

**BETA ADRENERGIC ACTIVITY OF *Laportea aestuans* LEAVES
EXTRACT ON THE SMOOTH MUSCLES OF THE RABBIT JEJUNUM**

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ABSTRACT

Laportea aestuans ethanol leaf extract (LALE) was screened for beta adrenergic activity in vitro on isolated rabbit jejunum. Results obtained indicate that LALE at all doses administered inhibited significantly ($p < 0.05$) the rhythmic contractions of the smooth muscle preparations with the least (14.28 $\mu\text{g/ml}$) and highest (228.60 $\mu\text{g/ml}$) concentrations producing inhibitions of 38.57% and 83.00% respectively. The inhibitory effect of LALE compared favourably with that of noradrenaline which produced 44.39% and 65.31% at doses 0.014 $\mu\text{g/ml}$ and 0.229 $\mu\text{g/ml}$ respectively. LALE had no effect on acetylcholine induced contractions but significantly ($p < 0.05$) blocked that of propranolol (a non selective beta receptor blocker). The results therefore suggest that the extract may contain substances with potent beta adrenergic property which may be of value in the management of conditions associated with increased motility of the gastrointestinal tract and by extension those caused by excessive activity of beta receptor blocking agents. This may serve as a template for the development of yet another synthetic beta adrenergic agent of clinical significance.

KEY WORDS: *Laportea aestuans*, Beta-adrenergic Activity, Smooth muscle, Rabbit.

INTRODUCTION

The role of the adrenergic receptors in the maintenance of physiological balance via the autonomic nervous outflows is well established. The interactions of adrenergic hormones and neurotransmitters with these receptors tend to produce a counter effect on the contractility induced by the cholinergic outflow. The sum effect is the maintenance of normal gastrointestinal tone, motility and peristaltic waves [1,2]. The interplay between these cholinergic (parasympathetic) and adrenergic (sympathetic) arms of the autonomic nervous system mediated by endogenous acetylcholine and epinephrine/ norepinephrine respectively accounts for the physiological balance [2]. Hyperactivity of the adrenergic arm in the gastrointestinal tract causes constipation [3]. These disorders have been shown to result from excessive inhibitory activity on the smooth muscles of gastrointestinal tract associated with excess release of epinephrine/norepinephrine, the neurotransmitters which mediate adrenergic functions [2].

Beta-adrenergic agonists are substances which mimic the action of epinephrine and norepinephrine in the heart, lungs and smooth muscle tissues. These substances act on beta receptors to activate the enzyme, adenylate cyclase which in turn leads to the activation of the secondary messenger cyclic adenosine monophosphate and induces smooth muscle relaxation and contraction of cardiac tissues. These effects of beta adrenergic agonists account for their use in the treatment of disease conditions including bradycardia, asthma, heart failure, chronic obstructive pulmonary disease and as antidote to beta blocker poisoning. Currently many plants are being studied for the presence of bioactive substances. Hence, the exploitation of wild plants for medicinal purposes has continued to grow globally [4]. These studies in addition to providing alternative sources of healing, may become a means of new drug discovery and rational drug design. *Laportea aestuans* is indeed one of such plants that are currently being studied.

Laportea aestuans is an annual little branched herb of family urticaceae. It has an erect fleshy and slightly woody stem densely covered with stinging hairs and grows up to 1-3m high. The leaves are alternate and are restricted to the top of the stem [5]. The plant is widely distributed in tropical Africa from Senegal eastwards to Eritrea and southwards to Angola, Zimbabwe, Mozambique and Madagascar. It is also found in Yemen, tropical Asia and tropical America [5,6]. Traditionally, the leaves have been used as an antihelminth and to treat oedema, ulcers, headaches, cough, gonorrhoea, indigestion, toothaches, inflammations, stomach ache, fever, filariasis, rheumatism and poisoning [5,6]. In Gabon, Nigeria and Ghana, the cooked leaves of *Laportea aestuans* are eaten as vegetables [7]. Previous works on *Laportea aestuans* leaf extract revealed strong antioxidant activity [7] and the phytochemical components include; alkaloids, tannins, saponins, steroids, terpenes, flavonoids and cardiac glycosides [8]. A recent study however revealed that the essential oil from the plant is dominated by methyl salicylate which has significant antioxidant and antimicrobial activities [9].

