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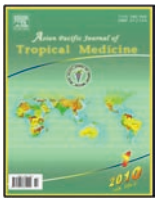

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The extract of *Mucuna pruriens* possesses histamine activity

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ABSTRACT

Objective: To investigate the activity of the active principle in the spines of the seed pods of Mucuna pruriens using contraction of guinea pig ileum as index of pharmacological activity. **Methods:** The active principle was extracted with 0.0015 M NaCl. Muscle strips of guinea pig ileum were prepared and contractile responses were measured using a Kymograph. Two sets of experiment were conducted: (1). The contraction of the ileum in presence of different concentrations of histamine, 2-methylhistamine and the extract of *Mucuna pruriens*. (2). The contractile response of the ileum in presence of different concentrations of the extract and antagonists including diphenhydramine, atropine and methysergide. **Results:** (1) The extract of *Mucuna pruriens* hair, 2- methylhistamine and histamine produced dose dependent contraction of guinea pig ileum (Extract ED_{50} = 13.0 μg/mL, 2-methylhistamine ED_{50}=8.5 μg/mL and histamine ED_{50}=10.0 μg/mL). (2) Diphenhydramine, an H_1 antagonist competitively blocked the contractile response of the Mucuna pruriens extract. (3) Coadminstration of the Mucuna pruriens extract either with different doses of antimuscarinic agent atropine or 5-hydroxytryptamine blocking agent methysergide did not alter the extract induced contractile response of the guinea pig ileum. **Conclusion:** These results demonstrate that the spines of *Mucuna pruriens* possess histamine activity which may contribute to its itching and painful irritation effects.

1. Introduction

Mucuna pruriens is a tropical plant. It possesses seed pods that are covered with barbed spines (hairs). The hairs of this plant seed are used in folk medicine for the treatment of worms in children, diagnosis and treatment of leprosy, arrow poison and cattle poisoning [1].

On contract with human skin, the hairs cause within seconds itching and painful irritation [2]. It is generally believed that these effects are brought about by the mechanical injury caused by the plant and more importantly by the irritating proteolytic enzyme (Mucunain) contained in the hairs (barbed spines) of the seed pods[2] However, this kind of explanation based on mechanical injury and presence of proteolytic enzyme in the hair is now unsatisfactory because careful examination of the *Mucuna pruriens* affected skin shows the presence of redness, swelling and wheal, thus suggesting that histamine and or autacoids like-substance might be involved in the actions of the spines of seed pods of this plant.

We therefore investigated the presence of autocoid activity in the extract of hairs of *Mucuna pruriens* using the contraction of guinea pig ileum as index of pharmacological activity of histamine and serotonin (5-hydroxytryptamine).

2. Materials and methods

2.1. Animals

Guinea pigs of both sexes weighing 250 – 450 g were used. The animals were obtained from the animal house of University of Port Harcourt and kept in the departmental animal house for at least 7 days before use. They were fed with chicken mash supplied by superfeeds Nig. Ltd. and were given drinking water ad libitum. Histamine acid phosphate and 2-methlhistamine were obtained from May & Baker Ltd. Dagenham England. Atropine sulphate, methysergide and diphenhydramine were obtained from sigma chemical Co. U.K.

2.2. Extraction

The barbed spines (hairs) covering the seed pods of

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Mucuna pruriens were collected and ground. 200 mg of ground *Mucuna pruriens* was dissolved in 50 mls of 0.0015 m NaCl and left for 2 days at room temperature in order to achieve maximal extraction. The orange coloured solution was filtered and then centrifuged at 3 000 rpm for 20 minutes. The supernatant solution was desalted by dialyzing in distilled water at room temperature. The dialysate was then heated to 45 ℃ under reduced pressure in a soxhlet extractor. The resultant paste was further dried in an oven at 37 ℃ to yield the solid extract. Concentrations of the extract used in the experiments were obtained by serial dilution.

2.3. Tissue preparation

The guinea pig was starved for 24 hours prior to each experiment. The animal was scarified by cervical dislocation and its ileum excised. The ileum was freed of faces and mesenteric attachments.

