ORIGINAL ARTICLE

COMPARISON OF EFFECTS OF AMMI VISNAGA EXTRACT AND VERAPAMIL ON CARBACHOL STIMULATED GASTRIC SECRETION IN RABBITS

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ABSTRACT

Background: Peptic ulcer is mostly produced due to over production of gastric acid. This study was undertaken to compare the effects of extract from fruits of medicinal plant Ammi visnaga which contains natural calcium channel blocker and verapamil on volume and acidity of carbachol induced gastric secretion.

Material & Methods: Thirty rabbits of local breed, weighing 1-1.5 kg were used. The animals were kept fasting for 48 hours, after which the pylorus of each animal was ligated. verapamil 10 mg/kg body weight, Ammi visnaga 500 mg/kg body weight and carbachol 600 mg/kg body weight were administered intraperitoneally.

Results: It was found that verapamil reduced the volume, free and total acidity of gastric secretion, which were statistically highly significant when compared with carbachol ($p<0.001$). The extract also had the same effects. When the difference of mean for verapamil was compared with that of extract, it was found statistically non-significant indicating that the extract has similar effect as that of verapamil on all parameters included in study i.e. free, combined and total acidity.

Conclusion: The extract of *Ammi visnaga* can be used effectively and safely in the treatment of hyperacidity conditions and peptic ulcer after evaluation in humans.

KEY WORDS: Ammi visnaga; Verapamil; Gastric secretion.

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INTRODUCTION

Peptic ulcer is a common disease. Increased acid production from gastric mucosa is responsible for peptic ulceration in the majority of patients. Ulcers are not found in achlorhydric patients and almost always occur in patients with Zollinger Ellison 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 Ammi visnaga have natural calcium channel blocker.[2] Khellin and visnagin were identified from Ammi visnaga fruit and were proved that all of them have calcium channel blocking mode of action.[3] Methanol extract from the fruits of Ammi visnaga showed significant calcium channel blocking activity.[4] In a study with the help of spectrophotometer and high performance liquid chromatography, it was observed that Khelin and visnagin are present in the fruits of Ammi visnaga.[5]

Carbachol being a cholinomimetic drug increases free intracellular calcium ions which, intern activate protein kinase by phosphorylation and lead to increased production of HCl. Calcium channel blocking agents like verapamil, nifedipine and diltiazem are commonly used in the treatment of hypertension, angina, myocardial infarction and supraventricular tachycardia.[6] Induction of hypercalcaemia through intravenous administration of calcium, is usually associated with increased gastric volume and acidity.[7,8] The acid stimulating ability of calcium; is well known and there is extreme sensitivity to calcium in patients with Z-E syndrome.[9,10] Calcium channel blocker Verapamil may interfere with H^+K^+ ATP ase due to its high affinity for the site H^+K^+ATP ase system which is accessible from luminal side of the stomach.[11] Histamine release from peritoneal mast cells is critically dependent upon extra cellular Ca^{++} concentration, so non-availability of Ca^{++} may cause reduced effects of histamine on acid production in the stomach. Calcium channel blockers have

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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.

This study was planned to evaluate the effects of extract from the fruits of Ammi visnaga having documented natural calcium blocker and Verapamil which is also a calcium channel blocker, on the volume and acidity of Carbachol induced gastric secretion.

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 Ammi visnaga + Carbachol treated.

The operative procedure was the one adopted by Vischer et al.[12] Animals were anaesthetized with ether, abdomen was opened and pylorus was ligated with silk suture. Then abdominal wall was closed with suture clamps and intraperitoneal (I.P) injection of carbachol 600 μg/Kg body weight were administered to group A, 10 mg/Kg body weight of verapamil to group B and 500 mg/Kg body weight of Ammi visnaga to group C, followed by carbachol 600 μg/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.[13] According to this method, One ml of centrifuged and filtered gastric secretion was titrated against 0.1N NaOH using Topfers reagent as an indicator for determination of free acidity and 1% phenolphthalein as indicator for combined acidity. The sum of the two titrations was total acidity.

The data was analyzed statistically using student "t" test. P-values less than 0.05 were considered as significant.

RESULTS

The volume, free acidity and total acidity of gastric secretion in group A (Carbachol treated group) was 28.7±0.650ml, 6.39±0.408 mEq/dl and 7.64±0.408 mEq/dl respectively. The volume, free acidity and total acidity in group B (Verapamil and Carbachol treated) was 13.64±0.564ml, 2.34±0.195 mEq/dl and 3.52±0.264 mEq/dl respectively. These reductions noticed in all the parameters were found to be highly significant in group B and C when compared with group A ($p<0.001$). Similarly the volume, free acidity and total acidity in group C (Ammi visnaga and Carbachol treated) was 13.8±0.578 ml, 2.41±0.216 mEq/dl and 3.57±0.276 mEq/dl

Table 1: Comparison of verapamil and extract from fruits of Ammi visnaga on volume and acidity of carbachol induced gastric secretion in fasting rabbits.

