



SYMPATHETIC EFFECT OF EUPHORBA HIRTALINN ON THE GASTROINTESTINAL TRACT IN RABBITS

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This study investigated the effect on amplitude and number of rhythmic contractions of ethanolic extract of *Euphorbia hirta* Linn on the gastrointestinal tract using the rabbit jejunum, so as to ascertain its safety as feed and to investigate the claim of its usefulness in the management of asthma. The effect of the extract on isolated rabbit jejunum was compared to those of standard parasympathomimetic and sympathomimetic drugs including Acetylcholine and Noradrenaline. Fasted Rabbits were euthanized by stunning and the jejunum was carefully isolated. 2 – 3cm of this tissue was cut and mounted in a 35cm³ organ bath supplied with oxygen and maintained at 37⁰C. Graded doses of Acetylcholine, Noradrenalin and *Euphorbia hirta* Linn extract were administered at different times. The entire procedure was repeated 5 times to obtain mean Amplitudes and number of contractions per minute. Acetylcholine increased Amplitude and number of contractions while noradrenaline reduce same. The extract of *Euphorbia hirta* Linn exhibited the same effect as that of noradrenaline which suggested that the extract may have bound to adrenergic receptors. This sympathomimetic effect of *Euphorbia hirta* Linn extract may be the rationale for its use in Asthma management.

Key words: *Euphorbia hirta*, Acetylcholine, Adrenaline, Adrenergic, Sympathomimetic, Amplitude, Jejunum

INTRODUCTION

Traditionally *Euphorbia hirta* Linn has been used as fodder. Fodder feeding has always been a useful source of animal feed. Fodders are harvested and fed to animals under confinement and during inclement weather conditions. Fodders are also used as supplementary feeds during dry season when green forages become scarce (Marshall, 2002^[1]). When fodder is used the cost of concentrate feed is drastically reduced to about 50% (Marshall, 2002) ^[1]. Carefully selected fodder provides fresh and highly nutritional feed materials for livestock feeding. *Euphorbia hirta* Linn is one of such plants that have being in use. This may be because it is readily available and has sustained growth even with minimal rainfall. Unfortunately *Euphorbia hirta* Linn and other plants have been fed to animals without considering that such plants could have other deleterious effects.

Euphorbia hirta Linn is a plant belonging to phylum Angiospermia and family Euphorbiaceae. It is an annual, branched herb with branches up to 50cm long. The plant which is probably a native of India has parts that are hairy with simple leaves and unisexual flower and contains a high amount of latex (Sunil *et al.*, 2010^[2]: http://en.wikipedia.org/wiki/Euphorbia_hirta^[3]). *Euphorbia hirta* Linn has been used traditionally for the treatment of many ailments including the management of asthma and respiratory tract inflammations, ear infections, boils, sores and wounds (Sunil *et al.*, 2010)^[2]., induction of labor and the treatment of gastrointestinal disorders including parasites, diarrhea, peptic ulcers, heartburn, vomiting, amoebic dysentery and respiratory tract problems such as bronchitis, emphysema, coughs and colds (Duke and Ayensu, 1985)^[4]. In view of the use of *Euphorbia hirta* Linn as fodder, this present work was aimed at evaluating the effect of the plant extract on gastrointestinal tract considering its effect on the rhythmic activity of the rabbit jejunum and by so doing investigate its safety in fodder feeding and also throw light on its acclaimed use in Asthma management.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *Euphorbia hirta* Linn were collected in Umudike, Ikwano Local Government Area of Abia State, Nigeria and authenticated at the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike by Dr. M.C. Dike.

Animals

Six adult male rabbits (2.5 – 2.8kg) obtained from the livestock production unit of the Department of Veterinary Physiology, Pharmacology, Biochemistry and Animal Production, Michael Okpara University of Agriculture, Umudike were used for the study. They were fed with standard feed and water *ad libitum*, but starved for 12 hours prior to commencement of experiment. All animal experiments were conducted in compliance with NIH guidelines for care and use of laboratory Animals, as expressed by Akah *et al.*, (2009)^[5]. The study was conducted at the Veterinary Physiology Laboratory, Michael Okpara University of Agriculture, Umudike, Abia State Nigeria.

