

Full Length Research Paper

Phyto-chemical analysis, anti-allergic and anti-inflammatory activity of *Mentha arvensis* in animals

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Allergic diseases are fairly common in all parts of the world and involve all ethnic groups with bronchial asthma, allergic rhinitis, conjunctivitis and eczema being the commonest manifestations. Despite our efforts, their incidence is on an escalating path. The use of herbal remedies and standardized extracts for treatment of allergy and other diseases has been gaining momentum in recent years. The qualitative and quantitative determination of phyto-chemicals and assessment of the anti-allergic and anti-inflammatory activities using aqueous and organic extracts of different plant parts (root, stem and leaves) of *Mentha arvensis* in animals showed that all parts of *M. arvensis* (specifically, leaves) are rich source of secondary phytoconstituents, which impart their therapeutic effects against allergic and inflammatory diseases. These results support the claim about the use of this herb in folk medicines.

Key words: Anti-allergic, anti-inflammatory activity, medicinal plants, *Mentha arvensis*, Pakistan.

INTRODUCTION

Allergic diseases are fairly common in all parts of the world and involve all ethnic groups with bronchial asthma, allergic rhinitis, conjunctivitis and eczema being the commonest manifestations. Despite our efforts, their incidence is on an escalating path. There appear to be no authentic data on the prevalence of allergic diseases in Pakistan. Allergic diseases are quite common in Pakistan, but they remain under-diagnosed. The prevalence data of allergic rhinitis shows an alarming situation. These diseases were found to affect middle-class the most, while the lower and lower-middle class remains the least affected (Noori et al., 2007). The use of herbal remedies and standardized extracts for treatment of allergy and other diseases has been gaining momentum in recent years (Londonkar et al., 2010;

Venkatesh et al., 2010). Traditional Chinese system of medicines recommends the administration of traditional medicines for the treatment of allergic and inflammatory disease which indicates that traditionally used medicinal plants may contain anti-allergic and anti-inflammatory agents (Koyama et al., 2006; Jeon et al., 2008). The experimental evaluation has shown the action of some medicinal plants on allergic and inflammatory disorders (including respiratory diseases, particularly, asthma, scabies and cough). These plants include *Gleditsia sinensis* (Li et al., 2006), *Centella asiatica* (George et al., 2009), Pomegranate (Panichayupakaranant et al., 2010), *Cassia occidentalis* Linn (Sreejith et al., 2010) and seeds extract of *Syzygium cumini* are also reported to inhibit histamine and serotonin (5-HT), mediators in allergic and inflammatory processes (Brito et al., 2007). These studies also revealed that phyto-chemical constituents of these plants co-relates with their pharmacological activities and may act individually, additively or in synergy

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to improve health (Schutz et al., 2006; George et al., 2009). Flavonoids, lignans, terpenoids, glycosides and alkaloids are the major classes of phyto-chemicals, widely distributed in plant kingdom and scientifically analyzed for anti-allergic and inflammatory activities (Koyama et al., 2006; Zheng et al., 2009).

Mentha arvensis L. belongs to the family Lamiaceae, locally known as Pudina (Khan and Khatoon, 2008). It is distributed throughout the Western Himalayas and cultivated throughout the world. It is an erect aromatic herb that grows up to 60 cm in height with suckers; the stem is cylindrical and the leaves are simple and opposing type (Londonkar and Poddar, 2009). The leaves are mostly used as salad and medicinally used for stomach problems and allergy (Khan and Khatoon, 2008). It is also used for the treatment of liver and spleen disease, asthma and jaundice. The infusion of these leaves is used in indigestion, rheumatic pains, arthritis and as remedy for inflamed joints. Menthol derived from its essential oil, is used in pharmaceutical, perfumery and food industries. The oil content of leaves yields 40 to 50% menthol, which is antiseptic, carminative, refrigerant, stimulant and diuretic in properties, and is used against skin infections. Essential oil can be diluted and used as a wash for skin irritations, itching, burns, inflammations, scabies, ringworm or to repel mosquitoes. When applied on the skin, relieves pain and reduces sensitivity (Nair and Chanda, 2007; Vivek et al., 2009; Nascimento et al., 2010).

