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Antidiarrheal and Antinociceptive Effects of different inclusion of Methanol Stem Bark Extracts of *Acanthospermum hispidum* and *Gmelina arborea* in Castor oil Induced Wistar Albino Rats.

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ABSTRACT

Diarrhea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hurry, leading to an excess loss of fluid in the feces. Pain is an unpleasant sensation localized to a part of the body. This was aimed at determining the antidiarrheal and antinociceptive effects of different inclusion of methanol stem bark extracts of *Acanthospermum hispidum* and *Gmelina arborea* in castor oil induced wistar albino rats. Healthy wistar albino rats of both sexes between eight to twelve weeks of age weighing 120-140g were use. After 7 days of acclimatization, rats were randomized into five groups (n=6 each). Rats received the plant extract inclusions at the doses of 250 mg/kg b.w. of A. h. and 750mg/kg b.w. of G. a., 750mg/kg b.w. of A. h. and 250 mg/kg b.w. of G. a., 500 mg/kg b.w. of A. h. and 500 mg/kg b.w. of G. a. in the three test groups, loperamide was given to the positive control group and vehicle in the negative control group. Diarrhea was induced with castor oil administered one hour later. Each animal was observed for 4 hours (with no access to food or water). The result of the analysis revealed that rats in group administered the standard drug (Loperamide) had significant ($p<0.05$) increase in percentage inhibition of defecation followed by group administered 500 + 500 mg/kg b.w. of Ah and G.a. and group given 750 + 250 mg/kg b.w. of Ah and G.a. respectively. The administration of the combined methanol stem bark extract of *Acanthospermum hispidum* and *Gmelina arborea* in rats at doses of 500 mg/kg and 500 mg/kg respectively caused a significant reduction in the progression of charcoal meal, intestinal transit time and the volume of the intestinal content. the combined extract of *Acanthospermum hispidum* and *Gmelina arborea* could be said to act both peripherally and centrally in producing analgesia. Rats in group 2 that was given 3 mg/kg piroxicam had significant ($p<0.05$) increase in percentage inhibiton (80.11%) of writhing as a result of pains, followed by group administered 500 + 500 mg/kg b.w. of Ah and G.a. (75.14%) and group administered 750 + 250 mg/kg b.w. of Ah and G.a. (47.79%). The result from this study also showed that doses of 500 and 500 mg/kg b.w. of *Acanthospermum hispidum* and *Gmelina arborea* showed better antinociceptive activity when compared to other inclusions.

INTRODUCTION

According to the World Health Organization (WHO), diarrhea is the second leading reason of death of children less than five years of age (WHO, 2009). During diarrhea, the normal bowel movement becomes changed, which results in an increase in water content, volume, or frequency of the stools (Guerrant, 2001). The common reason for causing diarrhea is gastrointestinal infection by various types of bacteria, virus, and parasites. This infection can be spread out through food, drinking water, and unhygienic environment. Besides other pathological conditions, usually four major mechanisms are responsible for pathophysiology in electrolyte and water transportation, such as increasing of luminal osmolarity and electrolyte secretion, decreasing of electrolyte absorption, and acceleration of intestinal motility ultimately decreasing of transition time (Lutterodt, 1992). Despite the efforts of international organizations to control this disease, still the incidence of diarrhea is very high (Kouitcheu, 2006). Some antibiotics are used as antidiarrheal drug, but these drugs sometimes show some adverse effects and microorganisms tend to develop resistance towards them (Knecht, 2014).

Pain refers to an unpleasant sensation, or a feeling of discomfort resulting from stimulation of pain receptors in the body when tissue damage occurs or is about to occur (Prabhu, 2013). Pain is a valuable symptom of an underlying pathology and may be vital in the diagnosis of diseases. As an essential body's defense mechanism, pain serves as a warning of a problem particularly when it is acute. Pain aggravates distress and morbidity and if unchecked, it results in a vicious cycle of associated pathological conditions (Owolabi, 2013). Considering the rich diversity of the region, it is expected that screening and scientific evaluation of plant extract for their analgesic activity may provide new drug molecule that can combat various side effects of the commercially available synthetic drugs, moreover reducing the amount of medication.

