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# Effects of *Adansonia digitata* L fruit pulp and *Lawsonia inermis* L leaves extracts on some smooth muscle preparations

**Musab A. M. Abdelrahim**

Department of Pharmacology, Faculty of Pharmacy, University of Sinnar, Sudan.

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**Plant materials serve as valuable sources of new medicinal agents, with a considerable number of them being used medicinally. However, it is important to follow systematic research methodology to evaluate the pharmacological properties of these plant materials. The aim of this study was to evaluate the effects on some smooth muscle preparations of *Adansonia digitata L* (Bombacaceae) fruit pulp and *Lawsonia inermis L* (Lythraceae) leaves extracts. When tested on isolated rabbit jejunum and isolated rat uterus, the extract of *A. digitata* fruit pulp (5 mg/ml) induced contraction in both tissue preparations, which was further blocked by pre-addition of atropine and adrenaline, respectively. However, the addition of 5 mg/ml of *L. inermis* leaves extract to the same preparations slightly increased the muscle tone without clear contraction magnitude, and this effect was not affected by pre-addition of atropine but was antagonized by pre-addition of adrenaline. Additionally, it was observed that the combination of *L. inermis* leaves extract with acetylcholine resulted in an increased contractile response of the two isolated tissue preparations compared to the action of acetylcholine alone at the same doses for each preparation. The obtained results revealed that *A. digitata* fruit pulp extract elicited contractile responses on isolated rabbit jejunum and isolated rat uterus preparations, possibly through its action on muscarinic receptors since it was effectively blocked by pre-addition of atropine. In contrast, the stimulatory response elicited by the extract of *L. inermis* leaves was related to the direct action of the extract on the smooth muscle preparations and/or by potentiation of acetylcholine action in both isolated tissues.**

**Key words:** *Adansonia digitate*, Baobab, *Lawsonia inermis*, Henna, Rabbit jejunum, rat uterus.

## INTRODUCTION

The plant kingdom serves as a valuable source of new medicinal agents, with many plants known to possess various phytochemical principles responsible for their pharmacological activities (Aliyu and Chedi, 2010). However, it is crucial to follow a systematic research methodology to evaluate the pharmacological properties of these plant materials. Additionally, herbal medicines should be taken with adequate knowledge about their

E-mail: musab_awad@hotmail.com

toxicity, purity, dosage, appropriate solvent for extraction, and potential adverse effects (Moshfegh et al., 2016).

Baobab (*Adansonia digitata L*), belonging to the family Bombacaceae, is a native deciduous tree of African savannas. The tree can grow up to 25 meters in height, 10-14 meters in girth, and can live for several hundred years (Sugandha Singh et al., 2013). It is widespread throughout the hot, drier regions of tropical Africa, extending from northern Transvaal and Namibia to Ethiopia, Sudan, and the southern fringes of the Sahara (Gebauer et al., 2002). Baobab fruit pulp, leaves, and seeds are widely used as sources for foods and drinks, while other parts such as bark and roots are also utilized for domestic purposes (Sidibe and Williams, 2002; De Caluwé et al., 2010).

For medicinal purposes, baobab leaves, bark, roots, pulp, and seeds have been found to exhibit interesting pharmacological properties, including antioxidant, prebiotic-like activity, anti-inflammatory, analgesic, antipyretic activity, anti-diarrhea, antidysentery activity, and as an excipient (Kabore et al., 2011). Several classes of compounds have been identified from various parts of the baobab, including terpenoids, flavonoids, sterols, vitamins, amino acids, carbohydrates, and lipids (Kamatou et al., 2011). In general, baobab pulp is rich in vitamin C, the leaves are rich in proteins and minerals, especially magnesium as manganese, while the seeds are rich in fats (Chadare et al., 2009). Other constituents include mucilage, lupeol acetate, β-sitosterol, scopoletin, friedelin, and baueronol (leaves and bark), pectin (fruit pulp), polyunsaturated fatty acids, tannins, a trypsin inhibitor, and adansonine alkaloid in seed oil (Sidibe and Williams, 2002).

