COMPARISON OF EFFECTS OF EXTRACT OF MYRISTICA FRAGRANS AND VERAPAMIL ON THE VOLUME AND ACIDITY OF CARBACHOL INDUCED GASTRIC SECRETION IN FASTING RABBITS

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Background: Peptic ulcer is mostly produced due to the over production of gastric acid. This study was undertaken to find out the effects of extract from Myristica Fragrans which contains documented natural Calcium channel blocker and Verapamil on volume and acidity of Carbacol induced gastric section. Their effects were also compared to find out any difference in their efficacy. **Methods:** Thirty rabbits of local breed, weighing 1-1.5kg were used. The animals were kept on fasting for 48 hours, after which the pylorus of each animal was ligated. Verapamil 10mg/kg, Myristica fragrans 500 mg/kg and Carbachol 600µg/kg body weight were administered intraperitoneally. **Results**: It was found that extract from Myristica fragrans reduced the volume, free and total acidity of gastric secretion, which were statistically highly significant when compared with Carbachol ($P<0.001$). Verapamil had also the same effects. When the difference of mean for verapamil was compared with that of extract, all these differences were found statistically non significant indicating that extract has similar effect as that of Verapamil on all parameters included in study. **Conclusion:** The effect of Myristica Fragrans is similar to Verapamil and therefore it can be used effectively in the treatment of peptic ulcer and all other conditions that require calcium channel blockers for the treatment of these disorders.

Keywords: Myristica Fragrans, Verapamil, gastric acid secretion, Medicinal plant

INTRODUCTION

Peptic ulcer is one of the most common ailments, with which a physician comes across in the clinical practice. Increased acid production from gastric mucosa is responsible for peptic ulceration in majority of the patients. Ulcers are not found in achlorhydric patients and almost always occur in patients with Zollinger Ellison (Z.E) syndrome which is characterized by very high acid secretion.[1] Inhibition of over production of acid is a desirable therapeutic goal in the treatment of peptic ulcer. It has been documented that 38 medicinal plants including Myristica fragrans have natural calcium channel blocker activity.[2,3] The calcium channel blocking agents like Verapamil, nifedipine and diltiazem are commonly used in the treatment of hypertension, angina, myocardial infarction and supraventricular tachycardia.[4]

Induction of hypercalcaemia through intravenous administration of calcium is usually associated with increased gastric volume and acidity.[5,6] The acid stimulating ability of calcium; is well known and there is extreme sensitivity to calcium in patients with ZE syndrome.[7,8] Calcium channel blocker verapamil may interfere with H^+K^+ ATPase due to its high affinity for the K^+ site H^+K^+ATPase system which is accessible from luminal side of the stomach.[9] Histamine release from peritoneal mast cells is critically dependent upon extracellular Ca^{++}concentration, so non-availability of Ca^{++}may cause reduced effects of histamine on acid production in the stomach. Calcium channel blockers have been mainly used in CVS as inhibitors of muscle contraction. In the stomach, motility and acid secretion have been shown to be dependent upon calcium ions. So this study was planned to evaluate the effects of extract from Myristica fragrans and calcium channel blocker Verapamil on the volume and acidity of Carbachol induced gastric secretion. Their effects were also compared on these parameters.

MATERIAL AND METHODS

Thirty rabbits of local breed were selected for the present study. Healthy animals of both sexes weighing 1-1.5kg were used in the study. All the animals were kept fasting for 48 hours with free availability of water before they were subjected to experimental procedure. The animals were divided into 3 groups each containing 10 animals. Group A

was carbachol treated, Group B was Verapamil+ carbachol treated and Group C. was Myristica fragrans + carbachol treated.

The operative procedure was the one adopted by Vischer et al.[10] Animals were anaesthetized with ether, abdomen was opened and pylorus was ligated with silk suture. Then abdominal wall was closed with suture clips and intraperitoneal (IP) injection of Carbachol 600µg/Kg body weight was administered to group A, 10mg/Kg body weight of Verapamil to group B and 500 mg/Kg body weight of Myristica fragrans to group C, followed by Carbachol 600µg/Kg body weight after 15 minutes to group B and C. The rabbits were deprived of water for four hours after administration of drugs. Then the rabbits were sacrificed, the thorax and abdomen were opened, oesophagus was ligated and the stomach was removed quickly. The contents of the stomach were collected. The volume of gastric juice was measured. Then the contents were centrifuged, filtered and subjected to titration for estimation of free and total acidity by the method described by Varley.[11] One ml of centrifuged and filtered gastric secretion was titrated against 0.1 N NaOH using Topfers reagent for determination of free acidty and 1% phenoplphthalein as indicator for combined acidity. The sum of the two titrations was total acidity. The data was analyzed statistically using student's "t" test.

