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ASSESSMENT OF ANALGESIC, ANTI-INFLAMMATORY AND ANTI-PYRETIC ACTIVITIES OF *MALVA NEGLECTA* WALLR. IN ANIMAL MODEL

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Abstract

Analgesic, anti-inflammatory and anti-pyretic activities of n-hexane and aqueous ethanolic extracts of *Malva neglecta* in *Swiss* albino mice was evaluated. For each activity, the mice were divided into 4 groups: Group 1 served as control, Groups 2 and 3 were given n-hexane and aqueous ethanol extracts, respectively whereas Group 4 was treated with the standard drug. Analgesic activity was evaluated against acetic acid induced writhing, Eddy's hot plate method, and Formalin induced paw licking. Anti-inflammatory activity was evaluated by carrageenan-induced paw edema. The extracts were also examined for their anti-pyretic activities against yeast-induced pyrexia. Results showed that the n-hexane ($p < 0.05$) and aqueous ethanolic ($p < 0.005$) extracts of *M. neglecta* exhibited analgesic activity by reducing acetic acid-induced writhing, mean reaction time on hot plate model and formalin-induced paw licking in mice as compared to the control. The n-hexane extract ($p < 0.05$) as well as aqueous ethanolic ($p < 0.005$) extracts produced significant anti-inflammatory activity as compared to the control. Both the n-hexane and aqueous ethanolic extracts revealed significant antipyretic activity ($p < 0.005$ and $p < 0.05$, respectively) in mice model. The results of the present study demonstrated that the n-hexane and aqueous ethanol extracts of *M. neglecta* possess analgesic, anti-inflammatory and anti-pyretic activities.

Introduction

The problem of resistance and tolerance to the existing drugs has created a decreased efficacy of the currently available drugs. Attempts have been taken to overcome such problems by synthesis of new drugs, either by the use of proteomics (Qadir 2011), or synthesis from lactic acid bacteria (Masood *et al.* 2011), or from marine microorganisms (Javed *et al.* 2011). However, now a days, the trend is being changed to the use of herbal products or extracts to control the diseases. The plant kingdom still holds many species containing substances of medicinal value which have yet to be discovered; large numbers of plants are constantly being screened for their possible pharmacological value particularly for their anti-inflammatory, hypotensive, hepatoprotective, hypoglycaemic, amoebicidal, anti-fertility, cytotoxic, antibiotic, spasmolytic, bronchodilator, antioxidant and anti-Parkinsonism properties (Ahmad *et al.* 2012, Janbaz *et al.* 2012).

Malva neglecta belonging to Malvaceae, known as dwarf mallow and round leaf mallow, has been known in traditional medicine as a demulcent, emollient and good cooling agent. It has been used in chronic bronchitis, inflammation, bladder ulceration and can also be applied on skin in case of skin diseases (Ahmad *et al.* 2009). It is also used in abdominal pain as antispasmodic and also applied on affected joint in rheumatic pain (Sarper *et al.* 2009, Sher *et al.* 2011). *M. neglecta* has also been reported to have antibacterial, anti-ulcerogenic and antioxidant activities (Mavi *et al.* 2004, Gurbuz *et al.* 2005, Mansour *et al.* 2010).

Considering different uses of this plant in traditional systems of medicines, the present study was carried out to evaluate the *in vivo* analgesic, anti-inflammatory and anti-pyretic activity of n-hexane and aqueous ethanolic extracts of *M. neglecta* Swiss Albino mice.

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Materials and Methods

Arial parts of *M. neglecta* were collected from local market in July 2012. The plant samples were identified at the Department of Botany, University of Agriculture, Faisalabad, Pakistan, where a voucher specimen has been deposited (voucher number-10569). The shade-dried powdered plants were extracted by the method of cold maceration in order to prepare n-hexane and aqueous ethanolic (30:70) extracts. After soaking the powder for 48 hrs, it was passed though muslin cloth and then through filter paper for filtration. Rotary evaporator was used for the drying of these extracts. For bioassay, the extracts were dissolved in normal saline by using Tween-80 for administration (Saha and Ahmed 2009) to the mice.