Henna (*Lawsonia inermis L*), belonging to the family Lythraceae, is a fragrant shrub primarily found in the tropical Savannah and tropical arid zones of Africa, South Asia, and North Australia (Jiny et al., 2010; Donkor et al., 2013). Henna leaves are highly popular as a natural dye for fingers, hands, nails, and hair (Choubey et al., 2010). Lawsone, a naphthoquinone derivative, serves as the main ingredient and coloring matter in henna leaves, typically found in concentrations ranging from 0.5 to 1.5% (depending on physical conditions). Additionally, henna leaves contain various chemical constituents such as phenolic derivatives, coumarins, xanthones, tannins, flavonoids, aliphatic components, triterpenes, sterols, glucose, gallic acid, amino acids, mannitol, trace elements, and minerals (Jiny et al., 2010; Upadhyay et al., 2010). In traditional medicine, henna leaves have been utilized for treating a spectrum of ailments including skin diseases, venereal diseases, smallpox, and spermatorrhea. Furthermore, powdered henna seeds have been effective against dysentery and liver disorders, while henna bark is employed in addressing various conditions such as burns, jaundice, spleen enlargement, calculus, leprosy, and skin disorders (Mohamed et al., 2016). Various studies have reported pharmacological properties of L. inermis including hepatoprotective activity (Sanni et al., 2010), antioxidant activity (Hsouna et al., 2011), hypoglycemic and hypolipidemic activities (Syamsudin and Winarno, 2008), tuberculostatic activity (Jiny et al., 2010), anticarcinogenic properties (Endrini et al., 2007), and wound healing activity (Nayak et al., 2007).

From previously published data, various studies have explored the pharmacological properties of these plant materials; however, none have addressed their actions on smooth muscle preparations. Therefore, the aim of the current work was to investigate the effects of *A. digitata* fruit pulp and *L. inermis* leaves extracts on isolated rabbit jejunum and isolated rat uterus preparations, and to elucidate their possible mechanisms. This could aid in further understanding their therapeutic potentials through more well-designed studies.

## MATERIALS AND METHODS

### Plants materials

The plant materials (Fresh fruits of *A. digitate* and leaves of *L. inermis*) were obtained from the local market, Wad Medani, Sudan. The plant materials were identified at the Herbarium of the Phytochemistry and Taxonomy Department, Medicinal and Aromatic Plants Institute, National Center for Research, Khartoum, Sudan.

### Drugs and chemicals

17-β Estradiol (Sigma-Aldrich, USA), Acetylcholine bromide (Sigma-Aldrich, USA), Adrenaline (Shanghai, China), and Atropine (Sigma-Aldrich, USA) were utilized. The solvents and chemicals employed were of analytical grade, and the physiological solutions were prepared according to those specified by Kitchen (1984).

### Extraction of plants materials

The leaves of *L. inermis* were dried at room temperature and then ground into powder using a mortar and pestle, while the fruits of *A. digitata* were crushed to collect the powdered pulp. 100 g of dry powder from each plant were separately extracted by maceration using methanol (99%) as the solvent system in conical flasks (0.5 l) for 72 h, with intermittent shaking, and then filtered under vacuum using a Buchner funnel. The filtrate for each plant was then allowed to evaporate at room temperature for 7 days, collected separately, and stored in an amber glass container (in the refrigerator) until use.

### Ethical considerations

The study was ethically approved by the University of Gezira ethical committee. The experimental protocols and procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the National Academies (2011).

### Isolated rabbit jejunum preparation

The experiment was conducted following the method described by Kitchen (1984). A local strain rabbit weighing 1.7 kg, subjected to a 16-h fast, was used. The rabbit was euthanized, the abdomen

