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ANTIDIARRHEAL EFFECTS OF ROSEMARY OIL *(ROSEMARINUS OFFICINALIS)*

Muhammad Ibrar 1, Mansoor Ahmad2, Mehjabeen3, Noor Jahan3 and Kamran Ahmad Chishti4 *I Department* of *Pharmacy, University of Peshawar, Peshawar, Pakistan* 2 *Research Institute* of *Pharmaceutical Sciences, Department* of *Pharmacognosy, Faculty* of *Pharmacy, University of Karachi, Karachi-75270, Pakistan 3Department of Pharmacology, Faculty* of *Pharmacy, Faculty of Pharmacy, University (of Karachi, Karachi-75270, Pakistan. 4Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Faculty o/Pharmacy, University* of *Karachi, Karachi-75270, Pakistan*

ABSTRACT: The aim of this study was to investigate the antidiarrheal effect of pure volatile oil from the leaves of *Rosemarinus of jicinalis* (RO). *In vitro* experiments were performed by using the small intestine (ileum, jejunum) of rabbit. The results showed the dose dependent relaxing activity of intestine, which was found maximum at the dose of 25 mg/ml. It is also found that the extract exhibited both muscarinic and histaminic receptor blocking effect in comparison with control and standard drugs. When the tissue activity was blocked by calcium blocker (Verapamil I *flM)* and atropine I x lOA M and then treated with RO in a dose of 20 mg/m'. It showed further decrease in the activity in a cumulative way. Similarly upon blocking the adrenergic and muscarinic receptor the inhibitory effect of drug was also observed. The purpose of this work is to study the beneficial use of this oil in OIT disorder specifically as an antidiarrhoeal agent. Inhibition of smooth muscle contraction through the muscarinic and histaminic receptor and antagonist effect of the test drug exhibited a useful effect in the intestinal disorder.

INTRODUCTION

The Rosemary oil distilled from fresh herb of *Rosemarinus officinalis* contains I, 8-cineole, Upinine & camphor in 50-30-20% ratio. It is used in wide range of perfumery & cosmetic products. Rosemary is an evergreen woody shrub with aromatic, needle-like leaves and gray, scaly bark (Plouzek *et a1.l999).* Rosemary bushes can grow up to 6 ft (1.8 m) tall with a spread of 4-5 ft (1.2-1.5 m). The plants stay smaller in pots. The leaves resemble needles and are about 2.5 cm long with a pungent fragrance, somewhat reminiscent of pine. The flowers appear in winter and spring, are pale blue, about 2.5 cm long, and arranged in clusters of 2 or 3. Rosemary was originally from the Mediterranean region, where it grows ill dry, sandy or rocky soils in a climate characterized by warm summers and mild, dry winters. Rosemary leaves and flowers contain a volatile oil that increases blood flow just beneath the skin. Rosemary oils are known to have antibacterial properties. For centuries herbalists have prescribed rosemary for curing dozens of maladies. Most of these medicinal uses have not been verified by modern science, but probably many are effective. Rosemary leaves extracts has highly active antioxidants (Carnosic acid 20% and Carnosol 10%). Its aromatic oils are said to stimulate blood flow to the brain. The main constituents include camphor, cineole and verbenone. It is used in acne, alopecia, aphrodisiac, arterioscleosis, arthritis, asthma, bronchitis, bruises, cellulite, chilblains, circulatory stimulant, colds, constipation, cramps, cystitis, dandruff, decongestant, depression, dermatitis, digestive, disinfectant, diuretic, diverticulosis, dry skin, ear infections, fatigue, fainting, fibrositis, gout, hair loss, hangovers, headaches, immunity stimulant, influenza, inguinal hernia, insect repellent, lice, lower abdominal pain, lumbago, mature skin, muscular dystrophy, sinusitis, vaginal infections, varicose veins (Staff, 1970).

It is antiinfectious, antiseptic, antifungal, antirheumatic, antimicrobial, analgesic, antioxidant, antispasmodic, antidepressant, bactericidal, hypertensive, cicatrizant, cephalic, cytophylactic, decongestant, expectorant, fungicidal, insecticidal, rubefacient, circulatory and nervous system stimulant, mucolytic (Staff, 1970).