Presently, there is paucity of information on the gastrointestinal tract effects of *Laportea aestuans* hence, this study was designed to investigate the effects of the leaf extract of this medicinal plant on isolated intestinal smooth muscle strips of the rabbit.

MATERIALS AND METHODS

Collection of plant leaves and preparation of plant extract

Laportea aestuans leaves were collected from a bush in Uturu, Isuikwuato Local Government Area of Abia state, Nigeria. The leaves were air dried at room temperature for 7 days after which they were ground to coarse powder using a manual blender. Fifty grammes of the powdered material was Soxhlet extracted at 70 °C for 48 hours using ethanol as solvent. The ethanol was evaporated at 40 °C in an electric oven to obtain a crude extract.

Animals

Thirty five mice (20 – 25 g), 25 rats (90 – 140 g) and 5 rabbits (1.8 - 2.5 kg) obtained from the Animal Production Unit of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, were used for the study. They were housed under specific pathogen free (SPF) conditions with 13 H/11 H light/dark and were provided 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.* [10]. The study was carried out in the Physiology Laboratory of the Department of Physiology, Pharmacology and Biochemistry, Michael Okpara University of Agriculture, Umudike, Nigeria.

Acute toxicity test of *Laportea aestuans* leaf extract (LALE)

Thirty five mice of both sexes weighing 20-25g were divided into 6 groups of 5 mice each and were assigned graded oral doses of LALE reconstituted in water and administered in the order 500, 1000, 2000, 3000, 4000 and 5000mg/kg body weight. The mice were kept in aluminum cages after administration and allowed free access to feed and water. Observation was made for toxicity signs and number of deaths in

each group after 24 hours for LD₅₀ determination using the method of Karber, as expressed by Enevide *et al.* [11].

Preparation of intestinal smooth tissue for in vitro isometric contraction studies

The method of Uchendu [12] was adopted. Briefly, the rabbits were killed by stunning and decapitation. The abdomen was cut open and the jejunum was carefully isolated and transferred into tyrode solution that was continuously bubbled with air and maintained at 37⁰C (pH 7.4) The tyrode solution had the following composition: NaCl (8g), KCl (0.2g), CaCl₂ (0.2g), NaHCO₃ (1g), NaH₂PO₄ (1g), MgCl₂ (0.1g) and Glucose (2g). About 2 – 3 cm length of the jejunum was cut out and suspended vertically in a 35 ml organ bath by means of ligatures attached at one end to a tissue holder and at the other end to an isometric force displacement transducer connected to a digital physiological recorder (Medicaid Physiopac, India) and computer screen for displaying isometric contractions. Resting tension in the muscle strip was readjusted, just sufficient to remove the slack. The preparation was allowed to equilibrate within 30 minutes of mounting.

After regular rhythmic contractions were recorded, dose-response relationships were established for acetylcholine, noradrenaline and LALE. To study the mechanism(s) of LALE action, effective concentrations (EC₅₀) of the standard drugs were administered in the presence of their respective antagonists; atropine for acetylcholine and propranolol for noradrenaline. Same effective dose of acetylcholine was repeated in the presence of LALE before LALE was tested against the effect of Propranolol. For all administrations, a minimum time of 1 minute was allowed for individual tissue responses before being washed 3 times with Tyrode solution. Concentration of test substances given in the text are all final bath concentrations (FBC), except otherwise indicated.

Statistical analysis

Results were analyzed using one way Analysis of variance and presented as means ± standard errors of the means (SEM). P-values less than 0.05 were considered significant.

RESULTS

Plant extract yield

Extract yield following sohxlet extraction was dark green in colour, pasty in consistency, water soluble and weighed 13.20 g representing a yield of 26.40%.

Acute toxicity

No death was recorded at the end of the 24 hours of acute toxicity study, even at the highest dose administered (5000 mg/kg) body weight. No clinical signs of toxicity or physical disability were found in the animals in addition to their surviving the 24 hours period of observation.