The present study was conducted for qualitative and quantitative determination of phyto-chemicals and assessment of the anti-allergic and anti-inflammatory activities using aqueous and organic extracts of different plant parts (root, stem and leaves) of *M. arvensis* in animals.

MATERIALS AND METHODS

Collection of sample

The plant material, namely, root, stem and leaves of *M. arvensis* were collected from district Islamabad, Punjab in the month of May, 2010. All plant samples were air dried unrinsed, milled in a micro-hammer (without metal parts in it), and stored in clean paper bags (GACP and FCP, 2004). The plants were identified and voucher specimens were deposited at the Drugs Control and Traditional Medicines Division, National Institute of Health, Islamabad, Pakistan in its herbarium with the numbers DCTMD-1076.

Qualitative and quantitative analysis of secondary phyto-chemicals

The powdered plant samples (root, stem and leaves) were subjected to qualitative and quantitative evaluation of secondary phyto-chemicals, such as, alkaloids, polyphenols, flavonoids, tannins, saponins, cardiac glycosides and diterpenes, following the reported methods with modifications (Harnafi and Amrani, 2008; Roopashree et al., 2008; Ventura et al., 2008; Kaur and Arora,

2009). Alkaloid detection was carried out by Dragendorff's and Wagner's test. Polyphenols were identified by ferric chloride test. Alkaline reagent test and lead acetate test were used to identify flavonoids. The presence of tannins was confirmed by Geltin test. Saponins were tested by Froth and Foam test. Modified Borntrager's test was used to identify cardiac glycosides.

Diterpenes were tested by using copper acetate test. Folin ciocalteu method was used for quantitative estimation of total phenol content (polyphenols).

Extracts preparation

Crude ethanolic and aqueous extracts of the leaves, stem and roots of *M. arvensis* were prepared in triplicates by following the methods reported by Samee et al. (2009) and Londonkar et al. (2010). Dried samples (10 g) of leaves, stem and roots were immersed in 100 ml optimized 85% (v/v) ethanol and distilled water, stirred for 24 h at 25°C, followed by centrifugation and concentrated under vacuum using a rotary evaporator at 40°C. The concentrated sample (semi-solid mass) was lyophilized. Extracts at dose of 100 mg/kg were used for the treatment, and were compared with standard drug (positive control), disodium cromoglicate (DSCG) for anti-allergic activity and diclofenac sodium for anti-inflammatory activity. BALB-c mice weighing 40 to 45 g, housed in the standard cages at room temperature (25±°C) in 12 h dark/12 h light control, with both food and water *ad libitum* (Andrade et al., 2007).

Anti-allergic activity

Histamine release inhibition test

Inhibitory effect of ethanolic and aqueous extracts (leaves, stem and roots) of *M. arvensis* on histamine production by mast cells was evaluated using a modified method previously reported (Komasa et al., 2004). Histamine content, release rates and percent inhibitions of histamine release were determined by using the following Equations 1, 2 and 3, respectively.

$$\text{Histamine content (ng)} = \text{Histamine concentration (ng/ml)} \times 1.39 \times \frac{3}{2} \quad (1)$$

$$\text{Histamine release rate (\%)} = \frac{\text{Histamine content in the supernatant} \times 100}{\text{Histamine content in the supernatant} + \text{Histamine content in the sediment}} \quad (2)$$

$$\text{Inhibition of histamine release (\%)} = \frac{A-B}{A} \times 100 \quad (3)$$

A: Histamine release rate in the absence of any test substance (the rate in the presence of compound 48/80 – the rate in the absence of compound 48/80)

B: Histamine release rate in the presence of a test substance (the rate in the presence of compound 48/80 – the rate in the absence of compound 48/80).

