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STUDY OF ANTIDIARRHOEAL EFFECT OF AQUEOUS EXTRACT OF YOUNG LEAVES OF *COMBRETUM GLUTINOSUM* PERR. EX DC

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ABSTRACT

Combretum glutinosum Perr.ex DC of the family Combretaceae is a medicinal plant whose root, stem, bark and leaves are used in the ethno-medical practice of North-Eastern Nigeria for treatment of diverse medical ailments including diarrhoea. However, most of these uses were not validated. The aim of this study is to screen aqueous extract of young leaves of *Combretum glutinosum* for antidiarrhoeal activity in albino rats. The young leaves of *Combretum glutinosum* was collected from Hong Local Government area of Adamawa State, Nigeria. The leaves were shade dried and then pulverized into coarse powder which was defatted with petroleum ether and extracted by maceration using cold water. The extract obtained was then used for the antidiarrhoeal screening. The antidiarrhoeal potentials of the aqueous extract of young leaves of *Combretum glutinosum* was investigated in vivo using castor oil induced diarrhoea and charcoal meal transit time in albino rats. The extract showed dose dependent reduction reduction of diarrhoea castor oil induced diarrhoea with the highest effect observed, with the 600 mg/kg body weight which gave 80.4% protection. The pretreatment of rats with the extract caused a dose dependent and significant ($p<0.01$) delay in onset of diarrhoea, frequency of stooling and general diarrhoeal score in rats when compared with the negative control. The effect of extract on normal intestinal transit in rats was dose dependent with no statistical significant difference with the control ($p>0.05$). The results indicate that the aqueous young leaf extract of this plant is well tolerated and relatively safe having significant antidiarrhoeal effect which amply supports its traditional usage.

KEY WORDS: ***Combretum glutinosum*, Diarrhoea, Castor oil, Charcoal**

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INTRODUCTION

The use of plant parts in the treatment of human disease is as old as the disease itself and herbal medicine was the major form of medicine in Nigeria[1]. About 25% of the drugs and their derivatives in developed and developing countries are based on plant sources[2]. Most of the pathogens causing enteric infections have developed resistance to the commonly prescribed antibiotics which tends to increase the length of stay in the hospital and increased use of a particular antibiotic could lead to increased chances of bacterial resistance[3,4]. In Nigeria and the world at large, diarrhoea remains number one killer among children aged 1-5 years. The disease accounts for 4-5 million deaths among human annually[5] and more than 80% of the population, especially in the rural areas where infectious disease are endemic and modern health care facilities are inadequate depend on traditional system of medicine for their health problems. The ever increasing demand for safer and cheaper herbal recipes in developed countries has led to the extraction and development of several drugs and chemotherapeutic agent from plants as well as from traditional herbal remedies[6]. Combretum is a very large genus comprising of about 250 species found in both temperate and tropical regions of the world[7]. The roots, stem, bark and leaves of *Combretum glutinosum* are used in the treatment of scrotal elephantiasis, dysentery, ring worms, syphilis, typhoid fever, eye sore and ear ache[8]. This study is hereby embarked upon in order to evaluate the antidiarrhoeal activity of aqueous young leaves extract of *Combretum glutinosum* in laboratory animals.

MATERIALS AND METHODS

Experimental site, Source of Plant Material, Collection and Authentication

All experiments were conducted in the laboratories of the Faculty of Pharmacy and the Department of Physiology, University of Maiduguri, Borno State, Nigeria. Fresh young leaves of *Combretum glutinosum* were collected in February, 2013 from Hong local government area of Adamawa State, Nigeria. The plant was identified taxonomically by Prof. S.S Sanusi, Department of Biological Sciences, University of Maiduguri, Borno, Nigeria.

Preparation of Plant Extract

The fresh leaves of *Combretum glutinosum* were shade dried for one week in the laboratory away from sunlight, and then pulverized into coarse powder. About 100 g of pulverized sample was weighed and defatted with 500 ml of petroleum ether for 24 hours. The mixture was filtered and the residue cold macerated with 500 ml of water for 24 hours and filtered using filter paper. The aqueous filtrate was subjected to a Soxhlet rotary evaporator to recover the aqueous extract of the plant. The percentage yield (w/w) of extract was determined.

Experimental Animals

New Zealand rabbits and albino rats were acclimatized in the animal house facility of the Faculty of Pharmacy, University of Maiduguri, Borno State, Nigeria. The animals were maintained on standard feed and water *ad libitum*.

LD_{50} Determination

The method previously described by Lorke[9] was adopted using 13 rats. In the first phase, four doses of the aqueous extract (1, 10, 100 and 1000 mg/kg) was administered to four groups each containing one rat. In the second phase, more specific doses were administered to three groups each containing three rats each. The median lethal dose (LD_{50}) was determined as the geometric mean of the highest non-lethal dose and lowest lethal dose.