Ileum of 2 cm in length was then suspended in a 20 mL organ bath containing tyrode solution, bubbled with air and maintained at a temperature of 37 ℃ by a the mocirculator (Harvard manufacturers). The tissue was allowed to equilibrate for 60-90 minutes and concentrations were recorded on a Kymograph (Bisocience, Harbour Estate Sheerness Kent, M. 12 IRZ).

2.4. Experiments

Three types of experiments were carried out in this study: (1) Dose response experiments. In these experiments, increasing concentrations of Histamine, 2-methylhistamine and the extract of *Mucuna pruriens* were used for the graded contractile response of the guinea pig ileum. (2) Coadministration of fixed does of an antagonist with increasing concentrations of the extract. (3) Coadministration of fixed concentration of the extract and increasing concentrations of the antagonists. Each dose of the drug tested was repeated five times. The percentage contraction was calculated using the formula:

$$\% \text{ contraction} = (A-B)/A \times 100$$

where A = Maximal response of histamine;
B= Maximal response of the extract.

The contractile responses were reported as means± standard error (SE).

3. Results

Different concentrations of the extract, histamine and 2-methyhistamine were added into the medium and the contractions recorded with kymograph as described in the methods. As shown in Figure 1, histamine, 2-methylhistamine and the extract of *Mucuna pruriens* produced dose dependent contractile response of guinea pig ileum. The dose of histamine which produced half maximal response (ED_{50}) was 10 μg/mL whereas those of 2-methylhistamine and the extract *Mucuna pruriens* were 8.50 μg/mL and 13.0 μg/mL respectively.

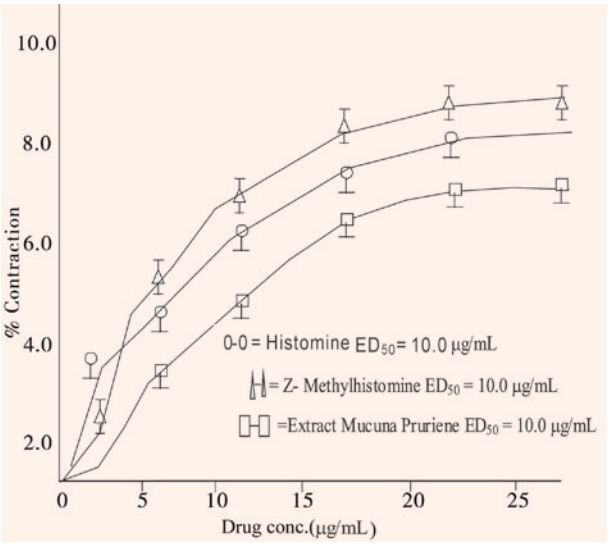

Figure 1. The Effect of *Mucuna pruriens* extract, histamine and 2-mthylhistamine on the contraction of guinea pig ileum.

10 μg/mL or 20 μg/mL of diphenhydramine was added to different concentrations of the extract as describe in the methods. The data were plotted using logarithmic dosage scale.

$$\overline{X} + S_{\bar{x}}, n=5$$

Figure 2 shows the competitive antagonism observed where doses of the extract (*Mucuna pruriens*) were coadministered with two doses of dlphenhydramine, an H_1 antagonist.

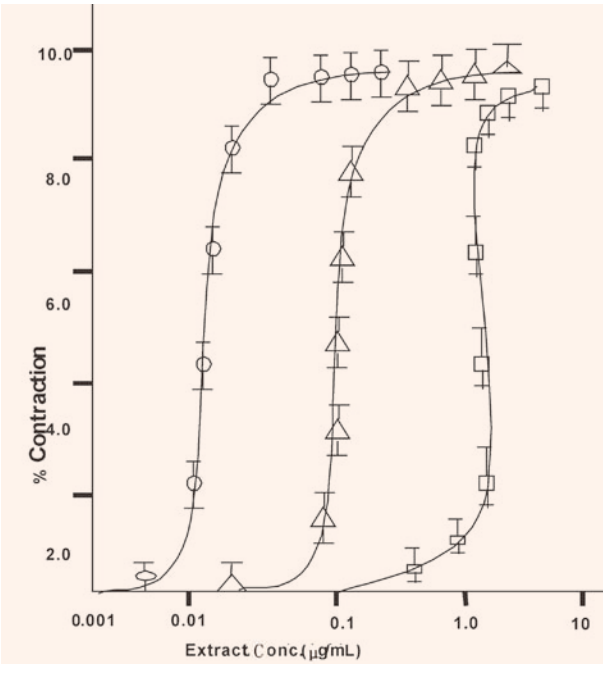

Figure 2. The effect of diphenhydramine on mucuna pruriens extract induced contraction of guinea pig ileum.

