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Research Article

INVESTIGATION OF ANTI-INFLAMMATORY AND ANTINOCICEPTIVE EFFECTS OF AQUEOUS EXTRACTS OF *ARTEMISIA AFRA* IN WISTAR RATS

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ABSTRACT

Objective: The objective of the study was to evaluate the anti-inflammatory and antinociceptive effects of *Artemisia afra*.

Methods: Animals were randomly divided into five groups of six animals each and administered with normal saline (2 ml/kg), indomethacin (10 mg/ kg), and *A. afra* at doses of 100, 200, and 400 mg/kg, respectively. For the anti-inflammatory activity, carrageenan-induced paw edema was used while the hot plate and acetic acid induced-writhing tests were used to assess the antinociceptive activity.

Results: Pretreatment with *A. afra* at a dose of 100 mg/kg did not show any significant biological effects ($p>0.05$) for any of the three tests, when compared against saline-treated control group. At a dose of 200 mg/kg, *A. afra* demonstrated significant effects ($p<0.01$), during the 5th h reducing carrageenan-induced paw edema by 12%. The highest dose (400 mg/kg) of *A. afra* demonstrated more potent effects by decreasing the carrageenan-induced paw swelling ($p<0.001–0.05$) during the 3rd, 4th, and 5th h, by up to 38% when compared against saline-treated control group. Both the 200 and 400 mg/kg, *A. afra* doses achieved a significant increase ($p<0.05$) in reaction time in the hot plate test. In the acetic acid-induced writhing test, pretreatment with *A. afra* (400 mg/kg) significantly reduced pain by 39% ($p<0.01$) by comparison with the saline control.

Conclusion: Experimental data demonstrated that aqueous extract of *A. afra* possesses anti-inflammatory and antinociceptive properties in experimental acute inflammation and pain. These findings support the usage of *A. afra* in managing inflammation and pain in traditional practice.

Keywords: *Artemisia afra*, Antinociceptive, Analgesic, Anti-inflammatory, Medicinal plants.

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INTRODUCTION

Inflammation is a local defensive reaction of a living body to eliminate or limit the spread of injurious agent [1]. Injurious agents may include infections, chemicals, and thermal and mechanical injuries [2]. Inflammation is often characterized by the classical features of swelling, heat, redness, and often pain. More mechanistic definitions of inflammation have now been established that involve invading macrophages and lymphocytes and induction or activation of inflammatory mediators such as kinins and pro-inflammatory cytokines tumor necrosis factor (TNF-α), interleukin-1 (IL-1), interleukin-2, interleukin-6 (IL-6), interleukin-8, and interleukin-12, and chemokines [3]. The majority of these molecules are locally produced, and their involvement in tissue inflammation is well established and is therefore worthy targets for therapeutic intervention in a variety of diseases [4].

Inflammation and nociception are functionally linked at multiple levels such as formation of exudates, tissue swelling, and inflammatory mediators which are responsible for "inflammatory pain." Indeed, both are non-specific manifestations of many kinds of diseases [5]. In addition, prostaglandins can lower the threshold of pain sensation by increasing the sensitivity of nociceptors [6]. There is evidence to suggest that pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α are involved in the process of pathological pain [7]. Indeed, all pain is hypothesized to originate from inflammation and the inflammatory response [8]. Therefore, efforts meant to manage inflammation can resolve pain.

Prolonged inflammation has been now implicated in the pathogenesis of most chronic illness which including cancer, cardiovascular diseases, diabetes, obesity, pulmonary diseases, and eurologic diseases [9]. There are numerous other inflammatory disorders including rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, and psoriasis [10]. Many occur when the immune system mistakenly triggers inflammation in the absence of infection [4].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed drugs worldwide in managing pain and inflammation [11]. Nevertheless, NSAIDs and other anti-inflammatory drugs are associated with numerous side effects including gastrointestinal ulcers, bleeding, and renal disorders [2,12]. It is, therefore, necessary to continue searches of anti-inflammatory agents that are effective and have minimal side effects.

