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*Full Length Research Paper*

# Spasmogenic, spasmolytic and antihypertensive activity of *Forsskalea tenacissima* L.

**Syed Wadood Ali Shah[1], Samir Kamil[1,2], Waqar Ahmad[1] and Niaz Ali[1*]**

[1]Department of Pharmacy, University of Malakand, Chakdara, Dir (Lower) N-W.F.P Pakistan.
[2]Cellulose and Paper Department, National Research Center, Dokki, Cairo, Egypt.

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**Crude methanolic extract of *Forsskalea tenacissima* was screened for possible spasmogenic, spasmolytic and anti hypertensive activities. The extract was studied in isolated rabbit's jejunum preparations. The extract was also screened for *in vivo* antihypertensive effect in spontaneous hypertensive Wistar rats. Crude methanolic extract produced spasmogenic activity (15 ± 2% of control, n = 6, p ≤ 0.05) at a test dose of 0.1 mg/ml of the cumulative test dose(s) tried as 0.01, 0.03, 0.1, 0.3, 1, 5 and 10 mg/ml. However, at higher dose 5.0 mg/ml, maximum spasmolytic activity was observed ($EC_{50}$ = 2.7 ± 0.06 mg/ml, n = 6). The extract also produced bradycardiac effects at test doses of 100 and 300 mg/kg. Statistically significant fall in blood pressure (BP) was 19 and 21 mm Hg for test doses of 30 and 100 mg/kg, respectively (p≤ 0.05). Our current work confirms the folkloric uses of *F. tenacissima* as spasmogenic, antispasmodic (in large doses) and antihypertensive drug.**

**Key words:** *Forsskalea tenacissima,* spasmogenic, spasmolytic, antihypertensive, $EC_{50}$ values.

## INTRODUCTION

Urticaceae family consists of 45 genera that are mostly distributed in the tropical and sub tropical regions throughout the world (Ghafoor, 1983). Most of its plants carry medicinal properties that are of great commercial importance (Wagner et al., 1989; Balzarini et al., 1992). Some plants of this family have antiviral (Wagner et al., 1989), galactagogue (Westfall, 2003) and anti-inflammatory activities (Obertreis et al., 1996; Teucher et al., 1996). As example, *Urtica dioica* is used in benign pro-static hyperplasia (Lopatkin et al., 2005) and increases free testosterone by occupying sex-hormone binding globulin (Schottner et al., 1997). Entire plant of *Urtica cannabina* has been used in the treatment of kidney problems, diabetes, respiratory, digestive disorders and excessive menstrual bleeding (Huang, 2005). Reported activities for *Forsskalea* genus are diuretic, calculolitic, antiflu (Darias et al., 1986; Perez et al., 1993) and anti-septic (Darias et al., 2001). *Forsskalea tenacissima* is an annual herb that belongs to the Urticaceae family. Reported activity for *F. tenacissima* are antimicrobial, antioxidant, cytotoxic, diuretic, kidney diseases and antifungal activities (Qaisar et al., 2003). Folkloric uses of *F. tenacissima* in Peshawar region, North West Frontier Province (N-W.F.P) of Pakistan, are anti-inflammatory, spasmogenic, anti-diabetic and antipyretic. Keeping in view the reported activities and the folkloric uses of this specie, we carried out this work for further investigations.

*Corresponding author. E-mail: niazpharmacist@yahoo.com.

## MATERIALS AND METHODS

### Plant materials

The whole plant material of *F. tenacissima* (Urticaceae) was collected in April, 2007 from Hayatadad, Peshawar and nearby hills of Malakand regions of the N-W.F.P, Pakistan. Plant was identified by Jahander Shah, Plant Taxonomist, Ex Vice Chancellor, University of Malakand. A voucher specimen (FT-01-07) has been deposited in the herbarium of University of Malakand.

### Extraction and isolation

The whole plant material (25 kg) was subjected to shade drying and grinded into fine powder weighing approximately 16 kg. The powdered drug was extracted three times with commercial grade methanol (90 - 95%). The extracts were combined and evaporated (40˚C) to get crude greenish residue using rotary evaporator. The residue was fractionated successively with n-hexane, chloroform, ethyl acetate and *n*-butanol.