Acanthospermum is from the Greek words 'acantha' (thorn) and 'sperma' (seed) and refers to the prickly fruit while hispidum is Latin which means rough, bristly or prickly (David *et al.,* 1989). Ethnomedicinally, *A. hispidum* is used in the treatment of yellow fever, malaria and stomach disorder (Mann *et al*., 2003). It is also used in some parts of South America as sudorific and diuretic. The plant has been scientifically investigated for its antibacterial and antiviral (Kamanzi *et al*., 2002). Hoffman *et al*., (2004), abortive and teratogenic (Lemonica and Alvarenga, 1994), antifeedant (Rai and Achanya, 1999), antimalarial (Gafon *et al*., 2012), immunostimulatory (Summerfield and Sallmuller, 1998), antitrypanosomal, antileishmania (Ganfon *et al.*, 2012) activities.

Gmelina arborea Roxb belonging to the family Verbenaceae fast growing deciduous tree found throughout India and also in Pakistan, Bangladesh, China, Japan, Myanmar, Nepal, Pakistan, Sri Lanka, Thailand. It is a one of the herbs mentioned in all ancient scriptures of Ayurveda. It is known to have been used in traditional Indian medicine. It is an important timber-yielding tree that grows naturally in the tropical and subtropical regions of Southeast Asia and has also been introduced as a plantation species outside these regions (Asolkar, 1992).

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Materials and method

Collection of Plant Material

Stem bark of *Acanthospermum hispidum* and *Gmelina arborea* was collected by hand-plucking from parent plants at different locations within Rufus Federal Polytechnic Nekede, Owerri, Imo State, Nigeria. They were identified by a taxonomist, Dr. Duru C. N. Of the department of Biological Sciences, Federal University of Technology Owerri, Imo State, Nigeria.

Preparation of the extracts

The Stem bark of *Acanthospermum hispidum* and *Gmelina arborea* was chopped into small pieces and was air dried at room temperature for 21 days. The dry stem bark was ground into fine powder using an electric grinder. The powdered plant material (1 Kg) was sequentially extracted by cold extraction method with 1.5 L of methanol for 8 h. This was left at room temperature for 72 hours. The extract was filtered through muslin cloth then Whatman No. 1 filter paper and was concentrated with a rotary evaporator at 40 0C to dryness. The dried extract was transferred to sample bottles which will be placed in a dessicator containing anhydrous sodium sulphate to remove any traces of water that could have been present. The dry extract was kept in tightly stoppered bottles in a refrigerator for further analysis.

Experimental Animals

Healthy wistar albino rats of both sexes between eight to twelve weeks of age weighing 120 -140g were used for the study. They were purchased from the Laboratory Animal House of College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria. The rats were acclimatised in different cages of six per cage (standard laboratory metal animal cage). The animals were maintained under good laboratory practice (12 hr light and 12 hr dark cycle at uniform temperature of 28 -33^{0}C). All animals had free access to food (vital feed grower, Ibadan) and water. All the investigation involving the experimental animals was conducted in accordance with the accepted principles for laboratory animal use and care.

Treatment

Prior to every tests, rats were fasted for 16-18 hours but allowed free access to drinking water. In all cases, the extracts inclusions, all drugs and chemicals, as well as the charcoal meal in the motility test were administered once, by gavage, at a volume of 10 ml/kg.