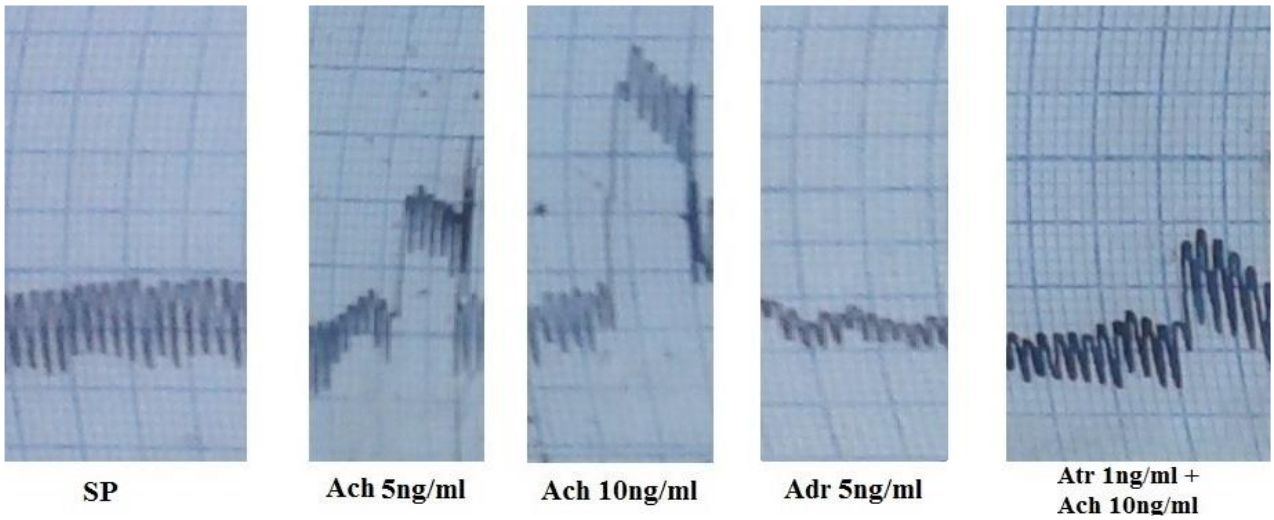


**Figure 1.** Effects of conventional drugs on isolated rabbit jejunum. SP: Spontaneous contraction, Ach: Acetylcholine, Adr: Adrenaline, Atr: Atropine.

opened, and the jejunum exposed. The first 3 cm of the jejunum were removed and transferred to a Petri dish containing Tyrode solution, where the mesentery, connective tissues, and fats surrounding the muscle were trimmed away. Threads were passed through one wall of the jejunum at both the top and bottom. The bottom thread was attached to the tissue holder, and then the mounted tissue was transferred to an organ bath (50 ml) containing aerated Tyrode solution maintained at 37°C. The top thread was attached to a Harvard isotonic transducer (under 0.5 g tension), which was connected to a Harvard Universal Oscillograph recorder (Harvard Apparatus Limited, UK) with a speed of 0.25 mm/second. The experiment was allowed to settle for 30 min as an equilibrium period, and the responses to different drugs (acetylcholine at 5 and 10 ng/ml, adrenaline at 5 ng/ml, atropine at 1 ng/ml, *A. digitata* fruit pulp, and *L. inermis* leaves extracts) were then recorded. The dose used for each plant extract was 5 mg of plant material dissolved in 1 ml distilled water, based on the method described by Kitchen (1984) and Hassan et al. (2013). The contact time for each concentration was 60 s, followed by washing three times and a resting period of 10 min before the next addition.

#### Isolated rat uterus preparation

The preparation was conducted following the method outlined by De Jalon et al. (1945) and Kitchen (1984). A female Wister albino rat weighing 160 g was selected and brought to the estrus status by administering 17β-estradiol (2 mg/kg) subcutaneously 72 h prior to the experiment (Hassan et al., 2013). The rat was euthanized by slaughtering and exsanguination. The abdomen was opened, and the two uterine horns were exposed by gently pulling aside the intestine. Each horn was carefully freed from surrounding fats and mesenteric attachments, then cut out separately and transferred to a Petri dish containing De Jalon solution. A longitudinal cut was made to form a sheet of muscle. Threads were passed through one wall of the uterus at both the top and bottom. The bottom thread was attached to the tissue holder and transferred to a 50 ml organ bath containing aerated De Jalon solution maintained at 32°C, while the top thread was attached to a Harvard isotonic transducer connected to a Harvard Universal Oscillograph recorder. A 30-min equilibration period was allowed under 0.5 g tension for the preparation. At the end of the equilibration period, the effects of the reference drugs (acetylcholine at 50 ng/ml, adrenaline at 100 ng/ml, and atropine at 100 ng/ml) and the plant extracts (5 mg/ml) were investigated with a contact time of 60 s for each concentration, followed by washing three times and a 10-min rest before the next addition.

