

Full Length Research Paper

Anti-inflammatory and analgesic activity of the methanol extract of *Malva parviflora* Linn (Malvaceae) in rats

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Malva parviflora Linn Malvaceae is a medicinal plant used for the treatment of wounds and other related ailments by the Xhosa people of South Africa. The anti-inflammatory activity of the stem extract of this plant was assessed using carrageenan-induced paw oedema and histamine-induced paw oedema. The analgesic effect was determined using the acetic acid writhing method as well as formalin test. The extract at 100 and 200 mgkg⁻¹ body weight reduced significantly, the formation of oedema induced by carrageenan and histamine. In the acetic acid-induced writhing model, the extract showed a good analgesic effect characterized by reduction in the number of writhes when compared to the control. The extract caused dose-dependent decrease of licking time and licking frequency in rats injected with 2.5% formalin, signifying its analgesic effect. These results were also comparable to those of indomethacin, the reference drug used in this study. Since the plant extract reduced significantly the formation of oedema induced by carrageenan and histamine as well as reduced the number of writhes in acetic acid-induced writhing models and dose-dependent decrease of licking time and licking frequency in rats injected with 2.5% formalin, it is concluded that the use of *M. parviflora* for the treatment of inflamed purulent wounds, swellings, bruises and broken limbs may have been justified.

Key words: Anti-inflammatory, analgesic, carrageenan, histamine, *Malva parviflora*

INTRODUCTION

Inflammation is a pathophysiological response of mammalian tissues to a variety of hostile agents including infectious organisms, toxic chemical substances, physical injury or tumor growth leading to local accumulation of plasmic fluid and blood cells (Sobota et al., 2000). Although a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain and aggravate many disorders. The use of non-steroidal anti-inflammatory drugs (NSAIDs) in the treatment of diseases associated with inflammatory reactions has adverse effects which pose a major problem in their clinical use. Hence, new anti-inflammatory and analgesic drugs lacking such effects are being searched for as alternatives to NSAIDs. According to Kumara (2001),

plant based drugs in traditional medicine are being paid much attention because of their minimal side effects, cheapness and also the fact that 80% of the world population still relies on them.

Malva parviflora Linn (family: Malvaceae), a prostrate perennial herb, with a deep strong tap root system, found in Europe, has become a cosmopolitan weed species in gardens throughout South Africa and Namibia (Henderson and Anderson, 1966). The leaf of this plant is used in the treatment of boils (Grierson and Afolayan, 1999) and inflamed purulent wounds (Watt and Breyer-Brandwijk) by the Xhosa people of South Africa. The poultice from its leaves is also used to treat wounds and swellings. In Lesotho, the plant is incorporated into a lotion to treat bruises and broken limbs and the dried powder or an infusion made from the leaves and roots is used by herbalists to clean wounds and sores (Shale et al., 1999). In Ethiopia, the root of *M. parviflora* is used in

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the treatment of asthma and wounds (Abate, 1989). Grierson and Afolayan (1999) showed that *M. parviflora* possessed an inhibitory effect on some fungi but was ineffective against some species of bacteria. In contrast, Shale et al. (1999) reported the antibacterial activity of the hexane and methanol extracts of the roots but noted poor activity of the methanol leaf extract.

The present study was undertaken to investigate the anti-inflammatory and analgesic potentials of *M. parviflora* in experimental animals in order to validate its folkloric use in the treatment of inflamed purulent wounds.

MATERIALS AND METHODS

Plant material and preparation of extracts

Young plant of *M. parviflora* was collected from the Nkonkobe municipality of the Eastern Cape province, South Africa in May 2007. The plant was identified by Prof. D. Grierson of the Department of Botany, University of Fort Hare, Alice, South Africa and a voucher specimen (S/N Aboyade 001) deposited at the Giffens Herbarium of the same institution. The plant material was air-dried at room temperature, ground to a powder (200 g) and extracted in methanol (1 l) by shaking for 24 h. The extract was filtered using a Buchner funnel and Whatman no 1 filter paper and concentrated to dryness under reduced pressure at 40 °C.

