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In vivo and *In vitro* spasmolytic Effect of *Ficus sur* Forssk Ethanol Leaf Extract on the Gastrointestinal Tract

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Authors' contributions

This work was carried out in collaboration between all authors. All Authors designed the study and author SNI managed literature searches. All authors performed the experiments. Author CUE performed statistical analysis, wrote the protocol and managed analysis of the study, while author SCA wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Sequel to the report that *Ficus sur* ethanol leaves extract (FELE) has anti-diarrheal property and has been used in some parts of Nigeria for the treatment of the disease, this

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work examined the effect of *Ficus sur* ethanol leaves extract (FELE) on gastrointestinal motility.
Experimental Design: *In vivo* and *in vitro* Animal experiments were employed on whole rats and isolated intestinal tissues.
Methods: In the *in vivo* work, 25 adult rats were divided into 5 groups of 5 rats each. Group 1 was given 0.2ml normal saline and served as control. Group 2 received Atropine (0.1mg/kg), while groups 3, 4 and 5 were treated with 150, 300 and 500mg/kg of FELE respectively. Thirty minutes later, 0.2ml of charcoal meal was administered to all the rats used. The rats were sacrificed in another 30 minutes time. Each animal was opened to measure the distance travelled by the charcoal meal against the whole length of the small intestine. For the *in vitro* study, isolated guinea pig ileum and rabbit jejunum were used to study the effects of FELE on the gastrointestinal tract.
Results: In the *in vivo* studies, FELE exhibited a significant ($P<0.05$) dose dependent reduction in gastrointestinal tract motility as 150, 300 and 500mg/kg inhibited intestinal motility in treated rats by 58.36, 62.06 and 63.35% respectively and compared favorably with Atropine which inhibited same by 53 30%. On the isolated tissues, FELE exhibited relaxation effects and also significantly ($P<0.05$) blocked Acetylcholine induced intestinal contractions.
Conclusion: The relaxation effect of FELE on the gastrointestinal tract suggests that the extract contain principles with anticholinergic property and could be valuable in the management of diarrhea and gastrointestinal problems associated with hyper parasympathetic innervation. The results therefore agree with traditional claim that *Ficus sur* leaves extract has antidiarrheal property.

Keywords: Acetylcholine; atropine; Ficus sur; gastrointestinal; receptor.

1. INTRODUCTION

As the search for more effective ways of combating diseases continues, man has continued to derive benefits from the use of medicinal plants in disease management. This may be because herbal medicine is relatively cheap, safe and sometimes offer better therapeutic value than synthetic drugs [1]. The increasing discovery of more medicinal plants has demanded for increased scientific scrutiny of their bioactivity so as to provide data that will help physicians and patients make wise decision before using them [2]. *Ficus sur* is one of such plants that are commonly used in Nigeria to treat dysentery, infertility, gonorrhea, diarrhea, anemia and respiratory disorders [3]. *Ficus sur* is a medium sized tree up to 6-9m high, which grow cylindrical extending to the ground with brown bark and foliage leaves 2.5-15 cm long [3]. It is found on river banks and also in drier wood lands. Phytochemical tests reveal the presence of alkaloids, tannins, anthraquinones, phlobatannins, cardiac glycosides and sugars [3].

Diarrhea is a disease condition characterized by frequent discharge of semi-solid or watery faecal matter occasioned by uncontrolled peristalsis of the intestines. It may be acute or chronic and can be very serious in infants and the elderly because of the risk of severe, potentially fatal dehydration. It ranges from a mild and socially inconvenient illness to a major cause of death probably due to additional effect of malnutrition among children in less developed countries [4]. The condition is often assumed to be microbial, but may also be caused by anxiety, food, drugs or other toxins. Drugs have a place in its management, but the first priority is to restore or preserve fluid and electrolyte balance. No wonder the World

Health Organization (WHO) has formed a Diarrhea Disease Control Program (CDD) in an attempt to wipe out the problem of diarrhea in developing countries [4,5]. Although, a number of synthetic drugs have emerged over the years, none has formed a place in routine management of diarrhea [6]. The use of medicinal plants that possess potential anti-diarrheal activity could be of benefit in combating widespread diarrhea infections especially in third world countries [7].

The aim of the study is to evaluate the effect of *Ficus sur* ethanol leaves extract (FELE) on intestinal motility as a means of validating the traditionally acclaimed antidiarrheal property.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Material

Fresh leaves of *Ficus sur* were collected from a farm settlement in Ozuitem Community, Bende Local Government Area, Abia State, Nigeria and were identified as *Ficus sur* leaves by Dr. M. C. Dike, Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia state. A sample was deposited in the laboratory with voucher I.D MOUAU/CVM/HB 008. The leaves were air-dried at room temperature after which they were ground into powder using an electric blender. Thirty five (35) grams of the powdered material was subjected to soxhlet extraction at 70℃ for 48 hours using ethanol as solvent. The extract was thereafter concentrated to dryness over a hot air oven at 40℃. 12.3g of crude extract was obtained which represented a yield of 35.1%.

