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Gastrointestinal tract profile of laboratory animals treated with ethanol extract of *Acacia ataxacantha* (Leguminosae) D.C. stem bark

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Abstract
Acacia ataxacantha and its various parts had been documented as herbal remedy for treatment of pain, stomach ailments and dysentery. The major processes occurring in the GI system are: motility, secretion, regulation, digestion and circulation. Abnormal motility or abnormal sensitivity in any part of the gastrointestinal tract can cause characteristic symptoms and hence induce disease(s) in the GIT. The study is aimed at investigating the GIT profile of animals treated with the ethanol extract to validate the ethno-botanical use in the treatment of diseases of the stomach. The methods employed include; ethanol/HCl-induced ulcer in rats, gastrointestinal motility and charcoal screening test in rats, and isolated rabbit ileum test. The ethanol extract of *Acacia ataxacantha* stem-bark significantly ($p \leq 0.05$) and dose dependently reduces the ulcer induced by 40% and 60% at doses of 500 mg/kg and 1000 mg/kg body weight respectively, when compared with the normal saline control group. The groups pre-treated with ethanol extract exhibited a significant ($p \leq 0.05$) decrease in the distance travelled by charcoal meal and a considerable increased gastro intestinal transit time, in a dose dependent manner, in the charcoal meal transit test. Administration of ethanol extract of Acacia ataxacantha stem-bark on rabbit ileum at lower concentrations of (1mg/ml), (10 mg/ml) and (100 mg/ml) produced no observed pharmacologic effect, while at higher concentration of 200 mg/ml, a relaxation was observed on the isolated rabbit ileum test. The ethanol extract of *Acacia ataxacantha* stem-bark possess GIT anti-motility and gastro protective effect.

Keywords: *Acacia ataxacantha;* GIT Motility; Ulcer

INTRODUCTION

Peptic ulcer is one of the most common disease of the gastro intestinal tract (GIT) worldwide and it results from in balance between some gastric aggressive (gastric acid, pepsin, reactive oxygen species) and mucosa defensive (prostaglandins, histamine, mucus, bicarbonate) factors leading to damage of the mucosa epithelia of the GIT [1-3]. Some of the common causes of peptic ulcer includes stress, chronic use of drugs (NSAIDS), alcohol, tobacco and infections of the GIT with *Helicobacter pylori* [2]. The symptoms of peptic ulcer include; epigastric pain and cramps, nausea, vomiting, bloody stool, weight loss [4]. Different classes of drugs are used in the treatment of peptic ulcer but most of these drugs exhibit serious side effects like diarrhea, abdominal pain, nausea arrhythmias, gynaecomastia,

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impotence, arthralgia, hypergastrinemia and haemopoeitic changes [5]. Hence, majority of people leaving in developing countries rely on herbal medicine for the treatment of peptic ulcer, due its ease of accessibility, affordability, lesser side effects [6, 7].

Acacia ataxacantha is a shrubby scrambler, with stem measuring up to 10cm long forming thickets 4-5 cm deep. The common names of the plant in English are, flame thorn, Benin rope acacia and benirope. In Nigeria, it is called dufuwa, sarkakiyaa (Hausa), uke (Igbo) and ewon adele in Yoruba [8]. The pods and seeds of *Acacia ataxacantha* plant have been documented to be used as herbal remedy for treatment of stomach ailments and dysentery [9]. There is no scientific research on the gastro intestinal properties of stem bark of *Acacia ataxacantha;* hence, the present study was designed to evaluate the gastro intestine profile of the stem bark of the plant.

EXPERIMENTAL

Plant collection and extraction. The stem-bark of *Acacia ataxacantha* plant was collected in Basawa, Zaria. The plant was authenticated at the Department of Biological Sciences, Ahmadu Bello University, by Mallam Namadi Sanusi and the herbarium specimen number; voucher number (v/no) 2417 was made and kept for further reference. The plant material was cleaned and air-dried at room temperature until a constant weight was obtained and seized reduced using mortar and pestle. 350 grams of the coarse powder was extracted (cold maceration) with 70% w/v solution of ethanol in water. It was left standing for 72 hours; filtered and washed with the solvent into an evaporating dish. The extract was concentrated at a temperature of 40-50°C, until a constant weight (a solid greenish-black mass of the crude drug) was obtained, packed in an airtight container and placed in a desiccator until when used.

