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Evaluation of the antispasmodic potential of leaf extracts of *Albizia zygia* on isolated rabbit jejunum

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Abstract
Medicinal plants have been proven to be effective in the management of numerous ailments across the globe over the years. Many African plants have also been identified with gastrointestinal-relaxation properties. *Albizia zygia* is used traditionally to treat various illnesses, including those related to the digestive system. This study aimed to compare the antispasmodic effect of aqueous and crude leaf extracts of *Albizia zygia* (ALEAZ and CLEAZ) on an isolated rabbit jejunum. Aqueous and crude extracts of the plant material was prepared via Soxhlet extraction and maceration respectively. The antispasmodic effect was evaluated *in vitro* (37 ºC, pH 7.4 with continuous oxygenation). Both ALEAZ and CLEAZ caused spontaneous relaxation of the rabbit's jejunum with a maximal effect at 0.2ml (0.15g/ml). This study further showed that the mechanism of action of the relaxation was not dependent on the muscarinic, histaminergic receptors nor through voltage-gated calcium channel (VGCs) blockade. These suggests that aqueous and crude leaf extracts of *Albizia zygia* have spasmolytic effect on isolated rabbit jejunum, thus authenticates its folkloric use in the treatment of gastrointestinal disorders.

Keywords: Antispasmodic, gastrointestinal tract, *Albizia zygia*, relaxation, spasmolytic effect, folkloric

1. Introduction
Globally, gastrointestinal disorders have been categorized among the most frequently encountered diseases in humans that is often characterized by unexplainable GIT dysfunctions via organic abnormality, though marked by persistent or recurrent excruciating pain in the abdomen. The potential effect the world's population is between 15 to 20% [1].
Albizia is a large genus belonging to the Mimosaceae plant family with about 150 species as trees and shrubs that are distributed across Asia, Australia and Africa, especially in the tropics [2]. In traditional medicine, its roots bark is used against cough, while its stem bark is used as a purgative, antiseptic, aphrodisiac, to treat gastritis, fever, conjunctivitis, as well as to fight worms and overcome female sterility [3]. The methanol extract of its stem bark has been found to possess strong activity against P. falciparum K1 strain and Trypanosoma brucei rhodesiense [4]. Reports from phytochemical evaluation of stem bark and leaf extracts of *Albizia zygia* by Schoppa and Pachaly (1981) revealed lupen-20(30)-3b-ol, 14a-stigmast-5-en-3b-ol and 5a-stigmast-7,22-dien-3b-ol also including four additional compounds from the leaf extract. Furthermore, Albiziaprenol and phytol as well as three flavonoids [40, 7-dihydroxyflavanone; 30, 40,7-trihydroxyflavone; 3-O-methylfisetin (30, 40, 7-trihydroxy3-methoxyflavone)] have also been isolated from the bark [3, 6] according to other reports. Albizia is contain saponin compounds with a large number of sugar moieties [7] and flavonoids, alkaloids and tannins [8]. The methanol extract of its stem bark has been reported to exhibit strong activity against P. falciparum K1 strain and Trypanosoma brucei rhodesiense [9]. Despite its folkloric use in the treatment of digestive tract disorders, to our best knowledge, not many studies on antispasmodic activity of *Albizia zygia* is known. This study aimed to compare the antispasmodic effect of aqueous and crude leaf extracts of *Albizia zygia* (ALEAZ and CLEAZ) on an isolated rabbit jejunum.

2. Material and methods
2.1 Plant collection and identification
Fresh plant sample of *Albizia zygia* was collected from Mazah village, Jos North Local Government, Plateau State. The plant was identified, and a specimen was registered in the herbarium of the Faculty of Forestry Technology, College of Forestry, Jos, Plateau state, with the number: FHJ32321 as reported by Okoye *et al.*, (2021) [10].