2.2 Drugs and Chemicals

2.2.1 The drugs and chemicals used include

Acetylcholine, Atropine(Sigma Co. USA), Sodium chloride (NaCl), Sodium hydrogen carbonate $NaHCO_3$, Glucose, Sodium dihydrogen phosphate (NaH_2PO4), Potassium chloride (KCl), Magnesium chloride ($MgCl_2$), Calcium chloride ($CaCl_2$) (BDH Chemicals) and activated Charcoal.

2.3 Animals

Adult mice (20-25g), Rats (80-140g), Guinea pigs (180-270g) and Rabbits (1.8-2.4kg) were obtained from the Animal House, University of Nigeria, Nsukka and maintained for two weeks in the Department of Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia state, Nigeria. The animals were fed with standard animal feed and clean water *ad libitum*. The mice were used for LD_{50} determination, the rats for the determination of charcoal transit, while the rabbits and guinea pigs were used for the *in vitro* studies.

2.4 Acute Toxicity Test

Thirty (30) mice of both sex were randomly grouped into 6 with 5 mice in each group and each group was assigned an intraperitoneal (I.P) dose of ethanol extract of *Ficus sur* in the order 500, 1000, 1500, 2000, 2500 and 3000mg/kg body weight. The Number of deaths in

each group was noted at the end of 24 hours and LD_{50} value determined using the Karber's method, expressed by Enegide et al. [8] as

$$LD_{50} = LD_{100} - \frac{\sum (Dd \times Md)}{N}$$

Where:

LD_{50} = Dose that killed 50% of animals in a group
LD_{100} = Dose that killed all animals in a group
$\sum(Dd \times Md)$ = Summation of all products of dose difference and mean deaths
N = Number of animals in each group

2.5 Effect of FELE on Small Intestinal Transit Using Activated Charcoal

Twenty five (25) rats of both sex weighing 90-140 were divided into five groups of 5 rats each. Group 1 was administered 0.2ml of normal saline. Group 2 was administered 0.1mg/kg of atropine (I.P), while groups 3, 4 and 5 were given 150, 300, and 500mg/kg body weight intraperitoneal doses of FELE respectively. After 30 minutes of the administration, 0.2ml of activated charcoal meal was given to all rats in each group by the oral route. Thirty (30) minutes later, the rats were sacrificed by suffocation in a Chloroform chamber. Each was opened and the length of the small intestine was measured. The distanced travelled by the charcoal meal in the intestine of each rat was also measured. Percentage inhibition of charcoal meal movement was calculated for each rat using the formula:

$$\text{Percentage Inhibition} = \frac{D_1 - D_2}{D_1} \times 100$$

Where, D_1 = Distance moved by charcoal meal in control, and D_2 = Distance moved by charcoal meal in test.

2.6 Effects of the Extract on Isolated Guinea Pig Ileum and Rabbit Jejunum

The abdominal cavity of stunned adult guinea pig was opened and the ileum was carefully isolated and transferred into a beaker containing tyrode solution (composed of NaCl - 8g, KCl - 0.2g, $CaCl_2$ - 0.2g, $NaHCO_3$ - 1g, NaH_2PO_4 - 1g, $MgCl_2$ - 0.1g, Glucose - 2g per liter of water) at 37℃ and aerated. 2-3cm of the ileum was cut and mounted in a 35ml organ bath by attaching the tissue at one end to a tissue holder and the other end to a force transducer connected to a digital recorder (Physiograph). The mounted tissue was supplied with air and also maintained at a temperature of 37℃. The tissue was allowed to equilibrate for 30 minutes before drug administrations. A dose response relationship was established using Acetylcholine and was repeated in the presence of Atropine ($1.4X10^{-6}$g/ml) and FELE ($1.4 X10^{-3}$g/ml). The effects of FELE and Acetylcholine were also tested on an isolated rabbit jejunum.

2.7 Statistical Analysis

The experimental results were expressed as mean **±** standard error of the mean (SEM) and analyzed using ANOVA. P-values less than 0.05 at 95% level of significance were considered as being significant.

