Journal of Inflammation Research

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ORIGINAL RESEARCH

Evaluation of Analgesic and Anti-inflammatory Potential of 80% Methanol Leaf Extract of *Otostegia integrifolia* Benth (Lamiaceae)

This article was published in the following Dove Press journal:
Journal of Inflammation Research

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Introduction: Pain and inflammatory disorders are the most prevalent syndromes. Different herbs were used for treatment of pain and inflammation including members of the genus *Otostegia*. As a result, this research investigated the *in vivo* analgesic and anti-inflammatory effects of 80% methanol leaf extract of *Otostegia integrifolia* in mice.

Methods: The analgesic and anti-inflammatory effects of the plant was evaluated using hot plate method, acetic-acid induced writhing test, and carrageenan and formalin induced paw edema. Three experimental groups (100, 200, and 400 mg/kg) received the extract while morphine 10 mg/kg and aspirin 150 mg/kg were used as a positive control for analgesic and anti-inflammatory tests accordingly. Distilled water (10 mL/kg) was used as negative control.

Results: From the experimental groups, OI400 displayed significant analgesic and anti-inflammatory activities ($P<0.001$). In acetic acid induced writhing tests, the number of writhes decreased significantly ($P<0.001$) in all experimental groups. Similarly, OI400 reduced the mean paw edema significantly in carrageenan and formalin induced paw edema ($P<0.05$ and $P<0.001$, respectively).

Conclusion: In general, the results obtained in this study demonstrated that the extract exhibited significant analgesic and anti-inflammatory potential in mice.

Keywords: analgesic activity, anti-inflammatory, hot plate, *Otostegia integrifolia*, carrageenan

Introduction

Pain is a universal concept[1] and according to the International Association for the Study of Pain (IASP), pain is defined as "unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage".[2] In addition, it can also be expressed during inflammation. Inflammation is a prevalent condition where a coordinated unspecified reaction occurs toward tissue failure. It is undertaken by the nonspecific and acquired immune systems to fight various triggering factors.[2] Inflammation manifests in a form of warm inflamed site as a result of surge in blood flow towards the region, erythema, and swelling due to vascular permeability.[3]

Pain is the foremost cause of disability and disease burden universally.[1] Particularly when chronic, it significantly reduces the health and quality of life of individuals.[4] Furthermore, it predisposes to suicide. It is, therefore, a grave and expensive public health problem.[5]

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submit your manuscript | www.dovepress.com
DovePress
http://doi.org/10.2147/JIR.S285932

Wide ranges of medicines are available such as non-steroidal anti-inflammatory drugs (NSAIDs), opioids and steroids. However, the treatment of pain is far from being adequate leading to poor pain control and suffering.[6] Owing to the prevalence of pain, prolonged use and higher doses of analgesics will prompt patients to the toxicity and adverse effects of such drugs.[7,8] As a result of rise in fatigue levels, the social interaction and routine activities will be impaired. Moreover, the patient will result in poor functional ability and diminished quality of life.[9]

Traditional medicines especially herbs are considered as the main source of numerous medicinal agents.[10] They also serve as a lead for the synthesis of new molecules with better pharmacological profile.[10,11] Several medicines of herbal sources are still in use lacking major untoward effects.[12] Thus, it is crucial to search for novel medicinal agents that are more efficacious and cost effective.

The family Lamiaceae comprises 236 genera and more than 7000 species. The genus *Otostegia* consists of about 15 species. It is endemic to the northern part of tropical Africa and South-western and Central Asia. Five species of this genus have been reported to occur in the flora of Ethiopia including *Otostegia integrifloia*.[13]

O. integrifolia Benth known by its Amharic name "Tinjut" is an erect perennial shrub with long oval grey-green leaves. It has flowers that are green-white in color and fruits that are small nutlets within the calyx. The plant is endemic to Ethiopia, Eritrea, and Yemen.[13]

In Ethiopia, the plant is claimed to have insecticidal properties and often used as fumigant for pots and houses.[14–16] Other reports indicated that the leaves of the plant are used for tonsillitis, uvulitis, lung diseases, stomachache, malaria, and hypertension.[12,17–19]