Animals. Swiss albino mice (17-22g), adult Wistar rats (170-200g), Rabbit (1.3kg) were obtained from Animal House, Department of pharmacology and Therapeutics. Animals were maintained under standard condition of temperature, humidity and light. They were allowed access to standard feed and water *ad libitum.*

Acute toxicity studies. The acute toxicity studies, that is the LD_{50} was conducted using Lorke's method [10]. Twelve mice were used for the experiment. The study was divided into two phases. In the first phase, mice were grouped into three groups of three mice, each group treated with extract at doses of 10, 100 and 1000 mg/kg per oral respectively. The animals were observed for signs of toxicity including death over a period of 24 hours. In the second phase, 3 groups of one mouse each were treated with the ethanol extract dissolved in distilled water and given in doses of 1600, 2900 and 5000 mg/kg (per oral) respectively, and also observed for toxicity including death 24 hours. The median lethal dose (LD_{50}) was determined as the geometric mean of the highest non-lethal dose and the lowest lethal dose.

Ethanol/HCl-induced ulcer in rats. The experiment was conducted according to the method described by Mizui and Doteuchi [11]. Twenty-five (25) rats were grouped into five groups of five rats in each group. Food and water was withdrawn 24hrs prior to commencement the experiment. The rats in group 1 and 2 were treated with distilled water (10 ml/kg) and omeprazole (20 mg/kg) body weight respectively, while the rats in groups 3, 4 and 5 were administered with 250, 500 and 1000 mg/kg body weight of the extract respectively. One hour post-treatment, 0.2 ml of Ethanol/HCl mixture (60% ethanol and 0.3M HCl) was administered to the animals in all groups. All drugs were administered orally. After one hour post-treatment with Ethanol/HCl mixture, the rats were sacrificed. The stomachs were removed

and opened along the greater curvature. Macroscopic examination was carried out using a hand lens and the ulcer score was calculated for each animal according to the scale used by Singh *et al*. [12]. Where; 0 = no lesion, 1 = hyperemia, 2 = one or two slight lesions, 3 = very severe lesions, 4 = mucosal full of lesions. While the Ulcer index was calculated as mean ulcer scores [13].

Gastrointestinal motility and charcoal screening test. Animals were fasted for 18 – 24 hours prior to commencement of the experiments. Mice were separated into five groups of six animals each (n = 6). Group 1 and 2 were administered with normal saline (10 ml/kg) and Atropine (5 mg/kg), which serves as the negative and positive control respectively. While groups 3, 4 and 5 were treated with methanol extract of *Acacia ataxacantha* leaf, at doses of 250, 500, 1000 mg/kg body weight respectively. All treatments were administered orally. Then, 60 minutes after drug administration, each animal was administered 0.3 ml of charcoal meal (suspended in distilled water comprising of 10% gum Acacia and 10% charcoal). After 30 minutes, all animals were humanely sacrificed using cervical displacement and dissected. The dissected animal was placed on a clean surface and the whole small intestine was detached and measured according the method described by Marona and Lucchesi [14]. The distance moved by charcoal was also measured and the percentage moved by the charcoal was calculated and recorded.

$$\text{Peristalsis Index} = \frac{\text{Distance travelled by charcoal}}{\text{Total length of the intestine}} \times 100$$

Isolated rabbit ileum experiment. A rabbit (1.3kg) was humanely sacrificed and its abdomen was dissected to reveal the lower intestinal region. The ileum was then quickly removed and cut into a 3cm piece. The piece was placed into a physiological solution (Tyrode solution).The tissue was mounted on a 25ml organ bath containing the Tyrode solution maintained at 35^0C and aerated with oxygen with the aid of an oxygenator. A 60 minutes equilibrium period was allowed while the physiological solution was changed every 15 minutes. At the end of the equilibrium period, the effect of the ethanol extract of *Acacia ataxacantha* stem-bark was investigated. The contact time for each concentration was one minute, which was followed by washing three times. The tissue was allowed resting period of 15 minutes before the next addition of extract.

Statistical analysis. All values were expressed as Mean ± SEM. Statistical analysis of data was carried out using one-way analysis of variance (ANOVA) followed by Dunnet-t post hoc test. $P \leq 0.05$ were considered to be statistically significant.