Preparation of Plant extract

This was done using the method of Akah, *et al.*, (2009)^[5]. The collected leaves of *Euphorbia hirta* Linn were dried under shade at room temperature for 7 days and were pulverized to fine powder using a manual blender. 35g of this powdered material was introduced into the extraction chamber of the soxhlet extractor. Extraction was done using 95% ethanol as solvent with temperature maintained at 58°C for 48 hours. At the end of the period, the ethanol was evaporated at 40°C in a 400ml beaker placed in an electric oven to obtain a crude extract weighing 11.6g which represented a yield of 33.1%.

Drug Dilutions and Calculations of Final Bath Concentrations (FBC)

This was done using the method of Qayum *et al.*, (1982)^[6] 1g of the extract was dissolved in 10ml of tyrode solution to give a stock concentration of 1×10^{-1} g/ml. Serial dilutions were subsequently made from this stock to obtain lower concentrations. Same procedure was carried out for Acetylcholine and Noradrenalin which were used as reference drugs.

Final Bath Concentrations (FBC) was calculated using the formula:

$$FBC = (C1V1)/V2$$

Where:

C1 = initial drug concentration

V1 = initial drug volume

V2 = Final volume (volume of organ bath). (Arthur, 2012^[9]).

Tyrode solution was prepared per liter of water by the dissolution of NaCl - 8g, KCl - 0.2g, CaCl₂ - 0.2g, NaHCO₃- 1g, NaH₂PO₄ - 1g, MgCl₂ - 0.1g, Glucose - 2g.

Effect of *Euphorbia hirta* Linn extract on the Rhythmic contractions of Rabbit Jejunum

Each fasted rabbit was euthanized by stunning in compliance with NIH guideline for care and use of laboratory animals (NIH, 1985, in Akah *et al.*, 2009^[5]). The jejunum was isolated and transferred into a beaker containing tyrode solution maintained at 37°C in a water bath and aerated with via an electronic aerator. About 2 – 3cm of the jejunum was cut and mounted in a 35ml organ bath containing tyrode solution also supplied with oxygen and adequate temperature (37°C). The tissue was allowed to equilibrate for 15 minutes before dose response relationships were established to Acetylcholine, Noradrenalin and *Euphorbia hirta* Linn extract. Tracings were recorded using kymograph and its accessories. The procedure was repeated using the remaining 5 Rabbits.

Statistical Analysis

Results were expressed as Mean + Standard Error of Mean (SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA) using Student's t-test at 95% level of significance to assess significant difference between amplitude and number of contractions in Basal rhythmic activity and responses in treated tissues.

RESULTS

Effects of Acetylcholine, Noradrenaline and *Euphorbia hirta* Linn extract on the Amplitude of contraction of the Rabbit Jejunum

Acetylcholine produced a dose-dependent increase in the Amplitude of contractions of the rabbit jejunum with FBC of 2.7×10^{-19} /ml and 2.9×10^{-69} /ml increasing Amplitude from 12.00 ± 0.00 mm and 11.50 ± 0.34 mm in Basal to 15.67 ± 0.33 mm and 34.33 ± 0.34 mm respectively. Noradrenaline on the other hand decreased the Amplitude of contraction with same doses as Acetylcholine lowering the amplitude from 12.00 ± 0.00 and 11.50 ± 0.34 mm in basal to 8.33 ± 0.42 mm and 0.17 ± 0.17 mm respectively. The effects of acetylcholine and noradrenaline on Amplitude of contractions were significantly ($P < 0.05$) different from the basal values. The ethanol extract of *Euphorbia hirta* Linn mimicked the action of Noradrenalin by reducing the Amplitude of contraction significantly ($p < 0.05$) when compared to the basal Amplitude, with the same doses as in noradrenaline reducing amplitude of contraction from 12.00 ± 0.00 and 11.50 ± 0.34 mm in basal to 9.50 ± 0.43 mm and 0.33 ± 0.21 mm respectively. The effect of *E. hirta* Linn was also dose dependent.