## RESULTS

### Effects on isolated rabbit jejunum preparation

The effects of conventional drugs (acetylcholine, adrenaline, and atropine) on rabbit jejunum were depicted in Figure 1. The tissue exhibited evident contraction upon addition of 10 and 5 µg/ml of acetylcholine, while relaxation was observed upon addition of adrenaline (5 ng/ml). The contraction induced by acetylcholine was blocked upon pre-treatment with 1 ng/ml of atropine, indicating the integrity of the tissue endothelial cells. Addition of methanolic extract of *A. digitata* fruit pulp (5 mg/ml) to the tissue resulted in pronounced contraction (Figure 2), achieved in less than one minute, which was further antagonized by pre-addition of adrenaline (5 ng/ml) and blocked by pre-addition of atropine (1 ng/ml).

Conversely, addition of 5 mg/ml of *L. inermis* leaves methanolic extract to the same preparation slightly increased the muscle tone (indicated by the raised baseline of tissue spontaneous contraction) without clear contraction magnitude, and this effect was unaffected by pre-addition of atropine (1 ng/ml), but antagonized by pre-addition of 5 ng/ml of adrenaline (Figure 3), indicating physiological antagonism exerted by the sympathetic nervous system. Additionally, it was observed that the combination of *L. inermis* leaves methanolic extract (5 mg/ml) with acetylcholine (5 µg/ml) resulted in an

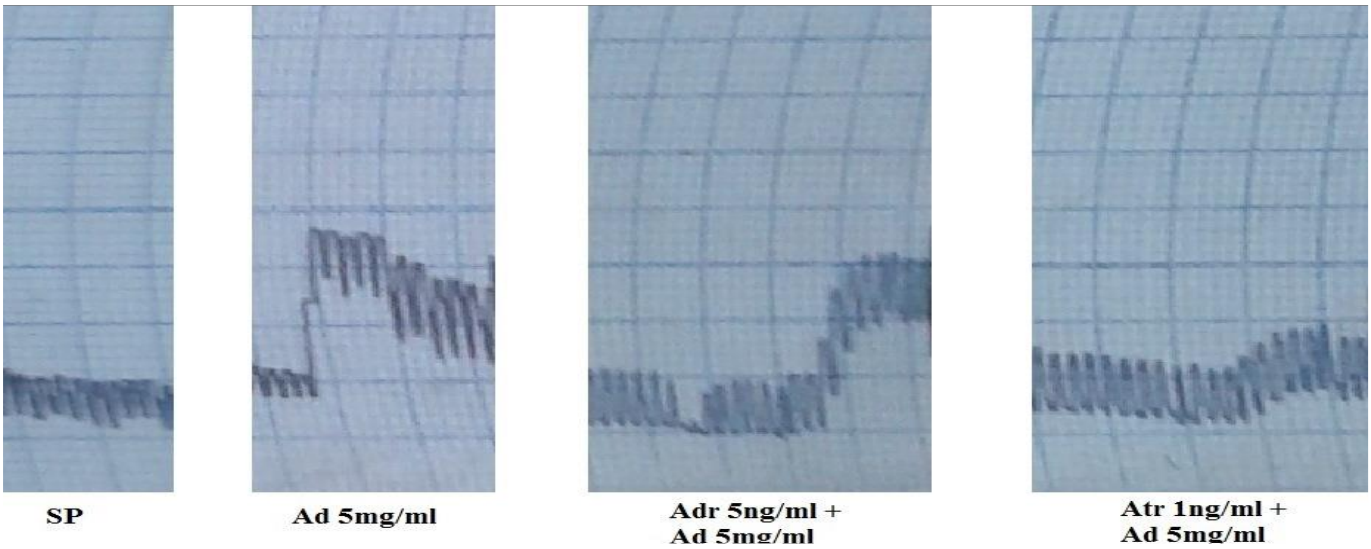


**Figure 2.** Effects of *A. digitata* fruit pulp extract on isolated rabbit jejunum. SP: Spontaneous contraction, Ad: *A. digitata*, Ach: Adr: Adrenaline, Atr: Atropine.