RESULTS

The ethanol extract of *Acacia ataxacantha* stem-bark significantly ($p \leq 0.05$) and dose dependently reduces the ulcer induces by 40% and 60% at doses of 500 and 1000 mg/kg respectively, when compared with the normal saline (negative control) group, in the Ethanol/HCl-induced ulcer test. The groups pretreated with ethanol extract of *Acacia ataxacantha* stem-bark exhibit a significant ($p \leq 0.05$) decrease in the distance travelled by charcoal meal and a considerable increased gastro intestinal transit time in a dose dependent manner, in the charcoal meal test.

Administrations of ethanol extract of *Acacia ataxacantha* stem-bark on rabbit ileum at lower stock concentrations of (10 mg/ml) and (100 mg/ml) produced no observed pharmacologic effect. However, at a higher dose of 200 mg/ml, a relaxation was observed on the rabbit ileum with the ethanol extract of *Acacia ataxacantha* stem-bark.

Table 1: Effect of ethanol extract of *Acacia ataxacantha* stem-bark on Ethanol/HCl-induced ulcer in rats

Treatment	Dose (mg/kg)	Ulcer indices ± SEM	% Inhibition
D/W	1 ml/kg	6.23 ± 1.20	-
Omeprazole	20	0.00 ± 0.00***	100
EEAA	250	4.80 ± 1.10	20
EEAA	500	2.50 ± 3.10*	40
EEAA	1000	1.20 ± 3.20**	60

D/W = Distilled Water; EEAA = Ethanol extract of *Acacia ataxacantha*; n = 6,
* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$

Table 2: Effect of ethanol extract of *Acacia ataxacantha* stem-bark on the gastrointestinal motility (charcoal meal test) in mice

Treatment	Dose (mg/kg)	Total length of intestine (cm)	Distance travelled by charcoal (cm)	Peristalsis index
D/W	10 mg/ml	56.19 ± 0.23	45.51 ± 0.55	80.99
Atropine	5	48.41 ± 1.03	18.52 ± 0.15**	38.58
EEAA	250	52.36 ± 0.91	27.08 ± 0.48*	51.72
EEAA	500	50.22 ± 0.06	25.16 ± 0.13*	50.10
EEAA	1000	49.09 ± 0.63	24.90 ± 0.22*	50.72

D/W = Distilled Water; EEAA = Ethanol extract of *Acacia ataxacantha*; n = 6; * = $p \leq 0.05$, ** = $p \leq 0.01$