Anti-diarrheal and anti-nociceptive effects in rats with castor oil-induced diarrhea

Castor oil-induced diarrhea was performed according to the method described by Karim and colleagues (Karim *et al.,* 2010). After 7 days of acclimatization, rats were randomized into five groups (n=6 each). Rats received the plant extract inclusions at the doses of 250 mg/kg b.w. of A. h. and 750mg/kg b.w. of G. a., 750mg/kg b.w. of A. h. and 250 mg/kg b.w. of G. a., 500 mg/kg b.w. of A. h. and 500 mg/kg b.w. of G. a. in the three test groups, loperamide was given to the positive control group and vehicle in the negative control group. Diarrhea was induced with castor oil administered one hour later. Each animal was thereafter placed in an individual cage and observed for 4 hours (with no access to food or water). Thereafter, there were observed carefully every one hour for four hours for the presence of diarrhea defined as watery (wet) stool. Percentage of inhibition of defecation was calculated as follows:

% inhibition defecation = [(A-B)/A] × 100

Where A represents the mean number of feces with vehicle

B mean number of feces with loperamide or plant extract inclusion.

Assessment of enteropooling induced with castor oil

Enteropooling was determined by the method of Robert and colleagues (Robert *et al.,* 1976). One hour after treatment, diarrhea was induced by the administration of castor oil. One hour later again, the rats was sacrificed by cervical dislocation under anesthesia. The small intestine was ligated both at the pyloric sphincter and the ileocecal junction. Its content was expelled into a graduated measuring cylinder. The activity of each treatment was expressed as the percentage of inhibition of intraluminal fluid accumulation calculated as follows:

% Inhibition of intraluminal fluid accumulation=[(A-B)/A] × 100

Where A represents the volume of intestinal fluid with vehicle and

B represents the volume of intestinal fluid after treatment with loperamide or plant extract inclusion.

Assessment of gastrointestinal motility

Gastrointestinal motility was evaluated using the charcoal transit method (Dosso *et al.*, 2012). Rats were randomized into five groups (n=6 each) and given the plant extract inclusion at the doses of 250mg/kg b.w. of A. h. and 750mg/kg b.w. of G. a., 750mg/kg b.w. of A. h. and 250mg/kg b.w. of G. a., 500mg/kg b.w. of A. h. and 500mg/kg b.w. of G. a., atropine sulfate as the positive control and the vehicle as the negative control. One hour after treatment, each rats received a freshly prepared charcoal meal (10% active charcoal in 100 mL of 5% aqueous methylcellulose). One hour later, the rats were sacrificed by cervical dislocation under anesthesia. The small intestine from the pylorus to the cecum was isolated and measured (cm). The distance traveled by the charcoal meal from the pylorus was measured (cm) and expressed as a percentage of the length of the small intestine according to the following equation:

Distance travelled by the charcoal meal (%)= (Distance travelled by charcoal meal/Length of small intestine) (cm) × 100

Screening of antinociceptive activity

Acetic acid induced writhing syndrome. The intraperitoneal injection of acetic acid result in constriction of abdominal muscle together with stretching of hind limb known as writhing syndrome. In this test the antinociceptive activity of the inclusion of the extracts was studied on the chemically induced pain sensation in female nonpregnant albino rats (Koster *et al*., 1959). Plant extract, standard drug and vehicle were administered orally 30 min. prior to intraperitoneal injection of acetic acid (10ml/kg of 0.6% v/v solution). Total number of stretching episodes for 20 min. immediately after the acetic acid injection in all the groups were recorded and antinociception was expressed as percent reduction in writhing numbers compared between the vehicle treated control and animals pretreated with the extract or piroxicam.

Statistical Analysis

Data generated from the study was presented as mean ± SEM of four determinations. Statistical analysis was done by one way analysis of variance using the SPSS version 21.0. This was followed by student's t-test of significance. The mean difference at $P<0.05$ will be considered statistically significant.

Results

Table 1: Effect Effect of combined methanol stem bark extract of *Acanthospermum hispidum* and *Gmelina arborea* on mean weight of stool and percentage defecation.