Fig 1: Albizia zygia [11]

2.2 Plant preparation

Fresh matured leaves of *Albizia zygia* were harvested from same location, washed with clean water, dried under room temperature in the Pharmacology Laboratory, College of Medicine and Allied Health Sciences of Bingham University, Jos campus, Nigeria. The dried leaves sample was grinded into fine powder using a blender after which 490g of same was weighed and extracted via a Soxhlet apparatus using distilled water as solvent while the crude extract was prepared by dispensing 10ml of distilled water into 1.5g of the powdered leaves sample for 72 hours. The soxhlet extract was evaporated into dryness via a rotary evaporator. Both the aqueous Soxhlet and crude extracts were stored at 4 ^{o}C in the refrigerator until used.

2.3 Rabbit jejunum preparation.

An adult male rabbit was acclimatized for 2weeks after which it was sacrificed under anesthesia. The abdominal cavity was dissected and a piece (3cm long) of intestinal jejunum was isolated and mounted using cotton thread in a 50ml glass tissue bath and maintained with Tyrode's solution, 37 °C, 95% oxygen and 5% Carbon (IV). The tissue was connected to microdyna Nometer via an isotonic force transducer (Harvard Apparatus, USA). Tissue contraction was established with Acetylcholine (1 µM), while muscle inhibitory effect of the extract with respect to amplitude and frequency was compared to the baseline muscle contractility. A potential spasmolytic response from the test extracts was further investigated on contractions sustained in rabbit jejunum with Ach (1 x 10^{-6}g/ml), Histamine (1 x 10^{-6}g/ml) and KCl.

2.4 Statistical Analysis

Tissue responses were expressed as standard error of the mean (SEM) while the statistical significance was determined with two-way analysis of variance (ANOVA) with $p < 0.05$ using GraphPad, USA.

3. Result and Discussion

Result shows that the extracts did not significantly reduce the amplitude of contraction produced by 2KCl, Acetyl choline and Histamine – Table 1.

Table 1: Effect of Aqueous and Crude Leaf Extracts of Albizia zygia on Amplitude of Contraction of Isolated Rabbit Jejunum

Treatment	Mean	SEM±	Sign. (< 0.05)	P Value
2MKCl	2.97	0.43		
AE+2MKCl	2.82	0.39	NS	0.59
CE+2MKCl	3.12	0.47	NS	0.59
Ach	4.35	0.60		
AE + Ach	3.80	0.23	NS	0.53
CE + Ach	4.00	0.058	NS	0.75
Histamine	1.80	0.16		
AE + Histamine	3.43	0.27	**	0.003
CE + Histamine	3.38	0.30	**	0.003

2MKCL; 2 Molar Potassium Chloride. AE+2MKCl; Aqueous Extract+2 Molar Potassium Chloride. CE+2MKCL; Crude Extract + 2 Molar Potassium Chloride. Ach: Acetylcholine. AE + Ach; Aqueous Extract +Acetylcholine. CE + Ach; Crude Extract +Acetylcholine. AE + Histamine; Aqueous Extract + Histamine. CE + Histamine; Crude Extract + Histamine. Mean; Amplitude of Contraction.*; significantly different Compared to Histamine at (p<0.05). NS; No Significant difference compared to 2MKCl and Ach at (p<0.05).

Result also reveals that the extracts have no significant effect on the frequency of contraction produced by 2KCl, Acetylcholine and Histamine – Table 2.

Table 2: Effect of Aqueous and Crude Leaf Extract of Albizia zygia on Frequency of Contraction of the Isolated Rabbit Jejunum

Treatment	Mean	SEM±	Sign (p<0.05)	P Value
2MKCl	0.15	0.077		
AE + 2MKCl	0.057	0.057	NS	0.25
CE + 2MKCl	0.12	0.062	NS	0.83
Ach	0.20	0.10		
AE + Ach	0.21	0.11	NS	0.74
CE + Ach	0.20	0.10	NS	1.0
Histamine	0.18	0.01		
AE + Histamine	0.18	0.01	NS	1.0
CE + Histamine	0.18	0.023	NS	0.99

2MKCL; 2 Molar Potassium Chloride. AE+2MKCl; Aqueous Extract +2 Molar Potassium Chloride. CE+2MKCL; Crude Extract + 2 Molar Potassium Chloride. Ach; Acetylcholine. AE + Ach; Aqueous Extract + Acetylcholine. CE + Ach;

Crude Extract +Acetylcholine. AE + Histamine; Aqueous Extract + Histamine. CE + Histamine; Crude Extract + Histamine. Mean; Frequency of Contraction. NS; No Significant difference compared to 2MKCl, Ach and Histamine. The aqueous and crude extracts (0.15g/ml) exercised relaxation of the rabbit jejunum muscle with a maximal effect e at 0.2ml. A higher dose (0.4ml) did not produce higher relaxation effect either. The foregoing could be due to 'therapeutic window' effect as suggested by Tripathi [12], in other words, it could be that the extract has attained its maximum effect at 0.2ml (Figure 2).