Upon pharmacological investigation, *O. integrifolia* exhibited antibacterial, antioxidant,[20,21] antidiabetic,[22] and antimalarial activities.[13] It consists of terpenes in the form of monoterpenes, sesquiterpenes, and diterpenes. The reported terpenes include axinyssene, otostegindiol, pre-tostegindiol, pentatriacontane, and stigmasterol.[23] In addition, Tesso and König reported trans-Sabinol, β-cyclo-citral, dihydroedulan, and theaspirane from the leaves of *O. integrifolia*.[17] From these compounds otostegindiol was found to possess antimalarial activity.[13] From the genus *Otostegia, O. fruticosa* and *O. persica* exhibited essential analgesic and anti-inflammatory activities.[24,25] Accordingly, the aim of this research was to examine whether the experimental plant possesses analgesic and anti-inflammatory effects. Besides, this investigation may serve as a benchmark for future examination and isolation of different phytochemicals responsible for the observed effects.

Materials and Methods

Drugs and Chemicals

All chemicals, drugs and reagents used in the study were analytical grade; methanol (Carlo erba Group Reagents, Italy), glacial acetic acid (Sigma-Aldrich Laborchemikalien, Germany) while morphine, acetyl salicylic acid, distilled water are obtained from Ethiopian pharmaceuticals manufacturing factory, Ethiopia.

Plant Material

Fresh leaves of *O. integrifolia* were collected from Tulu Dimtu, North West Shewa, Oromiya, around 29 km south-east of Addis Ababa, in December 2017. The plant was authenticated by a senior botanist and a voucher specimen (AD001) was kept at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University. The leaves were cleaned under a running tap water and dried under shade. Then a grinder was used to coarsely powder the dried leaves.

Extraction

Dried powdered leaves of *O. integrifolia* (500 g) were macerated in 80% methanol (3×2 L, 72 h each). Then the solution was filtered with Whatman no. 1 filter paper and concentrated under reduced pressure using Rota evaporator (Buchilabortechnik AG, Switzerland) at a temperature not more than 40°C. The concentrated extract was freeze-dried using a lyophilizer (Heto Power Dry LL3000 freeze-dryer, USA) to yield a sticky and brown extract weighing 30.12 g (16.6%).

Phytochemical Screening

The extract was tested for the possible constitution of various natural products, such as alkaloids, phenols, tannins, terpenes, and saponins as stated elsewhere.[26]

Experimental Animals

Experimental animals were acquired from the School of Pharmacy, Addis Ababa University and about 120 healthy Swiss albino mice of either sex (25–35 g, 6–8 weeks of age). Then a standard pellet and water ad libtum were fed to animals under a controlled environment (12 h light–dark cycle and temperature of 23–25°C). Acclimatization was

carried out a week prior to commencement of the investigation. The animals were handled in accordance with international guidelines[27] and approved by the Institutional Review Board of the School of Pharmacy (reference no. ERB/SOP/120/11/2017).

Animal Grouping and Dosing

Swiss albino mice of either sex (n=120) were selected for all the four experiments. The animals were indiscriminately distributed into 5 groups, each comprising 6 animals. Group I received distilled water as a negative control. While the test groups, Group II, Group III and Group IV received 100mg/kg, 200mg/kg and 400mg/kg of the test substance, respectively. The concentrations were selected according to acute toxicity study results conducted elsewhere.[13] Group V assigned as a positive control received the standard drugs. This grouping was used for all the four experiments. Morphine (10 mg/kg, s.c.) was used for hot plate method. While aspirin 150 mg/kg was used for acetic acid induced writhing test, carrageenan and formaldehyde induced paw edema. Oral route was used to deliver all the drugs using entragastric gavage.

Analgesic Effect

Hot Plate Test

Hot plate test was used for assessing the central analgesic effect of *O. integrifolia*.[28] The Swiss albino mice were screened and mice taking more than 15 seconds of latency time on a hot plate kept at 50±0.1°C were excluded. Induced analgesia was recorded at 0, 30, 60, and 120 min after the animal received the plant extract and standard drug. Cutoff time of 15 seconds was fixed for the animals to avoid tissue injury. The following formula was used to calculate percent analgesia.

$$\text{Max.Analgesia} = \frac{\text{Reaction time for the test} - \text{reaction time for saline}}{15\ \text{sec} - \text{reaction time for saline}} \times 100$$

Writhing Test

Acetic acid-induced writhing test has been used for investigating peripheral analgesic activity.[29] Acetic acid (0.6% v/v, 10 mL/kg, i.p.) was administered to every animal one hour after the animals were provided with the extract, distilled or standard drug according to their groups. Peripheral analgesia was assessed by recording the number of writhes for every animal for 30 min using a latency time of five minutes, characterized by stretching of the abdomen with simultaneous stretching of at least one hind limb. The writhing inhibition percentage was calculated by this equation.