In vitro effect of LALE on an isolated rabbit jejunum

Responses of the rabbit jejunum to acetylcholine were characterized by a dose dependent increase in the amplitude of the rhythmic contractions (Table 1), while noradrenaline on the other hand exhibited a relaxation effect which also appeared to be dose dependent (Table 2). LALE induced a dose dependent inhibitory effect on the smooth muscles of the isolated jejunum with minimum (14.28 µg/ml) and maximum (228.60 µg/ml) doses producing inhibitory effects of 38.52 % and 83% respectively. The effect of LALE compared favorably with that of noradrenaline, an adrenergic agonist (Table 3). The effect of acetylcholine (0.035 µg/ml) was effectively blocked by atropine (0.029 µg/ml), while that of noradrenaline (0.012 µg/ml) was blocked by propranolol (0.029 µg/ml). LALE (EC₅₀ = 28.84 µg/ml) had no effect on acetylcholine induced contractions but significantly ($p < 0.05$) blocked the effect of propranolol; a beta adrenergic antagonist (Fig. 5).

Table 1: *In vitro* effect of Acetylcholine on an isolated rabbit jejunum

FBC (µg/ml)	Mean basal amplitude (mm)	Amplitude in response to Ach (mm)	% rise in amplitude
0.014	7.00 ± 0.00	10.33 ± 0.52*	47.57
0.029	7.00 ± 0.00	13.24 ± 0.48*	89.14
0.057	7.00 ± 0.00	17.67 ± 0.17*	152.43
0.114	7.00 ± 0.00	22.00 ± 0.52*	214.29
0.229	7.00 ± 0.00	24.15 ± 0.34*	245.00

*= p< 0.05 for test versus basal values

Table 2: *In vitro* effect of Noradrenaline on isolated rabbit jejunum

FBC (µg/ml)	Basal amplitude (mm)	Amplitude in response to NA (mm)	% inhibition
0.014	11.15 ± 0.13	6.20 ± 0.49*	44.39
0.029	13.20 ± 0.42	7.00 ± 0.81*	46.07
0.057	14.10 ± 0.23	7.12 ± 0.25*	49.50
0.114	11.00 ± 0.19	6.18 ± 0.11*	43.82
0.229	12.25 ± 0.09	4.25 ± 0.20*	65.31

*= p< 0.05 for test versus basal values

Table 3: *In vitro* effect of LALE on isolated rabbit jejunum

FBC (µg/ml)	Basal amplitude (mm)	Amplitude in response to LALE (mm)	% inhibition
14.28	14.00 ± 1.25	8.60 ± 0.28*	38.57
28.57	13.00 ± 1.90	6.55 ± 0.33*	49.62
57.14	09.00 ± 0.52	3.50 ± 0.80*	61.11
114.30	08.00 ± 1.14	2.80 ± 0.55*	65.00
228.60	09.00 ± 0.23	1.53 ± 0.09*	83.00

*= p< 0.05 for test versus basal values

DISCUSSION

All doses of LALE administered to the mice during the acute toxicity study period produced no death or other observable toxicity symptoms, even at an oral dose of 5000 mg/kg body weight and suggests that *Laportea estuans* leaf extract (LALE) has high margin of safety and can be well tolerated following oral administration. This may account for the use of the plant over the years for the management of diseases.

It is established that the smooth muscles of the gastrointestinal tract is host to numerous alpha and beta adrenergic receptors which interact with adrenergic agonists to cause relaxation of the smooth muscles of the intestine, thereby slowing down the onward movement of intestinal contents along the gastrointestinal tract [13,14,15]. The administered noradrenaline therefore produced the observed intestinal smooth muscle relaxation by binding to the beta receptors to activate the enzyme, adenylate cyclase which in turn leads to the activation of the secondary messenger cyclic adenosine monophosphate. The inhibitory effect of exogenous nordrenaline on gastrointestinal smooth muscle contractions have also been reported [16].

Propranolol (0.057 µg/ml), a non selective beta blocker significantly inhibited the relaxations induced by noradrenaline in the experiments conducted by competitively binding to the beta receptors to which noradrenaline molecules should bind to and so reduced the effect of the noradrenaline [17]. Acetylcholine on the other hand induced contractions of the isolated jejunum by binding to the numerous muscarinic receptors also present in the smooth muscles of the isolated jejunum. This contractile effect of acetylcholine was significantly blocked by atropine (0.029 mg/ml), a muscarinic receptor blocker.

