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SPASMOGENIC AND SPASMOLYTIC ACTIVITY OF *GRATIOLA OFFICINALIS* LINN

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ABSTRACT

In present study, *Gratiola officinalis*, ethanolic extract and its various fractions were investigated for its spasmolytic and spasmogenic effects on smooth muscles of rabbit intestine. The crude extract showed maximum spasmogenic response (51.51 %) at 20 mg/ml concentration ($p<0.05$). Highest spasmogenic response was observed with aqueous, ethylacetate and *n*-butanol fractions. Antispasmodic (spasmolytic) effect was observed with *n*-hexane and chloroform fractions (52.22 % and 46.25 % respectively at 15 mg/ml). The fractions were further treated with different concentrations of standard drugs: Atropine, acetylcholine and adrenaline. Spasmogenic effects of different fractions were inhibited by atropine ($1x10^{-2}$M).) On the other hand, it was observed that adrenaline ($1x10^{-4}$M) has not altered the increased contraction of smooth muscles. For that, it can possibly be suggested that the different fractions (*n*-butanol ethylacetate and aqueous) of

G. officinalis produced spasmogenic response by the stimulation of muscarinic receptors. However, crude extract showed negligible spasmolytic effect at low dose.

Keywords: *Gratiola officinalis* L., Spasmogenic, Spasmolytic, Smooth Muscles

INTRODUCTION

Decrease in the gastrointestinal tract movements may results in several health related problems. Some of the very common problems are constipation, indigestion, heartburn and oesophageal injury **[1]**. For this purpose, a number of synthetic medicines are available **[2]**. Since many studies have shown the advantage of herbal medicine for the cure of gastrointestinal tract problems **[3]**. Therefore, present study is designed to evaluate the role of *Gratiola officinalis* for treatment of colic, spasm and other GIT disorders.

Gratiola officinalis L (Scrophulariaceae) was traditionally used as a cathartic, diuretic and emetic **[4]**. It contains a number of medicinally important constituents such as flavonoid, glycoside, gratioside, triterpene glycoside **[4-5]**. It also contains Cucurbitacins: gratiogenin, 16-hydroxygratiogenin, cucurbitacins E and I, the glycosides gratiogenin-3β-D-glucoside, gratioside (gratiolin, gratiogenindiglucoside), elaterinide, desacetylelaterinide **[6]**. Although a lot of phytochemical work has been done on *G. officinalis*, no any pharmacological study has been carried out yet. Keeping the above interest in view, therefore, *G. officinalis* has been selected.

MATERIAL AND METHODS

Plants Material

Whole plant of *G. officinalis* was collected from Khadagzai, Dir District, KPK, Pakistan, during september 2002. A voucher, specimen (OG-01/2002) was deposited in the Department of Botany herbarium after identification of plant.

Extract Preparation

Air-dried plant (4.0 kg) of *G. officinalis* Linn. was percolated in 80% ethanol at room temperature for 15 days. The percolate was filtered through Whatman filter paper. The process was repeated for two times and the three residues obtained after filtration of the percolates were combined.

Ethanol was evaporated under reduced pressure at 40^{o}C. The crude extract obtained was lyophilized and 164g extract was left. Crude extract (14 g) was kept for biological and pharmacological screening whereas 150 g of crude extract was subjected to further fractionation **[7]**.

Fractionation

The crude ethanolic extract (150 g) was suspended in distilled water (1000 ml) and sequentially partitioned with *n*-hexane (3 x 1000 ml), chloroform (3 x 1000 ml), ethyl acetate (3 x 1000 ml) and *n*-butanol (2 x 1000ml) to yield *n*- hexane (25.5 g), chloroform (30.4 g), ethyl acetate (10 g), *n*-butanol (40 g) and aqueous (44.1 g) fractions, respectively **[7]**.

Spasmogenic And Spasmolytic Activity Test

Smooth Muscles Preparation

Rabbits of either sex weighing approximately 1.0 to 1.5 kg were used in the experiments. The animals were obtained from bred stock available locally. A blow on the back of the neck sacrificed the rabbit. The abdomen was made open immediately and caecum was pulled forward to display the length of small intestine. The intestine was then cut from animal and placed in a Petri dish or beaker containing Tyrode's solution **[8-9]**.