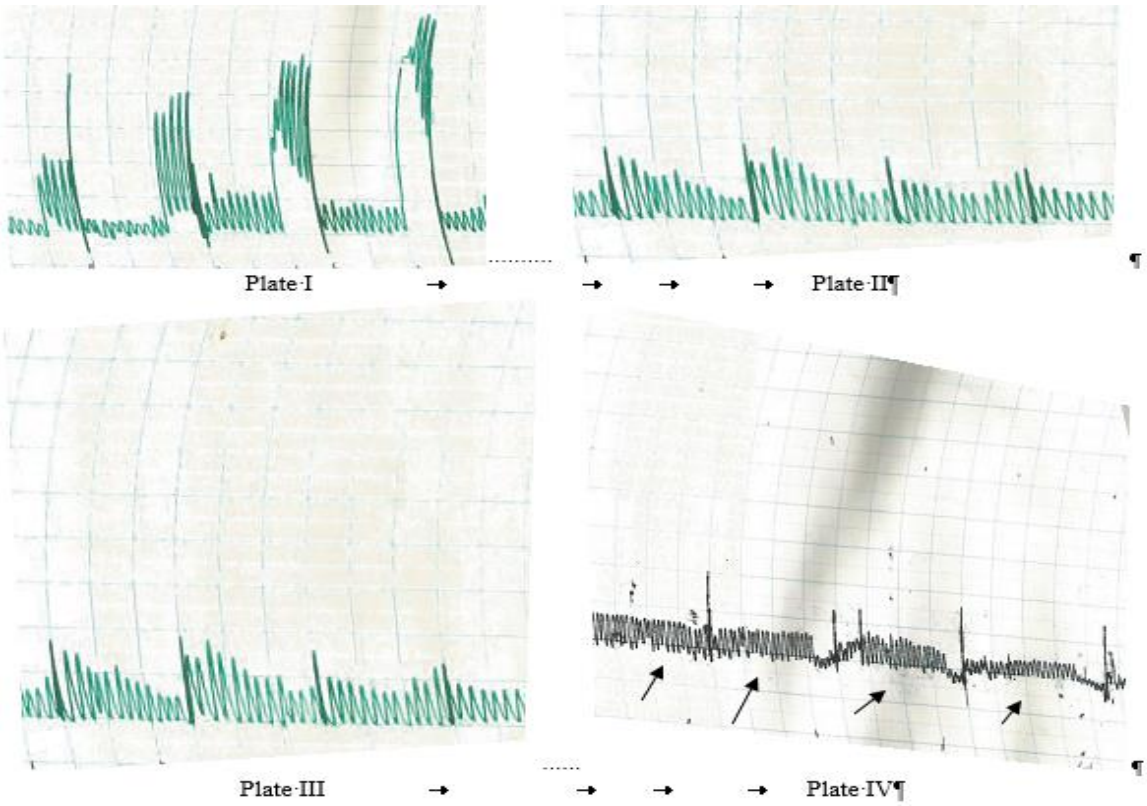

Plate I: Effect (contraction) of Acetylcholine at OBC (0.04 - 0.32ug/ml) on isolated rabbit ileum
Plate II: Effect (No observed effect) of *A. ataxacantha* extract at OBC (0.04 - 0.32µg/ml) on isolated rabbit ileum
Plate III: Effect (No observed effect) of *A. ataxacantha* extract at OBC (0.4- 3.2µg/ml) on isolated rabbit ileum
Plate IV: Effect (Relaxation) of *A. ataxacantha* extract at OBC (0.8- 6.4µg/ml) on isolated rabbit ileum

DISCUSSION

The oral lethal median dose (LD_{50}) was estimated to be ≥ 5000 mg/kg, which shows that the extract is practically non- toxic [10]. It has been shown that drugs, which are effective against Ethanol/HCl-induced gastric lesions, can possess gastric mucosal membrane protective action [5]. Ethanol/HCl-induced gastric damage ranging from endothelial microvascular damage to development of macroscopic gastric mucosal lesions [15], which is attributed mainly to the inhibition of biosynthesis of prostaglandin resulting in overproduction of leukotrienes, generation of oxidative stress, initiation of lipid peroxidation and inflammation, neutrophil infiltration and induction of apoptosis [16,17]. These agents break the mucosal barrier, provoke an increase in gastric mucosal permeability to H^+ and Na^+ ions, reducing the transmucosal difference and induce formation of erosions and ulcers in the gastro intestinal tract [18]. The protective effect of the extract against the gastric damage induced by Ethanol/HCl may be due to their action against 5-lipoxygenase pathway, as well as stimulation of prostaglandin synthesis, which in turn protects the gastric mucosa [5]. Omeprazole is a proton pump inhibitor (PPI) which inhibits both proton pump and acid secretion on the parietal cells, as well as offers mucosal protective effect via vasodilation in ethanol-induced ulcer [19,20]. Hence, the pharmacological effect of Omeprazole is related to its anti-ulcer activity.

The gastrointestinal motility test using charcoal meal as marker had been a simple and effective model to assess the effects of laxatives in laboratory animals [21]. It measures the maximum distance traveled by the marker in the small intestine after its administration in the presence of extract [22]. The decrease gastro intestinal tract motility effect of the extract can be attributed to its anti-motility effect and hence supports its use reported in ethnobotanical study for the treatment of diarrhoea. Atropine is a muscarinic receptor antagonist and produces its effect in the GIT through reduction in the tone and propulsive movement on the smooth muscles, and hence increases duodenal transit time [23, 24].

Acetylcholine is a chemical transmitter for nerves of the parasympathetic, somatic, preganglionic sympathetic and parts of the central nervous system that has contractile effect on rabbit ileum [25]. Acetylcholine has been reported and was demonstrated to contract the isolated rabbit ileum in a dose dependently [26]. From the isolated tissue experiment, at higher dose of the extract used, the extract showed gastro intestinal tract anti-motility (relaxation) effect, which supports the result obtained from charcoal meal test.

Previous preliminary phytochemical screening of the extract of *Acacia ataxacantha* stem-bark showed the presence of carbohydrate, terpenes, unsaturated sterol, cardiac glycoside, saponins, tannin, flavonoids and alkaloid [27]. Flavonoids and saponins contained in the extract had been linked to ulcer protective effect suggested to be due to selective inhibition of prostaglandin F2α and hence protecting gastric mucosa [9].

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