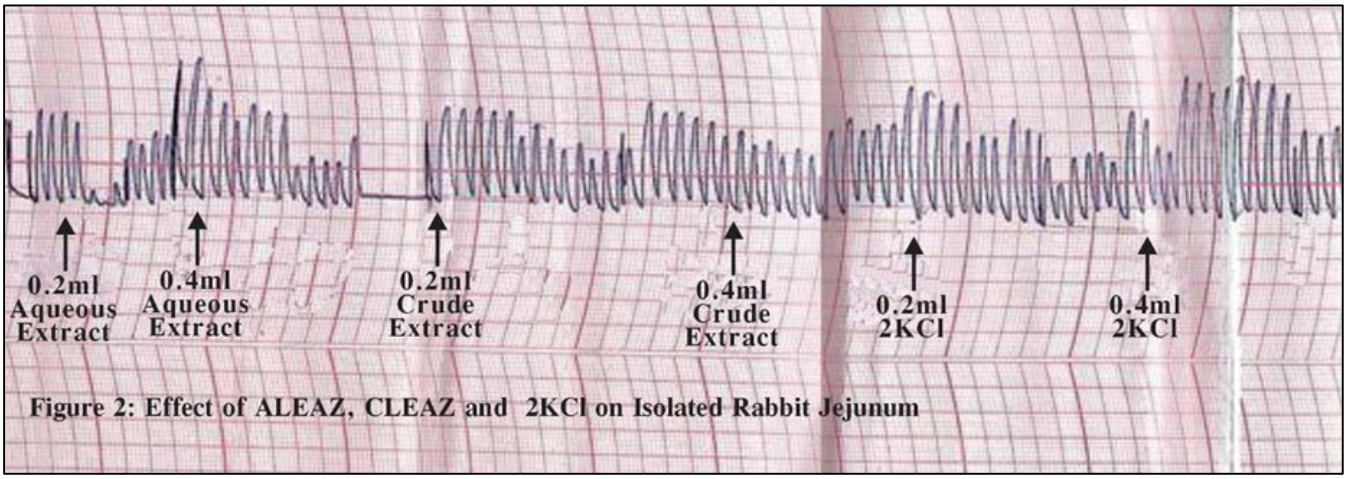

Fig 2: Effect of ALEAZ, CLEAZ and 2KCL on Isolated Rabbit Jejunum

Correlating research has also shown that K^+ at high concentrations (> 30 mM) is capable of opening voltage-dependent Ca^{2+} channels (VDCs) resulting in smooth muscle contractions and essentially causing a contractile effect by influx of extracellular Ca^{2+} which depicts inhibition of potassium-induced contraction, thus Ca^{2+} channel blockers [13, 14]. This study further reveals that ALEAZ and CLEAZ did not affect the contraction of jejunum smooth muscle induced by KCl (2M) solution and thus do not act through Ca^{2+} channels (VDCs) (Tables 1,2; Figures 2,4).