Isolated Intestine Segments Preparation

The segments of small intestine (jejunum or ileum) about 3-4 cm long were dissected immediately from isolated intestine, later it was placed in petri dish or beaker containing Tyrode's solution and aerated with oxygen (95% O_2 and 5% CO_2). For experimentation a piece of isolated smooth muscles was mounted in an organ bath of 70 ml capacity, filled with Tyrode's solution. Organ bath circulating water temperature was maintained to 37°C throughout the experiment. The perfusion solution was bubbled with a mixture of 95% oxygen and 5% carbon dioxide **[8-9]**.

Assay Method

The intestine segment was allowed to equilibrate before starting the experiments. The spontaneous movements of intestine were recorded on oscillograph or polygraph using isotonic transducer **[8-9]**.

To determine the effects of plant extract on spontaneous movements of intestine, crude extract and their fractions were dissolved in 1 ml of distilled water and thereafter, it was added to the organ bath after equilibration period. The effects of crude extract and their fractions on the contraction and relaxation pattern of isolated rabbit intestine (smooth muscles) were recorded.

STATISTICAL ANALYSIS

The results were expressed as mean±S.E.M. All statistical comparisons were made by means of Student's *t*-test and a *P* value smaller than 0.05 was regarded as significant.

RESULTS

This experiment was carried out on isolated intestine (jejunum) of rabbit and spontaneous movements of intestine were recorded on

Oscillograph using isotonic transducer (Harvard Isolated Organ Bath apparatus). The effects of crude extract and fractions were compared with standard drugs i.e. acetylcholine, adrenaline and atropine **(Figure 1a-m)**. The crude extract and fractions were given in different concentrations to observe the maximum effect at lowest concentration.

Table 1 and **2** show the response of smooth muscle activity of crude extract, ethylacetate, chloroform, *n*-hexane, *n*-butanol and aqueous fractions of *Gratiola officinalis*, the effects of crude extract and fractions were observed with 1, 5, 10, 15, 20 and 25 mg/ml and 5, 10 and 15 mg/ml doses respectively.

Table 1: Dose Related Response of Crude Extract of *G. officinalis* on Isolated Rabbit Intestine

Dose (mg/ml)	Control (cm)	Response (cm)	Response in Percentage	t- value
01	1.03 ± 0.032	0.96 ± 0.032	6.796	1.59
05	0.8 ± 0.00	0.83 ± 0.032	3.75	-0.937
10	0.7 ± 0.00	0.83 ± 0.032	18.57	-4.06*
15	0.63 ± 0.032	0.93 ± 0.032	47.62	-6.81**
20	0.66 ± 0.032	1.00 ± 0.00	51.51	-10.625**
25	0.86 ± 0.032	1.06 ± 0.032	23.25	-4.54*

The results are expressed in ± S.E.M, at P ≤ 0.05; * Significant, **highly significant

Table 2: Effects of Different Fractions of *G. officinalis* on Isolated Rabbit Intestine

Fractions	Dose (mg/ml)	Control (cm)	Response (cm)	Response in Percentage	t- value
Ethyl-acetate	05	0.73 ± 0.032	0.86 ± 0.032	17.80	-2.95
	10	0.8 ± 0.032	1.06 ± 0.032	32.5	-5.90
	15	0.76 ± 0.032	1.33 ± 0.032	75	-12.95
Chloroform	05	0.6 ± 0.00	0.53 ± 0.032	11.66	2.187
	10	0.6 ± 0.00	0.43 ± 0.032	28.33	5.31
	15	0.8 ± 0.00	0.43 ± 0.032	46.25	11.56
n-Hexane	05	0.76 ± 0.032	0.5 ± 0.032	34.21	5.90
	10	0.8 ± 0.00	0.46 ± 0.032	42.5	10.62
	15	0.9 ± 0.00	0.43 ± 0.032	52.22	14.68*
n-Butanol	05	0.76 ± 0.032	1.33 ± 0.032	75	-12.95**
	10	0.6 ± 0.057	1.5 ± 0.057	150	-11.25**
	15	0.73 ± 0.032	1.33 ± 0.032	82.19	-13.63**
Aqueous	05	0.766 ± 0.066	1.46 ± 0.066	92.1	-7.626
	10	0.76 ± 0.032	1.8 ± 0.057	136.8	-16.25**
	15	0.66 ± 0.032	1.93 ± 0.057	192.4	-19.53**

The results are expressed in ± S.E.M, at P ≤ 0.05; * Significant; **Highly significant