Animals

The animals used in this study were male wistar rats weighing between 120 and 290 g. They were maintained at the experimental animal house of the Agricultural and Rural Development Research Institute (ARDRI), University of Fort Hare in rat cages and fed on commercial rabbit cubes (EPOL Feeds, East London, South Africa). The animals were allowed free access to clean fresh water in bottles *ad libitum*. All experimental protocols were in compliance with University of Fort Hare Ethics Committee on research in animals as well as internationally accepted principles for laboratory animal use and care.

Chemicals

Carrageenan, Tween 80, carboxymethylcellulose and acetic acid were obtained from Sigma-Aldrich (Chemie GmbH, Steinheim, Denmark). The standard drugs used in the various experiments were indomethacin and histamine obtained from Sigma-Aldrich. All the chemicals and drugs used were of analytical grade.

Anti-inflammatory activity

Carrageenan-induced paw oedema

16 animals used in this study were divided into 4 groups of 4 animals per group. The first group served as the control, the second, third and fourth group received respectively indomethacin (10 mgkg⁻¹ body weight) and the *M. parviflora* extract at 2 doses of 100 and 200 mgkg⁻¹. The plant extract was suspended in carboxymethylcellulose while indomethacin was suspended in 3% Tween 80 in normal saline. Carrageenan solution (0.1 ml of 1% carrageenan solution) was injected into the sub plantar region of the right hind paw of the rats 1 h after intraperitoneal administration of 3% Tween solution, indomethacin and extract (Moody et al., 2006). The paw volume was measured at 0 h and at 1, 2 and 3 h after administra-

tion of drug and extract using a micrometer screw gauge.

The anti-inflammatory effect of the extract was calculated by the following equation; anti-inflammatory activity (%) = (1 - D/C) x 100, where D represented the average paw volume after the extract was administered to the rats and C was the paw volume in the control groups. The percentage inhibition of the inflammation was calculated from the formula: % inhibition = D0 - Dt/D0 x 100 where D0 was the average inflammation (hind paw oedema) of the control group at a given time and Dt was the average inflammation of the drug treated (that is, extracts or reference indomethacin) rats at the same time (Gupta et al., 2005; Sawadogo et al., 2006).

Histamine induced paw oedema

Using the method of Perianayagam et al. (2006), the paw oedema was produced by sub-plantar administration of 0.1% freshly prepared solution of histamine into the right hind paw of the rats. 16 rats divided into 4 groups of 4 rats per group were used. The paw volume was recorded before 0 and 1 h after the histamine injection. The 4 groups of the rats were pretreated with the plant extract (100, 200 mgkg⁻¹), 2 mlkg⁻¹ of normal saline (vehicle control) or 10 mgkg⁻¹ indomethacin (reference drug). These were administered intraperitoneally 1 h before eliciting paw oedema. The anti-inflammatory activity was calculated as described for carrageenan-induced oedema.

Analgesic activity

Acetic acid-induced writhing in rats

The acetic acid-induced writhing test measures abdominal constrictions together with stretching of the hind limbs resulting from intraperitoneal (i.p.) injection of 0.7% acetic acid (10 mlkg⁻¹). This was carried out according to the procedures described by Sawadogo et al. (2006). 4 groups of 4 animals per group were used in this study comprising the control (2 mlkg⁻¹ normal saline solution), indomethacin (10 mgkg⁻¹), or plant extract (100, 200 mgkg⁻¹). After 30 min, acetic acid was administered i.p. The number of writhing movements was counted for 30 min.