$$\text{\% inhibition of writhing} = \frac{\text{Mean no. of writhes (control)} - \text{mean no. of writhes (test)}}{\text{Mean number of writhes control}} \times 100$$

Anti-inflammatory Activity

Carrageenan Induced Paw Edema

Acute inflammation was induced via subplantarly administering carrageenan (0.03 mL of 1% w/v in normal saline) to the mice right hind paw.[30] The extract, the standard drug or the vehicles were administered one hour prior to administration of the phlogistic agent, carrageenan. The inflammation was measured in milliliters, via quantifying the displaced water by edema using a digital plethysmometer (Ugo Basile Company: Cat. No. 7140, Italy) at time zero, one, two, three, and four hours after carrageenan injection.[31] Acetylsalicylic acid (150 mg/kg) was used as a standard drug.[21] The following formula was used to calculate the percent inhibition of edema in comparison to the control groups:[32]

$$\text{\% inhibition of paw edema} = \frac{(\text{Vt} - \text{Vo})\text{control} - (\text{Vt} - \text{Vo})\ (\text{Treated})}{(\text{Vt} - \text{Vo})\text{control}} \times 100$$

Where: Vt: is the right hind paw thickness volume (in milliliters) at time t,

Vo: is the right hind paw thickness volume (in milliliters) before carrageenan injection,

Vt –Vo: control and treated edema or paw size after carrageenan injection for control and drug-treated groups respectively.

Formalin-induced Paw Edema

Subacute inflammation was induced by subplantar administration of formalin (0.02 mL of 2% v/v, in distilled water) into the right hind paw of mice at the first and third days of observations. Then a mark was placed at the level of lateral malleolus on the right hind paw before formalin induction. Thus, during the observation period, the injected paw would be immersed to the same extent in the measurement chamber of the plethysmometer. After that, each test substance (extract, the standard drug and the vehicle) was administered one hour before formalin injection for seven consecutive days according to their grouping. After one hour of administration the paw volume was measured daily using the

plethysmometer until the seventh day and the percentage of edema inhibition was calculated using the above formula.[33]

Statistical Analysis

The analysis was conducted using statistical package for social science (SPSS) version 25 and GraphPad prism version 8.1. One way ANOVA was used to analyze the data followed by Tukey's post hoc test to determine statistical significance. All the data were expressed as mean ±standard error of the mean (SEM). A *P*-value ≤0.05 was taken as statistically significant.

Results

Phytochemical Screening

The phytochemical analysis exhibited that 80% methanol leaf extract of *O. integrifolia* contains phenols, flavonoids and saponins, whereas steroids, tannins and alkaloidal compounds were lacking.

Pain and Inflammation Induction

On hot plate and acetic acid writhing tests, the minimum latency and maximum writhe was observed on negative control groups compared to positive control and the experimental groups as depicted in Tables 1 and 2. Similarly, injection of carrageenan and formalin induced inflammation corroborated by the maximum edema observed in negative control mice whereas the minimum being the positive control (Figures 1 and 3).

Analgesic Activity

Hot Plate Test

During the observation prolonged reaction time was recorded for the experimental groups (OI200 and OI400) and positive control (MO10) in the hot plate test (Table 1). However the lower dose (OI100) compared to the negative control showed no significant change in the latency time. The latency time observed was variable across the experimental groups. MO10 and OI400 significantly enhanced the latency time with $P<0.05$ at 30 min compared to DW group. At 60 min all the mice receiving the test substance were bettered by the positive control (MO10). However, the OI200 and OI400 at 90 and 120 min revealed significant upturn in latency. At 120 min OI400 and MO10 bring about significant rise ($P<0.01$) in latency compared to negative control.

Table 2 Effect of 80% Methanol Leaf Extract of *Otostegia integrifolia* on Acetic Acid Induced Writhing Test in Mice

Group	Mean No. of Writhing ±SEM	Percent Inhibition
DW	165±6.28	–
ASA150	49.8±4.52[a]**,[b]*	69.1%
OI100	96.2±4.85[a]**	41.6%
OI200	54.6±5.88[a]**	66%
OI400	62.2±5.05[a]**,[b]*	67.3%

Notes: Values are expressed as mean ±SEM (n=6); analysis was performed with one-way ANOVA followed by Tukey's post hoc test; [a]against the control; [b]against OI100. *$P<0.05$; **$P<0.001$. Doses are given in mg/kg.
Abbreviations: ASA, acetylsalicylic acid; OI, 80% methanol leaf extract of *Otostegia integrifolia*.