MATERIAL AND METHOD

Extraction of crude extract of rosemary oil

The fresh plant material of *Rosemarinus officinalis* (Labiatae), was collected from Sawat, and cultivated in botanical garden of Department of Pharmacy, University of Peshawar. The pure volatile oil was

Table I
Dose Related Response of Crude Extract of Rose Mary Oil

DOSE (mg/ml)	Control (cm)	Response (cm)	% of response	t- value
01	0.8 ± 0.058	0.57 ± 0.033	28.75	3.43 *
05	0.6 ± 0.033	0.53 ±0.033	11.67	1.48
10	1.03 ± 0.088	0.73 ±0.134	29.12	1.86
15	1.1 ± 0.058	0.73 ±0.058	33.63	3.36 *
20	1 ± 0.058	0.5 ±0.058	50	6.098 **
25	0.9± 0.153	0.27±0.121	70	16.58 **

The results are expressed in :t S.E.M, at P < 0.05 *significant, ** highly significant

obtained through steam distillation method, according to standard procedure given in USP. It was stored in air tight container atthe temperature of 25 :t *2°C.*

Animals: Rabbits of either sex (1-1.5 kg) were obtained from the laboratory animal house of the Department of Pharmacy, University of Karachi. They were kept at 26 :t *2°C* and relative humidity 45-55% under the light and dark cycle of 12 h each for 8-10 days before starting the experiment. Animals were provided a standard pellet diet and waJ:er *ad libitum.* The food was with drawn 10-12 h before the experiment only water was allowed (Principle, 1985).

Standard drugs: The chemicals used were acetylcholine hydrochloride (Sigrr,a), atropine (Boehringer Sohn Ingelheim), Histamine (Sigma), Verapamil (Abbott) and Adrenaline (Sigma).

Preparation of isolated smooth muscle: The rabbit was killed by a blow on the head followed by the incision of the abdomen. Immediately remove the segments of intestine from the sacrificed animal and follow the method of Magnus (1904). The tissue about 2 cm long was mounted in a 20 fl11 organ bath containing Tyrode's solution, maintained at 37 :t *1°C* and continuously bubbled with oxygen. The segments of intestine were allowed to equilibrate for 30 min. in an organ bath, under the resting tension. The isotonic movement of the intestine was recorded on an oscillograph. The response of the drug was recorded after the each time addition of 0.5 ml of the extract solution or standard drug.

Investigation of drug activity: The tissues were stabilized and normal rhythmic contraction and relaxation patterns of the intestine were recorded, then the activity of the. drug was investigated from the lowest concentration, at which the effect of the drug is started up, to the maximum effect of the drug is observed. Further investigation was carried out to explore the possible mechanism of action of drug. For this the test drug was treated with different standard drugs. Each time the fresh tissue was used to expose the combine effect of test drug and standard drugs. Acetylcholine and histamine produced contraction, where as atropine, adrenaline and verapamil caused a relaxation of intestine or inhibit the intestinal activity.

Statistical analysis: The experimental results were expressed as mean :t S.E.M. The student *t-test* method was used for calculation and determination of significant value i.e. P < 0.05.