The animals used were male *Swiss* albino mice of weight 20-30 g. They were kept in propylene cages. Temperature and humidity of the animal house were maintained. Animals were given clean and adequate food and free access to water. The animals were divided into 4 groups each containing 6 animals. For each activity, Group 1 served as control, Group 2 was given n-hexane extract, Group 3 received aqueous ethanolic extract and Group 4 served as standard treated animals.

After 30 min of oral extract treatment, each mouse was intraperitoneally injected with 0.2 ml of 3 % acetic acid to induce writhing. Acetic acid causes stretching of hind limb along with abdominal constrictions, which was measured between 5 to 15 min after acetic acid administration. After that, the response of different extracts was compared with the responses of animals in control groups (Okokon *et al.* 2010).

After 1 hour of oral administration of different doses of extracts, the mice were placed on hot plate. The temperature of the plate was kept at 55-56 ºC. The reaction time was the time taken by the animal to lick the hind paw or jump out of the place and was measured at 0, 30 and 60 min (Yadev *et al.* 2011).

The 2.5% Formalin solution was injected under the surface of hind paw of mice after 1 hr of administration of extracts and the responses were observed immediately for 30 min after the injection (Shylaja *et al.* 2008).

Extracts of *M. neglecta* were examined for their anti-inflammatory activities against Carrageenan-induced paw edema as described by Nawafor and Odwuasoba (2003). After 1 hr of treatment, 0.1 ml of freshly prepared Carrageenan suspension (1%) was injected into the sub plantar surface of hind paw to produce inflammation. The circumference was measured at 0, 1, 2 and 3 hrs after administration of injection with the help of Vernier caliper.

The plant extracts were also examined for their anti-pyretic activities against yeast-induced pyrexia as described by Okokon and Nawafor (2010). Pyrexia was induced by subcutaneous injection of 20% w/v aqueous suspension of Brewer's yeast 2 ml/kg. After 24 hrs, rectal temperatures were noted (pre-treatment values). The rectal temperature for all the groups was taken at 1 hr interval after the treatment.

The values of each bioassay are given as Mean ± SEM and the ANOVA test was used to determine the statistical significance of the test. The $p < 0.05$ was considered significant.

Results and Discussion

Results of analgesic activity of extracts of *M. neglecta* against acetic acid-induced writhing presented in Table 1 showed that the number of writhing (27 ± 1.89, $p < 0.05$) noted for n-hexane and aqueous ethanolic extract (15.3 ± 1.8, $p < 0.005$) between 5 to 15 min after administration of acetic acid was statistically significant as compared to the control mice. Thus, the aqueous ethanolic extract of *M. neglecta* showed more pronounced effect than that of n-hexane extract.

Analgesic activity of extracts of *M. neglecta* by hot plate method is shown in Table 2. The n-hexane extract displayed reaction time of 2.6 ± 0.4 and 3.1 ± 0.7 min after 30 and 60 min of treatment whereas the aqueous ethanolic extract showed 5.8 ± 0.4 and 8.2 ± 0.5 min, respectively. Thus, the aqueous ethanol extract of *M. neglecta* showed more significant effect than the *n*-hexane extract.

Table 1. Analgesic activities of extracts of *M. neglecta* on acetic acid-induced writhings.

Treatment	Number of constrictions/writhings (Mean ± SEM)	Per cent inhibition
Control (water) 2 ml/kg bw	33.3 ± 2.1	-
n-hexane *(M. neglecta*) 100 mg/kg bw	27 ± 1.89*	18%
Aqueous ethanolic (*M. neglecta*) 100 mg/kg bw	15.3 ± 1.8**	53%
Aspirin 100 mg/kg bw	14 ± 0.96**	57%

* $p < 0.05$, ** $p < 0.005$ compared to control.

Table 2. Analgesic activity of extracts of *M. neglecta* on hot plate model.

Treatment	Reaction time in sec (Mean ± SEM)	
	After 30 min	After 60 min
Control (water) 2 ml/kg bw	4.6 ± 0.8	5.6 ± 0.8
n-hexane extract 100 ml/kg bw	2.6 ± 0.4	3.1 ± 0.7 *
Aqueous Ethanol 100 ml/kg bw	5.8 ± 0.4	8.2 ± 0.5 *
Aspirin 100 ml/kg bw	16.8 ± 1.5**	4.5 ± 1.4 **

* $p < 0.05$, ** $p < 0.005$ compared to control

Results of analgesic activity on formalin induced hind paw licking on mice model of *M. neglecta* extracts are shown in Table 3. In the specified time (30 min), the number of licking of hind paw by the n-hexane and aqueous ethanolic extract was found to be of 189 ± 13.7 ($p < 0.05$) and 114 ± 9.9 ($p < 0.005$), respectively as compared to the control group.