Anti-inflammatory activity

Histamine induced paw edema in mice

Anti-inflammatory activity of ethanolic and aqueous extracts (leaves, stem and roots) of *M. arvensis* was determined by histamine induced paw edema in mice, according to the procedures described by Suralkar (2008). Animals (BALB-c mice) were divided into three groups (n = 12), starved overnight with water *ad libitum*

Table 1. Qualitative analysis of secondary phytochemicals from *M. arvensis*.

S/N	Phytochemicals	Name of the test	Leaves	Stem	Root
1	Alkaloids	Dragendorff's test	+	+	+
		Wagner's test	+	+	+
2	Flavonoids	Alkaline reagent test	+	+	+
		Lead acetate test	+	+	+
3	Phenols	Ferric chloride test	+	+	+
4	Tannins	Geltin test	+	+	+
5	Saponins	Froth test	-	-	-
		Foam test	-	-	-
6	Diterpenes	copper acetate test	+	+	-
7	Cardiac glycosides	Modified Borntrager's test	+	+	+

- = Absent, + = present, (n=3).

Table 2. Quantitative estimation of secondary phytochemicals from *M. arvensis*.

S/N	Plant sample	Alkaloids (%)	Flavonoids (%)	Phenols (%)	Tannins (%)	Cardiac glycosides (%)
1.	Leaves	0.095 ± 0.2	22.86 ± 0.3	3.51 ± 0.1	15.79 ± 0.5	5.49 ± 0.06
2	Stem	4.31 ± 0.1	19.9 ± 0.16	1.55 ± 0.1	9.6 ± 0.2	6.8 ± 0.3
3	Roots	0.23 ± 0.01	19.09 ± 0.04	1.95 ± 0.03	16.84 ± 1.2	5.61 ± 1.2

Values are expressed as mean ± SD (n = 3). P < 0.05, ANOVA followed by student's unpaired t-test.

respectively. Basal paw volume of left paw is measured prior to the day of experiment. The control group receives vehicle orally, while other group receives ethanolic and aqueous extracts (leaves, stem and roots) and standard drug (Diclofinac sodium), plethysmographically. The animals were given drug treatment. After 1 h dosing, all groups were challenged by a subcutaneous injection of 0.1 ml of 1% solution of histamine into the sub-plantar side of the left hind paw. The paw volume was measured again at 1, 2, 3 and 4 h after histamine challenge. The increase in paw volume is calculated as percentage when compared with the basal volume. The percentage inhibition of the paw edema (inflammation) was calculated using the formula, compared with control group and evaluated statistically.

$$\text{Inhibition of paw edema (\%)} = \frac{(V_c - V_t)}{V_c} \times 100$$

where V_t is edema volume in the plant extract treated groups and V_c is edema volume in the control group.

Statistical analysis

The results were presented as the mean (\pm standard deviation (SD)) of twelve animals per group. Statistical analysis of data was performed by using the student's unpaired t-test and by analysis of variance (ANOVA). Significance of results was considered relative to control readings at different P-values, 0.05 and 0.01.

RESULTS

Qualitative and quantitative analysis of secondary phyto-chemicals of *M. arvensis*

The qualitative phyto-chemical investigation of various extracts of leaves, stem and root of *M. arvensis* showed the presence of different phyto-chemical compounds, such as alkaloids, flavonoids, polyphenols, tannins, cardiac glycosides, which indicates their distribution in whole plant (Table 1). Test for saponins showed their absence from leaves, stem and root extracts. Furthermore, results indicate that diterpenes are present in leaves and stem extracts, while absent in root extracts. The data for the quantitative determination of secondary phyto-chemicals from leaves, stem and roots of *M. arvensis* is as shown in Table 2. This showed that phytoconstituents level is high in leaves as compared to stem and roots. Among all phyto-chemicals, flavonoid content is high in leaves, stem and roots (22.86>19.9>19.09%), respectively. Significant amount of total phenol (Polyphenols), 3.51% is present in leaves. Alkaloid content is more in stem extracts as compared to leaves and root. *Mentha* root extracts contains high tannins and cardiac glycosides content, 16.84 and

Table 3. Anti-allergic effect of crude ethanolic and aqueous extracts of leaves, stem and roots of *M. arvensis* by histamine release inhibition test.