Acetic Acid Induced Writhing Test

The writhing test assessed the peripheral activity of the test substance, all doses of the extract revealed significant inhibition with $P<0.001$ against acetic acid induced writhing compared to negative control (Table 2).

ASA150 yielded a significant inhibition of writhing than DW ($P<0.001$) and OI100 ($P<0.05$). However, compared with OI200 and OI400 the change was found to be insignificant. In addition, the percent protection from writhing was comparable amongst ASA150, OI200 and OI400.

Anti-inflammatory Activity

Carrageenan Induced Paw Edema

As it is illustrated by Figures 1 and 2, in anti-inflammatory test conducted using carrageenan-caused edema revealed

Table 1 Effect of 80% Methanol Leaf Extract of *Otostegia integrifolia* on Hot Plate Test in Mice

Latency (Sec) and Maximum Possible Protection (%)									
Group	**0 Min**	**30 Min**	**%**	**60 Min**	**Percent**	**90 Min**	**Percent**	**120 Min**	**Percent**
DW	5.2±1.77	5±1.095		5.6±1.029	±	4±0.77		4.4±0.77	
MO10	4.8±0.37	12.6±2.18[a]*	19	12.6±3.38[a]***,[b]**,[c]**,[d]*	47.2	13.6±1.54[a]***,[b]**	40.4	13.4±2.2[a]**	21.6
OI100	5.2±0.20	8.2±1.11	8	9.4±1.50	9.64	8.6±1.07	11.2	11.2±2.2	16.7
OI200	3.8±0.58	7.8±0.97	7	9.8±1.31	10.6	12.8±1.42[a]*	21.4	11.6±1.53[a]*	17.7
OI400	5.00±0.71	11.4±1.69[a]*	16.25	12.2±4.47[a]*	16.7	12.6±1.42[a]*	21.4	13.2±0.81[a]**	22.6

Notes: Values are expressed as mean ±SEM (n=6); analysis was performed with one-way ANOVA followed by Tukey's post hoc test; [a]against the control; [b]against OI100; [c]against OI200; [d]against OI400. *$P<0.05$; **$P<0.01$; ***$P<0.001$; Control received distilled water (10 mL/kg), whereas standard received morphine (10 mg/kg) orally.
Abbreviations: OI, 80% methanol leaf extract of *Otostegia integrifolia*; MO, morphine; DW, distilled water.

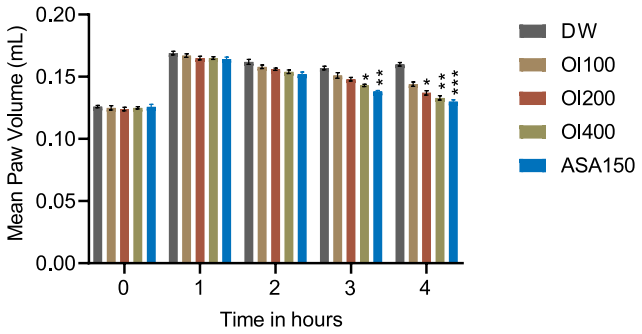

Figure 1 Mean paw volume in carrageenan induced paw edema. Data represent mean ±SEM (n=6). *P<0.05, **P<0.01, ***P<0.001; relative to control.
Abbreviations: ASA150, aspirin 150 mg/kg; OI, *O. integrifolia* at doses of 100, 200, and 400 mg/kg.

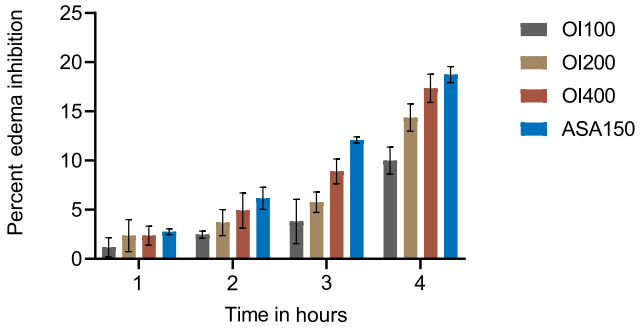

Figure 2 Percent edema inhibition of 80% methanol leaf extract of *Otostegia integrifolia* in carrageenan-induced paw edema in mice. Analysis was performed with one-way ANOVA followed by Tukey's post hoc test. Data was expressed in mean ±SEM (n=6).
Abbreviations: OI, 80% methanol leaf extract of *O. integrifolia* at doses of 100, 200, and 400 mg/kg; ASA150, aspirin 150 mg/kg; DW, distilled water (10 mg/kg).