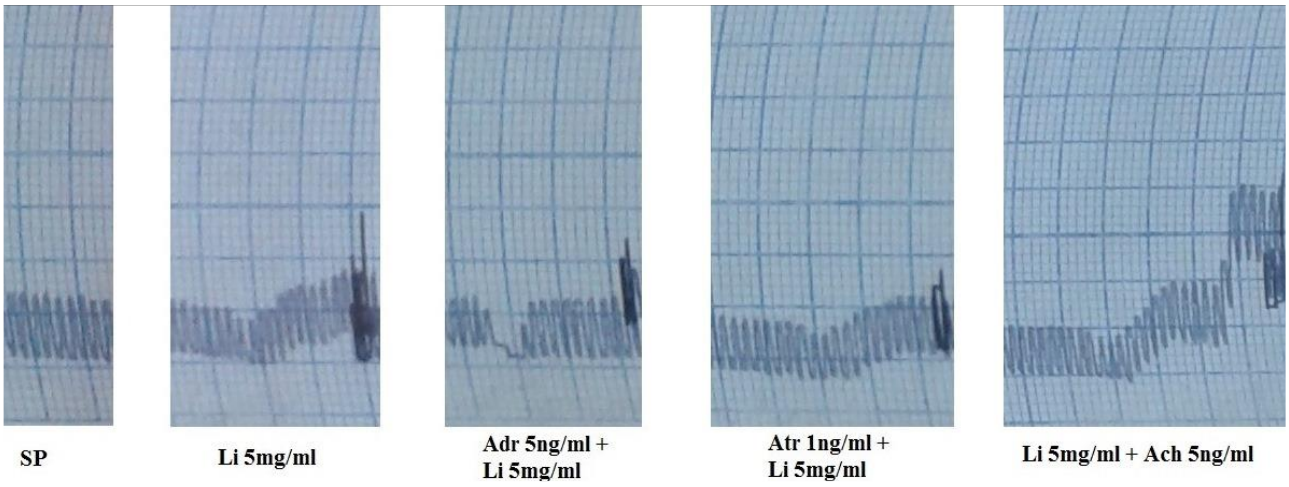


**Figure 3.** Effects of *L. inermis* leaves extract on isolated rabbit jejunum. SP: Spontaneous contraction, Li: *L. inermis*, Adr: Adrenaline, Atr: Atropine, Ach: Acetylcholine.

increased contractile response of the isolated rabbit jejunum compared to the action of acetylcholine alone at the same dose (Figure 3).

## Effects on isolated rat uterus preparation

Figures 4 to 6 illustrated the effects of conventional drugs (acetylcholine and adrenaline), *A. digitata* fruit pulp extract, and *L. inermis* leaves extract, respectively, on isolated uterus taken from 72 h estradiol-treated female Albino rats. Treatment with estradiol (2 mg/kg subcutaneously) increased the sensitivity of uterine muscle to the action of the tested drugs. Initially, the isolated rat uterus was tested with acetylcholine and adrenaline. Addition of acetylcholine (50 ng/ml) induced contraction, while addition of adrenaline (100 ng/ml) induced relaxation. The contraction elicited by acetylcholine (50 ng/ml) was reduced when the tissue was pre-treated with adrenaline (100 ng/ml), indicating physiological antagonism exerted by the two autonomic nervous systems. Subsequently, when the methanolic extract of *A. digitata* fruit pulp (5 mg/ml) was tested on the rat uterus preparation, it resulted in a clear contraction response achieved in less than one minute, which was further blocked by pre-addition of adrenaline

