

ANTISECRETORY AND SPASMOLYTIC POTENTIAL OF ETHANOLIC LEAF EXTRACT OF *DATURA STRAMONIUM* LINN IN ALBINO WISTAR RAT AND GUINEA PIG ILEUM MODELS

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Abstract

Background: The goal of this study was to investigate the effects of ethanolic leaf extract of *Datura stramonium* on the gastrointestinal tract. This was evaluated by examining the effects of the extract on gastric acid secretion in albino rats and its spasmolytic potential on the isolated guinea pig ileum.

Materials and Methods: *Datura stramonium* Extract (DS) was evaluated for possible antisecretory potential. Firstly, spasmolytic activity on guinea pig ileum using an isolated organ bath set up was evaluated and compared with known spasmogens (acetylcholine and histamine). Secondly, the effect of various concentrations of DS on basal gastric acid secretion and histamine-induced gastric acid secretions were tested and compared with standard antisecretory agents such as Omeprazole and Cimetidine in male albino rats.

Results: DS at various concentrations showed a dose dependent reversible blockade of the cholinergic receptors as shown in the isolated tissue set up but its blockade on the histaminergic receptors was not significant ($p < 0.05$). DS 100 mg/kg caused a marked reduction (55% inhibition) in histamine induced gastric acid secretion ($p = 0.012$). However, omeprazole was a more effective inhibitor showing almost 90% inhibition of histamine induced gastric acid inhibition. We also observed that subsequent administration of histamine in animals that had received DS (100mg/kg) 2 hours earlier significantly induced gastric acid secretion showing that the effect of DS100 inhibition was not long lasting as compared to omeprazole where within 6 hours of last omeprazole dose, no histamine induced gastric acid secretion was observed.

Conclusion: *Datura Stramonium* may be useful as antispasmodic agent in various medical conditions. Results from this study suggest that its actions are mainly mediated via the cholinergic signalling pathways.

Keywords: *Datura stramonium*, Gastric acid secretion, ilea smooth muscles, contraction, spasmogens, cholinergic signalling pathways

Abstrait

Contexte: Le but de cette étude était d'étudier les effets de l'extrait de feuilles éthanoliques de *Datura stramonium* sur le tractus gastro-intestinal. Ceci a été évalué en examinant les effets de l'extrait sur la sécrétion d'acide gastrique chez les rats albinos et son potentiel spasmolytique sur l'iléon isolé du cobaye.

Matériaux et méthodes: L'extrait de *Datura Stramonium* (DS) a été évalué en fonction du potentiel antisécrétoire possible. Tout d'abord, l'activité spasmolytique sur l'iléon de Guinée utilisant une configuration de bain d'organe isolée a été évaluée et comparée à des spasmogènes connus (acétylcholine et histamine). Deuxièmement, l'effet de diverses concentrations de DS sur la sécrétion d'acide gastrique basique et les sécrétions d'acide gastrique induites par l'histamine ont été testés et comparés avec des agents antisécréteurs standard tels que l'Omeprazole et la Cimetidine chez les rats albinos mâles.

Résultats: la DS à différentes concentrations a montré un blocage réversible dépendant de la dose des récepteurs cholinergiques comme indiqué dans le tissu isolé mis en place mais son blocage sur les récepteurs histaminergiques n'était pas significatif ($p < 0.05$). DS 100 mg / kg a entraîné une réduction marquée (55% d'inhibition) de la sécrétion d'acide gastrique induite par l'histamine ($p = 0.012$). Cependant, l'omeprazole était un inhibiteur plus efficace qui présentait près de 90% d'inhibition de l'inhibition de l'acide gastrique induite par l'histamine. Nous avons également observé que l'administration subséquente d'histamine chez des animaux qui avaient reçu une DS (100 mg / kg) 2 heures plus tôt a induit une sécrétion d'acide gastrique induite de manière significative, ce qui montre que l'effet de l'inhibition de DS100 n'a pas duré longtemps par rapport à l'oméprazole, où dans les 6 heures de la dernière dose d'oméprazole, aucune sécrétion d'acide gastrique induite par l'histamine n'a été observée.