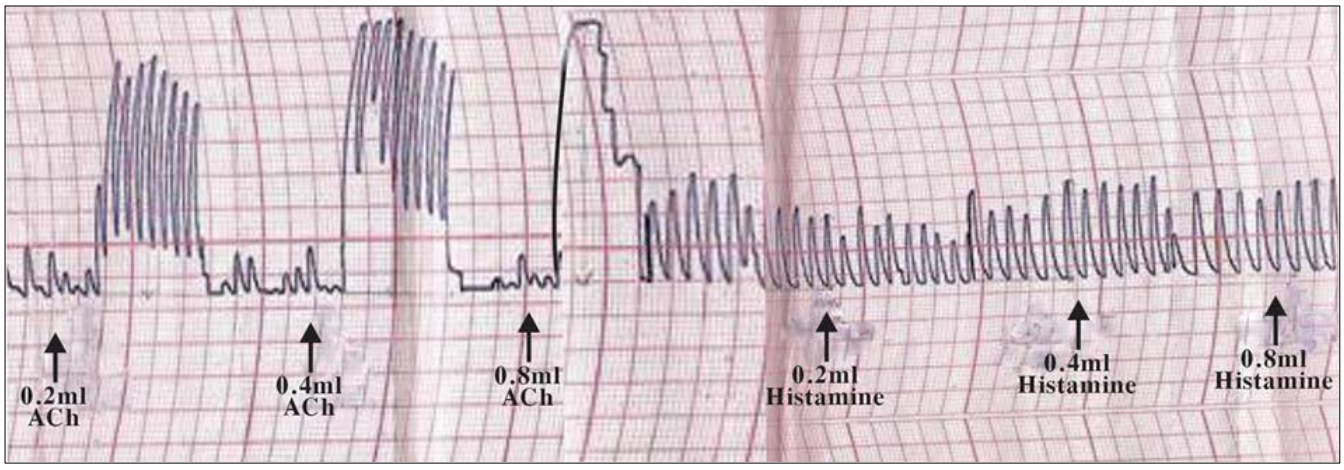

Fig 3: Effect of Ach and Histamine on Isolated Rabbit Jejunum

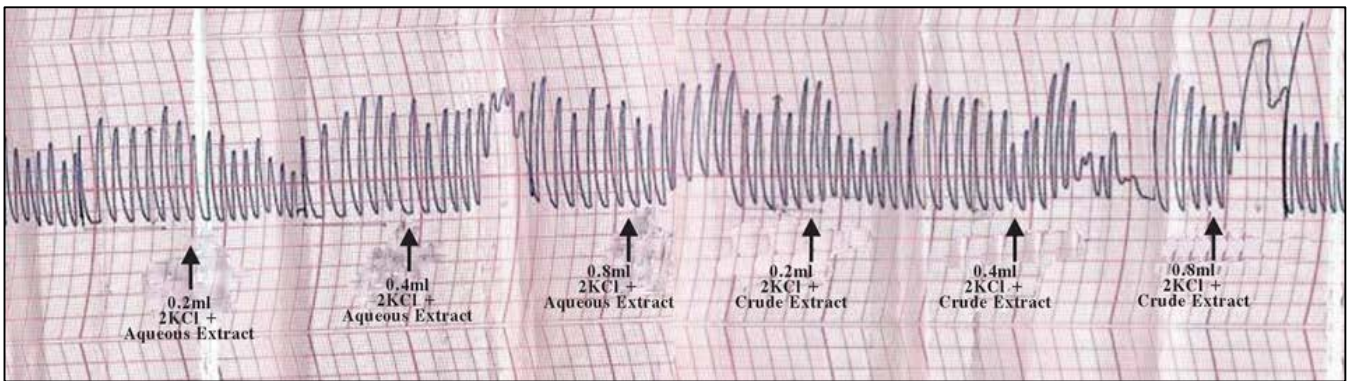

Fig 4: Effect of 2KCL in the presence of ALEAZ, CLEAZ on Isolated Rabbit Jejunum

There was no significant difference($P<0.05$) in the amplitude and frequency of contraction produced by acetylcholine alone and acetylcholine in the presence of ALEAZ and CLEAZ (Tables1 & 2; Figures 3 & 5). This suggests that the extracts

may not have acted through muscarinic receptors (M_2, and M_3). According to previous reports, histamine as a aminergic neurotransmitter and plays an important role in the regulation of several (photo) physiological processes that exerts a varying effect on smooth muscle cells depending on the animal species, often via binding to four GPCRs that are differentially expressed throughout the body and designated as the H_1 - H_4 receptors thus resulting in the control of gastrointestinal contractility and motility in complex manner, also capturing GIT contractility via increasing calcium availability at the sarcoplasmic level and receptor binding that eventually stimulates the activation of c-Fos, c-Jun, Protein Kinase C (PKC) and p70S6kinase [15-18].