Since ancient times, people in the Eastern Cape of South Africa, like people of other traditions elsewhere, have relied on traditional medicine, including medicinal plants, as prophylactic to restore, and maintain health. Plants are well known to be an important source of many biologically active compounds, especially for the treatment of pain and inflammatory diseases [13]. *Artemisia Afra* is one of the most widely used medicinal plants to treat inflammation and assorted forms of pain in the Eastern Cape of South Africa. It is also referred to as African wormwood (*wilde-als* in Afrikaans, *lengona* in Sotho, and *umhlonyane* in Xhosa and Zulu) [14]. This plant is widely used in traditional practice for the treatment of a variety of ailments including colds, fever, cough, asthma, diabetes, blocked nose, malaria, and other chest complaints [15,16]. In previous studies, it has been shown that dichloromethane extract of *A. afra* contains antimycobacterial activity which can inhibit both rapid growing *Myobacterium aurum* and virulent *Myobacterium tubercolosis* replication [13]. It also demonstrated

antimalarial, antioxidant, antimicrobial, antinematodal, cardiovascular (hypertensive), and sedative and cytotoxic effects [16,17].

METHODS

Plant collection and preparation of extract

A. afra whole plant material was collected from the outskirts of Mthatha town. The plant was identified and authenticated by a botanist of the Department of Botany, Walter Sisulu University. The material was then air dried at room temperature and grounded into powder using a blender. The powered plant material was macerated in distilled water. During extraction, the mixture was intermittently kept shaking using a laboratory shaker for approximately 48 h in a flask. The decoction was then filtered using Whatman filter paper. The filtrate concentrated and dried on a hot air oven at 37°C. The dried extract was named AAE to denote *A. afra* extract.

Animals

Adult male Wistar rats weighing 200 g–250 g were procured from the South African Vaccine Producers PTY (Ltd) in Johannesburg, South Africa, and were transported to Mthatha by air and kept in the animal holding facility of the Department of Human Biology, Walter Sisulu University. Rats were kept in grouped cages and subjected to a 12 h light: 12 h dark cycle at room temperature. Animals were maintained on standard rodent chow with *ad libitum* access to clean tap water.

For each of the three experimental protocols below, animals were randomly divided into five groups of six animals. Animals of Groups I and II served as negative and positive control groups and were treated with normal saline (2 ml/kg) and indomethacin (10 mg/kg), respectively. Groups III, IV, and V were administered with *A. afra* extract at doses of 100, 200, and 400 mg/kg, respectively.

Anti-inflammatory activity: Carrageenan-induced rat hind paw edema

Animals were starved overnight before the start of the study. Rats were divided into five groups and pretreated as described above. Edema was induced 30 min later in all the rats by subplantar injection of 0.05 ml of 1% carrageenan in freshly prepared normal saline, to the left hind paw. Paw volumes were measured using a plethysmometer (model 7140, Ugo Basile, Italy) before administration of carrageenan (time 0) and at 1, 2, 3, 4, and 5 h, after carrageenan, injection. Results obtained were compared with those obtained from the control groups, which received vehicle only at the respective times [18].

Antinociceptive activity: Hot plate test

To assess the effects of *A. afra* extract on thermally induced pain, a hot plate was used according to the modified protocol as previously described [19]. Rats were divided into five groups of six animals each and pretreated as described above. The animals were placed on the hot plate apparatus whose temperature was maintained at 55±1°C. The time period between placing the animals on the heated surface and beginning of licking of paws or jumping was considered the reaction time and recorded. Each time, animals were kept on the heated surface for a maximum period of 15 s to avoid tissue damage. Readings were conducted before treatment and at 30, 60, 90, and 120 min after treatment.

Antinociceptive effects - Acetic acid-induced writhing

This test was carried out using a modified method previously described elsewhere [20]. Animals were randomly divided into five groups of six each and pretreated as described earlier. After 30 min, the pain was induced by intraperitoneal administration of 10 ml/kg of 0.07% aqueous solution of acetic acid to all rats. Each rat was immediately placed in a transparent observation cage and resulting contractions of abdominal muscle together with the stretching of the hind limbs that occurred between 5 and 20 min after acetic acid injection were counted [21].

Data presentation and statistical analysis

The data were presented in the form of tables and graphs. Results were presented as a mean±standard error of the mean. One-way analysis of variance was used to analyze and compare the data followed by Dunnett's test for multiple comparisons among groups. p</0.05 was taken as the limit of significance in all cases. Statistical analyses were made using GraphPad Prism version 5.00 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com).