Conclusion: *Datura Stramonium* peut être utile en tant qu'agent antispasmodique dans diverses conditions médicales. Les résultats de cette étude suggèrent que ses actions sont principalement médiées par les voies de signalisation cholinergique.

Mots-clés: *Datura stramonium*, sécrétion d'acide gastrique, muscles lisses ilea, contraction, spasmogènes, voies de signalisation cholinergique

Introduction

Several decades of research into the field of plant based therapy has established the fact that there is a wide arsenal of plant material that are being used in the management of several ailments and diseases with very promising results. Recent studies have also postulated possible mechanisms of these therapeutic activities right from the crude plant material to isolates from various plant extracts.

Datura stramonium (DS) plant also referred to as Jimson weed or Thornapple in English and "Gegemu" in Yoruba language belongs to the family Solanaceae. It is a poisonous plant with many case reports of intentional and accidental human poisoning following its ingestion (Kurzbaum *et al.*, 2001; Arouko *et al.*, 2003; Spina and Taddie, 2007; Adegoke and Alo, 2013). There have been reported cases of delirium with hallucinations; jerky, myoclonic movements; choreoathetosis; hyperthermia; coma; respiratory distress; rare seizures; and central stimulations followed by depression. Hallucinations are reported in as many as 83% of cases; typically, they are simple visual images in natural colours, but infrequently also tactile hallucinations of crawling insects (Amy, 1995). Although poisoning may lead to fatal medullary paralysis, arrhythmias and cardiovascular collapse, Jimson Weed-related deaths are mainly as a result of impaired judgement and coordination resulting in risk-taking activities associated with accidental death. All parts of the plant are poisonous (Ma *et al.*, 2015). Attempts to explore the hallucinogenic effect of the plant may also contribute to the incidence of toxicity (Gaire and Subedi, 2013).

Despite the toxicological profile, it's been reported that *Datura stramonium* is one of preferred species used as anti-epileptic agent in communities of sub-Himalayan region of Uttarakhand, in India. It is widely used in Southern Africa as an anti-asthmatic agent (Pretorius and Marx, 2006). It is reportedly used as topical treatment for insect bites and stings (Das *et al.*, 2012).

Chemical analysis of the plant shows high levels of alkaloids particularly hyoscyamine, scopolamine and atropine (Soni *et al.*, 2012) which are mostly implicated in its poisoning. Over 60 alkaloids have been identified from various parts of the plant (Strahil *et al.*, 2006). The seeds also contain lectins. Steroids have also been isolated from aerial parts of the plant (Fang *et al.*, 2013). Atropine and scopolamine have been detected in biological samples, including hair following *Datura stramonium* ingestion (Ricard and Abe, 2012).

The pharmacological and toxicological profile of the plant has been reviewed (Soni *et al.*, 2012). It has been reported to possess anti-asthmatic effect which may be due to cholinergic blockade (Charpin *et al.*,

1979). In evaluating the acute toxic effects, 100 mg/kg of total alkaloid content was found safe (Bouzidi *et al.*, 2011). Even though no clinical trials involving DS is reported in published literature, it has however been proposed as an alternative to atropine in hospital management of cholinergic poisoning in settings where atropine supplies might be limited. Interestingly, despite widespread knowledge of atropine-like contents and well established anticholinergic toxicity, fewer studies have investigated cholinergic activity of DS extracts on the gastrointestinal tract.

The cholinergic system is known to modulate gastrointestinal function. It directly regulates gastric acid secretion as well as gastro-intestinal motility. The anti-diarrhoeal activity of *Datura stramonium* was shown in mice (Abbas, 2013) while spasmolytic activity of *Datura lanosa* has also been reported (Rojas *et al.*, 1999). Though the anti-ulcer effects of withesonalides isolated from another *Datura* species has been reported, no reports on possible effects of ethanol extracts of DS have been published. Significant differences might occur in components of different species (Jakabová, 2012) and hence influence activity, underscoring need for this study. We therefore hypothesize that since DS contains atropine-like alkaloids, it is likely to be a useful spasmolytic and anti-ulcer agent.