### Drugs and standards

Analytical grade chemicals were used in the bioassay techniques. Source of acetylcholine was BDH chemicals, Poole England. Atropine and other chemicals were purchased from E. Merck Germany. All the solutions were freshly prepared in distilled water on the same day of experiments.

### Animals and data recording

Rabbits of either sex were breeded locally. Their average weight was in the range of 1.0 - 1.5 kg. They were maintained at the "Animal House of University of Malakand" as per the Byelaws of University of Malakand Scientific Procedures issue-I. Animals were given free access to standard diet along with fresh water. Before the start of experiments, animals were given only water and were kept without food for 24 h. Intestinal responses were recorded using Teaching Force Transducer attached to Power lab (Model No: 4/25 T) AD Instruments, Australia.

### Spasmogenic activity

Crude extract of *F. tenacissima* was screened for possible cholinomimetic and spasmolytic activities as per procedure mentioned in our previous work (Bashir et al., 2009). Tyrode's solution was prepared having the following concentration (mM): KCl 2.68, NaCl 136.9, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, $NaH_2PO_4$ 0.42, $CaCl_2$ 1.8 and glucose 5.55. The animals were then slaughtered and their abdomens were opened. Rabbit's jejunum portion(s), of about 1 - 1.5 cm length, was isolated and mounted in the tissue bath containing 10 ml of Tyrode's solution maintained at 37˚C and supplied with carbogen gas (5% Carbon dioxide and Oxygen mixture). These portion(s) were kept in Tyrode's solution previously aerated with the carbogen gas (Qayum, 2004). Earlier, the tissues were stabilized for normal activity for a period of about 25 - 40 min. This was achieved by giving sub-maximal doses of acetylcholine (0.3 µM) to the tissues till it produced a reproducible response(s). For possible pharmacological screening on the tissues through series of experiments, crude extract(s) of *F. tenacissima* were tried at dose(s) of 0.01, 0.03, 0.1, 0.3, 1, 5 and 10 mg/ml. All the doses were applied in cumulative manner and the results were recorded (Farre et al., 1991). The spasmogenic activity was recorded as percent of the activity of acetylcholine given at the dose of 10 µM.

### Spasmolytic activity

We used the procedure described by Farre et al. to screen spasmolytic activity. Contractions in the intestine portions were produced by high KCl (80 mM) to depolarize the intestine portions (Farre et al., 1991). The extract was applied in the similar fashion to relax the tissues and percent relaxation response on KCl induced contractions was recorded (Van Rossum, 1963).

### Antihypertensive activity

As the plant extract relaxed the high KCl induced contractions, a characteristic of calcium channel blocking activity, therefore, we screened the extract for possible antihypertensive activity using invasive techniques (Gilani and Aftab, 1992). Spontaneous hypertensive Wistar rats of either sex were anaesthetized with Pentothal sodium 80 mg/kg given intraperitoneally. The trachea was cannulated for the maintenance of spontaneous respiration. For each rat, the carotid artery was cannulated and attached to Power lab Model 4/25T AD Instruments (Australia) connected with bridge pod amplifier model number ML221 to record the arterial blood pressure.

In the similar way on the opposite side, the jugular vein was cannulated to administer the different doses of test samples. A soft tissue paper soaked in the normal saline was placed on the exposed incised portion of the rat under the light and heat of an ordinary table lamp to keep the exposed portion of rat moist and warm. The fall in the arterial pressure and heart rate of the test animals were recorded at test doses 1, 3, 10, 30, 100 and 300 mg/kg. Earlier the vascular flexibility was checked with standard solution of Noradrenalin and Acetylcholine. The animals were given Atropine at a dose of 2 mg/kg and the effects of the extract at the said doses were recorded in Atropine pre treated animals.