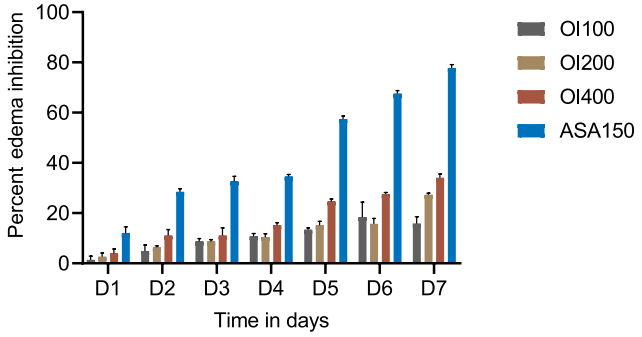

Figure 3 Percent edema inhibition of 80% methanol leaf extract of *Otostegia integrifolia* in formalin-induced paw edema in mice. Analysis was performed with one-way ANOVA followed by Tukey's post hoc test. Data was expressed in mean ±SEM (n=6).
Abbreviations: OI, 80% methanol leaf extract of *O. integrifolia* at doses of 100, 200, and 400 mg/kg; ASA150, aspirin 150 mg/kg; DW, distilled water (10 mg/kg).

that the hydroalcoholic plant extract endowed promising anti-inflammatory attributes. After administration of carrageenan, until three hours both the test substance as well as ASA150 did not exhibit remarkable anti-inflammatory activity. Later on as depicted on Figure 1, at three hours, OI400 (P<0.05) and ASA150 (P<0.01) demonstrated significant reduction in paw edema. The percent protection of paw edema at three hours was 24.5%, 50.8%, and 53.8% for OI200, OI400, and ASA150, respectively (Figure 2). At four hours, OI200 (P<0.05), OI400 (P<0.01) and ASA150 (P<0.001) produced significant paw edema reduction (Figure 1). Percent protection of paw edema at four hours was 24.2%, 50.4%, 75.7%, and 80.6% for OI100, OI200, OI400 and ASA150, respectively (Figure 2).

Formalin-induced Paw Edema

All doses of 80% methanol extract of *O. integrifolia* (100, 200, 400 mg/kg) resulted in significant decrease (P<0.001) in paw volume across all days starting from day two (Table 3). OI400 significantly reduced paw edema compared to the other doses of the extract (P<0.001) while OI200 showed better effect than OI100 (P<0.05) at the seventh day. Furthermore, higher percentage inhibition of edema was observed as 15.8%, 27.3%, and 34.1% for OI100, OI200 and OI400 respectively (Figure 3). However, all doses of the extract showed lower effect than the positive control (ASA150).

Discussion

The search for newer analgesic agents with higher efficacy and fewer side effects seems imperative due to the cost incurred for relief of pain and the numerous untoward side effects of available analgesics.[34] As a result, herbs as a component of traditional medicine are commonly used for treatment of various pain conditions with promising analgesic activity.[35] *O. integrifolia* is commonly used in Ethiopian traditional medicine for the treatment different pain and inflammatory conditions.[22] However, to the best of authors' knowledge, no prior pharmacological investigation regarding its analgesic and anti-inflammatory activities in animal models has been reported so far. Accordingly, hydroalcoholic extract of *O. integrifolia* exhibited significant analgesic and anti-inflammatory activity as attested by enhanced latency time, reduced writhing and inhibition of edema development.

