica* at a dose rate of 200, 400 and 600mg/kg on diarrhea. Twenty five (25) Wister Albino rats of both sexes weighing between 100-170g were used for each experiment. The results showed that the fraction insignificantly ($P>0.05$) reduced the number of unformed faeces at all the doses tested. There was a insignificant ($P>0.05$) decrease of 6.13% in the distance travel by the charcoal at 200mg/kg when compared to the control, while at 400mg/kg the extract significantly ($P<0.05$) decrease the distance travel by the charcoal by 29.8% and at 600mg/kg the distance travelled by the charcoal was decreased insignificantly ($P>0.05$) to 12.49% when compared to the control. The result of the study showed that the ethyl acetate fraction of *Acacia nilotica* possesses antidiarrhoeal activity since it reduces the number of unformed faeces decreased the intestinal transit of charcoal. [New York Science Journal 2010;3(8):16-20]. (ISSN: 1554-0200).

KEY WORDS: *Acacia nilotica,* gasterointestinal, enteropooling, charcoal, diarrhea

INTRODUCTION

Diarrhea occurs when the amount of water and other intestinal contents reaching the colon exceeds the ability of the colon to store the faeces and adequately remove the excess water (1). The major mechanism by which diarrhea occurs includes: increased permeability, hyper secretion and osmosis of the gastro intestinal tract. *Acacia nilotica* is one plant that has been reported to be of medicinal value. It has been reported that the bark has been used in the treatment of hemorrhages, cold, diarrhea, tuberculosis and leprosy, while the roots have been used as an aphrodisiac and the flowers for treating syphilis lesions (2). In the north eastern Nigeria, the bark is used in the management of diarrhea. However, for most herbs including *Acacia nilotica* the specific ingredients that causes a therapeutic effect is not known because whole herbs contain many ingredients and it is likely that they work together to provide the desired medicinal effects (3). This study was therefore designed to evaluate the effect of the ethyl acetate fraction of *Acacia nilotica* pod on diarrhea in albino rats

MATERIALS AND METHOD

PLANT COLLECTION AND IDENTIFICATION

Fresh pods of *Acacia nilotica* was collected in june, 2007 from Potiskum, Yobe state of Nigeria. They were identified at the department of Biological Sciences, University of Maiduguri. The pods were dried under shade, crushed into fine powder with the help of pestle and mortar.

PRAPARATION OF ETHYL ACETATE EXTRACT

The plant ethyl acetate fraction was prepared according to the method of Cho *et al* (4) and Motohashi *et al* (5). 50g of the powdered pod was exhaustively extracted with ethyl acetate using reflux method until the organic layer was visibly clear to get the ethyl acetate fraction.

EXPERIMENTAL ANIMALS

Seventy five (75) Wister Albino rats of both sexes weighing between 100-170g were used. They were kept in plastic cages in the laboratory for two weeks before the commencement of the experiment to acclimatize. They were fed commercial chick mash (Vital feeds Nig. Ltd) and given water *ad libitum.*

EFFECTS OF ETHYL ACETATE FRACTION OF *Acacia nilotica* ON CASTOR OIL INDUCED DIARRHEA IN ALBINO RATS

The method of Offia and Chikwendu (6) was used. The rats were denied food for 18 hours but were allowed access to water. They were separated into five groups of five rats each. Group A rats were administrated 2 mls of normal saline orally. Groups B, C and D rats were administered orally 200, 400 and 600mg/kg body weight of *Acacia nilotica* extract respectively. Group E rats were administered 5mg/kg of diphenoxylate HCL intraperitoneally (IP). One hour after treatment, the rats in all the groups were treated with 1ml of castor oil orally. The rats were observed for six (6) hours for watery or unformed faeces. The watery feaces of each rat was counted.

EFFECTS OF ETHYL ACETATE FRACTION OF *Acacia nilotica* POD ON GASTROINTESTINAL ENTEROPOOLING IN ALBINO RATS

The method described by Robert *et al* (6) was used. The rats were fasted overnight. They were separated into five groups of five rats each. Group A rats were administrated 2 mls of normal saline orally (Control). Group B rats were administered 3mg/kg of Atopine sulphate intraperitoneally (IP). Groups C, D and E rats were administered orally 200, 400 and 600mg/kg body weight of *Acacia nilotica* extract respectively. One hour after treatment, the rats in all the groups were treated with 1ml of castor oil orally. One hour after the castor oil treatment, the rats were sacrificed and the small intestines removed, tied on both ends with a thread and weighed. The intestinal content was collected by milking and the volume measured. The intestine was there after weighed and the difference between full and empty intestine calculated.