### Statistical analysis

Microsoft-XL Sheet was used to calculate Mean and Standard Error Mean (SEM) of the data (not shown). Student's 't' test was used to compare the data and 'P' value less or equal to 0.05 was considered as statistically significant.

## RESULTS AND DISCUSSION

As per Figure 1, there is mild spasmogenic activity in the absence of atropine. This suggests that muscarinic receptors were not blocked and the extract produced agonistic activity on the receptors. Whereas, in the atropine pre treated tissues (Figure 1; with atropine), the spasmogenic activity (15 ± 2% of control, n = 6, $p \leq 0.05$) was abolished showing its mechanism through muscarinic receptors as atropine is an antimuscarinic drug (Gilman et al., 1990). Thus, the left shift in $EC_{50}$ values in the activity ($EC_{50}$ without atropine = 2.7 ± 0.06 mg/ml, n = 6 and $EC_{50}$ with atropine = 1.9 ± 0.02 mg/ml, n = 6) confirms the cholinomimetic activity. In another series of experiments, the tissues were depolarized with high potassium level (80 mM bath concentration) that produced a sustained contraction (Farre et al., 1991). The test samples were then tried in cumulative manner to observe the spasmolytic effect on the tissues.

As it has been postulated that contractions produced by potassium are mediated through calcium channels via influx of calcium from extra cellular fluid and a substance which will inhibit the contraction produced by KCl is considered to have calcium channel blockade (Bolton, 1979). Hence, the extract produced a dose-dependent spasmolytic response on the KCl-induced contractions and is considered to have calcium channel blocking activity. According to Figure 1, the extract produced a spasmolytic effect on the KCl depolarized tissues. However, at higher doses 0.3 mg/ml of extract, spasmolytic effect was observed and was maximum at 5.0 mg/ml ($EC_{50}$ = 2.7 ± 0.06 mg/ml, n = 6). KCl-induced contractions (80 mM) were relaxed by the extract in the similar doses. Positive relaxing effects on KCl induced contraction are mostly referred to calcium channel blocking activity (Gilani et al., 2005). Hence, the spasmolytic activity of *F. tenacissima* may be mediated through calcium channel blocking activity that warrants for further

