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A study of the smooth muscle relaxant actions of harman alkaloids isolated from the seeds of *peganum harmala*

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ABSTRACT

Introduction: The plant *Peganum harmala* is a shrub native to the arid parts of the world including India. It's therapeutic value is attributed to the presence of alkaloids harmala and harmine in the dried ripe seeds. In traditional medicine these seeds are considered to have anti-spasmodic, hypnotic, anodyne, emetic, emmenogogue, stimulant, aphrodisiac, lactogogue and antihelminthic actions.

Objectives: The present study was undertaken to find out the effects of the active principle extracted from the seeds of *Peganum harmala* on isolated smooth muscle and skeletal muscle preparations and the probable mechanism of action

Materials & Methods: The harman alkaloid isolated from *Peganum harmala* seeds was used for experiments on isolated preparations of rat uterus, guinea pig ileum, rabbit jejunum and frog rectus abdominis muscles and the responses were recorded on a rotating smoked drum on a kymograph.

Results: The active principle in *Peganum harmala* seeds was shown to inhibit spontaneous movements of isolated rat uterus, guinea pig ileum and rabbit jejunum. Also shown to inhibit contractions of oestranised rat uterus induced by oxytocin, contractions of guinea pig ileum induced by histamine and acetyl choline. Have not shown any effect on contractions of frog rectus abdominus muscle.

Conclusion: Harman alkaloids have anti-spasmodic, tocolytic, anti-cholinergic and anti-histaminic activities, it is unlikely that these alkaloids have a specific receptor antagonistic activity. Also these have found to inhibit the smooth muscle contractions but not the skeletal muscle contractions. The smooth muscle relaxant action may be due to calcium channel blockade.

Keywords: Harman alkaloids, smooth muscle, relaxant

INTRODUCTION

Peganum harmala is a perennial succulent shrub native to the arid parts of North Africa, the Mediterranean, the Middle East, Pakistan and India.The dried seeds of the plant constitute the active principle Harmal, used in India for various medicinal purposes. The seeds are of a dull, earthy-brown color with a reticulated seed coat and have a bitter taste. The therapeutic value of the plant is attributed to the presence of alkaloids which occur in varying amounts in seeds, roots, leaves, flowers, stem, bark and wood. The dried ripe seeds contain 3.8-5.8% alkaloids concentrated mainly in the coat, roots contain over 3%, bark 2.2% and wood 1.06%. Seeds contain Harmaline, Harmine, Harmalol and Vasicine with the first two predominating[1,2] . The first three known as harman alkaloids are closely related and contain an indole nucleus, while Vasicine is a quinazoline derivative. In the traditional medicine the seeds of the plant are considered to have anti-spasmodic, hypnotic, anodyne, emetic, emmenogogue, stimulant, aphrodisiac, lactogogue and antihelminthic actions[3,4,5].

OBJECTIVES

A perusal of literature shows that the few studies were done mostly on the smooth muscle using aqueous extract of the seeds of *Peganum harmala,* very few studies have been done using the pure compound isolated from the plant. The present study was undertaken to find out the effects of the active principle extracted from the seeds of *Peganum harmala* on isolated smooth muscle and skeletal muscle preparations and the probable mechanism of action.

MATERIALS & METHODS

Peganum harmala seeds were crushed and treated with dilute 3% acetic acid and pressed after 48 hours. To this liquid, sodium chloride was added and kept in the refrigerator for a day, to isolate the harman alkaloids, which are precipitated as yellow material. This is dried in air to get yellow powder, which is used in experiments on oestranised rat uterus, guinea pig ileum, rabbit jejunum and frog rectus abdominus muscle.

1) Using the isolated rat uterus, spontaneous contractions are recorded on a rotating smoked drum on a kymograph. The responses to Harman alkaloids and their effect on the action of oxytocin is also recorded.

2) Using the isolated guinea pig ileum, spontaneous contractions and the responses to Harman alkaloids and their

effect on the action of Acetyl-choline and Histamine are also recorded.

3) Using the isolated rabbit jejunum, spontaneous contractions and the responses to Harman alkaloids and their effect on the action of Acetyl-choline is also recorded.

4) Using the isolated frog rectus abdominus muscle, the responses to Harman alkaloids and their effect on frog rectus abdominus muscle contraction induced by Acetyl-choline is also recorded.

RESULTS

Experiment No.1:- Effect of Harman alkaloids on isolated rat uterus:

There was decrease in amplitude of spontaneous contractions with 5 micrograms/ml dose and there was complete inhibition of spontaneous contractions at the dose of 10 micrograms/ml of bath fluid.

Table 1: Effect of Harman alkaloids on isolated rat uterus

S. No.	DOSE	Amplitude of Contraction
1	0 µg/ml(spontaneous contractions)	6 cm
2	5µg of Harman alkaloid per ml of bath fluid	2 cm
3	10µg of Harman alkaloid per ml of bath fluid	NIL.