Fig. 1 Effect of propranolol on noradrenaline induced relaxations

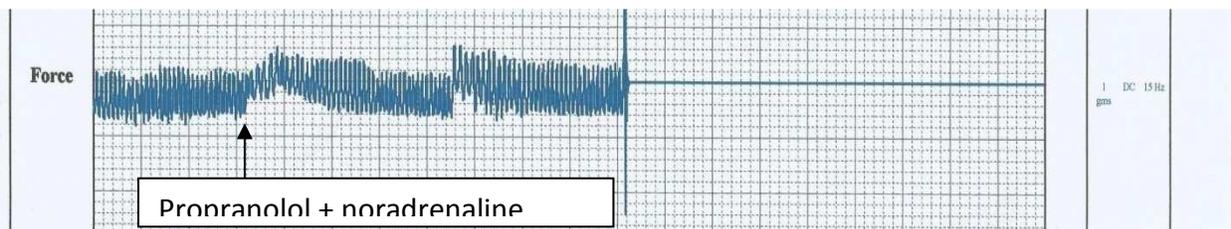
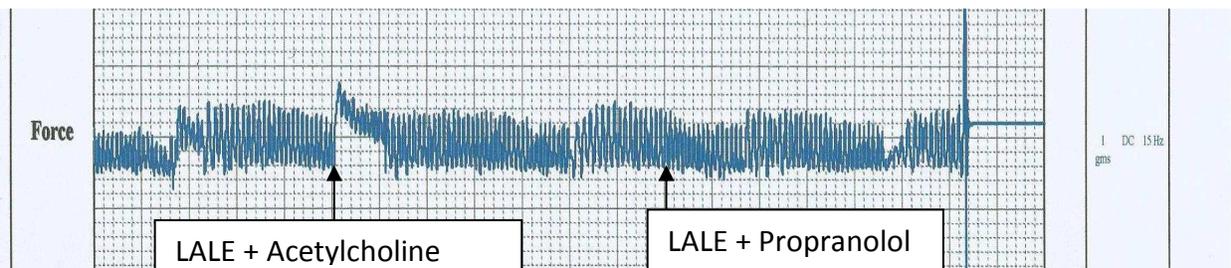


Fig. 2 Effects of LALE on the activities of Acetylcholine and Propranolol isolated rabbit jejunum



Laportea estuans leaf extract (LALE) like adrenaline exhibited a dose dependent inhibition of intestinal contractions in the *in vitro* experiments carried out. LALE also significantly ($p < 0.05$) blocked the slight rise in the amplitude of intestinal contractions due to propranolol ($0.057 \mu\text{g/ml}$) administration, but had no effect on acetylcholine induced contractions. These results suggest that LALE may contain active principles with beta adrenergic property. Temitope and Felix (2012) had reported the presence of various phytochemical agents including, alkaloids, tannins, saponins, steroids, terpenes, flavonoids and cardiac glycosides in leaf extract of *Laportea estuans*. Some of these phytochemicals have indeed been implicated in the relaxation of gastrointestinal smooth muscles. Udia *et al.* [18] had reported that flavonoids possess inhibitory effect on the smooth muscles of the gastrointestinal tract. These results suggest that LALE achieved its effects by binding to the beta receptors present in the gastrointestinal tract such that like noradrenaline it activated the enzyme, adenylate cyclase, leading to the activation of the secondary messenger- cyclic adenosine monophosphate thereby inhibiting intestinal peristaltic contractions. This explains the use *Laportea aestuans* leaves in traditional medicine for the management of diarrhea [9]. The role of LALE in gastrointestinal inhibition via the block of calcium channels remains an area for further investigation. Samra *et al.* [19], had reported the role of calcium channel blockade in the inhibition of gastrointestinal motility.

Conclusively, the inhibitory effect of *Laportea aestuans* leaf extract on the rhythmic contraction of the rabbit jejunum coupled with its significant block on the *in vitro* effect of propranolol on the isolated jejunum suggests that the extract may contain substances with potent beta adrenergic property and may be of value in the management of diseases associated with increased motility of the gastrointestinal tract and by extension those caused by excessive activity of beta receptors blocking agents. This study may further become a template for the development of yet another synthetic beta adrenergic agent of clinical significance.