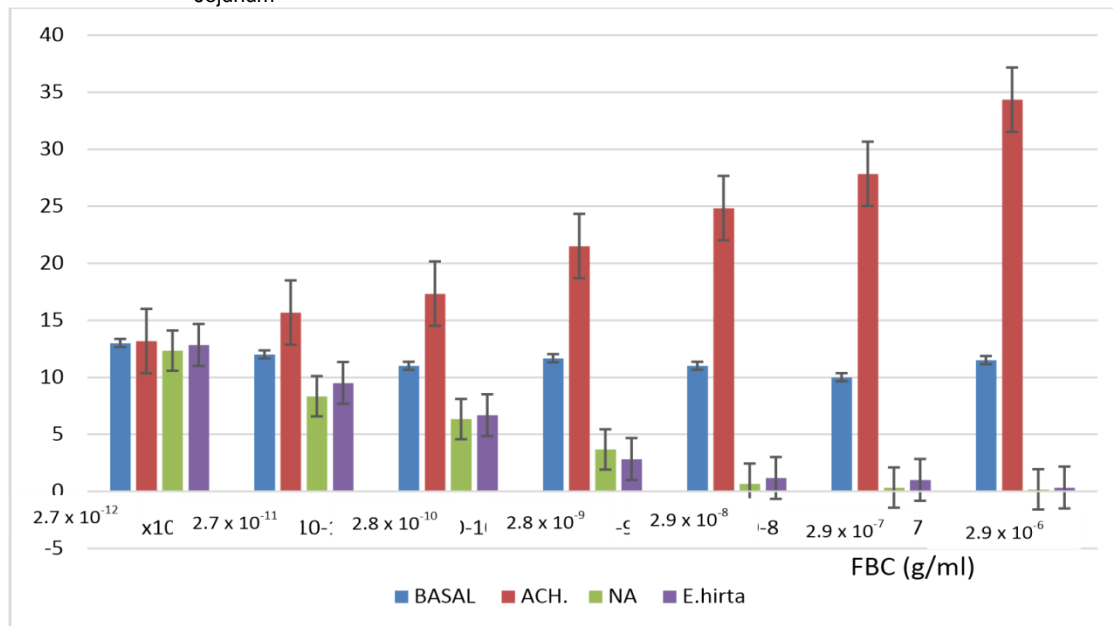
Effect of Acetylcholine, Noradrenaline and *E. hirta* Linn extract on the number of contractions per minutes of the Rabbit Jejunum

Acetylcholine significantly ($P < 0.05$) increased the number of contractions per minute of the rabbit jejunum. An FBC of 2.9×10^{-6} g/ml increased the number of contractions per minute from 12.00 ± 0.00 in basal to 49.17 ± 0.87 . Noradrenaline significantly ($P < 0.05$) reduced the number of contractions and abolished the contractions completely at an FBC value of 2.9×10^{-8} g/ml. The ethanol extract of *Euphorbia hirta* Linn significantly reduced the number of contractions at an FBC dose of 2.8×10^{-9} g/ml and abolished the contractions at a dose of 2.9×10^{-6} g/ml.

Table I: Effects of Acetylcholine, Noradrenaline and *Euphorbia hirta* Linn on the Amplitude of the Rhythmic contractions of Rabbit jejunum \pm SEM