Plant sample	Treatment	Concentration of test substance ($\mu\text{g/ml}$)	Histamine release (%) in the presence of compound (mean \pm SD)	Inhibition of histamine release (%)
	Control	-	49.6	-
Leaf	Ethanolic extract	100	23.6**	57
	Aqueous extract	100	31.5*	43
Stem	Ethanolic extract	100	42.8	26
	Aqueous extract	100	46.0*	25
Root	Ethanolic extract	100	25.7*	53
	Aqueous extract	100	38.8*	37
Standard	Disodium cromoglicate	10	2.3**	92.7

Significance relative to control reading: * $P < 0.05$, ** $P < 0.01$. Values are expressed as mean \pm SD (n = 6 mice per extract).

5.61%, respectively as compared to leaves and stem extracts. Results indicate that all plant parts (leaves, stem and root) are rich source of various important phytoconstituents.

Anti-allergic activity

Evaluation of inhibitory activity of ethanolic and aqueous extracts (leaf, stem and root) of *M. arvensis* was determined by histamine release inhibition test (Table 3) and was compared with standard drug, disodium cromoglicate. Ethanolic extract of the leaf exhibited high inhibitory effect on histamine release, that is, 23.6%. While it is also cleared that aqueous extract of all plant parts weakly inhibit histamine release from mast cells. Inhibitory potential of leaf aqueous extract is high among them showing 31.5% inhibition. Results revealed that ethanolic extracts of leaf and root possessed marked inhibitory activity expressed as percentage inhibition, that is, 57 and 53%, respectively.

Anti-inflammatory activity

The ethanolic and aqueous extracts of leaves, stem and roots of *M. arvensis* were tested and compared with standard drug, diclofinac sodium for anti-inflammatory activity by using histamine-induced paw edema test. The anti-inflammatory activity was expressed as "mean increase in paw volume \pm SD" in terms of milliliter and percentage inhibition in paw volume by different extracts. These extracts at the dose of 100 mg/kg significantly ($P < 0.05$) inhibited the paw edema at 1st, 2nd and 3rd phase of the experiment (Table 4). The percentage inhibition in

paw edema induced by histamine in mice model is as shown in Table 5. All extracts of *M. arvensis* has shown inhibitory effect against the acute allergic reaction. All ethanolic extracts of leaves, stem and roots showed more pronounced anti-inflammatory effect as compared to their respective aqueous extracts. Results of the present investigation revealed that leaf ethanolic extract of *M. arvensis* exhibited high inhibitory activity (68.30, 46.40, 36.50 and 24.50%) at the concentration of 100 mg/kg as compared to other ethanolic extracts. Aqueous extract of leaves also exhibited significant anti-inflammatory activity as it caused 55% inhibition, peaked at 3rd hour of experiment. Anti-inflammatory potential exhibited by ethanolic extracts of plant parts is leaf = 68.30 > root = 48.80 > stem = 10.70% and compared with percentage inhibitory potential of standard drug, diclofinac sodium which caused 77.87% edema inhibition.

DISCUSSION

The present study was conducted to determine phytoconstituents, evaluation of anti-allergic and anti-inflammatory activities of *M. arvensis*. Phyto-chemical analysis of the leaves, stem and root extracts of *M. arvensis* revealed the presence of alkaloids, polyphenols, flavonoids, tannins, cardiac glycosides and diterpenes. Gupta et al. (2010) has found alkaloids, glycosides, steroids and sugars in the aerial parts of *M. arvensis*, which substantiates our results. As the alkaloids constitute one of the largest groups of phyto-chemicals in plants and medicinally highly effective, thus has led to the development of powerful painkiller medicines (Igbiosa et al., 2009). Previous studies have shown that the leaves of *M. arvensis* are good source of polyphenols (Runnie et

Table 4. Anti-inflammatory effect of the administration of crude ethanolic and aqueous extracts of leaves, stem and roots of *M. arvensis* on histamine-induced paw edema assay.