3. RESULTS

3.1 Acute Toxicity

The acute toxicity (LD_{50}) value of 1975mg/kg showed that around this dose, *F.sur* leaf extract would produce fatal effects as all mice which received doses above 1000mg/kg showed physical signs of toxicity with deaths recorded in some groups at the end of 24 hours of the study (Table 1)

Table 1. LD_{50} determination for ethanol leaf extract of *Ficus sur* by Kaber's method

Group	Dose (mg/kg)	No of deaths	% mortally	Dose difference (Dd)	Mean death (Md)	Dd x MD
1.	500	0	0	250	0	0
2.	750	0	0	250	0.5	125
3.	1000	1	20	500	1	500
4.	1500	1	20	500	1.5	750
5.	2000	2	40	500	3	1500
6.	2500	4	80	500	4.5	2250
7.	3000	5	100			

Thus the LD_{50} value for Ficus sur was obtained as 1975 mg/kg body weight

3.2 Effect of FELE on Gastrointestinal Motility

FELE caused a significant ($P<0.05$) decrease in intestinal motility when compared to the control group, as 150, 300 and 500mg/kg body weight inhibited the distances travelled by charcoal meal by 58.36, 62.06 and 63.35% respectively. The effect of FELE was dose dependent and compared favorably with that of Atropine (Table 2).

Table 2. Effect of ethanol extract of *Ficus sur* on charcoal transit in rats' small intestine

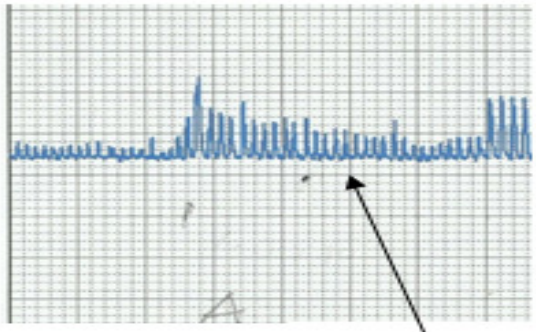
Groups	Treatment (mg/kg)	Length of intestine (cm)	Distance covered by charcoal (cm)	% Inhibition
1	0.2ml normal saline	90.00±3.40	85.67±1.80	
2	Atropin(0.1)	83.67±1.80	40.00±1.20*	53.31*
3	*Ficus sur*(150)	70.66±2.10	35.67±3.20*	58.36*
4	*Ficus sur*(300)	82.00±0.90	32.50±0.80*	62.06*
5	*Ficus sur*(500)	88.30±2.20	31.40±3.00*	63.35*

**P<0.05 when compared with control*

3.3 Effects of FELE on Isolated Guinea Pig Ileum and Rabbit Jejunum

On the isolated Guinea Pig ileum, Acetylcholine produced its usual dose dependent contractions with maximum contraction observed at a mean final bath concentration (FBC) of $1.4X10^{-6}$g/ml. This effect of Acetylcholine was significantly ($P<0.05$) blocked by FELE ($1.4 X10^{-3}$). The effect of FELE also compared favorably with that of Atropine ($1.4X10^{-6}$g/ml) (Fig. 1). FELE also significantly ($P<0.05$) inhibited the rhythmic contractions of the rabbit jejunum with a final bath concentration of $1.4x10^{-4}$g/ml achieving an inhibition of 72.15% (Table 3, Table 4 and Fig. 1A, B, C and D).

A



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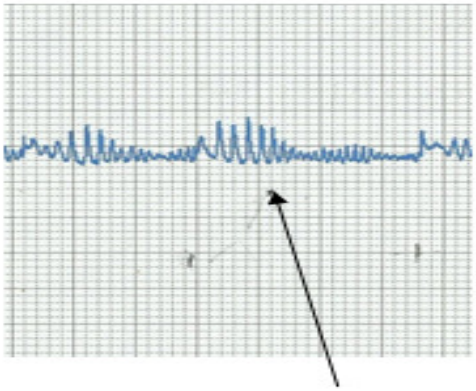

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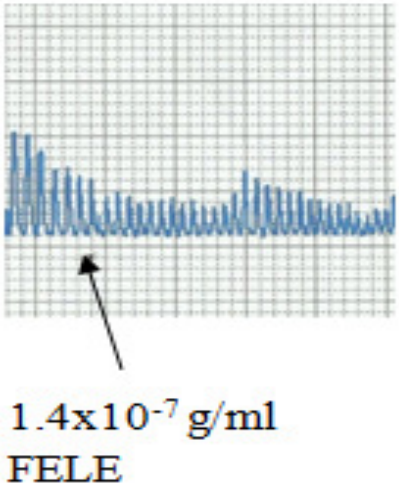