RESULTS

Carrageenan-induced rat hind paw edema

Table 1 illustrates results of the effects of administration of *A. afra* extract on rat hind paw edema induced by Carrageenan. The saline control group attained a peak mean paw volume of 1.9±0.07 by the 2nd and 4th h from the mean baseline value of 1.1±0.09 ml. The paw volume waned off to 1.6±0.07 by the 5th h. *A. afra* administered at a dose of 100 mg/Kg did not show any significant effects against carrageenan-induced edema at any of the times. However, at a dose of 200 mg/kg, the test extract demonstrated significant effects (p<0.01), during the 5th h reducing paw volume by 12%. More potent effects were demonstrated by *A. afra* at a dose of 400 mg/kg, which significantly reduced the paw volume (p<0.001–0.05) during the 3rd, 4th, and 5th h, respectively, by 21%, 35%, and 38%.

Hot plate test

The saline control group had the reaction time reduced from 5.2±0.5 to 3.8±0.4 s 30 min after treatment and remained low at 4.0±0.4 s on the 120-min mark. The extract of *A. afra* at the lower dose of 100 mg/ kg did not affect the latency period when compared with the values of the saline control group. However, treatment with *A. afra* at a dose of 200 mg/kg significantly increased (p<0.05), the reaction time during 60 and 120 min readings. The dose of *A. afra* of 400 mg/kg demonstrated more potent effects (p<0.05), increasing the reaction time from the 30 min mark through to the 120 min mark. The pattern of effects of the *A. afra* (400 mg/kg) was similar to a reference standard, indomethacin whose treatment resulted in an increase from 30 min to 120 min although p<0.01. Results of this experiment are presented in Table 2.

Antinociceptive activity: Acetic acid-induced writhing

The aqueous extract at a dose of 400 mg/kg exhibited a marked reduction in the number of abdominal constriction induced by the injection of an aqueous solution of acetic acid. As shown in Fig. 1, the mean number of writhing was significantly lower (21.2±3.3, p<0.01) in rats given *A. afra* extract compared with the control (34.5±2.7).

Table 1: Effects of acute treatment of *A. afra* extract on carrageenan-induced paw edema

Treatment	Paw volume (ml)					
Groups	0	1	2	3	4	5
Control	1.1±0.09	1.6±0.07	1.9±0.05	1.9±0.07	1.7±0.18	1.6±0.06
Indo	1.1±0.03	1.1±0.05**	1.0±0.10***	1.0±0.04***	1.0±0.06**	1.1±0.09***
AAE100	1.1±0.05	1.5±0.10	1.7±0.07	1.8±0.10	1.6±0.17	1.6±0.07
AAE200	1.1±0.12	1.4±0.11	1.8±0.10	1.8±0.06	1.5±0.07	1.3±0.06*
AAE400	1.2±0.11	1.4±0.11	1.7±0.14	1.5±0.13*	1.1±0.10**	1.0±0.05***

Data were expressed as mean±SEM, n=6. *p<0.05 versus saline control, **p<0.01 versus control, ***p<0.001 versus control, p<0.05, ***p<0.01, p<0.001. AAE100, AAE200, and AAE400, respectively, denote *A. afra* extract at doses of 100, 200, and 400 mg/kg. Indo=Indomethacin (10 mg/kg). *A. afra: Artemia afra*, SEM: Standard error of the mean

Table 2: Effects of acute treatment of *A. afra* extract on reaction time on hot plate test

Treatment groups	Reaction time (s)			
	0 h	30	60	120
Control	5.2±0.5	3.8±0.4	3.8±0.5	4.0±0.4
Indo	5.5±0.7	6.7±0.5**	6.5±0.5**	7.0±0.9**
AAE100	5.0±0.4	5.2±0.3	5.0±0.3	5.7±0.5
AAE200	5.2±0.6	4.0±0.6	6.0±0.7*	6.0±0.3*
AAE400	5.5±0.6	6.2±0.5*	6.0±0.4*	6.2±0.4*

Data were expressed as mean±SEM, n=6. *p<0.05 versus saline control, **p<0.01 versus control. AAE100, AAE200, and AAE400, respectively, denote *A. afra* extract at doses of 100, 200, and 400 mg/kg. Indo: Indomethacin (10 mg/kg). *A. afra: Artemisia afra*, SEM: Standard error of the mean