When 10.0 μg/mL diphenhydramaine was coadministered with the extract the log dose response curved shifted to the right. The parallel shift of the log dose response curve was further shifted to the right at the dose of 20.0 μg/mL.

As demonstrated in table 1, antimuscarinic agent atropine or antiserotonergic agent methysergade did not block the effects of *Mucuna pruriens* extract. For example, when 15.0 μg/mL of the extract was administered alone, (76.0 ±1.5)% of contraction of guinea pig ileum was produced, but when the same dose of the extract was administered with 10 × 10^{-6} mol/L methysergide, the magnititude of the contraction of the ileum remained unchanged. Similar results were obtained with atropine.

Table 1
Effect of co-administration of *Mucuna pruriens* extract and atropine on the contraction of guinea pig ileum.

Drug	Dose	% Contraction
Extract	15 μg/mL	75 ± 2.5
Atropine + extract	2.5×10^{-6} mol/L 15.0 μg/mL	74 ± 5.0
Atropine + extract	5×10^{-6} mol/L 15.0 μg/mL	76 ± 2.8
Atropine + extract	10×10^{-6} mol/L 15 μg/mL	75 ± 4.2
Atropine + Extract	12.5×10^{-6} mol/L 15 μg/mL	77 ± 3.7

All values are Mean ± S.E.M. of five determinations. The ileum was prepared and mounted inside an organ bath under physiological conditions. Different concentrations of Atropine were coadministered with 15.0 μg/mL of the extract and the results recorded as described in the methods.

4. Discussion

The results of this study demonstrate that the extracts of *Mucuna pruriens* produce identical effects on guinea pig ileum as histamine and histamine analogues. The results further confirm that the active principle in the extract or histamine competitively blocked the effects of the active principle. This H_1 receptor specificity is further confirmed by the fact that neither 5-hydroxytrytamine blocking agent nor cholinergic blocking drug blocked the contractile response of guinea pig ileum induced by active principle of *Mucuna pruriens*.

The active principle in the extract of *Mucuna pruriens* may act in two ways via: (1) The active principle may act directly on the receptors of histamine and thus elicit the itching and wheal effects observed and (2) The active principle may upon contact with the skin evoke the immediate release of histamine from mast cells which then acts on the receptors and elicit the itching and wheal effects. Since the extract of *Mucuna pruriens* possesses an irritating proteolytic enzyme [2], the later suggestion seems likely because it has been reported that some enzymes notably phospholipase A2 and chemotrypsin have pronounced histamine releasing activity of pathological relevance [3,4]. In this regard, the findings of this study are consistent with the report that the itching and painful irritation induced by *Mucuna pruriens* are as a result of the mechanical injury and the action of a highly irritating profeolytic enzyme called Mucunain[2].

E-hydroxytryptamine is lodge in tehgastraintestinal tract mainly nervous system. Small quantity is found in the mast cells of rodents such as guinea pig but not in man [5,6]. These facts emphasize that if the active principle of *Mucuna pruriens* acts on and degranulates mast cells which result in the liberated to contribute to the histamine induced contractile response of the guinea pig ileum would have been blocked partially by the 5-hydroxytryptamine antagonist-methysergide. On the contrary methysergide did not affect the induced contractile response. This observation can be explained by (a) the amount of 5-hydroxytryptamine in the rodent mast cell as stated above is very small to elicit any measurable contractile response and (b) it may be that the active principle in the extract of *Mucuna pruriens* acts on mast cells but not on enterochromaffin cells of the guinea pig ileum.

Finally, the other possible mechanism of action of this extract may be that it contains histamine or histamine analogue which acts directly on histamine receptors to elicit the observed irritation, itching, wheal and contraction of guinea pig ileum [7]. The strong evidence for this is that the action of the extract in the contraction of the ileam muscle stripe when added to the organ bath was immediate i.e. with latent period in seconds. Further work is required to elucidate and identify the contents of this extract.

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