D

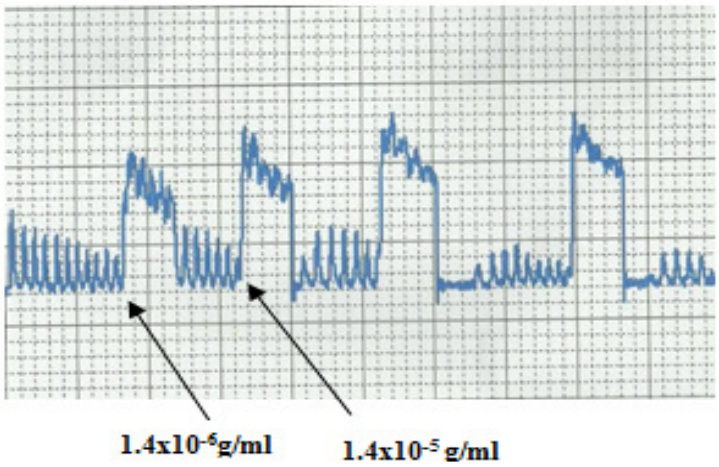

Fig. D. Effects of graded doses of Acetylcholine on isolated rabbit jejunum
Fig. 1A, B, C: Effect of FELE on the rhythmic contractions of rabbit jejunum

Table 3. Effect of FELE (1.4 x10^{-3}) and Atropine (1.4 x10$^{-6)}$ on Ach. Induced contraction on the Guinea pig ileum

FBC	Response to Ach (mm)	Response to Ach + Atr (mm)	Response to Ach + FELE (mm)
1.4x10^{-10}	0.00±0.00	0.00±0.00	0.00±0.00
1.4x10^{-9}	2.67±0.11	0.09±0.01*	1.90±0.02*
1.4x10^{-8}	4.81±0.19	2.52±0.10*	2.27±1.30*
1.4x10^{-7}	7.60±0.09	4.76±0.10*	32.08±0.15*
1.4x10^{-6}	12.82±1.02	6.20±0.09*	7.35±0.10*
1.4x10^{-5}	23.00±1.56	10.09±0.13*	14.62±0.11*
1.4x10^{-4}	21.80±0.91	12.03±0.18*	11.89±0.16*

**p<.05 for tests when compared to response to Ach*

Table 4. Effect of graded doses of FELE on the rhythmic contraction of the rabbit jejunum

FBC	Basal height (mm)	Response to FELE (mm)	% Inhibition
1.4x10^{-10}	13.00±0.21	11.23±0.20	13.62
1.4x10^{-9}	14.00±1.12	10.20±0.13*	27.14
1.4x10^{-8}	13.00±0.69	9.30±0.15*	28.46
1.4x10^{-7}	12.00±0.23	7.21±0.40*	39.92
1.4x10^{-6}	12.00±0.28	5.33±0.12*	55.58
1.4x10^{-5}	11.00±0.11	4.11±0.11*	62.63
1.4x10^{-4}	13.00±0.09	3.62±0.69*	72.15

**p< .05 for tests when compared to response to basal contraction*

4. DISCUSSION

The acute toxicity result indicates that FELE could moderately be tolerated at lower doses but toxic and deleterious at very high doses beyond 1000mg/kg body weight. The toxicity signs noticed during the acute toxicity study indicate that the extract could contain principles which if taken at high doses could lead to toxicity. Wisdom et al. [3] had reported the presence of saponins, glycosides, tannins, anthraquinones, phenols and steroids in the leaf extract and some of these substances have been implicated in plants toxicity. On the gastrointestinal tract, FELE inhibited intestinal motility in a dose dependent manner by its effects on charcoal transit in rats and on isolated guinea pig ileum and rabbit jejunum. The small intestine normally undergoes both segmental contractions and peristaltic waves in order to cause onward movement of its contents [9]. Stimulation of the parasympathetic arm of the autonomic nervous system usually causes these contractions by releasing acetylcholine which increases the activity of the myenteric plexus or by direct excitatory effect on the smooth muscle of the gut [10]. Both endogenous and exogenous acetylcholine usually achieve this contractile effects on the gastrointestinal tract by binding to the numerous muscarinic receptors present in the smooth muscles of the gut [11]. Any agent therefore that can block the effect of acetylcholine by binding to these muscarinic receptors would cause reduction in the contractions of the gastrointestinal tract leading to delayed movement of its content. This is the mechanism through which atropine inhibited intestinal contractions [12]. FELE also inhibited acetylcholine induced contractions in the *in vivo* and *in vitro* experiments and suggest that FELE contain active principles with anticholinergic property which acted by blocking the effect of acetylcholine by binding to the available muscarinic receptors in the gastrointestinal tract. These results agree with the findings of Kunle et al. [13], who reported that *Ficus sur* extract has spasmolytic effect on the rabbit jejunum and therefore corroborates with the claim in traditional medicine that the leaves extract may be used to treat diarrhea.

5. CONCLUSION

Ficus sur ethanol leaves extract contain principles with anticholinergic properties, capable of inhibiting intestinal motility and could be valuable in the management of diarrhea, incontinence and disease conditions associated with excessive parasympathetic innervation. Results therefore agree with the traditional use of *Ficus sur* leaves extract as agent for the treatment of diarrhea.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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