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RESEARCH ARTICLE

COMPARISON BETWEEN THE EFFECTS OF THYMOQUINONE OBTAINED FROM THE SEEDS OF *NIGELLA SATIVA* AND VERAPAMIL ON THE VOLUME AND ACIDITY OF GASTRIC ACID SECRETION

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ABSTRACT

Background and Objectives: The over production of gastric acid results in peptic ulcer. This study was done to compare the effects of thymoquinone and Verapamil on volume and acidity of carbachol induced gastric secretion.

Methods: There were 24 rabbits used, weighing 1-1.5 kg. The rabbits were kept on fasting for 48 hours. After fasting, the pylorus of each rabbit was ligated. Thymoquinone 5 mg/kg, Carbachol 600µg/kg and Verapamil 10 mg/kg body weight were administered intraperitoneally. Pylorus ligation method was used for getting gastric contents and titration method was used for finding out acidity.

Results: Verapamil has been proved very effective for the treatment of many diseases. The drug verapamil inhibits the release of histamine, acetylcholine and gastrin. Verapamil has also shown effects in reducing the secretion of gastric acid. It was found that Thymoquinone reduced the volume, free and total acidity of gastric secretion, which were statistically highly significant when compared with Carbachol (P=0.000) but when we compared the results of Thymoquinones with that of Verapamil, it was non-significant.

Conclusions: It was concluded that Thymoquinone can be used for the treatment of peptic ulcer and all other diseases which are caused by increased gastric acidity like dyspepsia, gastritis and reflux esophagitis.

Keywords: Gastric secretion, Thymoquinone, Verapamil.

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INTRODUCTION

In clinical practice, peptic ulcer is one of the most common medical complain. In majority of the patients, peptic ulcer is caused by elevated acid production from gastric mucosa. In patients who are achlorhydric, ulcers are not found. Ulcers mostly occur in Zollinger-Ellison (Z.E) syndrome which is caused by excess acid secretion¹. The goal of treatment of peptic ulcer is to inhibit the over production of acid. *Nigella sativa* is a part of the botanical family of Ranunculaceae. It is commonly cultivated in Europe, Middle East and Western Asia. All over the world, it is called by many names like habbat al-baraka or Kali jeera. In the light of Hadith "Use this Black seed, it has a cure for every disease except death" (Sahih Bukhar), The *Nigella sativa* (*N. sativa*) seeds, are mostly used in many Arab countries like Saudi Arabia, Middle East as a natural

remedy for many diseases. *Nigella sativa* seeds contain many active ingredients, the most important of which is thymoquinone (nigellone)². Because of the large variety of uses of *Nigella sativa*, many researchers have conducted various *in vitro* and *in vivo* studies on laboratory animals and human beings in order to find out their pharmacological activities. These include anti-inflammatory³, analgesic, anti-pyretic activity⁴, antimicrobial^{5,6,7,8}, antifungal^{9,10,11}, hypoglycemic¹² and anti-tuberculosis¹³.

Thymoquinone administration can prevent and improve the murine-DSS (dextran sodium sulfate) induced colitis. It could also serve as an effective therapeutic agent for the treatment of patients with inflammatory bowel disease. It also helps to prevent colitis and diarrhea in patients¹⁴.

MATERIALS AND METHODS

The active ingredient thymoquinone was obtained from Amidis chemical company PVT limited, China. Thymoquinone was extracted from the *Nigella sativa* plant by the company itself.

Source of chemical:

The chemical verapamil was purchased from VPL chemicals, private limited India.

Method

Twenty-four rabbits of local breed were selected for this study. The animals were healthy and were of both sexes. All the chemicals were administered through intraperitoneal route according to the body weight of the animals. The animals were not given any food for 48 hours. Only there was free availability of water before they were administered the drugs. The animals were separated into 3 groups each group had 8 animals. Group 1 was administered Carbachol. The dose of carbachol was 600 µg/kg body weight, Group 2 was administered Thymoquinone with a dose of 5mg/kg and group 3 was given Verapamil. The dose of verapamil was 10 mg/kg body weight. After 15 minutes Carbachol with a dose of 600 µg /kg body weight was administered to Group 2 and 3. The gastric juice was obtained from all rabbits by the method of pylorus ligation, as explained by Vischer *et al.*,¹⁵. The anesthesia was given with ether in a big glass desiccator, and all the animals were weighted. The abdomen was cut by an incision in mid-line and the

pylorus was disconnected with silk suture. Suture clamps were used to close the wall of abdomen.