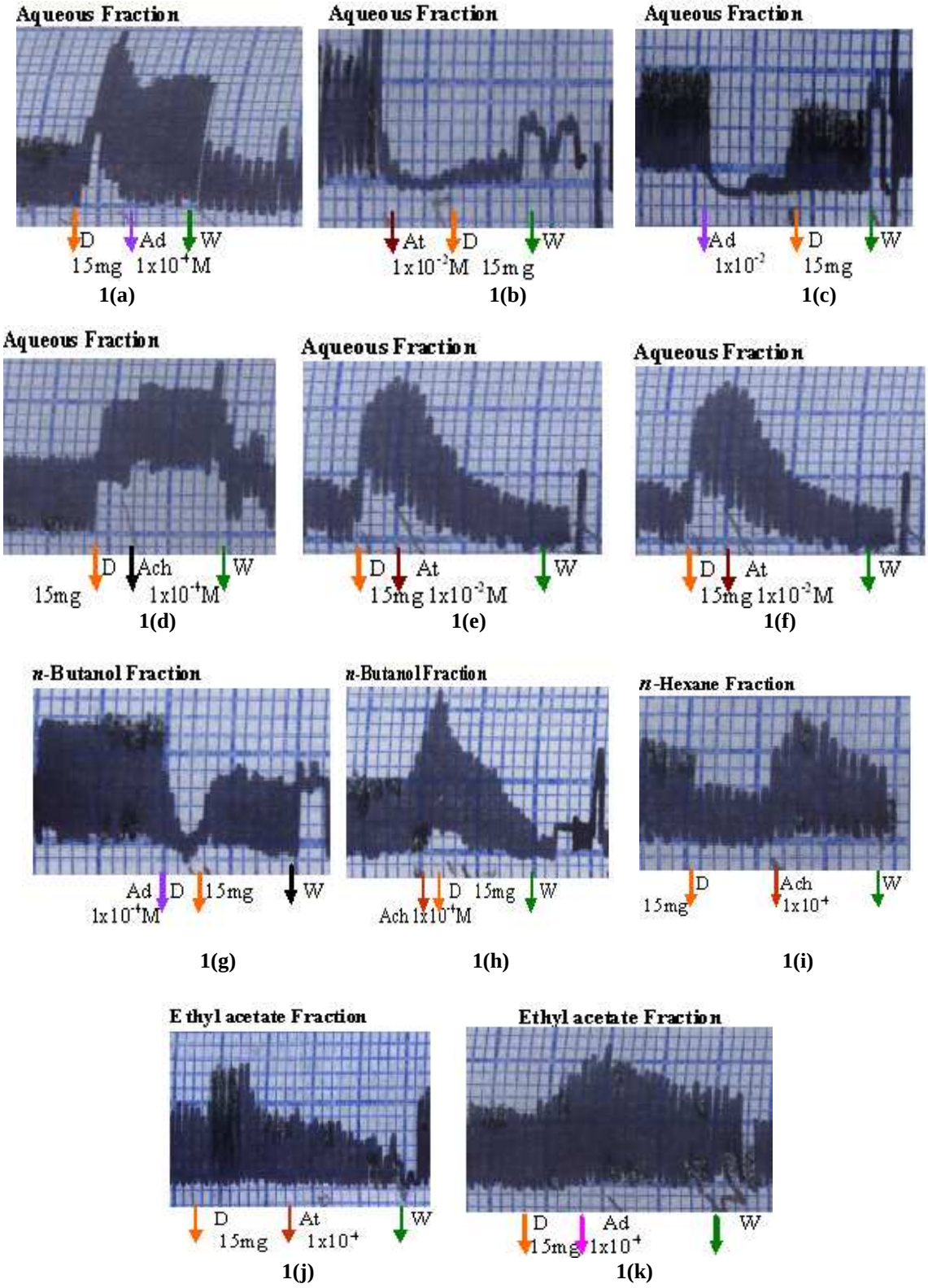

Figure 1a-k: Tracing of Smooth Muscle Activity of Fractions of *G. officinalis* with standard Drugs on Isolated Rabbit Intestine

DISCUSSION

Crude extract of *Gratiola officinalis* produced slight dose dependant increase in intestinal smooth muscles activity and has laxative effect. Where as, at low dose (1 mg/ml) it has antispasmodic effect.

