*Research Article*


ISSN Print 2231 – 3648
Online 2231 – 3656

**Available Online at: www.ijpir.com**

**International Journal of Pharmacy and Industrial Research**

# *IN VIVO* ANTI-INFLAMMATORY AND ANTI-NOCICEPTIVE ACTIVITIES OF AERIAL PART EXTRACTS OF *ZEHNERIA SCABRA*

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## Abstract

Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. Inflammatory disorders are a major course of morbidity for the working force. It is believed that current analgesia-inducing drugs, such as opiates and non-steroidal anti-inflammatory drugs (NSAIDs) are not useful in all cases, because of their side effects and low potency. Therefore the search for new analgesic and anti-inflammatory compounds has been a priority of drug researchers. In line with this notion, the anti-inflammatory and antinociceptive activities of *Zehneria scabra* (Cucurbitaceous family) were determined. Plant material was extracted with 80% methanol using maceration and then activities were performed *in vivo* using acetic acid and carrageenin for antinociceptive and anti-inflammatory activity respectively. The plant extract showed significant analgesic activity at doses of 50,100 and 200 mg/Kg, and it also showed remarkable anti-inflammatory activity at 100,200 and 300 mg/Kg. Hence, it is easy to conclude that 80% methanol extract of *Zehneria scabra* exhibit antinociceptive and anti-inflammatory activities.

**Keywords:** Antinociceptive, Anti- inflammatory, *Zehneria scabra*, Maceration.

## Introduction

Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. The wide spread use of herbal remedies could be attributed to the occurrence of cultural acceptability, physical accessibility and economic affordability, as well as efficacy against certain types of diseases, as compared to modern medicine[1,2]. The potential of medicinal plants can be assessed by finding new chemical entities of wide structural diversity. These new chemical substances can also serve as templates for producing more effective drugs through semi-synthetic and total synthetic procedure. According to World Health Organization (WHO), about 74% of 119 plant-derived pharmaceutical medicines or biotechnology medicines are used in modern medicine in ways that correlate directly with their traditional uses[3]. The primary benefit of using plant-derived medicine is that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments. People are reverting back to herbal

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preparations since the data provided by herbal professionals and health care policy makers have found the herbal products to exhibit high level of satisfaction[4,5].

Countries in Africa, Asia and Latin America use traditional medicine to help meet some of their primary health care needs. In Africa, up to 80% of the population uses traditional medicine for primary health care[6, 17]. In industrialized countries, adaptations of traditional medicine are termed "Complementary" or "Alternative" medicine. Based on current research and financial investments, medicinal plants will, seemingly, continue to play an important role as health aid[4].

Inflammatory diseases including different types of rheumatic diseases are very common throughout the world and hence inflammatory disorders are a major course of morbidity for the working force throughout the world[8]. It is believed that current analgesia-inducing drugs, such as opiates and non-steroidal anti-inflammatory drugs (NSAIDs) are not useful in all cases, because of their side effects and low potency[5]. Therefore the search for new analgesic and anti-inflammatory compounds has been a priority of drug researchers[5, 8]. As a matter of fact the study of plants that have been traditionally used as pain killers is still a fruitful and logical research strategy in the development for new analgesic drugs [9 - 11].

**NS**AIDs relieve pain, stiffness, swelling, and inflammation, but they do not cure the diseases or injuries responsible for these problems. They can also cause a number of side effects, some of which may be very serious. Most of the time inflammation is followed by bacterial infection, which needs combined use of antibacterial and anti-inflammatory agents[12]. Concomitant use of several drugs to treat inflammatory conditions that might be associated with some microbial infections may cause health problems especially in patients with impaired liver or kidney functions[5, 13]. Obviously, a plant exhibiting anti -inflammatory, analgesic and antibacterial activity would improve patient compliance and has economic importance[13]. Some patients who have had problems with effectiveness and side effects from antibacterial and anti-inflammatory drugs can benefit from traditional medicines as there are herbs exhibiting antibacterial and anti-inflammatory qualities[5, 14,15].