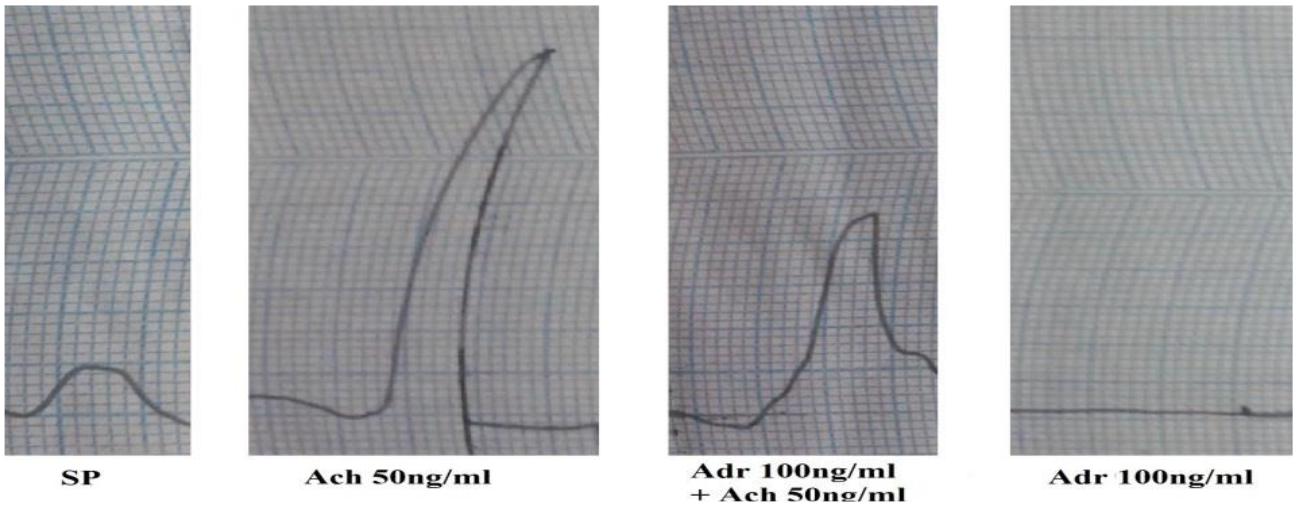


**Figure 4.** Effects of conventional drugs on isolated rat uterus. SP: Spontaneous contraction, Ach: Acetylcholine, Adr: Adrenaline.

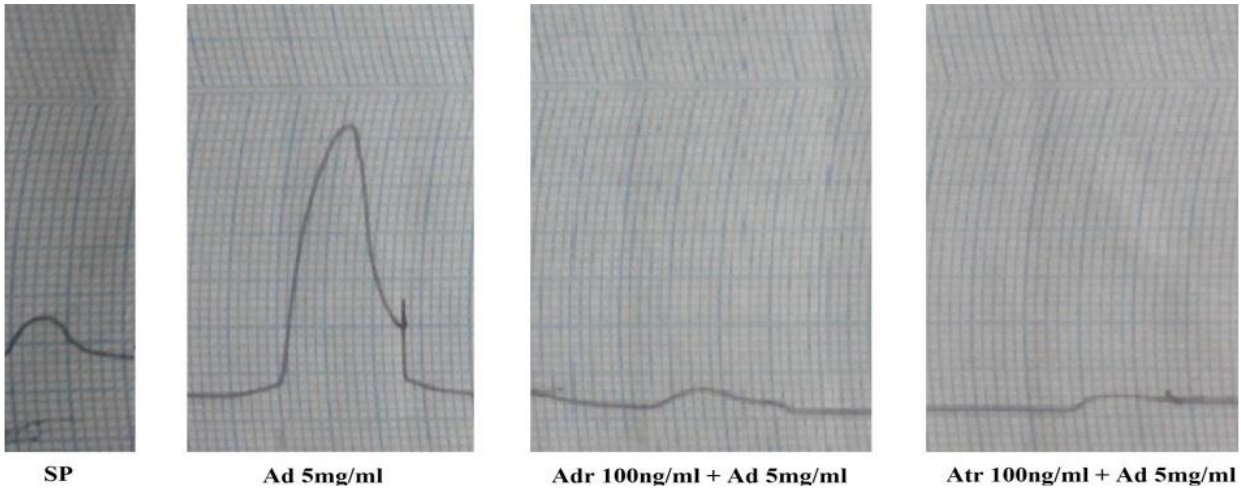


**Figure 5.** Effects of *A. digitata* fruit pulp extract on isolated rat uterus. SP: Spontaneous contraction, Ad: *A. digitata*, Adr: Adrenaline, Atr: Adrenaline.

