Original Research Article

Comparison between the effects of Thymoquinone obtained from the seeds of Nigella Sativa and Verapamil on volume and acidity of stimulated gastric secretion

Abstract:
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. It has been proved effective scientifically for many ailments. It inhibits the release of histamine, acetylcholine and gastrin and reduces secretion of gastric acid. There were 30 rabbits used of local breed, 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 & Verapamil 10 mg/kg body weight were administered intraperitoneally. Pylorus ligation method was used for getting gastric contents & titration method was used for finding out acidity.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. It was concluded that Thymoquinone can be used for the treatment of peptic ulcer, dyspepsia, gastritis and reflux esophagitis which are due to hyper gastric acidity.
Key words: Thymoquinone, Verapamil, Gastric secretion

Introduction:

In clinical practice, peptic ulcer is one of the most common medical condition which a physician encounter. In majority of the patients, peptic ulcer is caused by increased 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 secretion 1. The goal of treatment of peptic ulcer is to inhibit the excess production of acid.

Nigella sativa belongs to the botanical family of Ranunculacae. It commonly grows in Europe, Middle East and Westren Asia. In different countries it is called by different names for example, habbat al-baraka, Kali jeera. In the light of Hadeth*"Use this Black seed, it has a cure for every disease except death" (Sahih Bukhar),* The *Nigella sativa* (*N. sativa*) seeds, are frequently used in Saudi Arabia, Middle East and many other countries since ancient times as a natural remedy for many ailments. Nigella sativaseedscontains many active ingredientsincluding thymoquinone(Nigellone)[2]. Keeping in view, multiple uses of N.sativa many investigators conducted various in vitro & in vivo studies on laboratory animals & human beings in order to know their pharmacological actions. These include anti-inflammatory [3] Anti-inflammatory, analgesic and anti-pyretic activity[4] antimicrobial[5,6,7,8.]Antifungal[9,10,11]. Hypoglycemic effects[12], antituberculous[13.] TQ administration can prevent and improve murine DSS-induced colitis. It couldalso serve as a potential therapeutic agent for the treatment of patients with inflammatory bowel disease. It prevents colitis & diarrhea[14].

Materials & Methods

Twenty-four rabbits of local breed were selected for the present study. Healthy animals of both sexes were used in the study. All the agents were injected intraperitoneally (I.P) on the basis of per Kg body weight. All the rabbits 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 8 animals. Group 1 was treated with Carbachol 600 µg /kg body weight, Group 2 with Thymoquinone 5mg/kg & group 3 with Verapamil 10 mg/kg body weight. After15 minutes Carbachol 600 µg /kg body weight was injected to Group 2 & 3. Gastric juice was obtained from each rabbit by pylorus ligation method described by Vischer et al[15]. Animals were anaesthetized with ether in a big gloss desicator, weight was found out. Abdomen was opened by

a mid-line incision and pylorus was isolated & ligated with silk suture. Then abdominal wall was closed with suture clamps.

This enabled us to know about the inhibitory effect of the drug after stimulation by Carbachol. After termination of anesthesia, animals regained consciousness. After 4 hours, each animal was slaughtered, abdomen was reopened, cardiac end of the stomach was ligated & was cut from both ends outside the knot. Incision was given to stomach at greater curvature.

The gastric juice thus obtained was titrated against o.1 N NaOH solution by the method described by Varley [16]. For calculation of free, combined & total acidity. This method is being used successfully by various researchers since 1954. According to this method, one ml of centrifuged gastricjuice was titrated against 0.1 N NaOH using Topfer,s reagent as an indicator for determination of free acidity and 1% phenolphthalein as an indicator for combined acidity. Acidity of the gastric juice was calculated by using the formula NIVI=N2V2.

Total acidity was the sum of the two titrations. The data thus 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.

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) were13.625±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 and3.575±.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 & 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 & shown in Table 2.

Table 1

Effects of Thymoquinone &Verapamil on the volume and acidity of gastric secretion induced by Carbachol in fasting rabbits. Carbachol was injected 600 µg/kg body weight Thymoquinone 5 mg/kg & Verapamil 10 mg /kg body weight. All the drugs were injected intraperitoneally(I P).