Effect of extract on castor oil-induced diarrhoea

The rats were fasted for 12 hours prior to the commencement of the experiment and were randomly divided into five groups of five rats each weighing between 120-200 g. The rats in the first group received 0.2 ml normal saline intraperitoneally while the rats in the second

group received 5 mg/kg of Loperamide as a standard positive control. The third, fourth, and fifth groups were administered doses of 200, 400 and 600 mg/kg of the aqueous extract respectively. After 30 minutes of administration of the extract, castor oil 1 ml/rat was administered orally. The animals were placed on individual special cages over white clean Whatman filter paper. Four hours after castor oil challenge, the cages were inspected for the presence of the characteristic diarrhoea droppings. The absences were recorded as a protection from diarrhoea[10] and the percentage protections were calculated using the formula below[11].

Percentage protection = [(mean defecation of control – mean defecation of treated group) ÷ (Mean defecation of control group)] ×100

Charcoal meal test

Rats (each weighing between 120-200 g) were fasted for 18 hours divided into five groups of five animals each. Group 1 received 2 ml normal saline orally, group 3-5 received an aqueous extract of young leaves of *Combretum glutinosum* (200, 400 and 600 mg/kg orally) respectively while group 2 received atropine (3 mg/kg, i.p.). After 10 minutes one ml of marker (10% charcoal suspension in 5% gum acacia) was administered orally to each rat. The rats were sacrificed after 1 hour and the distance traveled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum[12].

% Gastrointestinal transit (GIT) = Movement of charcoal (cm) × 100
Total length of intestine (cm)
Percentage inhibition = [(Vehicle GIT-Test GIT) / (Vehicle GIT)] × 100
Vehicle GIT = Control, and test GIT = test group

Effects of aqueous extract of young leaves of Combretum glutinosum on rabbit jejunum

The rabbit was sacrificed by a blow on the head. Segments of the jejunum, about 3.0cm long, was removed and dissected free of adhering mesentery. The intestinal contents were removed by flushing with Tyrode's solution of the following composition in millimoles (mM): NaCl, 136.8; KCl, 2.7; CaCl, 1.3; $NaHCO_3$, 12.0; MgCl, 0.5; $NaPO_4$, 0.14; glucose, 5.5. The tissue was mounted in a 25 ml organ bath containing Tyrode's solution maintained at 35 ± 1.0 ºC and aerated with air. A load of 0.5 g was applied. A 1hour equilibrium period was allowed during which the physiological solution was changed every 15 minutes. At the end of the equilibrium period, the effect of acetylcholine (2 µg/ml), histamine (40 µg/ml) and extracts of *Combretum glutinosum* (50 mg/ml) was investigated non-cumulatively. The contact time for each concentration was 1 minute, which was followed by washing three times. The tissue was allowed a resting period of 15 minutes before the next addition. Responses were recorded isometrically using a Kymograph.

Statistical analysis

Results were expressed as mean ± S.E.M. The significance of difference between mean was determined using one way ANOVA (plus a post hoc Dunnett or Turkey tests). Values of $p<0.05$ were considered significant. Computer software GraphPad InStat® @ USA, 2003 was used.

RESULTS ANALYSIS

Acute toxicity study

The aqueous extract at doses of 10, 100 and 1000 mg/kg showed no sign of toxicity in rats within and after 24 hours following oral administration. No deaths were recorded even at the highest dose of 5000 mg/kg body weight (Table 1).

Effect of extract on castor oil induced diarrhoea

After 4 hours of administration, it was observed that all the rats in negative control group produced copious diarrhoea. Those in groups 3, 4, and 5 that were pretreated with aqueous extract of 200, 400 and 600 mg/kg respectively showed a significant dose dependent antidiarrhoeal activity ($p<0.01$) by causing a delayed in onset of diarrhoea and frequency of stooling. The highest anti-diarrhoeal effect (protection of 80.4%) was obtained with the dose of 600 mg/kg body weight of the extract which was comparatively less than 100% protection produced by the reference drug (loperamide) (Table 2).

Effect of extract on charcoal transit time

The extract (200, 400 and 600 mg/kg) produced significant effect on the normal intestinal transit in rats. The reference drug (Atropine 3 mg/kg) a significantly reduced the intestinal transit time in the rat. The extract (200, 400 and 600 mg/kg) showed a dose dependent reduction in the distance transverse by the charcoal compared to the rats treated with normal saline. The highest effect 58.5% inhibition was obtained with a dose of 600 mg/kg body weight of extract (Table 3).