The 80% methanol extract of *Zehneria scabra (*Linn.f.) sond exhibits antimicrobial activity against one of the most common bacterial pathogens, i.e. *staphylococcus aureus* and *E.coli* [16] and the plant has a traditional claim for treatment some inflammatory and painful conditions[17]. Hence, it is a wise approach to evaluate the credibility of anti-inflammatory and analgesic traditional claims of *Zehneria scabra* on a scientific basis so as to know whether the plant has analgesic and anti-inflammatory activities in addition to its proven antibacterial activity. To put this end, this study focuses on *in-vivo* anti-inflammatory and anti-nociceptive activities of aerial part extract of *Zehneria scabra.*

## Materials and methods

### Materials

Acetylsalicylic acid , carrageenan, acetic acid, saline, distilled water, plethysomometer (7140 Ugo, Italy), 1 ml syringes, needles, feeding tube, vials, electronic balance, stopwatch, gloves and other laboratory glass wares.

### Collection of Plant Material

Enough amount of the aforementioned plant was collected from North east part of Ethiopia for the experiment and it was identified and deposited in the national herbarium of the botanic laboratory (Faculty of Biology, Addis Ababa University, Ethiopia).

### Extraction

The leaves of *Zehneria scabra* were dried at room temperature and then powdered. The powder was extracted successively with 80% methanol by maceration at room temperature for 24 hours, and then it was filtered. The solvent was removed from resulting solution under vacuum in a rotary evaporator.

### Animals

The Swiss albino mice (18–25 g) were all kept in polypropylene cages in a room under controlled condition. All animals were fed according to a standard diet ad libitum and had free access to drinking water.

### Antinociceptive activity testing

The antinociceptive activity of the extract was determined using writhing test[18]. Nociception was induced by an intraperitoneal injection of acetic

acid to the mice. The animals were divided into group, was treated with 10mL/kg of vehicle (saline solution or Ethanol: Tween 80: distilled in the ratio of 0.5: 0.25: 4.25) intraperitoneally. The second group, the standard group, was treated with ASA (200 mg/kg, BW), as a reference drug. The last group was treated intraperitoneally with the 80% methanol extract of *Zehneria scabra* at different doses (50, 100, and 200 mg/kg). 30 minutes after the administration of these different substances, all the animals received 10mL/kg of 1% acetic acid intra-peritoneally. The number of abdominal writhes as a pain indicator was counted for 30 min, five minutes after the acetic acid injection. The antinociceptive activity was expressed as a percentage of inhibition of abdominal writhes.

***In vivo* anti-inflammatory testing**

*In vivo* anti-inflammatory activity was evaluated on the basis of inhibition of carrageenan-induced mouse hind paw[19]. Mice will be fasted for 12 hrs with free access to water until the experiment started. Extracts, standard drug and normal saline injections will be given into the gastrointestinal tract through oral gavage. 80% methanol extract was dissolved in water. The mice were divided into three groups of six animals each. The control group received 2.5mL/kg of vehicle (Ethanol- Tween80-distilled water in the ratio of 0.5: 0.25: 4.25). groups of six mice each. The first group, a control The standard group received acetyl salicylic acid (ASA) at a dose of 300mg/kg. The test groups received the methanolic extracts at doses of 100, 200 and 300mg/kg intraperitoneally. After thirty minutes, each rat was injected with 0.05mL of 1% carrageenan solution in saline as a phlogistic agent into the subplantar tissue of the left hind paw. The paw volume, up to the tibiotarsal articulation, was measured using a plethysmometer (model 7140, Ugo, Italy). The measurements were taken at 0, 1, 2, 3, 4, and 5 hours after the carrageenan injection. Edema was expressed as the increase in paw volume due to carrageenan injection as compared to the paw volume just before administration. The significance of differences between means (against control) was assessed by two-sample t-test, with a significance level of $p < 0.05$. The percent inhibition of edema volume was obtained from the ratio of the mean of edema volume of the test group to that of the control at a specific period of time.

## Result

**Extraction**

Dried and pulverized leaves of 200g of *Zehneria scabra* were extracted by maceration with 80% methanol and the yield was 30g (15%).