EFFECTS OF ETHYL ACETATE FRACTION OF *Acacia nilotica* POD ON GASTROINTESTINAL MOVEMENT OF CHARCOAL IN ALBINO RATS

The method of Chitme *et al* (8) was used. The rats were denied food for 18 hours but were allowed access to water. They were separated into five groups of five rats each. Group A rats were administrated 2 mls of normal saline orally (control). Group B rats were administered 3mg/kg of Atropine sulphate intraperitoneally (IP). Groups C, D and E rats were administered orally 200, 400 and 600mg/kg body weight of *Acacia nilotica* extract respectively. Ten minutes after drug and extract administration, 1 ml of 5% activated charcoal suspension in 10% aqueous solution of Acacia powder was given orally to each rat. The rats were sacrificed 30 minutes later and the abdomen opened. The distance travelled by the charcoal meal from the pylorus to caecum were measured and as percentage of total intestine.

STATISTICAL ANALYSIS

The Graphpad Instat 3.0 computer software (9) was used to analyse the data generated.

RESULTS

The results of the effect of ethyl acetate fraction of Acacia nilotica pod on induced diarrhea in rats are shown in tables 1-3.

The extract dose dependently decreased the number of mean unformed faeces. At 200mg/kg, the fraction insignificantly ($P>0.05$) reduced the mean number of unformed faeces to 6.50±2.38 when compared to that of control (8.25±3.40), while at 400mg/kg the extract insignificantly ($P>0.05$) decreased the mean number of unformed faeces to 5.00±3.32 and at 600mg/kg the number of unformed faeces was significantly ($P<0.05$) decreased to 3.20 ±2.38. Diphenoxylate, the standard agent, significantly ($P<0.01$) decrease the mean number of unformed faeces to 0.25±0.05 as compared to that of the control group (Table 1).

The extract at 200mg/kg produced an insignificant ($P>0.05$) increase in intestinal content (3.43±0.87) when compared with that of the control group (1.74±1.30). The group of rats treated with 400mg/kg of the extract insignificantly ($P>0.05$) produced 2.87±1.27 weight of intestinal content as compared to that of the control group of rats. The extract at 600mg/kg insignificanlyt ($P>0.05$) increased the intestinal content to 3.40±0.54 as compared to the control groups of rats. Atropine sulphate, the standard drug, produced insignificant ($P>0.05$) decrease in enteropooling (0.82±0.22) compared to that of the control group of rats (Table 2).

The ethyl acetate fraction of *Acacia nilotica* pod on gastro intestinal movement of charcoal in albino rats showed that the distance travelled by the charcoal in the rats treated with various doses (200, 400 and 600mg/kg) of the extract was shorter than that of the control. The mean distance travelled by the charcoal in the control rats was 84.54±13.84. The extract at

200mg/kg insignificanlyt (P>0.05) decrease the distance travel by the charcoal to 79.36±11.41 (6.13%) when compared to the control, while at 400mg/kg the extract significantly (P<0.05) decrease the distance travelled by the charcoal to 59.34±2.07 (29.8%) and at 600mg/kg the distance travelled by charcoal insignificantly (P>0.05) decreased to 73.98±13.04 (12.49%). The standard agent, Atropine significantly (P<0.001) decreased the distance travel by charcoal to 35.08±16.52 (58.51%) compared to the control (Table 3).

TABLE 1. EFFECTS OF ETHYL ACETATE FRACTION OF *Acacia nilotica* ON CASTOR OIL INDUCED DIARRHEA IN ALBINO RATS

GROUPS	[n]MEAN NUMBER OF UNFORMED FAECES
A Control (CO+ 2mls normal saline)	8.25 ± 3.40
B Extract(200mg/kg) + CO	6.50 ± 2.35
C Extract(400mg/kg) + CO	5.00 ± 3.32
D Extract(600mg/kg) + CO	3.20 ± 2.38*
E Diphenoxylate (5mg/kg) + CO	0.25 ± 0.50**