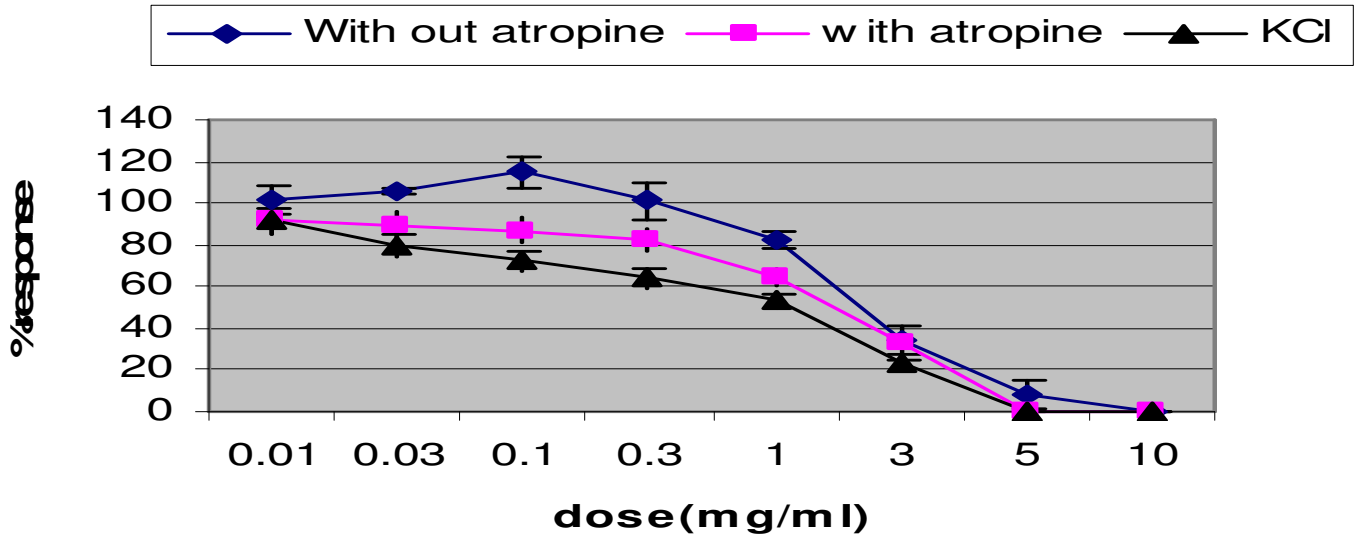


**Figure 1.** Effects of different concentrations of methanolic extract of *F. tenacissima* on atropinized and non-atropinized rabbit's jejunum preparations. Effects on KCl (80 mM) induced contractions are also shown (All values are mean ± SEM, n = 6).

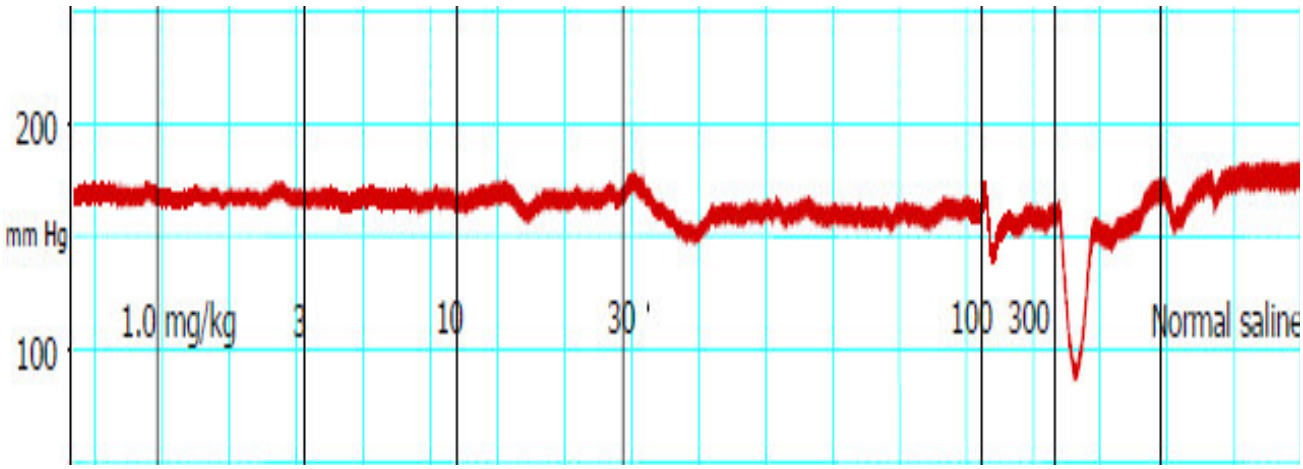


**Figure 2.** Representative graph tracing showing the blood pressure lowering effects of different doses of *Forsskalea tenacissima* extract in spontaneous hypertensive rats.(n = 6).

work to confirm its possible calcium channel blocking activity. Based on the spasmolytic activity and folkloric uses of the plant, we tried different doses of *F. tenacissima* for possible antihypertensive activity. The graph tracing of anesthetized spontaneous hypertensive rats are shown in Figure 2. Fall in blood pressure and heart rate are shown in Figure 3. The vascular flexibility and response of its endothelium was checked by giving quiescent doses of Noradrenalin and Acetylcholine as per procedure (Gilani and Aftab, 1992).