Formalin test in rats

The procedure was essentially similar to that described by Correa and Calixto (1993). Formalin solution (0.05 ml of 2.5% formalin) was injected into the sub-plantar of the right hind paw. The number of times and the time spent licking the injected paw was recorded and was considered as indicative of pain. The animals were pre-treated with indomethacin and plant extract (100 and 200 mgkg⁻¹) 30 min before being administered with formalin and the responses were observed for 30 min. The control group was pre-treated with normal saline.

Statistical analysis

The data were expressed as mean ± S.D. Where applicable the difference in response to test drugs was determined by student's t-test. P < 0.05 was considered significant.

RESULTS

Anti inflammatory activity

Carrageenan-induced paw oedema

When compared with the control, the extract and indome-

Table 1. Anti-inflammatory activity of the methanol extract of *M. parviflora* on carrageenan-induced oedema in the right hind-limb of rats. Data is presented as mean \pm S.D., n = 4.

Time (h)	Control	Extract (mgkg ⁻¹)		Indomethacin (10 mgkg ⁻¹)
		100	200	
1	21 \pm 0.3	11 \pm 0.5 (48.0)	0.7 \pm 1.0 (96.6)	4.0 \pm 0.5 (81.0)
2	28.9 \pm 0.1	9.7 \pm 0.7 (66.6)	5.8 \pm 0.2 (80.0)	5.1 \pm 0.4 (82.5)
3	30.1 \pm 0.1	14.5 \pm 0.9 (51.9)	4.8 \pm 0.7 (84.1)	3.1 \pm 0.4 (89.6)

Percentage inhibitions of the carrageenan-induced inflammation (oedema) are in parenthesis.

Table 2. Anti-inflammatory activity of the methanol extract of *M. parviflora* on histamine-induced oedema in the right hind-limb of rats. Data is presented as mean \pm S.D., n = 4.

Time (h)	Control	Extract (mgkg ⁻¹)		Indomethacin (10 mgkg ⁻¹)
		100	200	
1	18.5 \pm 0.3	18.2 \pm 0.5 (1.6)	13.0 \pm 0.5 (29.5)	17.0 \pm 0.3 (7.9)
2	14.4 \pm 0.2	14.2 \pm 0.5 (1.5)	5.5 \pm 0.1 (62.1)	11.7 \pm 0.2 (18.7)
3	9.7 \pm 0.1	7.9 \pm 0.9 (19.0)	5.5 \pm 0.2 (43.8)	3.4 \pm 0.12 (65.0)

Percentage inhibitions of the carrageenan-induced inflammation (oedema) are in parenthesis.

Table 3. Influence of methanol extract of *M. parviflora* and indomethacin on rat writhing reflex induced by acetic acid.

Group	Dose (mgkg ⁻¹)	Number of writhing per 20 min	Inhibition (%)
Control	3 ml/kg	50.4 \pm 2.2	0
Indomethacin	10	0 \pm 0.0	100
Extract	100	0 \pm 0	100
Extract	200	0 \pm 0	100

Data is presented as mean \pm S.D., n = 4.

thacin significantly reduced the paw oedema hours after carrageenan injection. For instance, the 100 mgkg⁻¹ produced its highest effect at 2 h (67%) after carrageenan injection while the 200 mgkg⁻¹ was more effective 1 h (97%) after injection. The reference drug produced time-dependent reduction as the effect was more pronounced at 3 h (90%) of carrageenan administration (Table 1).

Histamine-induced paw oedema

Inhibition of histamine-induced oedema was observed in this study. The antihistaminic activity of the 200 mgkg⁻¹ extract was most pronounced at 2 h (62%) while that of the 100 mgkg⁻¹ and indomethacin was at 3 h (Table 2).

Anti-nociceptive activity

Acetic acid-induced writhing

The effect of the methanol extract of *M. parviflora* on

writhing response in rats showed that the extract at 100 and 200 mgkg⁻¹ caused 100% inhibition on the writhing response induced by acetic acid. A similar inhibition was observed with indomethacin, the reference drug used in the study (Table 3).