Plant sample	Treatment	Dose (mg/kg)	Paw volume increase (ml) (mean ± SD)			
			1 h	2 h	3 h	4 h
	Control		0.7 ± 0.05	0.52 ± 0.4	0.52 ± 0.3	0.42 ± 0.4
Leaf	Ethanolic extract	100	0.4 ± 0.2*	0.33 ± 0.1**	0.3 ± 0.07**	0.2 ± 0.07**
	Aqueous extract	100	0.3 ± 0.2**	0.3 ± 0.2*	0.25 ± 0.2*	0.25 ± 0.2*
stem	Ethanolic extract	100	0.53 ± 0.2*	0.5 ± 0.25*	0.5 ± 0.2*	0.38 ± 0.3**
	Aqueous extract	100	0.75 ± 0.2	0.65 ± 0.3*	0.63 ± 0.3*	0.55 ± 0.4**
Root	Ethanolic extract	100	0.78 ± 0.3*	0.64 ± 0.3*	0.63 ± 0.3*	0.5 ± 0.3*
	Aqueous extract	100	0.625 ± 0.3*	0.5 ± 0.3*	0.48 ± 0.1	0.4 ± 0.4**
Standard	Diclofinac sodium	10	0.95 ± 0.5	0.86 ± 0.4	0.84 ± 0.4	0.72 ± 0.4

Significance relative to control reading: *P < 0.05, **P < 0.01. Values are expressed as mean ± SD (n = 12 mice per extract).

Table 5. Comparison of percentage inhibition of paw edema by different extracts of *M. arvensis* with standard drug.

Plant sample	Treatment	Percentage inhibition of paw edema (%) (mean ± SD)			
		1 h	2 h	3 h	4 h
Leaf	Ethanolic extract	68.30	46.40	36.50	24.50
	Aqueous extract	40.50	55	47.10	43.40
Stem	Ethanolic extract	10.70	10.70	3.80	0.47
	Aqueous extract	30.90	11.60	10.58	5.28
Root	Ethanolic extract	48.80	38.39	24.50	5.67
	Aqueous extract	19.03	11.60	13.50	10.30
Standard	Diclofinac sodium	77.87	70	65.95	59.90

Values are expressed as mean ± SD (n = 12 mice per extract). P < 0.05, ANOVA followed by student's unpaired *t*-test.

al., 2004). Tannins are also phenolic compounds (Suliaman et al., 2007) and effective for the treatment of inflamed tissues (Igbinosa et al., 2009). Among all phytoconstituents, flavonoids content was found high in all ethanolic and aqueous extracts of leaves, stem and root, respectively. Flavonoids have been referred to as nature's biological response modifier, because of strong experimental evidence of their inherent ability to modify the body's reaction to allergies; hence, possess anti-allergic potential (Aiyelaagbe and Osamudiamen, 2009). Therefore, it is possible that at least part of anti-allergic effects observed with *M. arvensis* extracts may be attributed to their flavonoids component. Previous studies reported that phyto-chemical prospection of ethanolic extract of *M. arvensis* leaves indicates the presence of

different secondary metabolites (Nascimento et al., 2010). The presence of significant amount of these important phytoconstituents bestow high medicinal activities (including, anti-allergic and anti-inflammatory) on plant extracts.