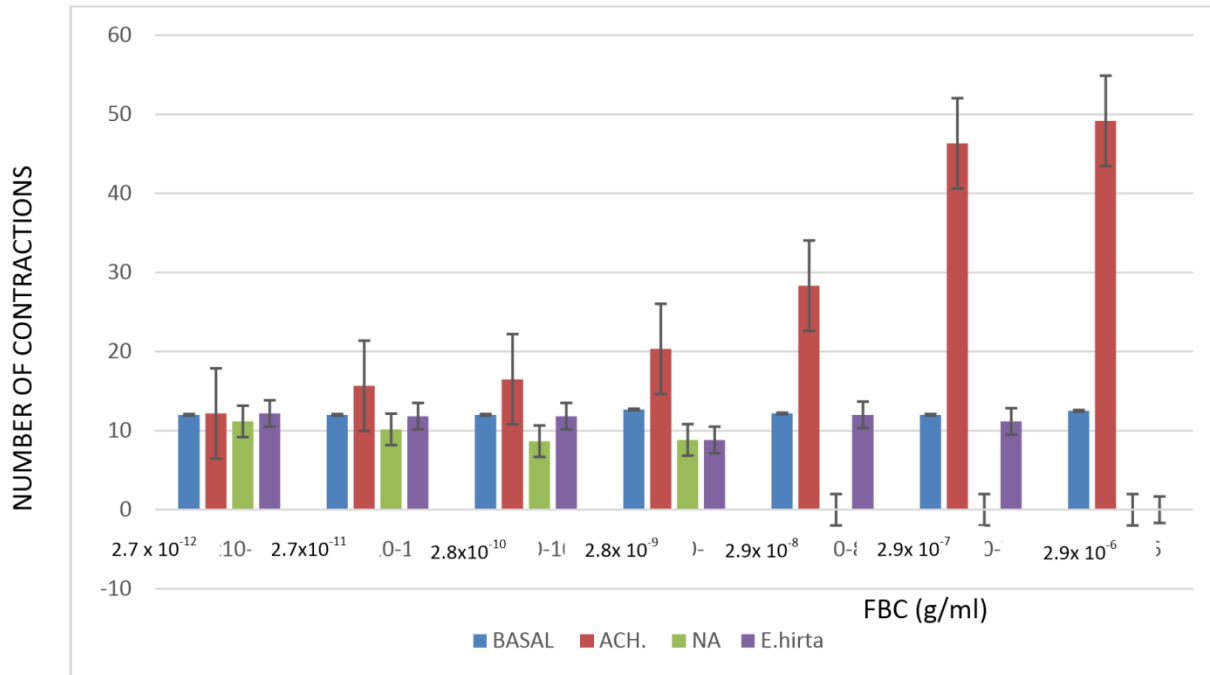
Conc. (g/ml)	FBC (g/ml)	Basal Amplitude (mm)	Ach.Amplitude (mm)	NA Amplitude (mm)	E.hirtaAmplitude (mm)
1×10^{-9}	2.7×10^{-12}	13.00 ± 0.00	13.17 ± 0.17	$12.33 \pm 0.49^*$	12.83 ± 0.16
1×10^{-8}	2.7×10^{-11}	12.00 ± 0.00	$15.67 \pm 0.33^*$	$8.33 \pm 0.42^*$	9.50 ± 0.43
1×10^{-7}	2.8×10^{-10}	11.00 ± 0.00	$17.33 \pm 0.95^*$	$6.33 \pm 0.33^*$	$6.67 \pm 0.49^*$
1×10^{-6}	2.8×10^{-9}	11.67 ± 0.33	$21.50 \pm 0.48^*$	$3.67 \pm 0.33^*$	$2.83 \pm 0.31^*$
1×10^{-5}	2.9×10^{-8}	11.00 ± 0.00	$24.83 \pm 0.30^*$	$0.67 \pm 0.33^*$	1.17 ± 0.31
1×10^{-4}	2.9×10^{-7}	10.00 ± 0.00	$27.83 \pm 0.60^*$	$0.33 \pm 0.21^*$	$1.00 \pm 0.37^*$
1×10^{-3}	2.9×10^{-6}	11.50 ± 0.34	$34.33 \pm 0.80^*$	$0.17 \pm 0.17^*$	$0.33 \pm 0.21^*$

* $P < 0.05$ when compared to Basal Amplitude

FIG. 1: Effects of Acetylcholine, Noradrenaline and *Euphorbia hirta* Linn on the Amplitude of the Rhythmic Contractions of Rabbit Jejunum**Table II:** Effects of Acetylcholine, Noradrenaline and *Euphorbia hirta* Linn on the number of contractions per minute of the Rabbit jejunum