RESULTS

The volume, free acidity and total acidity of gastric secretion for all the three groups are shown in table-1. The reductions in groups given verapamil or Myristica Fragrans noticed for all the parameters were found to be highly significant when compared with Carbachol ($P<0.001$). When we compared the mean values of volume, free and total acidity for Verapamil and extract it was observed that these differences in all the three parameters between two groups were found to be non significant. All these changes are shown in Table-2.

DISCUSSION

Acid secretion in the stomach is controlled at a variety of levels by neural, hormonal and paracrine mechanisms. When these regulatory mechanisms malfunction acid and pepsin autodigest the mucosa resulting in the ulceration of oesophagus, stomach and duodenum.[12]

Histamine, acetylcholine or Carbachol are potent secretogogues for the paricta cels of gastric mucosa leading to the production of HCl.[11]

Acetylcholine and gastrin act through calcium ions. Carbachol being a cholinomimetic drug increases free intracellular calcium ions. Which, in turn activate protein kinase by phosphorylation and lead to increased production of HCl. In this study we observed that Myristica fragrans reduced the volume free acidity and total acidity. This is due to the calcium channel blocking activity of natural calcium channel blocker present in the extract. All these reductions were statistically highly significant when compared with the mean values in Carbachol treated group.

Table-1:Comparison between the effect of Verapamil 10 mg/kg and extract from Myristica fragrans 500mg/kg on volume and acidity of Carbachol 600µg/kg body weight induced gastric secretion in rabbits.

Drug	Volume of gastric secretion(ml)	Acidity (m.Eq./dl of gastric secretion)	
		Free	Total
Carbachol	28.7±0.650 (10)	6.39±0.408 (10)	7.64±0.408 (10)
Verapamil + Carbachol.	13.64±0.564 (10)	2.34±0.195 (10)	3.52±0.264 (10)
P. Values	*<0.001*	*<0.001*	*<0.001*
Myristica fragrans+ Carbachol	15.33±0.597 (10)	2.9±0.331 (10)	3.86±0.426 (10)
P.Values	*<0.001*	*<0.001*	*<0.001*

Each value represents mean of total observations, Figures in parenthesis indicate the number of animals in each group, P. values when compared with Carbachol.

Table-2: Differences in the volume, Free and total acidity produced by Myrstica fragrans 500 mg/kg and Verapamil 10mg/kg in Carbachol 600μg/kg body weight induced gastric secretion in rabbits.

Drug	Volume of gastric secretion(ml)	Acidity (m.Eq./dl of gastric secretion)	
		Free	Total
Verapamil+ Carbachol	13.64±0.564 (10)	2.34±0.195 (10)	3.52±0.264 (10)
Myristica fragrans+ Carbachol	15.33±0.597 (10)	2.9±0.331 (10)	3.86±0.426 (10)
P.Values	N.S	N.S	N.S

Each value represents mean of total observations, Figures in parenthesis indicate the number of animals in each group , P. values when compared with Carbachol., N.S = Non significant.

Similar reduction were observed using Verapamil. All these reductions were found to be statistically highly significant when compared with Carbachol alone. Our study is consistent with other workers who concluded that Verapamil significantly reduces gastric acid secretion.[14,15.] It is due to the fact that Verapamil, a well known calcium channel blocker inhibits the calcium influx, which may be responsible for the observed reductions in volume and acidity of gastric secretion. Beside this, Verapamil inhibits lipoxygenase pathway during metabolism of arachidonic acid. So leukotriene, the injurous substance is not formed and all the arachidonic acid is metabolized through cyclooxygenase pathway. This will lead to the production of prostaglandin which couples with Gi protein and inhibits adenyl cyclase and thus decrease HCl production[16]

Release of histamine from mast cells is critically dependent on external calcium ions, so Verapamil by blocking calcium ions can block histamine release which is a potent agent for HCl secretion.[17]

When we compared the differences in the mean values of volume, free acidity and total acidity by Myristica fragrans and Verapamil, they were all found non significant. This indicates that extract is almost as effective as Verapamil in decreasing volume, free and total acidity of gastric secretion. Verapamil is also used in controlling contraction of cardiovascular smooth muscles[18], allergic reaction[19] and prevention of premature labor.[20]

All of these actions are due to the calcium channels blocking activity.