Experiment No.2:- Effect of Harman alkaloids on the action of oxytocin on isolated rat uterus:

Harman alkaloids at a dose of 10 micrograms and 15 micrograms per ml of bath fluid blocked the uterine stimulant action of 0.05 milliunits of oxytocin per ml of bath fluid

Table 2: Effect of Harman alkaloids on the action of oxytocin on isolated rat uterus

S. No.	DOSE	Amplitude of Contraction
1	0.05 millli units oxytocin per ml of bath fluid	8 cm
2	0.05 millli units oxytocin +10µg of Harman alkaloid per ml of bath fluid	0.9 cm
3	0.05 millli units oxytocin +15µg of Harman alkaloid per ml of bath fluid	NIL.

Experiment No.3:- Effect of Harman alkaloids on isolated Guinea pig ileum

There was a decrease in the amplitude of spontaneous contractions of guinea pig ileum.

Table 3: Effect of Harman alkaloids on isolated Guinea pig ileum

S. No.	DOSE	Amplitude of Contractions
1	0 µg/ml(spontaneous contractions)	1.5 cm
2	3µg of Harman alkaloid per ml of bath fluid	1 cm

Experiment No.4:- Effect of Harma alkaloids on the action of Acetyl-choline on isolated Guinea pig ileum.

Harman alkaloids at a dose of 6 miccrograms per ml of bath fluid reduced the intestinal contractions caused by Acetyl-choline 2micrograms per ml of bath fluid. At a dose of 12 micrograms per ml of bath fluid, Harman alkaloids blocked the intestinal contractions caused by Acetyl-choline 2 micrograms per ml of bath fluid.

Table 4: Effect of Harma alkaloids on the action of Acetyl-choline on isolated Guinea pig ileum.

S. No.	DOSE	Amplitude of Contractions
1	Acetyl-choline 2µg per ml of bath fluid	4.5 cm
2	6µg of Harman alkaloid + Acetyl-choline 2µg per ml of bath fluid	2.0 cm
3	12µg of Harman alkaloid + Acetyl-choline 2µg per ml of bath fluid	NIL.

Experiment No.5:- Effect of Harman alkaloids on the action of Histamine on isolated Guinea pig ileum:

There is dose dependent decrease in the amplitude of intestinal contractions caused by Histamine 0.1 microgram per ml of bath fluid, in the presence of Harman alkaloids in the dose of 6 micrograms and 12 micrograms per ml of bath fluid.

Table 5: Effect of Harman alkaloids on the action of Histamine on isolated Guinea pig ileum

S. No.	DOSE	Amplitude of Contractions
1	Histamine 0.1µg per ml of bath fluid	9.0 cm
2	6µg of Harman alkaloid + Histamine 0.1µg per ml of bath fluid	5.5 cm
3	12µg of Harman alkaloid + Histamine 0.1µg per ml of bath fluid	2.5 cm

Experiment No.6:- Effect of Harman alkaloids on isolated Rabbit Jejunum:

At a dose of 30 micrograms, 300 micrograms and 1 milligram of Harman alkaloids, there was a dose dependent decrease in the baseline indicating decreased tone of the rabbit jejunum.

Table 6: Effect of Harman alkaloids on isolated Rabbit Jejunum

S. No.	DOSE	Amplitude of Contraction
1	Spontaneous contractions	0.5 cm
2	3µg of Harman alkaloid per ml of bath fluid	with no decrease in baseline
3	30µg of Harman alkaloid per ml of bath fluid	0.5 cm with 1.1 cm decrease in baseline
4	300µg of Harman alkaloid per ml of bath fluid	0.5 cm with 1.5 cm decrease in baseline
5	1mg of Harman alkaloid per ml of bath fluid	0.5 cm with 3.2 cm decrease in baseline

Experiment No.7:- Effect of Harman alkaloids on the action of Acetyl-choline on isolated Rabbit Jejunum:

With small doses of Harman alkaloids 1, 3 and 10 micrograms no significant effect was observed. At a dose of 30, 100 and 300 micrograms there was decrease in amplitude of intestinal contractions caused by Acetyl-choline 300 nanograms. Harman alkaloids in doses of 1 and 3 milligrams caused significant decrease in the amplitude of intestinal contraction caused by 300 nanograms of Acetyl-choline.