Conc. g/ml	FBC g/ml	Basal(mm)	Ach (mm)	NA (mm)	E. hirta (mm)
1×10^{-9}	2.7×10^{-12}	12.00 ± 0.00	12.17 ± 0.31	$11.17 \pm 0.40^*$	12.17 ± 0.17
1×10^{-8}	2.7×10^{-11}	12.00 ± 0.00	$15.67 \pm 0.42^*$	$10.17 \pm 0.54^*$	11.83 ± 0.31
1×10^{-7}	2.8×10^{-10}	12.00 ± 0.00	$16.50 \pm 0.34^*$	$8.67 \pm 0.61^*$	11.83 ± 0.17
1×10^{-6}	2.8×10^{-9}	12.67 ± 0.33	$20.33 \pm 0.42^*$	$8.83 \pm 0.40^*$	$8.83 \pm 0.40^*$
1×10^{-5}	2.9×10^{-8}	12.17 ± 0.00	$28.33 \pm 5.09^*$	$0.00 \pm 0.00^*$	12.00 ± 0.25
1×10^{-4}	2.9×10^{-7}	12.00 ± 0.00	$46.33 \pm 0.67^*$	$0.00 \pm 0.00^*$	11.17 ± 0.40
1×10^{-3}	2.9×10^{-6}	12.50 ± 0.34	$49.17 \pm 0.87^*$	$0.00 \pm 0.00^*$	$0.00 \pm 0.00^*$

* $P < 0.05$ when compared to Basal number of Contractions per minute.

FIG. 2: Effects of Acetylcholine, Noradrenaline and *Euphorbia hirta* Linn on the number of contractions per minute of the Rabbit jejunum

DISCUSSION

Acetylcholine, a cholinergic agonist produced a dose dependent increase in amplitude and number of rhythmic contraction of the rabbit jejunum. This effect is typical of both endogenous and exogenous Acetylcholine and other parasympathomimetic agents (Guyton and Hall, 2002^[7]; Sembulingam and Prema, 2009^[8]). The result therefore agree with existing literatures which reports that the increase in amplitude and number of contractions shown by Acetylcholine on the rabbit jejunum is due to the binding of the drug molecules to the muscarinic receptors present in the smooth muscles of the jejunum (Rang, *et al.*, 2007^[9]). Noradrenaline also exhibited its typical sympathomimetic effect by relaxing the jejunum thus reducing both Amplitude and number of contractions per minute of the jejunum. This effect was achieved by the binding of noradrenaline molecules to the numerous adrenergic receptors in the smooth muscle of the jejunum (Rang *et al.*, 2007^[9]).

The ethanol extract of *Euphorbia hirta* Linn mimicked the effect of noradrenaline due to its dose dependent lowering of both the Amplitude and number of contractions per minute. The result therefore suggests that *Euphorbia hirta* Linn leaf extract may have achieved this by binding to the adrenergic receptors just like noradrenaline, and so may be seen as a sympathomimetic agent. Sympathomimetic agents are known for their ability to reduce intestinal motility with strong dilation of bronchial muscles (Rang, *et al.*, 2007^[9]; Avram *et al.*, 1974^[10]).

This finding therefore appears to be a good reason for the traditional use of the plant as a local agent for the management of asthma and diarrhea. However, small ruminant animal keepers have not considered the effect of including this plant in the diet of animals. Possibly the effect may be minimal and so go unnoticed in ruminants because of their enormous weights and complex stomach. Fermentation in the complex stomach may result in delayed access of the active principles to the animal. The case may be different in simple stomach animal like pigs and rabbits. In these species fodder feeding is also a common practice and the effect of the agent would be the constriction of blood vessels so as to enable blood supply to other very important tissues like the brain, inhibition of both the gastric and pancreatic activity and inhibition of intestinal motility. The extract may therefore be of use to Asthma patients. In asthmatics the constricted airways results in increasing airway resistance especially making exhalation difficult. The resistance may be increased by constrictions of bronchial smooth muscles, which narrow the passages and increase resistance. Incidentally the muscles are under autonomic control and sympathetic

stimulation brings about dilation while parasympathetic stimulation constricts them (Sembulingam and Prema, 2009^[8]; Arthur, *et al.*, 1998^[11]).

This probably explains why the leaves of *Euphorbia hirta* Linn are applied to asthmatics, thus increasing the sympathetic arm of the autonomic system and thereby providing bronchial dilation and bringing relief to the sufferer. This claim was corroborated by Suni *et al.*, 1994^[2], who reported that *E. hirta* Linn has an anti-asthmatic activity due to its relaxation effect on the bronchial tubes.

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