In traditional practice, decoction or infusions of medicinal plants are usually made with either alcohol or water as the solvent, which impart marked difference in phyto-chemical and pharmacological profile of alcoholic and aqueous extracts (Akinmoladun et al., 2007; Roopashree et al., 2008). Therefore, considering its reported traditional use, ethanolic and aqueous extracts of leaves, stem and roots of *M. arvensis* were prepared and analyzed comparatively, for their anti-allergic and anti-inflammatory potential. Determination of anti-allergic

activity using histamine inhibitory assay, revealed that ethanolic extracts of leaf and root possessed marked percentage inhibition on the release of histamine from mast cells. Whereas, aqueous extract of leaf also exhibited significant inhibition against histamine release and these results are consistent with the findings reported by Choi et al. (2000), stating that *M. arvensis* water extract, dose dependently inhibited the histamine released from rat peritoneal mast cells, activated by anti-DNP IgE antibody. It is obvious from results that ethanolic leaf extract showed 57% inhibition of histamine released from mast cells, which revealed that it has protective effect against mast cells degranulation. Allergic reaction is an IgE-mediated immune response, resulting in histamine secretion from deregulation of mast cells and blood basophils, and triggers allergy response (Tewtrakul et al., 2008). Results have shown that ethanolic and aqueous leaf extracts significantly inhibited deregulation of mast cells and this inhibition was statistically significant ($P < 0.01$ and $P < 0.05$), so inhibition of histamine released was effective in allergic disorders. The amount of histamine released depends upon deregulation of mast cells; so, inhibition of mast cell deregulation is promising for controlling allergic reaction. Evidently, mast cells are well recognized for their key role in allergic and anaphylactic reactions, but recent facts implicate that mast cells are critical for the development of inflammatory diseases (Theoharides and Kalogeromitros, 2006).

The anti-inflammatory activity of different extracts of *M. arvensis*, was also evaluated by using histamine-induced paw edema test. All extracts showed anti-inflammatory effect, but ethanolic and aqueous extract of *M. arvensis* leaves exhibited significant effect at early and late phases of inflammation. Histamine-induced paw edema is based upon the ability of compounds (or plant extracts) to inhibit the edema. It is a form of chemically induced inflammation in which histamine action results in swelling (Suralkar, 2008). The inflammation produced in sensitized subjects after exposure to a specific allergen produces an acute reaction, which is known as an early-phase reaction developed within minutes and in many subjects followed by a late-phase reaction leading to chronic allergic inflammation (Galli et al., 2008). The experimental evaluation revealed the l-menthol content of *Mentha piperita* anti-inflammatory effect on IL-1 beta production (Juergens et al., 1998). Studies have also shown that l-menthol, menthone and 1,8-cineole suppressed antigen-induced histamine release in rat peritoneal mast cells. Another *in vivo* evaluation (experimental guinea pigs) shows that intraperitoneal administration of menthol inhibited homologous passive cutaneous anaphylaxis (PCA) mediated by IgE antibody (Arakawa et al., 1992).

Furthermore, these results have shown that aqueous leaf extract exhibited significant anti-inflammatory activity peaked at 2 h after histamine challenge. Therefore, considering that histamine is the main mediator in paw

edema model (Andrade et al., 2007), it can be suggested that the ethanolic and aqueous extracts of leaves, stem and root of *M. arvensis* contain compounds that are capable of inhibiting histamine release from mast cells and/or block histamine receptors, thus exhibited anti-inflammatory potential. These finding justifies the usefulness of *M. arvensis* in the treatment of allergic and inflammatory diseases.

Conclusion

All parts of *M. arvensis* (specifically, leaves) are rich source of secondary phytoconstituents, which impart their therapeutic effects against allergic and inflammatory diseases. These results support the claim about the use of this herb in folk medicines. Therefore, it is proposed that bioactivity-guided studies should be undertaken to find the mechanism of action of plant extracts in various allergic and inflammatory disorders. Further investigation regarding isolation and purification of a number of phytoconstituents from leaves, stem and root of *M. arvensis*, may yield optimal combinations for treating allergic and inflammatory diseases.

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