REFERENCES

1. Osim, E. E. (2002). *Elements of Gastrointestinal Tract Physiology*. Helimo Associates Publishers, Calabar, Nigeria. pp87-88.

2. Sembulingam, K. and Prema, S. (2009). *Essentials of Medical Physiology*, 2nd Edition. 912-913.
3. Guyton, A. C and Hall, J. E. (1996). *Textbook of Medical Physiology*. W.B Saunders Company. Philadelphia, Pg. 771 – 778.
4. Oluwole, I. O. and Peter, F. A. (2011). Phytochemical analysis and hypolipidemic properties of *Jatropha tanjorensis* leaf extract. *European Journal of Medicinal Plants*, 194: 180 - 185.
5. Gard, B. (1965). Protabase Record on *Laportea aestuans*, vol. 21, 2000.
6. Bukil, H. M. (2000). *The useful plants of West Tropical Africa*. 2nd Edition, Addenda: Royal Botanic Gardens, New Rillmaid, UK. Pp 686.
7. Okereke, S. C. and Elekwa, I. (2014). Studies on the Antioxidant Activity of *Lapotea aestuans* Leaf Extract. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 8: 33 – 41.
8. Temitope, I. B. and Felix, O. O. (2012). Comparative Phytochemical Analysis of Medicinal Plants in Nigeria. *International Journal of Advanced Chemical Research*, 1(1): 011 – 018.
9. Ganiyat, K. and Olukoyejo, E. A. (2014). Phytochemical toxicity, Antimicrobial and Antioxidant screening of extracts obtained from *Laprtea aestuans* (Gaud). *Journal of Medical Sciences*, 14: 51 – 59.
10. Akah, J. A., Lemji, J. A., Salawu, O. A., Okoye, T. C. and Offiah, N. V. (2009). Effects of *Vernonia amygdalina* on biochemical and haematological parameters in diabetic rats. *Asian Journal of Medical Sciences*, 1(3): 108 – 113.
11. Enevide, C., David, A. and Fidelis, S. A. (2013). A new method for determining acute toxicity in animal models. *Toxicology International*, 20(3): 224 – 226.
12. Uchendu, C. N. (1999). Role of Ca²⁺ on the uterine force stimulated by glycoside from the root of *Dalbergia saxatilis*. *Indian Journal of Physiology and Pharmacology*, 43 (2): 171 – 178.
13. Wikberg, J. (1977). Localisation of adrenergic receptors in guinea pig ileum and rabbit jejunum to cholinergic neurons and to smooth muscle cells. *Acta Physiology Scandinavia*, 99 (2): 190 – 207.
14. Bauer, V. (1982). Distribution and types of adrenoceptors in the guinea pig ileum: the action of alpha and beta adrenoceptor blocking agents. *British Journal of Pharmacology*, 76 (4): 569 – 78.
15. Nakahata, N., Takagi, Y., Watanabe, E., Suuzuki, T. (1978). Adrenergic beta 1- and beta 2- receptors in the isolated rabbit jejunum and ileum. *Fukushima Journal of Medical Sciences*, 25 (3-4): 121 – 30.
16. Jun, J.Y., Choi, S., Yeum, C. H., Chang, I. Y., Park, C. K., Kim, M. Y., Kong, I. D., Insuk, S., Kim, K. W. and You, H. J. (2004). Noradrenaline inhibits pacemaker currents through stimulation of B1-adrenoceptors in a cultured interstitial cells of cajal from murin small intestine. *British journal of Pharmacology*, 14(4): 670 – 677.
17. Rang, H. P., Dale, M. M., Ritter, J. M., Flower, R. J. (2007). *Rang and Dale's Pharmacology*, 6th Edition. Churchill Livingstone, 131-136.
18. Udia, P. M., Braide, V. B. and Owu, D. U. (2009). Antispasmodic and Spasmolytic effects of methanolic extract from seeds of *Garcina kola* on isolated rat small intestine. *Nigerian Journal of Physiological Sciences*, 24(2): 111 – 116.
19. Samra, B., Raafia, M. and Anwar, H. G. (2011). Antispasmodic and Antioxidant Activities of *Valeriana hardwickii* wall Rhizome are putatively mediated through calcium channel blockade. *Evidence- Based Complimentary and Alternative Medicine*, 2011, Article I.D. 304960.