The inhibitory effect of the drug was better understood after the stimulation produced by carbachol. When the anesthesia was stopped, the animals became conscious. After a period of 4 hours, all the animals were slaughtered, their abdomen was again opened and the cardiac end of the stomach was ligated. It was cut from both ends outside the knot. The incision was given to stomach at greater curvature. The gastric juice was finally procured and was titrated against 0.1 N NaOH solution by the procedure explained by Varley¹⁶. This procedure is being performed since 1954, for the calculation of all forms of acidity i.e. free, combined and total. In this process, one ml of centrifuged gastric juice is titrated against 0.1 N NaOH using Topfer's reagent as an indicator for calculation of free acidity and 1% phenolphthalein as a standard for combined acidity. The acidity of the gastric juice was determined by using the normality equation formula-

$$N_1V_1 = N_2V_2$$

Where, N1 is the normality of unknown acid/base, N2 is normality of known acid/base, V1 is volume of unknown acid/base and V2 is volume of known acid/base. Total acidity was calculated by the sum of the two titrations. The data obtained was subjected to statistical analysis for any significance. The data was entered into SPSS-IBM Version 19. P value of <0.05 was considered to be statistically significant.

Table 1: Effects of Thymoquinone and Verapamil on the volume and acidity of gastric secretion induced by Carbachol in fasting rabbits. (P=0.000)

Drugs	Volume of gastric secretion (ml)	Acidity (meq/dl of gastric secretion)	
		Free	Total
Carbachol	28.125±2.031	6.225±1.188	7.650±1.243
Thymoquinone +Carbachol	13.625±1.355	2.412±.626	3.750±.8
P Values (When compared with carbachol)	0.000	0.000	0.000
Verapamil+ Carbachol	13.212±1.501	2.200±.575	3.575±.497
P Values (When compared with Carbachol)	0.000	0.000	0.000

Each value indicates mean of the total observation. P Value between Carbachol and drugs+ Carbachol

RESULTS

The volume, free acidity and total acidity in group 1 were 28.125±2.031 ml, 6.225±1.188 m.Eq./dl and 7.650±1.243 m.Eq./dl respectively. Similarly, the mean values for volume, free acidity and total acidity of gastric secretion in group 2 (Thymoquinone+ Carbachol treated group) were 13.625±1.355ml, 2.412±.626 m.Eq./dl and 3.750±.833 m. Eq./dl respectively. There was a reduction in all the parameters and was found to be highly significant when compared with Carbachol group (P=0.000). All these changes are shown in Table 1. Likewise, the

mean values for volume, free acidity and total acidity of gastric secretion in group 3 (Verapamil + Carbachol treated group) were 13.212±1.501 ml, 2.200±.575 m.Eq./dl and 3.575±.497m.Eq./dl respectively. There was a reduction in all the parameters and was found to be highly significant when compared with Carbachol group (P=0.000). All these changes are shown in Table 1. Similarly, when we compared the mean values for volume, free and total acidity of Verapamil+ Carbachol group to those of Thymoquinone + Carbachol group, p values were 0.392, 0.204, 0.412. All these changes are non-significant and shown in Table 2.

Table 2: Comparison between the effect of Thymoquinone and Verapamil on the volume and acidity of gastric secretion induced by Carbachol in fasting rabbits.