**Antinocicepive activity test**

**Table No. 01: The effect of 80% methanol extract of *Zehneria scabra* and Asprin on aceticacid induced writhing in mice**

| Treatment | Dose in mg/Kg | Mean no of writhes ± SEM | Inhibition in % |
|---|---|---|---|
| Control | 10mL/kg | 126 ±3.56 | 00 |
| Treatment | 50mg/Kg | 75±2.39 | 40.48% |
| | 100mg/Kg | 59±1.78 | 53.17% |
| | 200mg/Kg | 32±1.56 | 74.60 |
| Aspirin | 200mg/Kg | 17±1.25 | 86.5% |

**Anti-inflammatory activity test**

Anti-inflammatory of the crude extract of *Zheneria scabra* and aspirin on carrageenan induced mice paw edema was evaluated as shown in table 2 and 3. The test extract produced significant inhibition of paw edema as compared to the control.

**Table No. 02:** The anti-inflammatory effect of crude 80% extract of Zehneria scabra and aspirin

| Treatment | Dose (mg/Kg) | Volume before carrageenen | Mean paw edema ±SEM (ml) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1h | 2h | 3h | 4h | 5h |
| Control | 2.5 | 1.07 | 1.89±0.051 | 1.95±0.04 | 2.1±0.025 | 2.26±0.038 | 2.36±0.015 |
| Extract of Zehneria scabra | 100 | 1.04 | 1.52±0.071 | 1.54±0.067 | 1.61±0.033 | 1.72±0.023 | 1.81±0.041 |
| | 200 | 1.03 | 1.46±0.017 | 1.48±0.046 | 1.52±0.016 | 1.64±0.032 | 1.73±0.05 |
| | 300 | 1.09 | 1.48±0.024 | 1.50±0.028 | 1.55±0.013 | 1.68±0.017 | 1.74±0.029 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Aspirin | 300 | 1.05 | 1.41±0.018 | 1.43±0.065 | 1.46±0.013 | 1.58±0.041 | 1.66±0.034 |

**Table No. 03:** The calculated percentage of paw edema inhibition of crude 80% extract of *Zehneria scabra* and aspirin

| Treatment | Dose (mg/Kg) | Percentage of inhibition | | | | |
|---|---|---|---|---|---|---|
| | | 1h | 2h | 3h | 4h | 5h |
| Extract of | 100 | 41.46 | 43.18 | 44.66 | 42.86 | 40.31 |
| Zehneria | 200 | 47.56 | 48.86 | 52.43 | 48.74 | 45.74 |
| scabra | 300 | 52.44 | 53.40 | 57.80 | 50.42 | 49.61 |
| Aspirin | 300 | 56.09 | 56.82 | 60.19 | 55.46 | 52.71 |

## Discussion

*Zehneria scabra (*Linn.f.) sond, cucurbitaceae family, grows in Ethiopia, east and centeral Africa, Cameroon, Nigeria, Angola, South Africa and Botswana. It is a herbaceous climber up to 6m long with long thin branthlets. The flowers are dioecious[20]. 80% methanol extract of *Zehneria scabra (*Linn.f.) sond exhibits antimicrobial activity against most common bacterial pathogens, i.e. staphylococcus aureus and E.coli[16]. The masai in Kenya wash calves with the leaf pulp to get rid of fleas, in human medicine skin diseases are treated with it. It is also used in North-West Cameroon for treatment of opportunistic skin infections such as herpes zoster, kaposi's sarcoma and ringworm[21]. The roots are considered to ease abdominal pain, cold/flue and hypertension. The pare in Tanzania mix leaf ash with oil to treat skin diseases, especially infantile scabies, the leaves are also used against fever. The sukuma, too, use the plant for fever. In the south of Zambia the leaves are used as gentle laxative, the root decoction as a diuretic and emetic. *Zehneria scabra* is one of the species that commonly known and recognized as effective remedies in Ethiopia. The Amhara in Ethiopia crushed fruit and leaves with barely shoots to treat scabs and scabies with mixture. The plant is also used for alopecia[20].The leaves of the plant are boiled in water and the vapour is inhaled or the leaves are crushed and the juice is drunk with tea to treat *miche* (inflammation, pain[22] and malaria[23]. It is also used to treat fungal and bacterial infections in western Uganda[24]. In a nut shell, *Zehneria scabra* is a vital medicinal climber. In spite of the fabulous use of *Zehneria scabra* for different ailment, there is no adequate information about its toxic profile. Daniel, *et.al,*[25] laid bare that *Zehneria scabra* was the least toxic plant among the plants used in his experiment. More or less in line with this, Mainen, *et.al,*[26] demonstrates that *Zehneria scabra* has a moderate safety profile. Considering the traditional claim of *Zehneria scabra* and its safety profile into account, this research was carried out to evaluate its *in vivo* antinociceptive and anti- inflammatory activities.