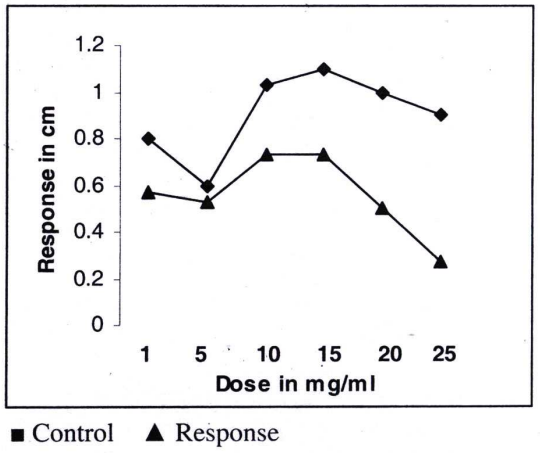

Figure 1: Relaxant effect of different doses of *R. officinalis*

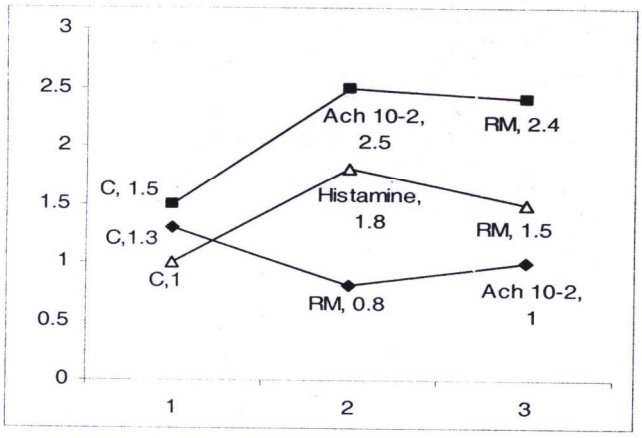

Figure 2: Effect of Crude Extract of Rosemary oil with Acetylcholine and Histamine

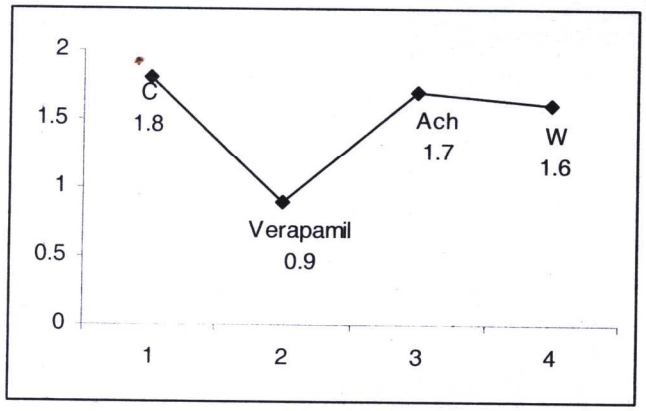

Figure 3: Response of drug after the treatment of tissue with Verapamil and Acetylcholine

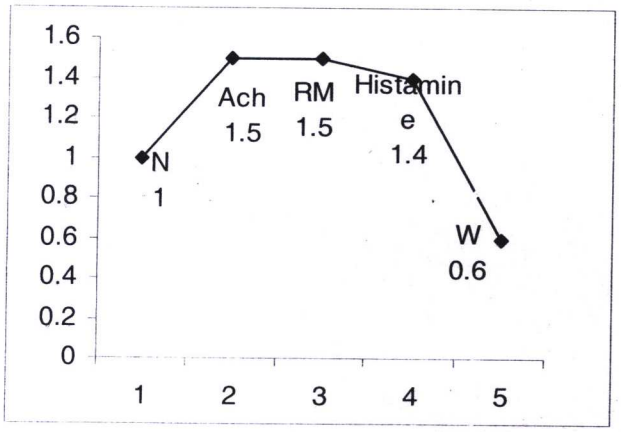

Figure 4: Shows the effect of the drug with pretreated tissue with Ach 1x 10^{-4} and post treated with Histamine 0.1 mg/ml

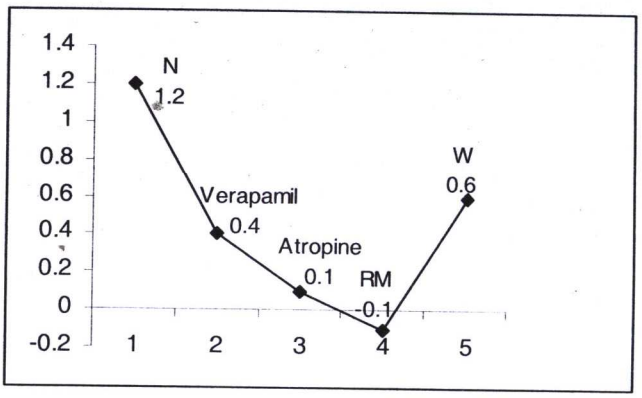

Figure 5: Cumulative relaxing effects of Rosemary oil, Pretreated with Verapamil 1μM and Atropine 1 x 10^{-2}.