1.1 Aims and objectives

The aim of this study was to investigate the effect of ethanolic leaf extract of *Datura stramonium* on the gastrointestinal tract. This was evaluated by examining the effects of the extract on gastric acid secretion in albino rats and its spasmolytic potential on the isolated guinea pig ileum.

2. Material and methods

2.1. Preparation of *Datura stramonium* Extract (DS)
Datura stramonium (DS) leaves were collected from a local garden in Ilorin, Kwara State, Nigeria. The plant was authenticated with voucher number: UILH/002/845 and voucher specimen deposited at the plant biology herbarium at the University of Ilorin. The plant leaves were air dried under shade, powdered and stored in airtight container. Ethanolic extract of DS leaves powder was prepared according to the method described by Harborne (1984). 2 litres of absolute ethanol was added to 500 g of powdered DS leaves and macerated by continuous shaking at 40 °C for 72 hours. The extract was filtered through a whatman filter paper No1 to get rid of residues. The filtrate was concentrated using a rotary evaporator at temperature below 40 °C and the concentrated extract was freeze dried, weighed and kept in sterile tight container.

2.2 Spasmolytic activity of DS

Male guinea pigs (750-850 g) obtained from the animal house facility of the College of Medicine University of Lagos was used for this study. They were fasted for 18-24 hrs and then sacrificed by a blow to the base of the skull. The abdomen was opened; the ileum was excised gently and freed from mesenteric attachments and cut into segments of 1-1.5cm length each after washing gently with warm tyrode solution. The tissue was connected with a thread to a transducer at one end and to a tissue holder at the opposite end inside a tissue bath containing tyrode solution (30ml). The tissue was mounted for tension recording. The mounted tissue was allowed to equilibrate for about 1 hr. The composition of Tyrode solution in mM concentration was: 136.0 NaCl, 5.0 KCl, 0.98 MgCl₂, 2.0 CaCl₂, = 0.36 NaH₂PO₄, 11.9 NaHCO₃, and 5.5 glucose at pH 7.4, maintained at 37 °C and bubbled with air (5% carbon dioxide and 95% oxygen)

A preload of 1 g was applied to each tissue, while intestinal contractions were measured using an isotonic transducer 50-6360 (Harvard Apparatus, Holliston, MA, USA) coupled with a student oscillograph (Harvard Apparatus)/PowerLab model ML-845, data acquisition system (AD Instruments; Sydney, Australia). Each tissue was allowed to equilibrate for at least 1 hr before the addition of any drug. In order to observe the effect of the test substance on basal tissue response, the plant extract was added in a cumulative fashion to obtain the concentration-dependent responses. The relaxation of the tissue preparation was expressed as the percentage of the control contraction mediated by the spasmogen (acetylcholine or histamine)

After a concentration/dose response curve was established for each spasmogen, the tissue was washed three times and allowed to rest for about 30-45 minutes. Three treatment concentrations of DS (1 mg/ml, 10 mg/ml and 100 mg/ml) were used as the test group against each of acetylcholine and histamine induced contractions. The effects of 3% DMSO on acetylcholine and histamine induced contraction was also tested since 3% DMSO served as the carrier for the extract.

2.3 Effects of DS on gastric acid secretion

Forty-five (45) Male Wistar rats weighing 180 – 250 g obtained from Central Animal House facility of University of Ibadan were used for the study. They were housed under standard conditions of temperature (23 ± 2°C); humidity (55 ± 15%) and 12 h light (7.00 am-7.00 pm). They were kept in wire meshed cages and fed with standard commercial rat

pellets (Ladokun Feeds Limited, Nigeria). Each animal was deprived of food 14 – 18 hours before the start of the experiment but allowed water *ad libitum*. Each animal served as its own control and received multiple drugs.

For each animal, basal acid secretion was obtained before administering the test drugs. In most experiments, each animal's response to histamine was always obtained. Where extract or antagonists were used, before terminating experiment, histamine response was again obtained to confirm integrity of experimental system. Rats that did not die before end of experiment were killed by pentobarbital overdose.