This study further shows that the pre-incubation of the tissue with ALEAZ and CLEAZ (0.15g/ml) did not elicit the blockade of the contractile activity of increasing doses of histamine, thus suggesting that the effect of the extracts may not be mediated through histaminergic receptors (Tables 1 & 2; Figures 3& 5).

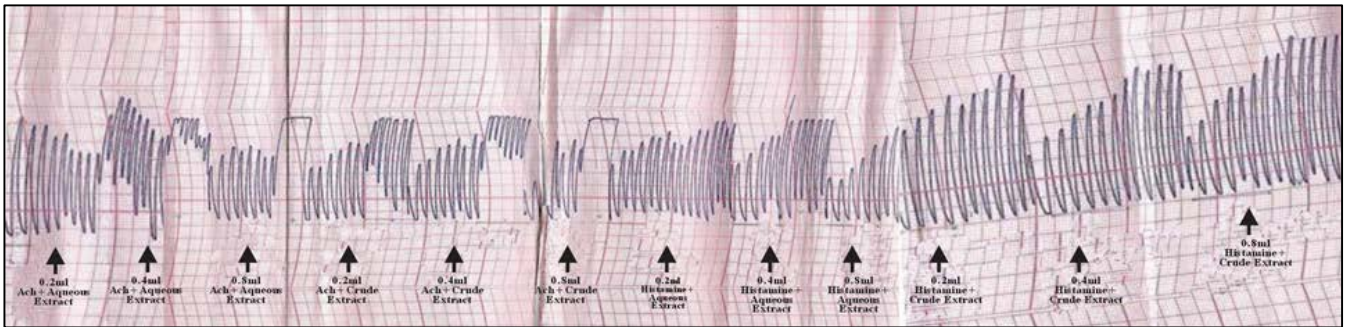

Fig 5: Effect of Ach and Histamine in the presence of ALEAZ, CLEAZ on Isolated Rabbit Jejunum

A non- significant difference ($p<0.05$) in the reparative effect was also observed in the aqueous extract compared to the crude extract.

Several phytochemical compounds such as flavonoids, saponins, and terpenes have been reported to possess relaxant effect on smooth muscles [19]. The relaxant effect of the extracts may be due to these constituents as previous phytochemical screening of ALEAZ and CLEAZ indicated the presence of terpenes and flavonoids [20, 21, 10].

4. Conclusion

The ACLEAZ demonstrated a significant spasmolytic effect by acting neither on muscarinic, histaminergic receptors nor through Ca^{2+}channel (VDCs). These results support the traditional usage of this plant to treat intestinal disorders. However, further studies to determining the mechanism of action of the plant is recommended.

5. Compliance with ethical standards

5.1 Acknowledgments

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6. Conflict of interest

The authors declare that they have no conflicts of interests

7. Statement of ethical approval

Authors Ogundeko TO, Okoye NP, Bulus Diyen and Kamoh L are licensed to handle laboratory animals. Standard protocols involving the maintenance and use of laboratory animals were strictly adhered to.

8. Ethical clearance

This was obtained from the Research and Ethical Committee of the Faculty of Pharmaceutical sciences, University of Jos, Nigeria, with the Reference No: F17-00379.