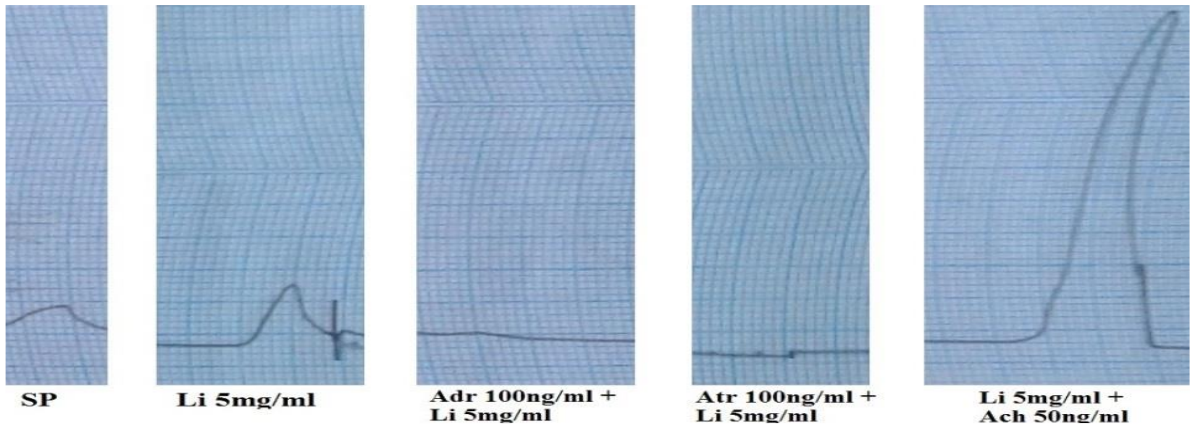


**Figure 6.** Effects of *L. inermis* leaves extract on isolated rat uterus (SP: Spontaneous contraction, Li: *L. inermis*, Adr: Adrenaline, Atr: Atropine, Ach: Acetylcholine.

(100 ng/ml) and atropine (100 ng/ml). On the contrary, addition of methanolic extract of *L. inermis* leaves (5 mg/ml) slightly increased the force of spontaneous contraction of the isolated rat uterus. This effect was antagonized by pre-addition of adrenaline (100 ng/ml) and atropine (100 ng/ml). Additionally, it was observed that addition of *L. inermis* leaves extract (5 mg/ml) followed by acetylcholine (50 ng/ml) on the rat uterus preparation potentiated the action of acetylcholine on the tissue.

## DISCUSSION

Plants materials may contain numerous active ingredients utilized for medicinal purposes, yet scientific evidence from trials assessing their effects remains limited (Alfa et al., 2018). Additionally, the suitability of the extraction method and solvent selection must be considered, as the target compounds may range from non-polar to polar and/or thermally labile (Musab and Elhadi, 2024).

The current study aimed to evaluate the effect of crude extracts of these plant materials as a preliminary screening test, which could facilitate further investigations to explore additional pharmacological properties and characterize their chemical constituents.

The observed contraction produced by the methanolic extract of *A. digitata* fruit pulp on isolated rabbit jejunum (evident by both elevation of muscle tone and magnitude of contraction) may likely be attributed to its action on muscarinic receptors, as the activity was clearly blocked by pre-addition of atropine, indicating its parasympathetic effect.

Conversely, the response observed with the methanolic extract of *L. inermis* leaves on isolated rabbit jejunum only affected the muscle tone (slight rise of the baseline), suggesting that the action may be attributed to a direct effect on the smooth muscle as it was not affected by pre-addition of atropine. However, when the extract was combined with acetylcholine, the contractile response was significantly increased compared to the action of acetylcholine alone at the same dose, indicating that the plant extract potentiated the acetylcholine-induced contraction of the tissue.