For gastric acid secretion measurement, the method of Ghosh and Schild (1958) amended by Amure and Ginsburg (1964) was used for the study. Briefly, animals were anesthetized by 60 mg/kg pentobarbital and placed on the wooden surgical surface after righting reflexes were confirmed to be lost. Most animals responded to this dose of anesthesia, but a few required additional doses. The trachea was exposed and cannulated. A small incision was made to aid breathing. Through a midline incision, the abdomen was opened to expose the stomach. An incision was made in the body of the fundus close to the pyloro-duodenal junction. Polystyrene rubber tubing was then fixed in place in the stomach and ligated by a thread. The whole surgical procedure lasted about 20 minutes. For continuous recording of gastric acid secretion, the stomach via the orogastric tube was perfused with warm 0.9 % normal saline at a rate of 1 ml/min using a Langerdorff apparatus maintained at 37 °C. Cotton wool soaked in normal saline was placed on opened abdominal cavity to prevent dehydration. Perfusate from the stomach was collected every ten minutes. Acidity of perfusate was determined by titrating with 0.0025N NaOH with phenolphthalein as indicator. The normality of acid was converted to milliequivalents per liter (mEq/L) multiplying by equivalent factor 0.025. Test drugs were used only when consistent basal secretion was established.

All administered drugs and extracts were given intraperitoneally. Histamine (1 – 10 mg/kg), cimetidine 50 mg/kg, omeprazole 4 mg/kg, DS (25, 75 and 100 mg/kg) were administered to rats. Each animal served as control as multiple drugs could be given to each animal during experiment.

2.4. Ethical Statement for animal studies

The study was conducted according to the Institutional guidelines for the care and use of animals and was approved by the Ethical Review Committee, College of Health Sciences, University of Ilorin.

2.5. Statistical methods

The results were expressed as Mean and Standard Error of Mean. Student's paired t-test was used and the level of significance was set at $p < 0.05$. P value less than or equals to 0.05 were regarded as significant.