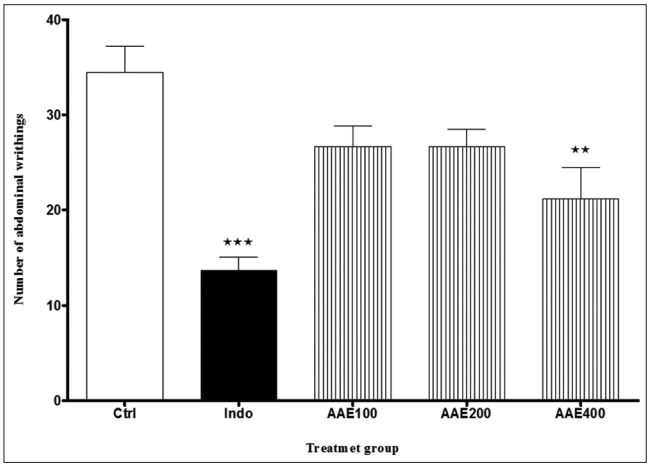

Fig. 1: Effects of treatment of *Artemisia afra* aqueous extract on acetic acid-induced writhings. Data are expressed as mean±standard error of the mean, n=6. **p<0.01 versus control; ***p<0.001 versus control. AAE100, AAE200, and AAE400, respectively, denote *A. afra* extract at doses of 100, 200, and 400 mg/kg. Indo: Indomethacin (10 mg/kg)

This represents a percentage inhibition of pain of 39%. The reference standard, indomethacin, on the other hand, reduced the number of abdominal writings to 13.7±1.4 by comparison with the saline-treated control group representing a percentage inhibition of 60%.

DISCUSSION

This study was aimed at evaluating the scientific basis for the traditional use of *A. afra* against inflammation and pain. The aqueous extract of *A. afra* was investigated for anti-inflammatory and antinociceptive activities using carrageenan-induced hind paw edema, acetic acid-induced abdominal contraction, and hot plate tests. The extract exhibited anti-inflammatory and anti-nociceptive potency in all the tests.

The carrageenan-induced rat paw edema is used widely as a working model of inflammation in the research for the new anti-inflammatory drug [22]. Development of inflammation due to carrageenan administration is believed to involve two phases - early and late phase [18,23]. The release of histamine and serotonin by mast cells characterize the first phase which lasts up to an hour while the second phase is mediated by the release of prostaglandin E_2 and other cytokines [24]. In the present study, *A. afra* demonstrated significant anti-inflammatory effects, p<0.05, against edema induced by carrageenan particularly from the 3rd through to the 5th h. This may suggest potential antiprostaglandin properties.

Acetic acid-induced writhings are a nonspecific pain model which is widely used in the assessment of peripheral analgesic activity [25]. Prostaglandin release is implicated in the pathogenesis of this pain model following acetic acid intraperitoneal administration [26,27]. As shown in Fig. 1, the administration of *A. afra* (400 mg/kg) significantly reduced abdominal writhings. Products that reduce the number of abdominal writhings often demonstrate antinociceptive properties through possible inhibition of prostaglandin synthesis [28].

A diverse number of medicinal plants with established therapeutic potential against pain and inflammation possess tannins, flavonoids, triterpenoids, and other secondary metabolites [29]. Although the present study did not determine the phytochemical composition of the test product, earlier studies elsewhere have reported that aqueous extract of *A. afra* possesses flavonoids [30]. Among others, flavonoids suppress cyclooxygenase activities and reduce the secretion of arachidonic acid [19].

Unlike the acetic acid abdominal writhing test which evaluates peripheral analgesia, the hotplate test is suitable for assessing analgesic agents that act centrally [31]. As shown in Table 2, administration of *A. afra* at doses of 200 and 400 mg/kg exhibited modest (when compared against indomethacin) but significant, p<0.05, analgesic effects, increasing the reaction time in the treated groups when compared against a saline control group. This suggests the possible involvement of supraspinal centers in mediating pain relief in addition to peripheral means. There are reports elsewhere of medicinal plants with analgesic properties that are mediated through central and peripheral means [32]. It has also been reported elsewhere that agents that act centrally to reduce pain do so by elevating the threshold of pain sensation [28].

CONCLUSION

The findings of the present study demonstrate that *A. afra* possesses anti-inflammatory and antinociceptive properties. These properties may be due to the possible presence of phytocompounds such as flavonoids which are known to suppress the synthesis of prostaglandins, a well-known mediator of inflammation and pain. The findings partly offer credibility to the traditional practice of managing inflammation and pain using *A. afra*.

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AUTHOR'S CONTRIBUTION

AM and LZ carried out the experimental work and drafted the manuscript. MG designed the study, MG, DK, EN, CS, MS, and JI supervised the work and edited the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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