Solvent or the extraction agent used in the preparation of phyto-pharmaceuticals must be suitable or dissolving the important therapeutic drug constituents. In addition, solvents used should be easy to remove, inert, nontoxic, and not easily flammable. It is hypothesized that alcoholic solvents efficiently penetrate cell membranes, permitting the extraction of high amounts of endocellular components in contrast to lower polarity solvents such as chloroform, which are limited to extracting mostly extra-cellular material. In this manner, alcohols dissolve chiefly polar constituents together with medium and low polarity compounds extracted by co solubilization. In general hydroalcoholic cosolvents such as 80 % methanol seem to possess the optimum solubility characteristics for initial extraction[21, 27]. Hence, 80% methanol was chosen as in the present study for extracting. The solvent also was removed by rota vapour to prevent its effects on experimental animal[15].The percentage yield of the extract was 15%. In the same solvent and method, Bruk and *et.al*[16] obtained a percentage yield of 12.7%. This difference could be emanated from geographical and seasonal variation of the collected plant materials.

Acetic acid is used to induce pain in this experiment. Acetic acid induced writhing model causes pain sensation by triggering localized responses. Increased level of $PGE_2$ and $PGF_{2\alpha}$ in the peritoneal fluid has been reported to be responsible for pain induction by administration of acetic acid[4, 15, 29]. The administration of the 80% methanol extract of the plant at doses of 50, 100, and 200mg/kg caused a significant reduction in the

number of the writhing episodes induced by acetic acid compared to the control. The percentage of inhibition of the writhing response was calculated 40.48% for 50mg/Kg, 53.17% for 100mg/Kg and 74.60% for 200mg/kg. Inhibition of writhing decrease as the dose of the extract increased in the studied range. The 200mg/kg dose of methanol extract (74.60%) has comparable antionociceptive activity to that of the reference drug ASA (86.5%). All the results are statistically significant with *P* value < .05.

The most commonly used primary test for screening of anti-inflammatory is Carrageenan induced paw edema model. Several hypotheses have been advanced to explain the carrageenan mechanism action. Various mediators are released by carrageenan in the rat paw to induce inflammation[30]. The edema formation has biphasic nature. Thus, while the first or initial phase is mainly associated with release of histamine and serotonin[15, 29, 30], the second or delayed phase is due to the release of prostaglandins [29, 30], bradykinin[15, 30], protease, and lysosome[29,30]. This would entail the probable mechanism of action of the extract. The extract has anti-inflammatory effect 1 to 5 hrs, the maximum effect being observed at 3hr. Hence, the plant extract mechanism of action probably could be via inhibiting the second phase. The plant extract exhibit significant anti-inflammatory activity at all dose, the maximum effect being observed at 300mg/Kg which is comparable to the standard reference ASA.

*Zehneria scabra* might contain Cucurbitacin (especially cucurbitacin B and E), a group of tetracyclic triterpenoids, as a number of species belonging to cucurbitaceous family have these compounds for their useful as well as toxic profile[31]. These compounds may be responsible for its antinociceptive and anti-inflammatory activities as flavonoids, polyphenolic compounds, terpenoids and alkaloids are the active ingredients in plants of which the active principles are determined for their the anti-inflammatory and antinociceptive activities[32].

## Conclusion

*Zehneria scabra* is very vital climber as clearly demonstrated in this experiment and in hooks of volumes of literatures. This experiment has attested that the traditional claim has sort of truth as the plant extract exhibit promising antinociceptive and anti-inflammatory activities. However, the exact mechanism of action, the active principle and its well established safety profile should be unraveled in future studies.

## Acknowledgment

The author would like to thank the Research and Community Service Core Process of University of Gondar for financing this research.

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