2. Result

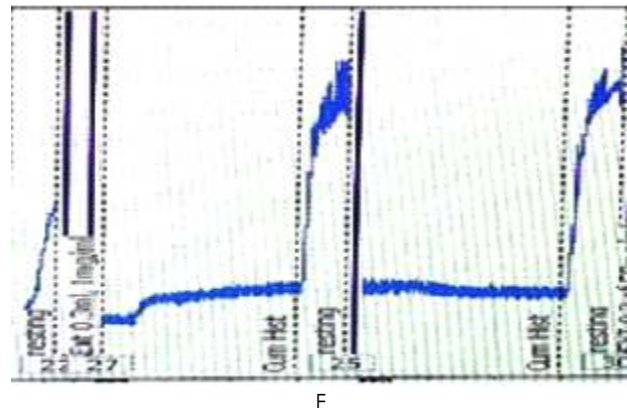
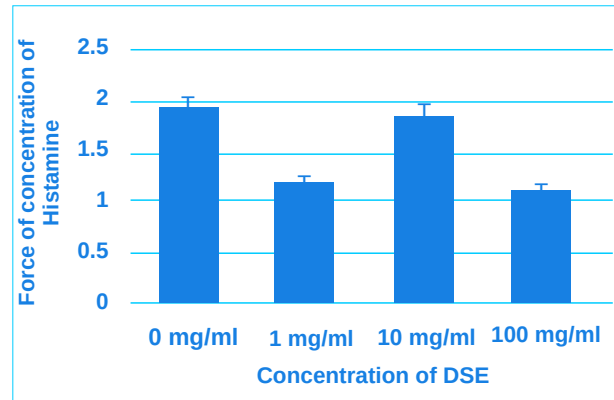
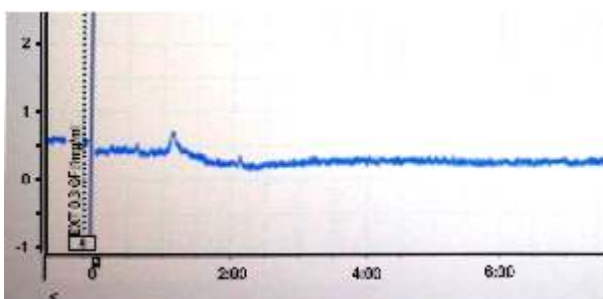
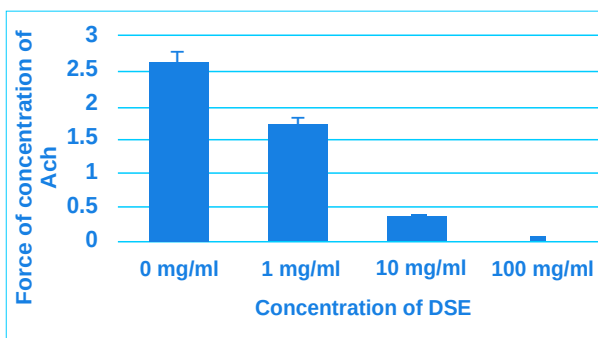
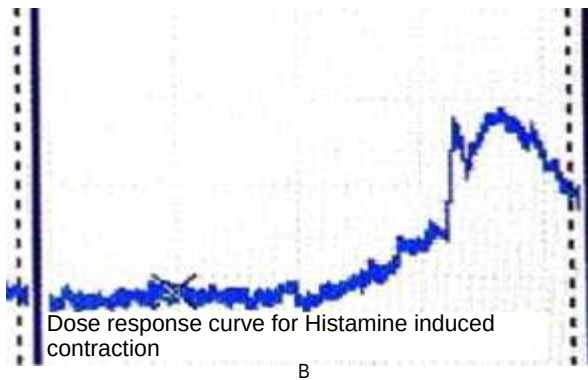
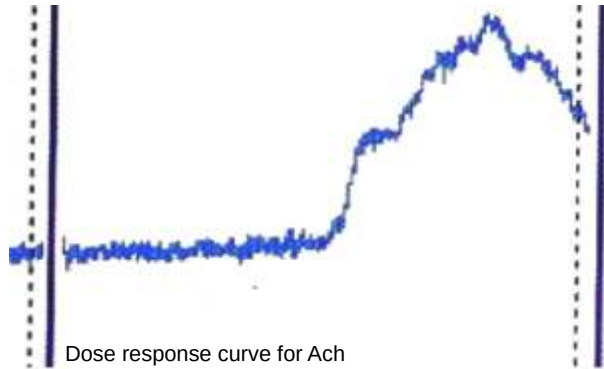


Figure1: Tracing showing tissue response to Ach (A) and Histamine (B). Effect of various concentration of DS on Ach and Histamine-induced contraction (C and E respectively) and their corresponding tissue responses (D and F), N= 12 (Number of experimental trials). Level of Significance was set at $P < 0.05$.

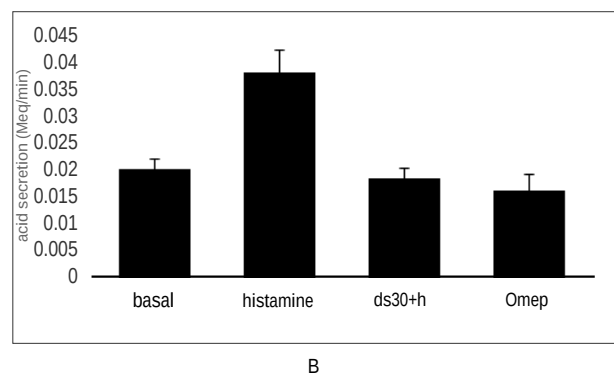
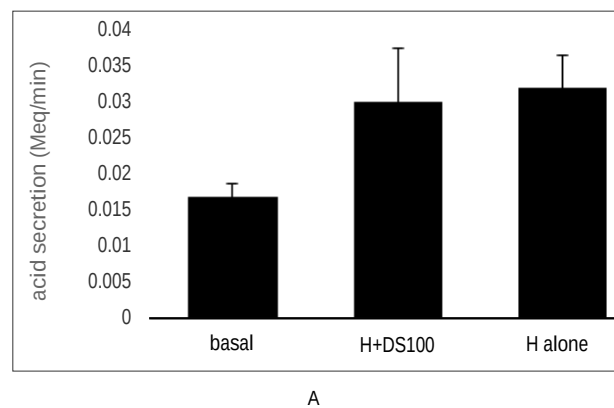