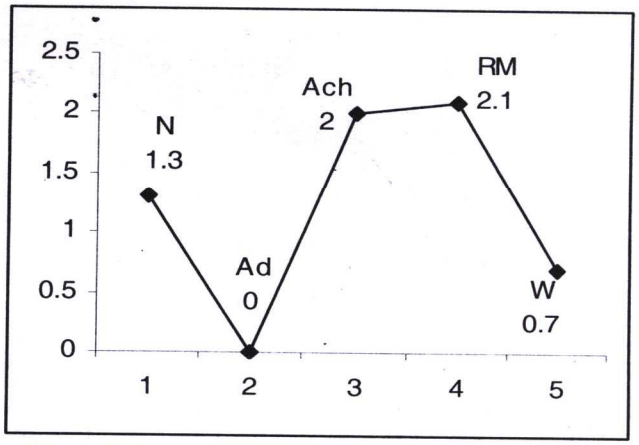

Figure 6: Effect of Rosemary oil after blocking the Adrenergic and Muscarinic Receptor.

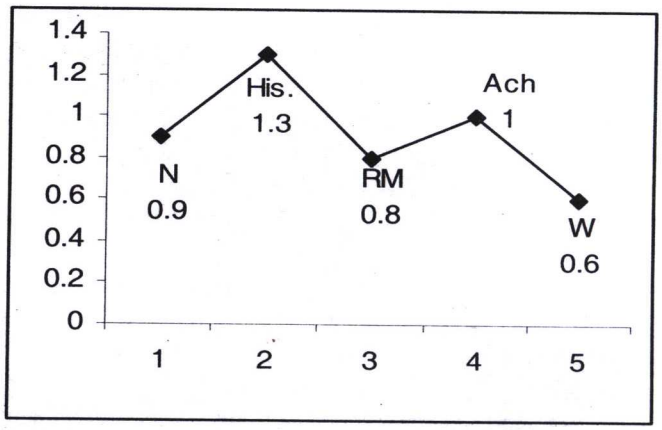

Figure 7: Effect of Rosemary oil, pretreated with Histamine 0.1 mg/ml and post-treated with Ach 1x10^{-2}.

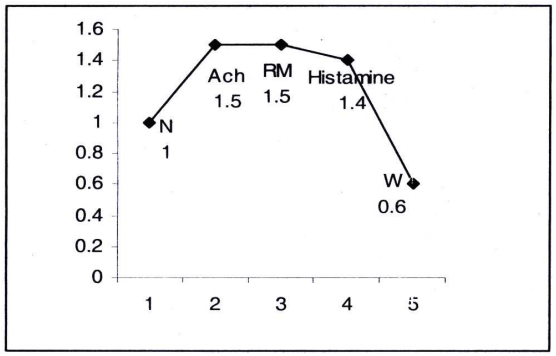

Figure 8: Effect of Rosemary oil, Pretreated with Acetylcholine 1 x 10-2 and Post treated with Histamine O.lmg/ml.

RESUL TS AND DISCUSSION

There is a basic rhythmic activity in the intestine of rabbit which can initiate action potential in the absence of extrinsic innervations (Kitchen, 1984). This activity is produced by adrenergic and cholinergic nerves. Therefore, whenever any change in its environment/condition occurs automatically its rhythm deviate from normal. Extract of rosemary is used in acne, alopecia, aphrodisiac, arteriosclerosis, arthritis, asthma, bronchitis, bruises, cellulite, chilblains, circulatory stimulation, colds, constipation, cramps, cystitis, dandruff, decongestant, depression, dermatitis, digestive, disinfectant, diuretic, divei-ticulosis, dry skin, ear infections, fatigue, fainting, fibrositis, gout, hair loss, hangovers, headaches, immu:1ity stimulant, influenza, inguinal hernia, insect repellent, lice, lower abdominal pain, lumbago, mature skin, muscular dystrophy, sinusitis, vaginal infections, varicose veins (Staff, 1970). During this study it is found that pure oil of rosemary inhibits the rhythmic movement of the rabbit intestine in the dose dependent manner. The inhibitory actions were found reversible after washing.