CO- Castor oil

*-significantly (P<0.05) lower than the control

**- Significantly (P<0.01) lower than the control

[n] – based on observation from five rats each

TABLE 2. EFFECTS OF ETHYL ACETATE FRACTION OF *Acacia nilotica* ON CASTOR OIL INDUCED ENTEROPOOLING IN ALBINO RATS

GROUPS [TREATMENT]	SAMPLE NUMBER	WEIGHT OF INTESTINE + CONTENT (g)	WEIGHT OF EMPTY INTESTINE (g)	MEAN WEIGHT OF INTESTINAL CONTENT	% CHANGE
A [Saline]	5	5.08 ± 1.01	3.34 ± 1.21	1.74 ± 1.30	-
B [Atropine sulphate (3mg/kg)]	5	5.66 ± 0.79	4.84 ± 0.76	0.82 ±0.22	-52.87
C [Extract(200mg/kg) + CO]	5	8.13 ± 1.01	4.70 ±0.26	3.43 ± 0.87	+98.83
D [Extract(400mg/kg) + CO]	5	7.80 ± 1.65	4.93 ± 0.38	2.87 ±1.27	+64.94
E [Extract(600mg/kg) + CO]	5	9.30 ± 2.03	5.90 ± 1.76	3.40 ±0.54	+95.40

- Percentage decrease

+ Percentage increase

TABLE 3. EFFECTS OF ETHYL ACETATE FRACTION OF *Acacia nilotica* ON MEAN GASTROINTESTINAL MOVEMENT OF CHARCOAL IN ALBINO RATS

GROUPS [TREATMENT]	SAMPLE NUMBER	TOTAL LENGTH OF INTESTINE (CM)	DISTANCE TRAVEL BY CHARCOAL (CM)	PERCENTAGE INHIBITION (%)
A [Control + CO]	5	101.34 ± 15.6	84.54 ± 13.82	-
B [Atropine sulphate (3mg/kg)]	5	98.74 ± 9.31	35.08 ± 16.52*	58.51
C[Extract(200mg/kg) + CO]	5	120.94 ± 4.77	79.36 ±11.41	6.13
D [Extract(400mg/kg) + CO]	5	109.06 ± 3.25	59.34 ± 2.07*	29.81
E [Extract(600mg/kg) + CO]	5	107.44 ± 3.07	73.98 ± 13.04	12.49

*P<0.05 significantly lower than the control

DISCUSSION AND CONCLUSSION

The ethyl acetate fraction of *Acacia nilotica* showed some degree of anti-diarrheal effect in this study since it reduced the GIT transit period of charcoal and decrease the number of unformed faeces. The antimotility property maybe due to the presence of some active principles in the extract. Phytochemical studies of the fraction revealed the presence of tannins, flavanoids, glycosides and carbohydrates (10). Tannins have astringent property which makes the intestinal mucosa more resistant and reduce secretion by contracting the GIT tract (13). Robertson *et al* (14) reported that the tannins bearing preparations are used for arresting diarrhea because of their ability to coagulate and precipitate proteinecious materials. This might also be one of the mechanisms of action of this extract. However, the extract could not inhibit intestinal fluid accumulation (enteropooling) caused by castor oil even though it contains pharmatocological active ingredients like flavonoids. Flavonoids was reported to have the ability to inhibit the development of fluids that results in diarrhea (14). Castor oil prevents reabsorbtion of water thus making the volume of intestinal content, thereby causing diarrhea (11). Atropine sulphate produced a non-significant decrease in enteropooling. This is possible due to its anticholinergic effect (12). It may be that the concentration of these active principles is not high enough to inhibit the action of castor oil.

The ethyl acetate fraction of *Acacia nilotica* was noticed to reduce the gastrointestinal transit period of the charcoal in this study. The antimotility property of the fraction appears to be due to the presence of some active principles. Tannins have astringent property which made the intestinal mucosa more resistant and reduce the secretion by contracting the gastrointestinal tract (13).

The combined effects of tannins, glycosides and flavonoids may be responsible for the antiperistaltic effects of the ethyl acetate fraction seen in this study. Atropine is an antimuscorinic drug with the capacity to reduce gastrointestinal motility and secretions. The antimotility effect of the extract in this study may also be via the muscorinic receptors. However, atropine was observed to be superior to the extract of *Acacia nilotica* in its ability to reduce the gastrointestinal movement of orally administered charcoal. The ethyl acetate fraction of *Acacia nilotica* possesses antidiarrhoeal activity since it reduces the number of unformed faeces and decreased intestinal transit of charcoal which may support the folkloric use of the pod as an antidiarrheal agent.

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