According to Figure 3, there is no statistical difference (Confidence interval = 95%) at test doses of 1, 3 and 10 mg/kg. However, statistically significant fall in B.P was 19 and 22 mmHg for test doses of 30 and 100 mg/kg, respectively (Confidence interval = 95%). There was relatively low lowering effect on heart rate as compared to fall in blood pressure. However, at a dose of 300 mg/kg, there was a major fall in blood pressure (73 mmHg) and heart rate (340 BPM). To explain the possible mechanism for this fall, the authors treated the rats with atropine 2 mg/kg dose to antagonize the cholinergic receptors. After atropine, when the same doses were tried, there was no fall in B.P and heart rate at dose of 30 mg/kg, meaning that the muscarinic receptors of the vessels were blocked and the extract has an action, which is although not muscarinic receptors. Similarly, fatal bradycardia was blocked in Atropine pre treated rats at a dose of 100 mg/kg. This suggests that the muscarinic receptors which were present at the heart level were also blocked by Atropine and hence the extract at

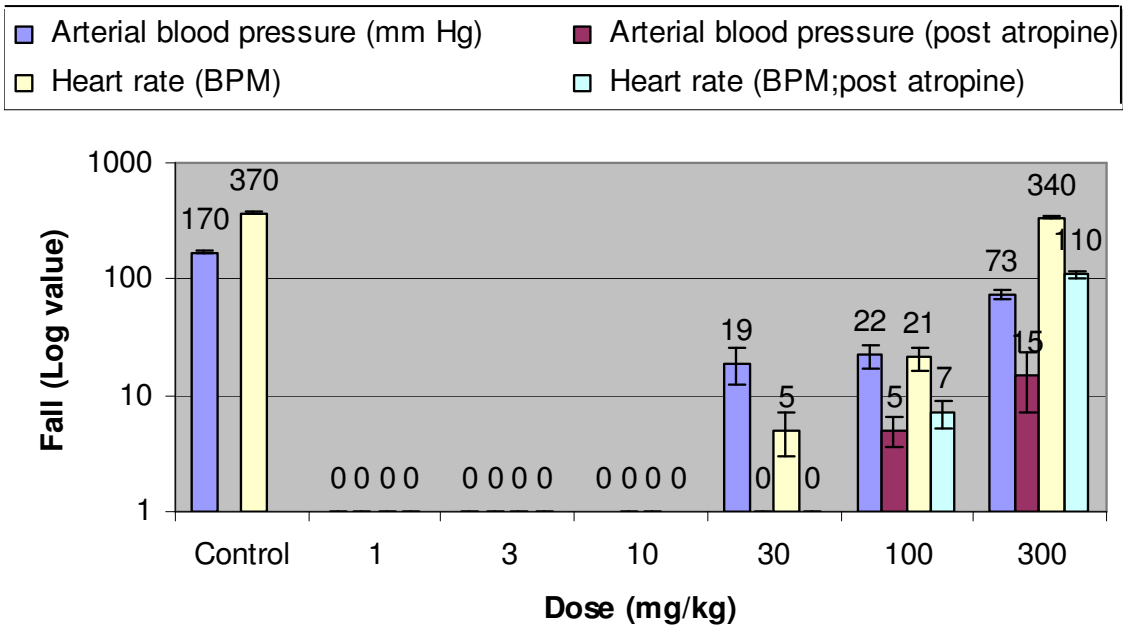


**Figure 3.** Effects of different doses of *Forsskalea tenacissima* extract on the arterial blood pressure and heart rate (BPM) in spontaneous hypertensive rats. The fall in arterial blood pressure and heart rate against control are also shown (All values are mean ± SEM, n = 6).

test dose 100 mg/kg could not produce fatal bradycardia. However, the effects at a dose of 300 mg/kg lasted for 3 - 4 min, followed by fatal bradycardia that was reversed by administration of 1 × $10^{-5}$ M solution of Noradrenalin indicating that the heart beat lowering effects were surmountable and mediated through the muscarinic cholinergic receptors (Furchgott and Zawadzki, 1990; Gilman et al., 1990; Arunlakhshana and Schild, 1959). This partial blockade by the higher doses of extract 300 mg/kg further confirms the competitive antagonism phenomenon of the muscarinic receptors as discussed (Gilani and Aftab, 1992).

## Conclusion

The results confirm the presence of acetylcholine like substances that are responsible for spasmogenic activity in small doses. The spasmolytic activity at higher doses was possibly by the presence of calcium antagonists. Moreover, the extract of *F. tenacissima* produced an antihypertensive action that confirms its folkloric uses.

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