CONCLUSION

It is concluded that the extract of Myristica Fragrans has effects similar to Verapamil and therefore may be beneficially used as a single drug therapy in patients having peptic ulcer concurrent with angina, myocardial infarction, prevention of premature labor or bronchial asthma. Further studies in this regard for evaluation of these effects are suggested in human subjects.

REFERENCES

1. Edward CRW, Bouchier IAD, Haslett C. Diseases of the stomach. In:Davidson's Principles and Practice of Medicine, Churchill Livingstone, London, 1995.p425-34.

2. Azhar I, Aftab K, Usmanghani K. Naturally occurring calcium channel blockers. Hamdard Medicus 1995;xxxviii:April-June:5-16

3. Ichikawa K, Kinoshita T, Sankawa U. The screening of Chinese crude drugs for calcium antagonistic activity. Identification of active principles from the arial parts of the pogostenum cablin and the fruits of prunus mume. Chem Pharm Bull 1989;37(2):345-8.

4. Fleckenstein A. History of calcium antagonists. Circ Res 1983;52(1):3-16.

5. Barreras RF. Calcium and gastric secretion. Gastroenterology 1973;64:1168-84.

6. Anderson JR. Diseases of the stomach. In: Muir's Text book of pathology, English Language Book Society/Edward Arnold, London, 1985, pp19.18-19.26.

7. Basso N, Materia A, Folini A, Jafe BM. Prostaglandin generation in the gastric mucosa of rats with stress ulcer. Surgery 1983;94:104-08.

8. Passaro E Jr., Basso N, Walsh JH. Calcium challenge in Zollinger- Ellision syndrome, Surgery 1972;72:60-7.

9. Nandi J, King RL, Kaplan DS, Levine RA. Mechanism of gastric proton pump inhibition by Calcium channel antagonists. J.Pharamacol Exp Ther 1990;252(3):1102-7.

10. Vischer FE, Seay PH, Tazelaar AP, Veldkamp Jr.W, Brook MJ. Pharmacology of famine Bromide. J Pharacol Expt. Ther 1954;110:188-204.

11. Varley H. Test of gastric function, occult blood. In practical clinical biochemistry, London, William Meinmann, 1962. pp 249-277.

12. Shambuerk RD, Schubert ML. Control of gastric acid secretion. Gastroenterol Clin North America 1992;21(3):527-50.

13. Negulescu PA, Matchen TE. Intracellular calcium regulation during secretogague stimulation of the parietal cells. Am J Physiol 1988;254:130-40.

14. Brogden RN, Carmine AA, Heel RC, Speight TM, Avery GS. Ranitidine: A review of its pharmacology and therapeutic use in peptic ulcer disease and other allied diseases. Drug 1982;24;267-303.

15. Brage R, Cortijo J, Esplugues JK, Esplugues J, Bomnati EM, Rondriguez. Effects of calcium channel blockers on gastric emptying and acid secretion of the rat in vivo. Br.J. Pharmacol 1986;89(4):627-33.

16. Kirkegaard P, Charistianson J, Peterson B, Olsen PS. Calcium and stimulus-secretion coupling in gastric fundic mucosa: effect of inhibition of calcium transport by verapamil on gastric acid secretion in the isolated guinea pig fundic mucosa and in healthy subjects. Scand. J. Gastroenterol 1982; 17:533-8, cited by Levine et al. Effect of verapamil on basal and pentagastrin stimulated gastric acid secretion. Clin Pharmacol Ther 1983;34(3):399-402.

17. Rogers C, Pihan G, Szabo S. Role of leukotriens in the pathogeneis of haemorrhagic gastric mucosal lesions induced by ethanol or HCL in the rat. Gastroenterol 1986;90:1797 cited by Ghanayem BI, Matthews HB, Maronpot RR. Calcium channel blockers protect against ethanol and endomethacin induced gastric lesions in rats. Gastroenterol 1987;92(I):106-11.

18. Main IHM, Pearce JB. Effects of calcium on acid secretion from the rat isolated gastric Mucosa during stimulation with histamine, pentagastrin, methacholine, and dibutyrl cyclic adenosine-3,5-monophosphate. Br, J.Pharmacol 1978; 64:359-68.

19. Flekanstein A. Specific pharmacology of calcium in pericardium, cardiac pacemaker and vascular smooth muscles. Ann Rev Pharmacol Toxicol 1977;17:149-66.

20. Latif A, Aamir K, Ali M, Siddiqi N. Deactivation of ripened uterine tissue by calcium channel blocker Verapamil.Pakistan J Pharmacol 2002;19(10):13-17.

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