Table 7: Effect of Harman alkaloids on the action of Acetyl-choline on isolated Rabbit Jejunum

S. No.	DOSE	Amplitude of Contractions
1	Ach - 100 nanograms	1.5 cm
2	Ach - 300 nanograms	5.2 cm
3	Ach - 1µg	7.5 cm
4	Harman alkaloid 1µg + Ach - 300 nanograms	5.2 cm
5	Harman alkaloid 3µg + Ach - 300 nanograms	5.2 cm
6	Harman alkaloid 10µg + Ach - 300 nanograms	5.2 cm
7	Harman alkaloid 30µg + Ach - 300 nanograms	4.7 cm
8	Harman alkaloid 100µg + Ach - 300 nanograms	4.5 cm
9	Harman alkaloid 300µg + Ach - 300 nanograms	4.2 cm
10	Harman alkaloid 1mg + Ach - 300 nanograms	1.5 cm
11	Harman alkaloid 3mg + Ach - 300 nanograms	0.9 cm

Experiment No.8:- Effect of Harman alkaloids on frog rectus abdominus muscle:

Harman alkaloids did not produce any effect on frog rectus abdominus muscle

Table 8: Effect of Harman alkaloids on frog rectus abdominus muscle

S. No.	DOSE	Amplitude of Contraction
1	Harman alkaloid 1µg	NIL
2	Harman alkaloid 3µg	NIL
3	Harman alkaloid 10µg	NIL
4	Harman alkaloid 30µg	NIL
5	Harman alkaloid 100µg	NIL
6	Harman alkaloid 300µg	NIL
7	Harman alkaloid 1mg	NIL
8	Harman alkaloid 3mg	NIL
9	Harman alkaloid 10mg	NIL

Experiment No. 9:- Effect of Harman alkaloids on the action of Acetyl-choline on frog rectus abdominus muscle.

There was no change in response to Acetyl-choline 100 micrograms in the presence of Haarman alkaloids in doses of 100 micrograms, 300 micrograms, 1 milligram and 3 milligrams.

Table 9: Effect of Harman alkaloids on the action of Acetyl-choline on frog rectus abdominus muscle

S. No.	DOSE	Amplitude of Contractions
1	Ach - 30µg	0.7 cm
2	Ach - 100µg	0.9 cm
3	Ach - 300µg	0.9 cm
4	Ach - 100µg + Harman alkaloid 100µg	0.9 cm
5	Ach - 100µg + Harman alkaloid 300µg	0.9 cm
6	Ach - 100µg + Harman alkaloid 1mg	0.9 cm
7	Ach - 100µg + Harman alkaloid 3mg	0.9 cm

DISCUSSION

In this study, Harman alkaloids isolated from *Peganum harmala* seeds were shown to inhibit the spontaneous contractions of isolated rat uterus and guinea pig ileum and the tone of isolated rabbit jejunum. The Harman alkaloids were also shown to inhibit the contractions of isolated oestranised

rat uterus induced by oxytocin, the contractions of isolated guinea pig ileum induced by Histamine and the contractions of rabbit jejunum induced by Acetyl-choline. These inhibitions were concentration dependent and reversible. Harman alkaloids have not shown any effects on frog rectus abdominus muscle on their own and they have also not shown any effect on the contractions of frog rectus abdominus muscle induced by Acetyl-choline[6,7].

The data suggests that Harman alkaloids isolated from *Peganum harmala* seeds have anti-spasmodic, tocolytic, anti-cholinergic and anti-histaminic activities. Contractions of the skeletal, cardiac and smooth muscles are dependent on an increase in the concetration of cytoplasmic free calcium which activates the contractile element. The source of activator calcium may be intracelluar or extracellular. The relative contribution of calcium from these two sources however depends largely upon the type of muscle tissue, the intracellular calcium from sarcoplasmic reticulum having a main role in case of skeletal muscle contraction and the extracellular calcium having a predominant role in case of cardiac and smooth muscle contractions[8]. The spontaneous movements of intestine are regulated by cycles of depolarisation and repolarisation. Action potentials appear at the height of depolarisation and constitute a rapid influx of calcium through voltage dependent calcium channels. Since, Harman alkaloids isolated from *Peganum harmala* seeds inhibit the spontaneous movements of intestine and uterus, they may be interfering with calcium influx via voltage dependent calcium channels or with membrane depolarisation which opens these channels[9,10].

Acetyl-choline and Histamine produce depolarisation and contractions of non-vascular smooth muscle and oxytocin produces depolarisation and contractions of uterine smooth muscle. These contractions are dependent on extracellular calcium which gains access to the cytoplasm either via opening of voltage dependent calcium channels or via receptor operated calcium channels. It is unlikely that Harman alkaloids have a specific receptor antagonistic effect because they antagonise the effects of many agonists like Acetyl-choline, Oxytocin and Histamine.

As the Harman alkaloids were found to inhibit the smooth muscle contraction and not the skeletal muscle contractions, they may be inhibiting calcium influx via receptor operated calcium channels or voltage dependent calcium channels.

CONCLUSION

It seems that the Harman alkaloids isolated from *Peganum harmala* seeds affect the smooth muscle contraction by blocking the calcium influx into the cell via voltage dependent calcium channels and receptor operated calcium channels. They do not affect the skeletal muscle contractions as it is dependent on sodium and potassium channels and calcium is released from sarcoplasmic reticulum inside the cells in response to depolarisation.

Hence, the smooth muscle relaxant actions of Harman alkaloids may be due to calcium channel blockade.

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