Figure 2: (A) Basal and histamine-stimulated gastric acid secretory response of the rat stomach to *Datura stramonium* (DS) extract (100 mg/kg). DS or vehicle was co-administered with histamine. All drug administration was via intraperitoneal route. Each bar represents mean $\pm$ SEM of five (5) rats per group. (B) Acid secretion following Histamine 1 mg/kg (H) alone compared with DS 100mg/kg and Omeprazole 30 minutes before histamine. All drugs were administered via intraperitoneal route. Each bar represents mean $\pm$ SEM of five (5) rats per group. DS, *Datura Stramonium* Ethanol Extract

3.1 Effects of various concentrations of DS on ACH and histamine induced contractions

Figure 1 shows the contractile effect of acetylcholine and Histamine on the isolated guinea pig ileum expressed as Force of contraction. Also, it shows a concentration dependent blockade of ACH induced contraction, statistical analysis showed a significant difference at $P < 0.05$. Also, the result showed that the extract at various doses did not significantly block histamine induced contraction as the statistical analysis showed no significant difference ($P > 0.05$). 3% DMSO did not have any effect on the contractility of the tissue and it served as the vehicle for dissolving the extract

3.2. Effects of various concentrations of DS on basal and histamine induced gastric acid secretions

3.2.1 Effect of Cimetidine and Omeprazole on Basal gastric acid secretion

We tested the effects of DS on basal gastric acid secretion. As shown in figure 2A though DS 100 mg/kg tended to lower basal gastric acid secretion by about 20%, the effect was not significant ($p=0.131$). Only omeprazole significantly lowered basal gastric acid secretion ($p=0.036$) as Cimetidine also lowered gastric acid secretion without a significant effect ($p=0.064$).

3.2.4 Effect of DS 100 mg/kg on histamine induced gastric acid secretion

Figure 2B shows the effect of co-administration of DS 100 mg/kg with Histamine. Our results showed that DS 100 mg/kg did not inhibit histamine induced gastric acid secretion. However, when DS 100 mg/kg was administered 30 minutes prior to Histamine, it completely inhibited histamine induced gastric acid secretion ($p=0.006$)

4. Discussion

This present study was carried out to find a valid experimental justification for the use of *Datura stramonium* for several gastro-intestinal disorders

including diarrhoea and spasms. This may be due to the fact that it contains alkaloids of established gastro-protective properties, despite its reported cases of poisoning in humans (Wiebe *et al.*, 2008).

In-vitro isolated organ bath experiment showed that the ethanolic leaf extract of *Datura stramonium* exerted reversible relaxant and antispasmodic effects on guinea-pig ileum with specific activity for the cholinergic receptors. The results also showed that the actions of the extract did not cause significant blockade of histaminergic receptors. This suggests that *Datura Stramonium* may not have direct antihistaminergic activity inferring from the dose dependent blockade of Ach-induced contraction which was statistically as compared with the effects of DS on histamine induced contraction which was not statistically significant.

Binding of acetylcholine to muscarinic receptors or histamine to H₁ receptor in smooth muscles results in opening of receptor operated channels, thereby allowing sodium influx, which causes a depolarization of the cell membrane. This depolarization opens voltage dependent calcium channels and calcium ions enter the cell to induce the release of calcium from the sarcoplasmic reticulum. The cytosolic calcium thus binds to calmodulin, which results in contraction (Rojas *et al.*, 1996). The results obtained on the isolated smooth muscle show a significant inhibition of acetylcholine but not histamine induced contractions, which suggests selective inhibition of the cholinergic receptors.

Increase in gastric acid secretion is observed in many diseases including duodenal ulcers and Zollinger syndrome and is amenable to pharmacological approaches which involve inhibition of different pathways involved in gastric acid secretion. Histamine is one of the most potent gastric acid secretagogues acting directly on the parietal cells. Histamine acts on histamine H₂ receptors to induce gastric acid secretion. Other gastric acid secretagogues include gastrin, acetylcholine, motilin amongst others (Goswami *et al.*, 2015).