Drugs	Volume of gastric secretion (ml)	Acidity (m.Eq/dl of gastric secretion)	
		Free	Total
Thymoquinone+Carbachol	13.625±1.355	2.412±.626	3.750±.833
Verapamil+Carbachol	13.212±1.501	2.200±.575	3.575±.497
P values	0.392	0.204	0.412

Each value indicates mean of the total observation. P Value between Thymoquinone+Carbachol and Verapamil+Carbachol

DISCUSSION

Nigella sativa seed and its constituents are mostly utilized as a natural cure for many diseases. A lot of scientific work has been conducted to determine the pharmacological activities of this plant. Most of the research has established its importance in traditional medicine as an analgesic, anti-inflammatory, anti-oxidant, anti-cancer, anti-microbial, anti-parasitic, antihypertensive and as an immune booster. The main neurotransmitters/hormones that directly increase the secretion by the gastric organs are acetylcholine, gastrin and histamine¹⁷. The stimulation of these neurotransmitters depends on the influx of Ca ions¹⁸. The increase in gastric volume and acidity is due to the intravenous administration of calcium which results in hypercalcemia¹⁹. During an *in vitro* study, it was explained that *Nigella sativa*, successfully stopped the release of histamine from mast cells, through lowering the intracellular calcium and blockage of protein kinase C. In a study relating to hypertensive rats, it was found that *Nigella sativa* extract produced a significant hypotensive effect when compared to that of 0.5 mg/kg/day of oral calcium channel blocker nifedipine²⁰. *Nigella sativa* antagonized methacholine induced contractions of isolated guinea-pig tracheal chain²¹. This study explained the anticholinergic effect of *Nigella sativa* which could be the reason for inhibiting the gastric acid secretion. Further, it was found that this study is reported to show significant response as concluded by other scientists who found that calcium channel blocker Verapamil significantly reduces gastric acid secretion^{22,23}. Calcium channel blockers block the calcium influx, which causes a reduction in volume and acidity of gastric juices. Also, the lipoxygenase pathway, a step-in metabolism of arachidonic acid, is also blocked by calcium channel blockers. As a result, the leukotrienes, the harmful substance is not formed and all the arachidonic acid is metabolized by cyclooxygenase pathway. This cause the production of prostaglandin which couples with guanine nucleotide binding protein (Gi protein) and blocks the adenylyl cyclase and thus decreases the gastric secretion²⁴.

Release of histamine from mast cells is critically dependent on external calcium ions, so by blocking calcium ions can block the release of histamine. Histamine is an important factor for increasing the gastric acid secretion²³. In this study it was investigated that thymoquinone, which is obtained from *Nigella sativa* showed maximal reduction in gastric secretion and acidity. This study can be correlated to the study done by El-Dakhkhani *et al.*,²⁵. El-Dakhkhani *et al.*, observed the effect of *Nigella sativa* oil on HCL secretion and ethanol-induced ulcer in rats. The gastroprotective and anti-secretory effects of *N. sativa* seed powder significantly reduced gastric secretion, volume, pH and gastric acid-output. This was explained as the acid reducing effect of thymoquinone is due to its antihistaminic effect. Histamine is the main stimulus for histamine release that cause high gastric hydrochloric acid secretion leading to peptic ulcer, gastritis etc. Thymoquinone inhibits histamine, also increase gastric mucous

secretion which is protective to stomach²⁶. Significant increase in mucin content, glutathione level as well as a significant decrease in mucosal histamine content and ulcer formation. From above discussion it is clear that thymoquinone obtained from the seeds of *Nigella sativa* can significantly reduce the gastric acidity by blocking the release of histamine from histamine H2 receptors. It also contains calcium channel blocking activity which reduces the release of acetylcholine and histamine.

CONCLUSION

It is concluded that thymoquinone obtained from the *Nigella sativa* seeds may be effectively used in patients with diseases of peptic ulcer and other medical conditions caused by excess secretion of HCL. For the evaluation of these effects, further experiments should be done in human subjects. Current study concluded that thymoquinone significantly decreased the Carbachol stimulated acid secretion. Further work on the mechanism of action is suggested.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

AUTHOR'S CONTRIBUTION

The manuscript was carried out, written and approved in collaboration with all authors.

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