The ethyl acetate fraction showed dose dependent increased contraction of intestinal smooth muscle and chloroform fraction showed dose dependent relaxing effect of intestine. The similar relaxating effect of tissue activity was observed with *n*-hexane fraction where as *n*-butanol fraction of *G. officinalis* also showed a significant dose dependent contraction of smooth muscle of rabbit intestine. The increased contraction of smooth muscle (spasmogenic effect) was highly significant in aqueous fraction at 15 mg/ml dose which was similar to the standard drug acetylcholine ($1x10^{-4}$M conc.). For the probable mechanism of action these fractions were further treated with standard drugs (acetylcholine, atropine and adrenaline).

Figure 1a-k showed the tracing of smooth muscle activity of fractions of *G. officinalis* with standard drugs. The cholinomimetic effect of aqueous fraction of drug post treated with adrenaline $1x10^{-4}$ M concentration was not completely inhibited. The aqueous fraction did not produce its effect because of occupancy of muscarinic receptor by atropine (Figure 1a). Figure 1c showed the effect of aqueous fraction of *G. officinalis* pretreated with adrenaline $1x10^{-2}$ M concentration. The drug produced its response but not at full strength. Figure 1d showed the effect of aqueous fraction of *G. officinalis* post treated with acetylcholine $1x10^{-4}$M concentration, it gives synergistic response or supportive action.

Figure 1e showed the effect of aqueous fraction of *G. officinalis* post treated with atropine $1x10^{-2}$M concentration, there was a gradual decrease in the response but did not completely block. Similarly the effect of *n*-butanol fraction pretreated with atropine $1x10^{-2}$M conc. showed that the effect of drug was not completely blocked and smooth muscle of intestine continued its activity but in reduced form. The same thing was observed with low concentration of adrenaline $1x10^{-4}$ M concentration **(Figure 1g)**. **Figure 1e** also showed the effect of aqueous fraction of drug post treated with atropine $1x\ 10^{-2}$M conc. The activity of tissue was observed normal but not blocked.

Figure 1g showed the effect of *n*-butanol fraction of *G. officinalis* pretreated with adrenaline $1x\ 10^{-4}$M concentrations. It block the tissue activity which was overcome by the test drug, indicates the possible involvement

of muscarinic receptors not adrenergic receptor.

Graph 1h showed the effect of *n*-butanol fraction of drug pretreated with acetylcholine $1x10^{-4}$M, at low concentration the synergistic effect of the drug was less potent as compared to the effect which was observed in **Figure 1d**. **Figure 1i** showed the effect of *n*-hexane fraction of *G. officnalis* post treated with acetylcholine $1x10^{-4}$M conc. The cholinergic response of acetylcholine was less significant as compared to its alone effect. Similarly the effect of chloroform fraction of *G. officinalis*, pre treated with acetylcholine $1x10^{-4}$M was decreased the effect of acetylcholine (might be the receptors are not fully occupied due to low conc.).

Graph 1j showed the effect of ethylacetate fraction post treated by atropine $1x10^{-4}$M concentrations. The cholinomimetic effect was slightly reduced by the atropine but with adrenaline $1x10^{-4}$M concentration **(Figure 1k)** effect of ethyl acetate fraction was not disappeared. All of above findings showed the possible involvement of both muscarinic and adrenergic or may be other receptor.

In *n*-hexane fraction acetylcholine produced its contractile response, although the receptors were blocked with drug and in chloroform fraction the effect of acetylcholine was also decreased. These results suggested that either the test drug replaces the standard drug or the standard drug replaces the test drug or partial occupancy of muscarinic and adrenergic receptors so that when another drug was given it partially produced the response.

There are muscarinic, adrenergic, some vasoactive peptides and different other neurotransmitters present in intestinal flora but the major influence is of muscarinic and adrenergic receptors **[1]**. These results showed that the contraction produced by aqueous fraction was not inhibited or decreased by adrenaline and vice versa. The inhibitory effect of adrenaline was overcome by aqueous fraction of *G. officinalis*, which showed in aqueous fraction the constituents present are possibly not works through adrenergic receptors. For this it is treated with adrenaline and atropine. The synergistic effects of acetylcholine and the decrease contraction of aqueous fraction by atropine showed that drug was replaced by atropine so the contraction of smooth muscle of jejunum was decreased. It is well known that pretreated tissue with atropine does not produces the effect of acetylcholine **[8, 10]**. The same thing was observed with different fractions of *G. officinalis*. On the other hand the crude extract at low dose and chloroform and n-hexane fractions exhibited the spasmolytic effect.

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