A conventional method of measuring basal acid secretion was used. This involved the aspiration of gastric acid content for measurement of alterations in gastric acid secretion. This model is deemed useful for pharmacological screening of potential gastric acid modulating drugs (Oluwole *et al.*, 2011). The effects of DS 75 mg/kg and 100 mg/kg on basal gastric acid secretion was initially evaluated and no significant effects was established, though the observed trend was a lowering of basal gastric acid secretion, albeit briefly. It was observed that the extract at the two dose levels tested as well as the standard drugs, omeprazole and cimetidine did not significantly inhibit basal gastric acid secretion.

The effect of co-administration of DS 100 mg/kg with histamine 1 mg/kg was evaluated. This resulted in a reduction of histamine induced gastric acid secretion, though not statistically significant. The inability of DS to inhibit gastric acid secretion when co-administered with histamine suggests that it does not act via histamine receptors directly. This corroborates our observation in the *in vitro* spasmolytic work.

Further to this, investigations were made to check if prior administration of DS 100 mg/kg would inhibit histamine-induced gastric acid secretion. The findings showed that DS 100 mg/kg caused a marked reduction (55% inhibition) in histamine-induced gastric acid secretion ($p=0.012$). The role of the cholinergic system in gastric acid secretion has been well described. Myenteric neurons in response to vagal stimulation release acetylcholine. Vagal stimulation is a very important input in modulating gastric acid secretion. There are at least two possible mechanisms for cholinergic influence on gastric acid secretion. Cholinergic agonists can induce histamine secretion which may thereafter stimulate gastric acid secretion. Histamine may act directly on parietal cells to induce gastric acid secretion. To buttress the role of histamine secretion in cholinergic control of gastric acid secretion, in histidine carboxylase null mice, carbachol-induced gastric acid secretion was almost nullified implicating role of receptor mediated action on gastric acid secretion (Furutani *et al.*, 2003). The precise role of individual cholinergic receptors has been investigated using knockout mice. Loss of M3/M5 but not M1 receptors was shown to be involved in basal and stimulated gastric acid secretion (Aihara *et al.*, 2005). Hence, since DS contains alkaloids with cholinergic antagonist properties, it is possible that both cholinergic induced histamine secretion as well as direct cholinergic stimulation of gastric acid from parietal cell was inhibited. This may explain its effects on histamine induced secretion. Administration of histamine in the same animals that earlier received DS 100 within 2 hours induced significant gastric acid secretion, showing that DS 100 inhibition was not long lasting, as compared with omeprazole where within 6 hours of last omeprazole dose, no histamine induced gastric acid secretion was observed.

Toxicological assessments of the ethanol leave extract of DS showed a calculated median lethal dose (LD_{50}) of 3185.25mg/kg (Al Ani and Abbas 2015) and a sub-acute study showed that the oral administration of DS at the highest dose of 200mg/kg for 5 weeks did not affect the feeding profile, organ weight, liver function test, serum biochemical and haematological parameters. Although, serum creatinine levels were significantly high at the highest dose (200mg/kg), serum AST, ALT, bilirubin, total protein, urea and electrolyte levels were not affected by the extract at

this dose (Gidado *et al.*, 2007). Considering its general CNS effect as reported in the case of its recreational use, further studies must be embarked on to elucidate its indication or contra-indication in patients suffering from depression, maniac, suicidal tendencies and other psychological disorders. Human safety assessment must also be carried out before considering its medicinal use.

Datura stramonium Extract (DS) contains active constituents with anticholinergic effects on the guinea pig ileum, as well as on gastric acid secretion, the effects were not selective for the histaminergic receptors. This extract has the potential of being developed as a potent antispasmodic agent for the management of a number of gastro-intestinal disorders such as diarrhoea and abdominal cramps

Acknowledgement

The authors are most grateful to Mr. Chijioke M. of the University of Lagos for his assistance in the set up and use of the isolated organ bath. Our gratitude also goes to Mr Adesua O. for his assistance in the extraction of *Datura stramonium* leaves.

Conflict of interest statement

We declare that we have no conflict of interest

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