Spontaneous contraction of the jejunum smooth muscle, initiated by calcium influx, and relaxation obtained by inhibiting calcium influx, are crucial for maintaining the basal tone of the smooth muscle (Chokri et al., 2010). Acetylcholine induces smooth muscle contraction via muscarinic receptors, causing the opening of receptor-operated channels, which allows sodium influx, leading to cell membrane depolarization. Subsequently, voltage-dependent calcium channels open, permitting calcium ions to enter the cell and induce the release of calcium from the sarcoplasmic reticulum. The cytosolic calcium then binds to calmodulin, ultimately resulting in muscle contraction (Bolton, 1979). Drugs acting on adrenoceptors, such as adrenaline, relax gastrointestinal tract smooth muscle through $\beta_2$ receptors, which stimulate adenylyl cyclase and increase cAMP levels, and/or by inhibiting parasympathetic activity through presynaptic $\alpha_2$ receptors (Katzung, 2007). Histamine binds to $H_1$ receptors on gastrointestinal smooth muscle, initiating a sequence similar to that of acetylcholine, which ultimately leads to muscle contraction (Aliyu and Chedi, 2010). Atropine causes reversible blockade of cholinomimetic actions at muscarinic receptors, and by binding to these receptors, it prevents the actions induced by muscarinic agonists (Katzung, 2007). According to reports by Kitchen (1984), rabbit intestine is insensitive to histamine action, and contractile responses (induced by ganglionic stimulants, parasympathomimetics, or drugs acting directly on the muscle) are observed above the myogenic movements, whereas relaxation produced by sympathomimetics or direct muscle relaxants is observed when there is inherent tone in the tissue.

Regarding the uterus preparation, the uterine smooth muscle is innervated by sympathetic and parasympathetic supply. Drugs that stimulate muscarinic receptors, such as acetylcholine, produce contraction of the uterus. The relative proportion of α- and β-adrenoceptors mediating contraction and relaxation, respectively, varies from species to species and between estrus and diestrus phases (Kitchen, 1984). Responses related to β-receptors remain constant (pregnant and non-pregnant) and mediate relaxation of the uterine muscle, while activation of α-receptors on the uterus produces contraction and is predominant during pregnancy (Ganong, 2003). The smooth muscle of the rat uterus is particularly insensitive to the action of histamine (Kitchen, 1984). Furthermore, 5-hydroxytryptamine (5-HT) produces contractions of the uterus, which are increased either during estrus or by the administration of estrogen, and 5-HT2A receptors are probably the primary target in the myometrium (Minosyan et al., 2007). Oxytocin is a neurohypophyseal polypeptide that produces contraction of uterine smooth muscles independent of autonomic innervation. There are specific oxytocin receptors that mediate the response mainly by depolarization of muscle fibers, influx of calcium ions, and intracellular release of calcium ions (Aliyu and Chedi, 2010). Uterine smooth muscle also responds to prostaglandins (PGs) depending on the state of the uterus and may cause contraction ($PGF_{2\alpha}$) or relaxation ($PGE_2$). It is also possible that the generation of prostaglandins contributes to the action of some agonists (Kitchen, 1984).

In the current study, the contraction elicited by the methanolic extract of *A. digitata* fruit pulp on the rat uterus preparation (Figure 5) may be attributed to its action on muscarinic receptors, as it was effectively blocked by pre-addition of atropine. Additionally, the findings depicted in Figure 6 showed that the methanolic

extract of *L. inermis* leaves, when combined with acetylcholine, significantly increased the contraction evoked by acetylcholine compared to the action of the drug alone. Therefore, these findings demonstrate that the plant extract exerted a stimulatory action on the isolated rat uterus by potentiating the acetylcholine-induced contraction of the tissue.

These observed actions could also be linked to the effects of the plants on isolated rabbit jejunum conducted in this study, which align with previous results. These findings seem to support the traditional use of some *A. digitata* parts for the expulsion of retained placenta in cattle and women, as reported by Chika et al. (2011). Furthermore, the obtained results correlate with the findings of Bello et al. (2010), who reported that *L. inermis* is used to induce first-trimester abortions and prevent and treat postpartum hemorrhage in traditional medicine due to the uterotonic activity of the plant.

## Conclusion

The obtained results showed that the extracts of *A. digitata* fruit pulp and *L. inermis* leaves caused stimulatory effects on both isolated tissue preparations, which could be through their actions on muscarinic receptors and by potentiating the acetylcholine-induced contraction of the tissues, respectively. The observed stimulatory effects of the plant materials could reflect their chemical constituents. However, the significance of these findings remains an area for further investigation to assess the effects of these plant materials on pregnant myometrial strips, since the responses to many pharmacological substances may vary between pregnant and non-pregnant uterus. Additionally, further research is needed to address the qualitative and quantitative chemical analysis of the extracts by HPLC method, which could highlight the chemical components responsible for these effects.

## CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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