The hot plate evaluation is a typical test that uses heat stimulation to induce pain and considered as a vital tool to examine centrally coordinated analgesic pathway. The main benefits of this model are the precision of the outcome, its sensitivity to most effective analgesics, minimal tissue injury, and limited time consumption.[36] In this

Table 3 Effect of 80% Methanol Leaf Extract of *Otostegia integrifolia* in Formalin-induced Paw Edema in Mice

Edema Volume (Mean ±SEM)							
Groups	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
DW	0.125±0.0008	0.126±0.0006	0.126±0.0008	0.129±0.0006	0.132±0.0005	0.133±0.0005	0.136±0.0006
OI100	0.124±0.0008	0.119±0.0009^{a***}	0.115±0.0006^{a***}	0.116±0.0006^{a***}	0.114±0.0004^{a***}	0.112±0.0006^{a***}	0.107±0.003^{a***}
OI200	0.122±0.0006^{a*}	0.118±0.0004^{a***}	0.115±0.0006^{a***}	0.115±0.0004^{a***}	0.112±0.0005^{a***} ^{b*}	0.111±0.0004^{a***}	0.099±0.0004^{a***} ^{b*}
OI400	0.12±0.0004^{a***} ^{b**}	0.112±0.0006^{a***} ^{b***} ^{c***}	0.112±0.0011^{a***}	0.11±0.0009^{a***} ^{b***} ^{c***}	0.099±0.0008^{a***} ^{b***} ^{c***}	0.096±0.0004^{a***} ^{b***} ^{c***}	0.09±0.0006^{a***} ^{b***} ^{c***}
ASA150	0.11	0.09	0.085	0.085	0.056	0.043	0.03
	±0.0009^{a***} ^{b***} ^{c***} ^{d***}	±0.0008^{a***} ^{b***} ^{c***} ^{d***}	±0.0008^{a***} ^{b***} ^{c***} ^{d***}	±0.0006^{a***} ^{b***} ^{c***} ^{d***}	±0.0006^{a***} ^{b***} ^{c***} ^{d***}	±0.0006^{a***} ^{b***} ^{c***} ^{d***}	±0.0006^{a***} ^{b***} ^{c***} ^{d***}

Notes: Analysis was performed with One-Way ANOVA followed by Tukey post hoc test; Data was expressed as mean ±SEM (n=6); [a]against the control; [b]against 100 mg/kg; [c]against 200 mg/kg, [d]against 400 mg/kg. *P<0.05; **P<0.01; ***P<0.001. Doses are given in mg/kg.

Abbreviations: ASA, acetylsalicylic acid; OI, 80% methanol leaf extract of *Otostegia integrifolia*.

model, paw licking and jumping response are taken as end points upon exposure of the animal to a heated plate at a constant temperature. Both are considered as supraspinal integrated response and determining the involvement of central antinociceptive mechanism.[37]

In the hot plate method, at 120 min the recorded latency significantly raised for morphine and OI400. Likewise comparable percentage inhibition was observed between the two groups. Pharmacokinetic difference to induce peak activity was also observed between OI400 and MO10 where MO10 peaked at 60 min while peak activity of OI400 was observed at 120 min. The lag time might be attributed to the time it takes for the extract to reach to the systemic circulation; furthermore perhaps it takes time for the metabolism of an active metabolite that has analgesic activity. Alternatively, the consistency of pharmacokinetics across the experimental groups might explain the direct relationship observed between the observation time and percentage inhibition. As the hot plate method is a specific central antinociceptive test, the extract may partly exert its effect via the periaqueductal gray matter (PAG) of the central nervous system which may induce the release of endogenous peptides.[38] These peptides such as met-enkephalin and leu-enkephalin, and β-endorphin along with their cognate receptors are integral parts of the pain modulatory circuit expressed in the brain and across the pain pathways.[39]

One of the classic tools for the assessment of peripheral analgesia is acetic acid induced writhing method.[37] The benefit of this method is to simulate an actual clinical pain conduction and it is also sensitive to mild analgesics thereby helping to scrutinize peripheral analgesic activity of investigational substances.[36] The procedure follows IP injection of acetic acid which causes injury to the peritoneal cavity and induces a very characteristic behavior in mice. Consequently the reaction of the mice follows with abdominal contraction and hind paw stretching. The release of prostaglandins specifically PGE2 and PGF2 as well as leukotrienes yielded from lipoxygenases are responsible for stimulation of chemosensitive nociceptors.[40]

In the writhing method the OI100, OI200 and OI400 groups brings about significant (P<0.001) analgesia revealed by decrement of percentage writhing number 41.6%, 66%, and 67.3% respectively compared to the negative control. The latter two groups also recorded significant (P<0.001) peripheral analgesia as comparable as ASA150; this perhaps explicates the rise in the level of secondary metabolites responsible for the recorded

activity. In addition, compared to the DW group all the experimental groups significantly reduced the writhing. The mechanism behind acetic acid induced abdominal writhing is mediated through increased production of prostaglandins. This may indicate that the extract may elicit its action on peripherally situated pain transduction pathways through suppression of prostaglandin synthesis.[41]