Formalin test

In this study, the extract caused a dose-dependent decrease in licking time and licking frequency by the rats injected with formalin. The indomethacin group showed better analgesic effect than the 2 doses. The analgesic effects of these groups were significantly different from that of control at $P < 0.05$ (Table 4).

DISCUSSION

The results of this investigation suggest that the methanol extract of *M. parviflora* possesses a dose dependent activity against carrageenan and histamine induced paw oedema in rats. The activity of the 200 mgkg⁻¹ of the

Table 4. Analgesic effect of methanol extract of *M. parviflora* and indomethacin on formalin test on mice.

Group	Dose (mgkg ⁻¹)	Licking time (s)	Licking frequency/30 min
Control	0	15 ± 2.5	25.3 ± 0.5
Indomethacin	10	5.8 ± 0.5	13.8 ± 0.3 ^a
Extract	100	6.4 ± 0.3	15.4 ± 0.6 ^a
Extract	200	2.3 ± 0.5	14.5 ± 0.2 ^a

Data is presented as mean ± S.D., n = 4.

^a Superscript is significantly different from the control at P < 0.05.

extract was comparable to that of indomethacin. The carrageenan-induced inflammation model which is a predictive test for anti-inflammatory agents acts by inhibiting the mediators of acute inflammation (Ozaki, 1990; Mossai et al., 1995; Silva et al., 2005; Sawadogo et al., 2006). Therefore, these results suggest that *M. parviflora* may be effective in the treatment of acute inflammatory disorders.

In the histamine-induced paw oedema, 200 mg kg⁻¹ of the extract exhibited a higher inhibitory effect than the reference drug at 2 h. It should be noted that the early phase (1 - 2 h) in the induced paw oedema model is mainly mediated by histamine, serotonin and the increase of prostaglandin (PG) synthesis in the surroundings of the damaged tissues. The late phase on the other hand, is mainly mediated by bradykinin, leukotrienes, polymorphonuclear cells and PGs produced in tissue macrophages (Antonio and Souza Brito, 1998; Cuman et al., 2001; Linardi et al., 2002; Vasudevan et al., 2007). The results of the present study suggest that the suppression of inflammation at the early phase was as a result of the antihistamine activity of the plant extract.

The acetic acid induced writhing test, a non specific but nevertheless sensitive method widely used for analgesic screening (Le Bars et al., 2001), indicated a 100% inhibition of writhing in the animals at both doses of the extract and also of the reference drug used in this study. Acetic acid has been found to cause an increase in peritoneal fluid levels of prostaglandins (PGE₂ and PGF₂), hence causing inflammatory pain by inducing capillary permeability (Amico-Roxas et al., 1984). The observed effects in the present study suggest that *M. parviflora* had an inhibitory effect on prostaglandins synthesis.

The formalin test has been described as a convenient method for producing and quantifying pain in rats (Dubuisson and Dennis, 1977; Tjolsen et al., 1992). The test employs an adequate painful stimulus to which the animals show a spontaneous response and it is sensitive to commonly used analgesics. The pain stimulus, a continuous rather than a transient one, may have resemblance to some kinds of clinical pain and observations are made on animals which are restrained only lightly or not at all (Hunskar et al., 1985; Ghannadi et al., 2005). Intraplantar injection of 2.5% formalin evoked a characteristic licking response in the Wistar rats. In this

study, the extract caused a dose-dependent decrease in licking time and licking frequency by the rats injected with formalin signifying the analgesic effect of the extract. Although the active doses of the plant extract were higher than those of the reference drug, it should be noted that the extract is made up of different compositions of several substances.

It is concluded that the plant extract reduced significantly the formation of oedema, reduced the number of writhes and dose-dependent decrease of licking time and licking frequency in rats. These results may have validated the basis for the traditional use of *M. parviflora* against inflamed purulent wounds, swellings, bruises and broken limbs among the Xhosa people of South Africa.

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