Drugs	Volume of gastric secretion (ml)	Acidity (m.Eq/dl of gastric secretion)	
		Free	Total
Carbachol	28.125±2.031 (8)	6.225±1.188 (8)	7.650±1.243 (8)
Thymoquinone ₊Carbachol	13.625±1.355 (8)	2.412±.626 (8)	3.750±.833 (8)
P Values (When compared with carbachol)	0.000	0.000	0.000
Verapamil+ Carbachol	13.212±1.501 (8)	2.200±.575 (8)	3.575±.497 (8)
P Values (When compared with Carbachol)	0.000	0.000	0.000

Each value indicates mean of the total observation.
Figures in parenthesis indicate the number of animals in each group.
± Indicates standard deviation
P Value between Carbachol & drugs+ Carbachol

Table 2
Comparison between the effect of Thymoquinone & Verapamil on the volume and acidity of gastric secretion induced by Carbachol in fasting rabbits. Carbachol was injected 600 µg/kg body weight Thymoquinone 5 mg/kg & Verapamil 1o mg /kg body weight. All the drugs were injected intraperitoneally(I P).

Drugs	Volume of gastric secretion (ml)	Acidity (m.Eq/dl of gastric secretion	
		Free	Total
Thymoquinone ₊Carbachol	13.625±1.355 (8)	2.412±.626 (8)	3.750±.833 (8)
Verapamil+ Carbachol	13.212±1.501 (8)	2.200±.575 (8)	3.575±.497 (8)
P values	0.392	0.204	0.412

Each value indicates mean of the total observation.
Figures in parenthesis indicate the number of animals in each group.
± Indicates standard deviation
P Value between Thymoquinone + Carbachol & Verapamil + Carbachol

Discussion

Nigella sativa seed and its components are frequently used as a natural remedy for many ailments. A lot of work has been done to evaluate the pharmacological basis of their uses. Most studies confirm its value in folk medicine as analgesic, anti-inflammatory,anti-oxidant, anti-cancer, anti-microbial, anti-parasitic, antihypertensive and as an immune stimulant.

The basic neurotransmitters or hormones that directly stimulate secretion by the gastric glands are acetylcholine, gastrin and histamine [17].

The release of acetylcholine, histamine and gastrin is dependent upon Ca ions influx [18]

The increase in gastric volume and acidity is due to the intravenous administration of calcium which results in hypercalcaemia[19].

In an *in vitro*study, it was demonstrated that *N. sativa*, effectively inhibited the release of histamine from mast cells, possibly through decrease in intracellular calcium and inhibition of protein kinase C.

According to a research*N. sativa* extract produced a significant hypotensive effect in spontaneously hypertensive rats comparable to that of 0.5 mg/kg/day of oral calcium channel blocker nifedipine [20].

N. sativa antagonized methacholine induced contractions of isolated guinea-pig tracheal chain [21]

This shows that Nigella sativa has also anticholinergic activity which could be the cause of anti-gastric secretory function.

Our study is in consistent with other workers who concluded that calcium channel blocker Verapamil significantly reduces gastric acid secretion[22,23].

Calcium channel blockersinhibit the calcium influx, which may be responsible for the observed reductions in volume and acidity of gastric secretion. Besides, calcium channel blockers block the lipoxygenase pathway during metabolism of arachidonic acid. So, leukotrienes, the injurious substance is not produced 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 the adenyl cyclase and thus reduces the HCl production[24]

Release of histamine from mast cells is critically dependent on external calcium ions, so by blocking calcium ions can inhibit, histamine release which is a potent agent for HCl secretion [23.]

In our study we observed that thymoquinone, obtained from Nigella sativa significantly reduced gastric secretion & acidity. Our study is in agreement with that of El-Dakhakhani et al [25]. They observed effect of *N. sativa* oil on gastric secretion and ethanol-induced ulcer in rats. Significant increase in mucin content, glutathione level as well as a significant decrease in mucosal histamine content and ulcer formation.

In our study we found out that thymoquinone significantly reduced Carbachol stimulated gastric acidity. From above discussion it is clear that thymoquinone obtained from the seeds of Nigella sativa can significantly decrease gastric acidity either by decreasing histamine release or blocking histamine H_2 receptors. It is also having calcium channel blocking activity which reduce the release of acetylcholine & histamine. Further work on the mechanism of action is suggested.

Conclusion.

It is concluded that this extract may be effectively used in patients having peptic ulcer & other diseases related to hyper gastric acidity conditions. For the evaluation of these effects, further experiments should be done in human subjects.

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