Inflammation follows a triphasic pattern where the first phase is acute inflammatory condition characterized by extravasation of exudates from blood to interstitial space. The second phase is the subacute stage distinguished by permeation of leukocytes to the tissue and the final phase is the chronic phase marked by granuloma formation.[42] Carrageenan-induced paw edema is considered as an important model to assess acute inflammatory conditions. Carrageenan being a typical phlogistic agent, this test was employed to assess the anti-inflammatory prospect of candidate substances.[43] Carrageenan works by releasing inflammatory mediators, in particular, histamine, serotonin, and prostaglandins from mast cells as well as paracrine cells followed by release and permeation of cytokines, nitric oxide and neutrophils to the site of inflammation.[44]

In carrageenan-induced paw edema, OI200 and OI400 revealed significant anti-inflammatory characteristics as it is corroborated with reduced paw edema and increased percent edema protection. As time increased the anti-inflammatory activity of the extracts was exposed particularly for OI200 and OI400, this perhaps indicates that the minimum dose to elicit the anti-inflammatory effect is more than 200 mg/kg. In addition, the delay in effect until three hours could be attributed to the pharmacokinetic dynamics via which the extract needs to undergo. The intensity of the extracts effect is comparable with aspirin (ASA150) both in terms of reduction of paw edema as well as percent edema protection (Figure 2).

Formalin is a commonly used irritant to induce acute and sub-acute inflammation. The activity of analgesic and antipyretic drugs, capable of inhibiting inflammation in the mouse foot after formalin, may well be owed to their antifibrinolysin action. It is also possible that inhibition of formalin induced inflammation is due to inhibition of the action of bradykinin released by the injured cells. This initiates the release of substances such as prostaglandins, serotonin, and histamine causing capillary hyperpermeability leading to edema.[45]

The increase in dose of the extract resulted in increased anti-inflammatory potential as shown by reduced paw edema volume and percentage inhibition. This could be attributed to the presence of sufficient concentration of active ingredient at the site of inflammation while the overall activity of the extract could be ascribed to inhibition of chemotactic factors and proinflammatory mediators through attenuating the lipoxygenase and the cyclooxygenases pathways.

As depicted in the result, the hydroalcoholic extract of *O. integrifolia* possess important phytochemicals which might explain the above pharmacological activities of the extract. For instance, quercetin and rutin are flavonoids which exhibit substantial anti-inflammatory and analgesic activity via inhibiting nuclear factor kappa B (NF-κB) in immune cells and activating nitric oxide mediated ATP-dependent potassium channels signaling pathways in neuronal cells.[46,47] Rutin, in particular, is also known to reduce carrageenan-induced paw edema in rats.[47] Furthermore, other phenolic compounds, saponins and terpenoids as depicted by numerous research demonstrated profound anti-inflammatory and analgesic activity through inhibition of prostaglandin synthesis and reduction in expression of proinflammatory cytokines, particularly interleukin 1 and interleukin 6.[48,49]

Conclusion

In conclusion, the extract possesses peripheral analgesic and central pain inhibition activity. The extract also demonstrated promising anti-inflammatory effect in both acute and subacute phases of inflammation. Thus, the potential of the plant material could be attributed to the suppression of diverse endogenous pain and inflammatory mediators due to the presence of natural products like flavonoids, polyphenols, and saponins. It is recommended that further investigations should be carried out to identify and isolate active principles responsible for the detected analgesic and anti-inflammatory effect.

Abbreviations

ASA, acetyl salicylic acid; DW, distilled water; IASP, International Association for the Study of Pain; MO, morphine; NF-κB, nuclear factor kappa B; OI, *Otostegia integrifolia*; PAG, periaqueductal gray matter; PG, prostaglandins; SEM, standard error of the mean.

Data Sharing Statement

The datasets used and/or analyzed during the current work are available from the corresponding author up on reasonable request.

Ethics Approval and Consent

The protocol was approved by Institutional Review Board of the School of Pharmacy with reference no. ERB/SOP/120/11/2017.

Acknowledgments

We would like to thank Addis Ababa University College of Health Sciences School of Pharmacy for allowing the study to be conducted with facility of the institution.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Funding

There is no funding to report.

Disclosure

The authors report no conflicts of interest in this work.

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