Results presented in Table 4 on the anti-inflammatory activity of the plant extracts against carrageenan-induced paw edema exhibited that the *n*-hexane extract of *M. neglecta* produced significant ($p < 0.05$) anti-inflammatory activity with 0.88 ± 0.01, 0.9 ± 0.01, 1.07 ± 0.03 and 1.29 ± 0.14 (cm paw volume) at 0, 1, 2 and 3 hrs, respectively. The aqueous ethanolic extract of the plant revealed much profound anti-inflammatory effect ($p < 0.005$) by giving the paw volumes measured by Vernier caliper as 0.91 ± 0.01, 1 ± 0.01, 1.05 ± 0.01 and 1.19 ± 0.03 (cm) at 0, 1, 2 and 3 hrs, respectively. Whereas, for standard drug (aspirin), the paw volume measurements were 0.79 ± 0.01, 0.86 ± 0.01, 0.92 ± 0.01 and 1.15 ± 0.18 (cm) at 0, 1, 2 and 3 hrs, respectively.

Table 3. Analgesic activity of *M. neglecta* extracts on formalin-induced hind paw lickings.

Treatment/Dose	No. of paw lickings (Mean ± SEM)	Per cent inhibition
Control (water) 2 ml/kg bw	288 ± 10.9	-
n-hexane extract 100 ml/kg bw	189 ± 13.7*	34%
Aqueous Ethanol 100 ml/kg bw	114 ± 9.9**	60%
Aspirin 100 ml/kg bw	94± 8.2**	67%

* $p < 0.05$, ** $p < 0.005$ compared to control

Table 4. Effects of *M. neglecta* extracts on carrageen induced paw edema.

Treatment	Inflammation (0 hr)	Inflammation (1 hr)	Inflammation (2 hr)	Inflammation (3 hr)
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Control (water) 2 ml/kg bw	0.83 ± 0.001	1.28 ± 0.01	1.63 ± 0.01	1.86 ± 0.01
n-hexane extract 100 ml/kg bw	0.88± 0.01*	0.9 ± 0.01*	1.07± 0.03*	1.29 ± 0.14*
Aqueous Ethanol 100 ml/kg bw	0.91 ± 0.01**	1 ± 0.01**	1.05 ± 0.01**	1.19 ± 0.03**
Aspirin 100 ml/kg bw	0.79 ± 0.01**	0.86 ± 0.01**	0.92± 0.01**	1.15 ± 0.18**

* $p < 0.05$, ** $p < 0.005$ compared to control

Table 5 shows the results of anti-pyretic activity of *M. neglecta* extracts against yeast induced pyrexia. Both the n-hexane and aqueous ethanolic extracts showed significant activity ($p < 0.005$ and $p < 0.05$, respectively). The rectal temperature was noted after the treatment. The *n*-hexane extract showed temperature 39.1 ± 0.29, 38.7 ± 0.17, 38.4 ± 0.3 and 38.4 ± 0.25ºC at 0, 1, 2 and 3 hrs, respectively. On the other hand, the aqueous ethanolic extract displayed the rectal temperature as 39.6 ± 0.23, 38.9 ± 0.2, 38.3 ± 0.13 and 38.6 ± 0.19ºC whereas the standard drug aspirin showed 38.5 ± 0.23, 37.6 ± 0.27, 37.8 ± 0.19 and 37.4 ± 0.36ºC at 0, 1, 2 and 3 hrs, respectively.

The folkloric claim of *M. neglecta* in pain, fever and inflammation has been reported along with several other traditional uses. Therefore, it was thought that scientific authentication is necessary for validation of the traditional claims. A quick literature review demonstrated that, no systemic pharmacological screening has been conducted on *M. neglecta* so far. Therefore, the pharmacological screenings of *M. neglecta* for analgesic, anti-inflammatory and anti-pyretic activities by using established methods in Swiss Albino mice model were carried out.

Table 5. Anti-pyretic activity of *M. neglecta* against yeast induced pyrexia.