Drug	Volume of gastric secretion (ml)	Acidity (mEq/dl of gastric secretion)	
		Free	Total
Carbachol (10 mg/Kg)	28.7±0.650 (10)	6.39±0.408 (10)	7.64±0.408 (10)
Verapamil + Carbachol (600 +µg/kg body wt)	13.64±0.564 (10)	2.34±0.195 (10)	3.52±0.264 (10)
p-value	<0.001	<0.001	<0.001
Ammi visnaga (500 mg/kg) + Carbachol (600 µg/kg body wt)	13.8±0.578 (10)	2.41±0.216 (10)	3.57±0.276 (10)
p-value	<0.001	<0.001	<0.001

Table 2: Differences in the volume and acidity produced by 500 mg/kg of extract and verapamil in carbachol body weight induced gastric secretion in fasting rabbits.

Drug	Volume of gastric secretion(ml)	Acidity (mEq/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	NS	NS	N.S

respectively. All these reductions were also found to be statistically highly significant when compared with the group A ($p<0.001$). (Table 1) 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 groups B and C were found to be non significant. (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 autodiagest the mucosa resulting in the ulceration of oesophagus, stomach and duodenum.[14] Histamine, acetylcholine or Carbachol are potent secretogogues for the parietal cells of gastric mucosa leading to the production of HCl.[15] Acetylcholine and gastrin act through calcium ions. Carbachol being a cholinomimetic drug increases free intracellular calcium ions which, intern activate protein kinase by phosphorylation and lead to increased production of HCl. In this study we observed that Ammi visnaga 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. Similar reductions were observed using Verapamil. All these reductions were found to be statistically highly significant when compared with Carbachol alone. Our study is in consistent with other workers who concluded that Verapamil significantly reduces gastric acid secretion.[16.17.] 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. Besides, Verapamil inhibits lipoxygenase pathway during metabolism of arachidonic acid. So leukotrienes, the injurious substance is not formed and all the arachidonic acid is metabolized through cyclooxygenase pathway. This will lead to the production of prostaglandin which couple with Gi protein and inhibits adenyl cyclase and thus decrease HCl production.[18]

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.[19]

When we compared the differences in the mean values of volume, free acidity and total acidity by Ammi visnaga and Verapamil, they were all found non significant. This indicates that the 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,[20] allergic reaction[21] and prevention of premature labor.[22]

All of these actions are due to the calcium channels blocking activity. As the extract also contains calcium channel blocker, so it can also be used for the treatment of the above mentioned diseases and peptic ulcer.

CONCLUSION

It is concluded that the Ammi visnaga extract may be beneficially used for treatment of peptic ulcer & all other diseases associated with hyper gastric acidity. 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. p. 425-34.
2. Azhar I, Aftab K, Usmanghani K. Naturally occurring calcium channel blockers Hamdard Medicus, 1995; 38:5-16.
3. Rauwald HW, Brehm O, Odenthal KP. The involvement of a calcium channel blocking mode of action in the pharmacology of Ammi visnaga fruits. Planta Med 1994; 60:101-5.
4. Rauwald HW, Brehm O. Screening of some medicinal plants for their possible calcium antagonistic activity. Planta Med 1991; 57:A59-A60.
5. Domiaty-el MM. Improved high-performance liquid chromatographic determination of khellin and visnagin in Ammi visnaga fruits and pharmaceutical formulations. J Pharm Sci 1992;81:475.
6. Fleckenstein A. History of calcium antagonists. Circ Res 1983; 52:3-16.
7. Barreras RF. Calcium and gastric secretion. Gastroenterology 1973; 64:1168-84.
8. Anderson JR. Diseases of the stomach. In: Muir's Textbook of Pathology, English Language Book Society /Edwardarnold, London, 1985, p.19.18-19.26.
9. Basso N, Materia A, Folini A, Jafe BM. Prostaglandin generation in the gastric mucosa of rats with stress ulcer. Surgery 1983; 94:104-8.
10. Passaro E Jr, Basso N, Walsh, JH. Calcium challenge in Zollinger Ellision syndrome. Surgery 1972; 72:60-7.
11. Nandi J, King RL, Kaplan DS, Levine RA. Mechanism of gastric proton pump inhibition by calcium channel antagonists. J Pharamacol Exp Ther 1990; 252:1102-7.
12. Vischer FE, Seay PH, Tazelaar AP, Veldkamp Jr W, Brook MJ. Pharmacology of famine bromide. J.Pharmacol. Expt Ther 1954; 110:188-204.
13. Varley, H. Test of gastric function, occult blood. In: Practical Clinical Biochemistry, London, Willium Meinmann, 1962. p 249-77.

14. Shambuerk RD, Schubert ML. Control of gastric acid secretion. Gastroenterol Clin North Am 1992; 21:527-50.

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

16. 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.

17. 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; 89:627-33.

18. 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.

19. Rogers C, Pihan G, Szabo S. Role of leukotrienes in the pathogeneis of haemorrhagic gastric mucosal lesions induced by ethanol or HCL in the rat. Gastroenterol 1986; 90:1797.

20. 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.

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

22. Latif A, Aamir K, Ali M, Siddiqi N. Deactivation of ripened uterine tissue by calcium channel blocker Verapamil. Pak J Pharmacol 2002; 19:13-7.

CONFLICT OF INTEREST
Authors declare no conflict of interest.
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None declared.