Group	Mean weight of stools after 4 hours (g)	% inhibition of defecation
Group 1 (0.2 ml normal saline)	9.02 ± 0.01^{e}	
Group 2 (3 mg/kg loperamide)	0.89 ± 0.01^{a}	90.13
Group 3 (250 + 750 mg/kg b.w. Ah and G.a)	2.52 ± 0.03^{d}	72.06
Group 4 (750 + 250 mg/kg b.w. Ah and G.a)	1.21 ± 0.02^{c}	86.59
Group 5 (500 + 500 mg/kg b.w.	0.98 ± 0.02^{b}	89.14

Ah and G.a)

Table 2: Effect of combined methanol stem bark extract of *Acanthospermum hispidum* and *Gmelina arborea* on volume of intestinal content of rats

Group	Volume of intestinal content (ml)	% inhibition of intraluminal fluid accumulation
Group 1 (0.2 ml normal saline)	3.72 ± 0.01^{e}	
Group 2 (3 mg/kg loperamide)	1.43 ± 0.01^{b}	61.56
Group 3 (250 + 750 mg/kg b.w. Ah and G.a)	1.85 ± 0.01^{c}	50.27
Group 4 (750 + 250 mg/kg b.w. Ah and G.a)	2.21 ± 0.01^{d}	40.59
Group 5 (500 + 500 mg/kg b.w. Ah and G.a)	1.16 ± 0.06^{a}	68.82

Table 3: Effect of combined methanol stem bark extract of *Acanthospermum hispidum* and *Gmelina arborea* on small intestinal transit of rats

Group	Mean distance travelled by charcoal (cm)	% inhibition of defecation
Group 1 (0.2 ml normal saline)	70.20 ± 0.01^{e}	
Group 2 (3 mg/kg atropine)	18.75 ± 0.01^{a}	73.29
Group 3 (250 + 750 mg/kg b.w. Ah and G.a)	34.25 ± 0.02^{c}	51.21
Group 4 (750 + 250 mg/kg b.w. Ah and G.a)	43.21 ± 0.03^{d}	38.45
Group 5 (500 + 500 mg/kg b.w. Ah and G.a)	22.14 ± 0.03^{b}	68.46

Table 4: Antinociceptive activity of *Acanthospermum hispidum* and *Gmelina arborea* in acetic acid induced writhing in albino rats

Group	Total No. of writhing	% inhibition
Group 1 (0.2 ml normal saline)	40.22 ± 0.01^{e}	
Group 2 (3 mg/kg piroxicam)	8.00 ± 0.03^{a}	80.11
Group 3 (250 + 750 mg/kg b.w. Ah and G.a)	31.21 ± 0.01^{d}	22.40

Group 4 (750 + 250 mg/kg b.w. Ah and G.a)	21.0 ± 0.15^{c}	47.79
Group 5 (500 + 500 mg/kg b.w. Ah and G.a)	10.0 ± 0.02^{b}	75.14

Discussion

The use of plants in the management and treatment of diseases started with life. In more recent years, with considerable research, it has been found that many plants indeed have medicinal values (Sofowora, 2010). Diarrhea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hurry, leading to an excess loss of fluid in the feces (Rouf *et al.,* 2003). Diarrhea induced by castor oil results from the action of ricinoleic acid which causes the irritation and inflammation of the intestinal mucosa leading to prostaglandins (PGE2α) release. The released PGE2 stimulates gastrointestinal motility and secretion of water and electrolytes (Rajat *et al.,* 2013), thus inducing an increase in the peristalsis and an intestinal hyper secretion of fluid. The inhibition of prostaglandins biosynthesis prolongs the time of induction of diarrhea by castor oil (Lenika *et al.,* 2005). The results of this study revealed that the combined extract of *Acanthospermum hispidum* and *Gmelina arborea* produced statistically significant protection against diarrhea, and was found to be comparable to loperamide, a drug widely used against diarrhea disorders which effectively antagonizes diarrhea induced by castor oil, prostaglandin and cholera toxin (Kan *et al*., 2006).