DISCUSSION

The relative less toxicity of the aqueous extract at the tested doses in albino rats observed in this study agrees with several related findings in which substances from natural sources was found to be less toxic in humans [6, 13-15]. The aqueous extract of young leaves of *Combretum glutinosum* possessed antidiarrhoeal activity in castor oil treated animals. The frequency and severity of castor oil induced diarrhoea was inhibited in a dose dependent manner but the percentage protection of loperamide (100%) was significantly higher than the extract ($p<0.01$). This agrees with several literature reports in which several medicinal plants was found to posseses antidiarrhoeal activity[12,13,16]. The presence of phytochemicals in this plant may be responsible for the observed effects as earlier reported by Marquet and Jansen [16] and Kumar and his colleagues[17]. The aqueous extract of *Combretum glutinosum* was noticed to reduce the frequency and severity of stools in castor oil induced diarrhoea as well as decrease gastrointestinal transit period of charcoal in this study. The prevention of intraluminal fluid secretion by *Combretum glutinosum* in this study which may be responsible for the anti-diarrhoeal activity of the extract may either be due to inhibition of prostaglandin biosynthesis with resultant decrease in secretion of fluid into the lumen or the promotion of absorption of water and electrolytes in the gut. Suppression of intestinal fluid accumulation by the extract might also suggest inhibition of intestinal function[13]. The charcoal meal study was done to study the effect of *Combretum glutinosum* on peristaltic movement. The extract and the reference drug atropine produced anti-diarrhoeal effects in which they were found to decrease the distance travelled by the charcoal plug in a dose dependent manner but the percentage inhibition movement of Atropine (86.6%) was higher than the extract ($p<0.01$). It can be suggested that if the active principle responsible for the observed effect in the crude extract is isolated, it may have a comparable antidiarrhoeal activity to atropine. Since an increase in dose of extract showed a more antidiarrhoeal effect, it is feasible to suggest that at a dose higher than 600mg/kg we may have a result comparable to atropine or even having a higher tendency to cause complete inhibition of gastrointestinal transit time. The inhibitory activity of flavonoids on intestinal motility in a dose related manner was earlier reported by Dicarlo *et al* [18]. Reduction of intestinal transit time may possibly be due to anticholinergic effects as reported by Brown and Taylor[14]. Previous phytochemical screening of the extract revealed the presence of ellagic acid, gallic acid, flavonoids, glycosides and 4 tannins. The tannins are 2, 3-(S)-hexahydroxydiphenoyl-D-glucose, punicallin and combreglutinin[16]. Tannins have an astringent property which makes the intestinal mucosa more resistant and reduce the secretion by contracting the gastrointestinal tract[17]. Flavonoids are known to relax pre

contracted intestinal smooth muscle and to delay intestinal transit. Flavonoids possess antibacterial, spasmolytic[15], anti-gastric activities as well as inhibit acid secretion[18, 19]. The combine effect of tannins, glycosides and flavonoid may be responsible for the anti-peristaltic activity of aqueous extract of *Combretum glutinosum* seen in this study.

Table 1
Acute toxicity study of aqueous extract of young leaves Of Combretum glutinosum in albino rats

Dose Level (Route)	*Effect (motality)*
10 mg/kg (IP and Oral)	Absent
100 mg/kg (IP and Oral)	Absent
1000 mg/kg (IP and Oral)	Absent
2000 mg/kg (IP and Oral)	Absent
3000 mg/kg (Oral)	Absent
5000 mg/kg (Oral)	Absent

Table 2
Effect of the aqueous extract of young leaves of Combretum glutinosum on castor oil induced diarrhea

Group	*Treatment(mg/kg)*	*Number of wet stools*	*Protection (%)*
1	Normal saline	19.4±0.93	-
2	Loperamide(5)	0.00	100
3	*C. glutinosum* (200)	7.2±1.88**	62.8
4	*C. glutinosum* (400)	6.2±0.49**	67.8
5	*C. glutinosum* (600)	3.8±1.11**	80.4

Values are expressed as mean ± S.E.M, - = no protection, **p < 0.01 was considered significant, **p < 0.01 was considered highly significant

Table 3
Effect of aqueous extract of young leaves of Combretum glutinosum on normal intestinal transit.

Group	*Treatment (mg/kg)*	*Total length of intestine*	*Charcoal meal length*	*% GI transit*	*% Inhibition*
1	Normal saline	93±7.22	61±5.16	65	-
2	Atropine (3)	95.3±7.22	8.3±8.3**	8.7	86.6
3	*C. glutinosum* (200)	100±8.60	33±5.87**	33	49.2
4	*C. glutinosum* (400)	101±6.40	26.5±5.96**	27.6	57.5
5	*C. glutinosum* (600)	110±11.52	28.75±75**	26	58.5

Values are expressed as mean ± S.E.M, **P < 0.01, considered highly significant from the control, GI = Gastrointestinal, C. glutinosum = Combretum glutinosum

CONCLUSION

The result of this study shows that aqueous young leaf extract of *Combretum glutinosum* is relatively safe and possesses significant anti-diarrhoeal activity and thereby supports its traditional usage by rural dwellers and Traditional Medicine Practitioners of Hong Local Government Area of Adamawa State, Nigeria.

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