The effect of the drug was observed in the dose of 125 mg/ml (Fig. 1) but the maximum and excellent inhibitory action was noted at 25mg dose. The mechanism of this inhibitory effect of the test drug was checked with the different standard drugs including Acetylcholine (1 x 10-2 M, 1 x 104M) Adrenaline (l x 10-2 M), Histamine (0.1 mg/ml) , Verapamil (lJlM), Atropine (l x 1O-2M).

It is observed that when the tissue was treated with rosemary (Fig. 2) and then with acetylcholine (lxlO-2 M), the contractile response of the Ach was not produced. It was the same as pretreated tissue. With atropine it has not produced any effect of the acetylcholine. In the reverse manner the tissue is treated first with acetylcholine then with Rosemary oil, it inhibited the tissue activity with the same tone at which the effect of standard Ach is produced, i.e. through the activation of muscarinic receptor Fig. 2) (Brown and Taylor, 1996). When the effect of the drug was checked, the tissue regained its normal activity. Similarly when treated the tissue with histamine it has increased the tone of the smooth muscle, followed by drug administration causes a slight decrease in the contracting effect (Fig. 2). When Verapamil is given in IJlM concentration, it has decreased the tissue activity, and when it was treated with Ach (l x 10-2 M), the tissue has not attained full contracting response, followed by the drug treatment, showed the straight line at the same tone of the muscle but has not declined the effect. Here the calcium channel and muscarinic receptors were blocked but still the effect of the drug in the form of complete inhibition of the tissue activity was observed that indicates there is an involvement of some other receptor therefore, the drug is further evaluated. To observed whether the relaxing effect of the crude extract is mediated through atropine like mechanism, the tissue was pretreated with Rosemary oil, the test drug, then with Ach (l x 10-2 M), this treatment showed the same effect which was observed with the pretreated tissue with atropine followed by Ach (Fig. 2). Another way used to confirm the effect of the drug is the treatment of tissue with Ach then test drug and histamine (0.1 mg/ml). If the effect of the histamine remains same that indicates the mechanism of the test drug is similar to Ach, but here histamine has not produced its effect (Fig. 4/8) that indicates the involvement of both histamine and muscarinic receptors. Another way to reconfirm the effect of the drug is the blockage of Ca+2 channel and muscarinic receptors (Fig. 5). A further decrease in the activity of the tissue was observed in a cumulative manner. Similarly tissue was treated with adrenaline (l x 102) and acetylcholine 1 x 10-2 to block both adrenergic and muscarinic receptor and then was treated with Rosemary oil it showed a straight line (Fig. 6). Upon washing the tissue regained its activity. For further
confirmation tissue was treated with histamine 0.1 mg/ml, the contraction produced by histamine was

decreased by Rosemary oil, when given with Ach 10-4, it showed a slight increasing effect (Fig. 7). Because of blocking of muscarinic receptor by Rosemary oil, Ach does not produce its full response. !n the reverse manner histamine does not produce contraction after the treatment of tissue with Ach and Rosemary oil (Bolton *et ai.,* 1979; Gilani *et ai.,* 2000).

The spontaneous movement of the intestine was regulated by cycles of depolarization and repolarization, Ach and histamif'e produces depolarization and tonic contraction of the smooth muscle depend upon the extra cellular Ca +2 which causes an opening of Ca+2 channel (Godfraind *et ai.,* 1986). Rosemary oil inhibits the tonic contraction of smooth muscle of intestine induced by histamine and Acetylcholine stimulation. This inhibition could involve the influx of Ca+2 (Rodger, 1 S'85) since this extract inhibits the effect of both histamine and acetylcholine thus it has a specific antagonist effect.

In conclusion it seems that Rosemary (IiI extract may block the Ca $^{+2}$ influx through voltage gated calcium channel or receptor operated calcium channel causing a contractile effect (Godfraind *et ai.,* 1986), it also blocked the muscarinic receptor up to some extent. Further experiments are needed to identify the active ingredient of this volatile oil extract of rosemary plant which has an antidiarrheal effect. Mor~over it blocks the histamine receptor as well, besides Ach receptor. Therefore it is concluded that it has an anti ulcer activity. In case of diarrhea the peristaltic movement of the intestine is usually increased and some time muscle cramp also occurred.

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