The result of the analysis revealed that rats in group administered the standard drug (Loperamide) had significant ($p<0.05$) increase in percentage inhibition of defecation followed by group administered 500 + 500 mg/kg b.w. of Ah and G.a. and group given 750 + 250 mg/kg b.w. of Ah and G.a. respectively. This indicated that the combination of the two plants at doses of 500 + 500 mg/kg b.w. reduced the weight of stool after four hours when compared to other combination thereby increasing the inhibiting defecation. Therefore, activity of the combined extract of *Acanthospermum hispidum* and *Gmelina arborea* showed a significant dose-dependent significant decrease in the frequency of defecation with a subsequent increase in the percentage of inhibition of defecation in castor oil treated animals.

Moreover, the administration of the combined methanol stem bark extract of *Acanthospermum hispidum* and *Gmelina arborea* in rats at doses of 500 mg/kg and 500 mg/kg respectively caused a significant reduction in the progression of charcoal meal, intestinal transit time and the volume of the intestinal content. This activity is comparable to that of atropine used here as reference drug and which is known to reduce intestinal motility (Longanga *et al*., 2000). The antidiarrheal effects of combined methanol stem bark extract of *Acanthospermum hispidum* and *Gmelina arborea* could thus result from a reduction of intestinal motility and an increase in the intestinal absorption of water and electrolytes. In fact, many previous studies have shown that drugs and natural products as well, can induce their antidiarrheal effect through antispasmodic activity (Rehman *et al*., 2009). Tannins, alkaloids, saponins, sterols and terpenoids present in plants have been shown to be responsible for antidiarrheal activity (Zia-Ul-Haq, *et al.,* 2012), and phytochemical screening of stem bark extract of *Acanthospermum hispidum* and *Gmelina arborea* revealed the presence of these two plants in appreciable amount (Daya *et al.,* 2013).

Presence of tannins in plant extract denatures proteins which form protein tannates that make the intestinal mucosa more resistant to chemical alteration thereby reducing secretion 36. Moreso, the antidiarrheal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydroelectric secretions which are known to be altered in diarrhoeic conditions (Tripathi, 1994). It is therefore obvious that the tannins and flavonoids content of the two plants may be the responsible mechanism of their antidiarrheal effect.

Pain is an unpleasant sensation localized to a part of the body. It is often described in terms of a penetrating or tissue destructive process (e.g.: stabbing, burning, twisting, tearing, and squeezing) and/or of a bodily or emotional reaction (e.g. terrifying, nauseating, and sickening) (Kumar and Elavarasi, 2016). From the result of the study, the combined extract of *Acanthospermum hispidum* and *Gmelina arborea* could be said to act both peripherally and centrally in producing analgesia. Rats in group 2 that was given 3 mg/kg piroxicam had significant ($p<0.05$) increase in percentage inhibiton (80.11%) of writhing as a result of pains, followed by group administered 500 + 500 mg/kg b.w. of Ah and G.a. (75.14%) and group administered 750 + 250 mg/kg b.w. of Ah and G.a. (47.79%). The decrease in writhing movement of the combination therapy could be as a result of inhibition of the release of prostaglandins (Wagner *et al*., 2004). The centrally acting analgesics such as pentazocine act through their receptors in the central nervous system (CNS) by increasing the pain threshold response to pain stimuli (Singh and Majumdar, 1995).

Conclusion

From the results, it showed that the combined extract of *Acanthospermum hispidum* and *Gmelina arborea* possesses antidiarrheal activity which may be due to the presence of flavonoids, tannins, terpenes, saponin, or steroids. The pharmacologically activity could result from their ability to increase the absorption of water and electrolytes from the gastrointestinal tract and to inhibit prostaglandin/histamine synthesis, intestinal motility and hydro-electrolytic secretions.

The result from this study also showed that doses of 500 and 500 mg/kg b.w. of *Acanthospermum hispidum* and *Gmelina arborea* showed better antinociceptive activity when compared to other inclusions.

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