Treatment/Dose	Rectal temperature (^{o}C)			
	Time after administration			
	0hr	1hr	2hr	3hr
Control (water) 2 ml/kg bw	39.2 ± 0.3	39.5 ± 0.26	39 ± 0.4	39.5 ± 0.22
n-hexane extract 100 ml/kg bw	39.1 ± 0.29	38.7 ± 0.17*	38.4 ± 0.8*	38.5 ± 0.3**
Aqueous Ethanol 100 ml/kg bw	39.6 ± 0.23*	38.9 ± 0.2	38.3 ± 0.13	38.6 ± 0.19*
Aspirin 100 ml/kg bw	38.5 ± 0.2	37.6 ± 0.3**	37.8 ± 0.19*	37.4 ± 0.4**

* $p < 0.05$, ** $p < 0.005$ compared to control

Results obtained in the present pharmacological screenings of the plant showed that the extracts exhibited analgesic activity by reducing acetic acid induced writhing, mean reaction in time on hot plate model and formalin induced paw licking in mice. In acetic acid-induced model, the analgesic property of the plant could be due to presence of some sterols and flavonoids (Bittar *et al.* 2000). In acetic acid induced abdominal writhing, the result showed that all doses of the test materials exhibited significant analgesic activity ($p < 0.05$). The biosynthesis of cyclooxygenase and prostaglandins causes the release of arachidonic acid which is the main cause of producing pain (Sawadago *et al.* 2006). These prostaglandins also cause inflammation by increasing capillary permeability (Amico- Roxas *et al.* 1984). In hot plate test, the extracts increased the latency time to heat stimulus. The effects began early at 30 min and persisted up to 60 min. Formalin test is mostly used for testing the analgesic activity. It produces a distinct two phase effect and various drugs of different nature act in a different way in early and late phases of this test. So, this test can be helpful to investigate the process of nociception of suggested analgesic drugs (Tjolsen *et al.* 1992). Formalin when administered produces an intense increase in spontaneous activity of the C fiber indicating that the pain differs in the form of paw licking by different animals (Ijeoma *et al.* 2011, Karthikeyan *et al.* 2011). The test extracts also demonstrated significant results in formalin induced paw licking test in mice.

In the carrageenan-induced edema test, the extracts of *M. neglecta* showed prominent effect at early stage of inflammation (1-2 hrs), by its main effects on histamine, serotonin and kinins as these are present at early stage of carrageenan-induced edema (Gorgewill and Nwankwoala 2010). The extracts also inhibited the later stage, mainly by inhibiting prostaglandins which are thought to mediate the second phase of edema (Vane and Booting 1987). The mediators of inflammation like histamine and serotonin are released within the first 30 min of injection of carrageenan, then kinins are released after 1 hr, prostaglandins are released after up to 2 hr, after induction of inflammation (Dirosa *et al.* 1971). However, aspirin, a cyclooxygenase inhibitor, significantly inhibited prostaglandins at the second stage of carrageenan-induced edema.

Antipyretics are drugs, which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point elevates (Goodman and Gilman 1996). The extracts of *M. neglecta* also showed significant antipyretic activity ($p < 0.05$) in mice model.

Malva neglecta consists of high concentration of alkaloids, terpenes and saponins (Mojab *et al.* 2003). So, these constituents mainly attribute to analgesic, anti-pyretic and anti-inflammatory activities. That is why, the plants may have NSAIDs like actions. Indomethacin, a NSAID, shows analgesic activity by reduction of sensitization of pain receptors at the inflammatory site (Dhara *et al*. 2000). The analgesic activities of plants are mainly produced to the presence of secondary metabolites like saponins, terpenes, flavonoids, and tannins. It is also evident that anti-inflammatory effect is shown due to the flavonoids, as they inhibit the cyclooxygenase pathway (Liang *et al.* 2001).

It may be concluded from the present study, that the n-hexane and aqueous ethanol extracts of *M. neglecta* possess statistically significant analgesic, anti-inflammatory and anti-pyretic activities. Hence, it may be supported to use this plant in ethnomedicine to relieve pain, fever and inflammation. However, further investigation with *M. neglecta* is required to elucidate the cellular mechanism and also to establish structural components of active ingredients for standardizing the plant, selectivity for COX-I and COX-II inhibition should also be investigated.

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