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COMPARISON BETWEEN THE EFFECTS OF EXTRACT FROM MEDICINAL PLANT OLEA EUROPEA AND VERAPAMIL ON STIMULATED GASTRIC SECRETION IN FASTING RABBITS

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ABSTRACT

Background: Peptic ulcer is mostly produced due to the over production of gastric acid. This study was designed to find out the effects of extract from the leaves of medicinal plant Olea europea (which contains documented natural Calcium channel blocker) and Verapamil on volume and acidity of Carbachol 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 fasting for 48 hours, after which the pylorus of each animal was ligated. Verapamil 10 mg/kg, Olea europea 500 mg/kg and Carbachol 600 g/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, all these differences were found statistically significant($p<0.05$) indicating that the extract has less effect as compared to Verapamil on all parameters included in the study.

Conclusion: The extract from the leaves of Olea europea therefore, may be used effectively in the treatment of peptic ulcer and all other conditions like angina, myocardial infarction, hypertension, prevention of premature labor and bronchial asthma which require calcium channel blockers for the treatment of these disorders.

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 Olea europea have natural calcium channel blocker effect [2]. Thirty percent ethanol extract from the leaves of Olea europea has significant calcium channel blocking activity [3]. The calcium channel blocking agents like Verapamil, nifedipine and diltiazem are commonly used in the treatment of hypertension, angina, myocardial infarction and supraventricular tachyeardia [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 Z-E syndrome [7,8]. 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 [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 cardio vascular disease (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 the leaves of Olea europea 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 1-1.5 kg 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 Olea europea+ Carbachol treated and Group C was Verapamil + Carbachol treated.

The operative procedure was the one adopted by Vischer et al. (1954) [10]. 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 600µg/Kg body weight were administered to group A, 500mg/Kg body weight of Olea europea to group B and 10 mg/Kg body weight of Verapamil 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, thorax and abdomen were opened, esophagus 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 (1962) [11] One ml of centrifuged and filtered gastric secretion was titrated against 0.1 N NaOH using Topfer,s 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.

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 m.Eq./dl and 7.64±0.408 m.Eq./dl respectively. The volume, free and total acidity in group B was 16.5±0.763 ml, 3.15±0.375 m.Eq. /dl and 4.02±0.353 m.Eq./dl respectively.

Similarly the volume, free and total acidity in group C, was 13.64±0.564ml 2.34±0.195m.Eq./dl and 3.52±0.264m.Eq./dl respectively. These reductions noticed in all parameters were found to be highly significant when compared with Carbachol ($P<0.001$). All these changes are shown in Table 1.

Table 1

Effects of extract from the leaves of *Olea europea* and Verapamil on the volume and acidity of gastric secretion induced by Carbachol in fasting rabbits. *Carbachol* was injected 600 µg/kg body weight and Olea *europea* 500 mg/kg body weight intraperitoneally,

Drugs	Volume of gastric secretion (ml)	Acidity (m.Eq/100 ml of gastric secretion)	
		Free	Total
Carbachol	28.7±0.650 (10)	6.39±0.408 (10)	7.64±4.408 (10)
Olea europea – Carbachol	16.5±0.763 (10)	3.15±0.375 (10)	4.02±0.353 (10)
Verapamil–Carbachol	*13.64*±0.574 (10)	2.34±0.195 (10)	3.52±0.264 (10)
P Values	<0.001	<0.001	<0.001

Each value indicates mean of the total observation
Figures in parenthesis indicate the number of animals in each group.
± Indicates standard err
P Value between Carbachol and *Olea europea*

When we compared the mean values of volume, free and total acidity for extract and Verapamil, it was observed that these differences in all the three

parameters between groups Band C were found to be significant($P<0.05$). All these changes are shown in Table 2.

Table 2

Shows Comparison between the effects of extract from *Olea europea* and the drug Verapamil on the volume and acidity of gastric secretion induced by Carbachol in fasting rabbits. *Carbachol* was injected 600 µg/kg body weight, *Olea europea* 500 mg/kg and Verapamil 10 mg/kg body weight intraperitoneally.

Drugs	Volume of gastric secretion (ml)	Acidity (m.Eq/100 ml of gastric secretion)	
		Free	Total
Olea europea, Carbachol	16.5±0.763 (10)	3.15±0.375 (10)	4.02±0.353 (10)
Verapamil Carbachol	13.64±0.574 (10)	2.34±0.195 (10)	3.52±0.264 (10)
P Values	P>0.05	P>0.05	P>0.05

Each value indicates mean of the total observation.
Figures in parenthesis indicate the number of animals in each group.
± Indicates standard error
P Value between *Olea europea* And Verapamil.

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[12]. Histamine, acetylcholine or Carbachol are potent secretogogues for the parietal cells of gastric mucosa leading to the production of HCl[13]. 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 Olea europea reduced the volume free 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 [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. Besides, Verapamil inhibits lipoxygenase pathway during metabolism of arachidonic acid. So leukotrienes, the injurious substance are 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 and total acidity by Olea europea and Verapamil, they were all found significant. This indicates that the extract can block gastric acidity significantly but it is less effective as compared to 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. 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 extract may have role as a single drug therapy in patients having peptic ulcer alone or concurrent with angina, myocardial infarction, prevention of premature labor & bronchial asthma. Large scale studies in human volunteers are recommended to accurately find out the role of this medicinal plant

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