9. References

1. Makrane M, Aziz M, Mekhfi H, Zyyat A, Bnouham M, Legssyer A, *et al*. EtoB. Antispasmodic and myorelaxant activity of organic fractions from *Origanum majorana L*. on intestinal smooth muscle of rodents. Europ. J Med. Plants. 2018;23(2):1-11.
2. Mimidis K, Tack J. Pathogenesis of dyspepsia. Dig. Dist. 2008;26:194-202.
3. Abdalla MA, Laatsch H. Flavonoids from Sudanese *Albizia zygia* (Leguminosae, subfamily Mimosoideae), a plant with antimalarial potency. African Journal of Traditional, Complementary and Alternative Medicines. 2012;9(1):56-58.
4. Kigondu EV, Rukunga GM, Keriko JM, Tonui WK, Gathirwa JW, Kirira PG, *et al*. Anti-parasitic activity and cytotoxicity of selected medicinal plants from Kenya. *J Ethnopharmacol*. 2009; 123:504-9.
5. Schoppa T, Pachaly P. Inhaltsstoffe von Albizzia zygia. Arch Pharm. 1981;314(1):18-25.
6. Pachaly P, Redeker F, Schoppa T. Inhaltsstoffe von *Albizzia zygia*, 2. Mitt. Constituents of *Albizzia zygia*, II. Arch Pharm (Weinheim). 1983;316(7):651-2.
7. Cheng ZQ, Yang D, Ma QY, Yi XH, Zhang NL. Triterpenoid saponins from Albizia mollis. Bul Korean Chem. 2011;32(4):1403-9.
8. Gupta RS, Chaudhary R, Yadav RK, Verma SK, Dobhal MP. Effect of saponins of *Albizia lebbeck* (L.) Benth bark on the reproductive system of male albino rats, J Ethnopharmacol. 2005;96(1-2):31-6.
9. Wanyama, PA, Kiremire BT, Murumu JE, Kamoga O. Textile dyeing and phytochemical characterization of crude plant extracts derived from selected dye-yielding plants in Uganda. Int J Nat Prod Res. 2011;1(2):26-31.
10. Okoye NP, Oto-Obong VI, Ogundeko TO, Bulus D, Kamoh NL, Ogbole EA, *et al*. Comparative effect of aqueous and crude leaf extract of Albizia zygia on gastric acid secretion in male wistar strain albino rats. Int. Journal of Pharmaceutical Research and Application. 2021;6(3):1350-1356.
11. Image of *Albiziazygia* [Cited 2022 Oct 2], Available from http://www.westafricanplants.senckenberg
12. Tripathi KD. Essential of Medical Pharmacology; Pharmacodynamics, 7th Ed. India: Jaypee brothers; c2013.

13. Aleem A, Janbaz KH. Dual mechanisms of anti-muscarinic and Ca^{++} antagonistic activities to validate the folkloric uses of *Cyperus niveus* Retz. as antispasmodic and antidiarrheal. J Ethnopharmacol. 2018;213:138-148.
14. Gilani AH, Shah AJ, Yaeesh S. Presence of cholinergic and calcium antagonist constituents in *Saussurealappa* explains its use in constipation and spasm. Phytother Res: PTR. 2007;21(6):541-544.
15. Deiteren A, De Man JG, Pelckmans PA, De Winter BY. Histamine H4 receptors in the gastrointestinal tract. British J of Pharmacol. 2015;17:1165-78.
16. Fabisiak A, Włodarczyk J, Fabisiak N, Storr M, Fichna J. Targeting histamine receptors in irritable bowel syndrome: A critical appraisal. Journal of Neuro-gastroenterology and Motility. 2017;23(3):341-345.
17. Kim H, Dwyer L, Song JH, Martin-Cano FE, Bahney J, Peri L, *et al*. Identification of histamine receptors and effects of histamine on murine and simian colonic excitability. Neurogastroenterology and Motility. 2011;23(10):949-959.
18. Shahid M, Tripathi T, Sobia F, Moin S, Siddiqui M, Ali Khan R. Histamine, histamine receptors, and their role in immunomodulation: An updated systematic review. The Open Immunol J. 2009;2:9-41.
19. R¨uegg JC. Calcium in Muscle Contraction: Cellular and Molecular Physiology, Springer Science & Business Media, Berlin, Germany; c2012.
20. Oloyede GK, Ogunlade AO. Phytohemical Screening, Antioxidant, Antimicrobial, and Toxicity Activities of Polar and Non-Polar Extracts of Albizia zygia (DC) Stem-Bark. Annual Review & Research in Biology. 2021;3(4):1020-1031.
21. Okoye NP, Oto-Obong VI, Ogundeko TO, Bulus D, Kamoh NL, Ogbole EA, *et al*. Comparative effect of aqueous and crude leaf extract of *Albizia zygia* on gastric acid secretion in male wistar strain albino rats. Int J Pharm. Res and Appl. 2021;6(3):1350-1356.