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EVALUATION OF OCIMUM BASILICUM FOR ANTINOCICEPTIVE EFFECTS: IN VIVO AND IN SILICO APPROACHES

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Abstract

Objective Pain is a major health issue. Pain has different forms. Pain affects about 30% of the global population, making it a significant public health concern.

Methods: The ethanolic seed extract was used for the *in vivo* trial. Assessment of the peripheral analgesic activity was conducted through the acetic acid-induced test, while the assessment of central antinociceptive activity was carried out using formalin-induced tests alongside heat-induced pain techniques, specifically the hot plate and tail immersion methods. Additionally, potential pathways involved in the analgesic effect were also investigated. Swiss ADME web server was used to predict compounds' ADME and Pyrex was used for molecular docking computational research.

Key Finding: In the acetic acid-induced writhing assay, the intensity of the writhing response was diminished to the administered dose. For both phases of the formalin-induced licking test, a maximum decrease in biting time and licking. A dose-dependent response was observed in the hot plate method and tail immersion approach. Mechanism involvement was also shown significant results but, terazosin did not affect the analgesic efficacy of EEBOS. The computational analysis identified 3 phytocompounds that demonstrated favourable interactions with 1SG1 receptor protein, showing high binding affinities and meeting the docking criteria.

Conclusion: The collective results of these studies showed that *O. basilicum* has both central and peripheral antinociceptive activities that lend credence to its traditional medical use.

Keywords: Nociceptive, cholinergic and K channel blockers, noradrenergic, docking parameters, opioidergic, pain.

Introduction

Pain is one of the most common conditions reported globally [1]. Chronic pain (CP) affects around 1.9 billion people globally [2]. According to epidemiological data, the prevalence of chronic pain worldwide is estimated to be between 47% and 63%, with neuropathic pain making up 2–17% of cases [3] [4]. In the United States, chronic pain prevalence ranges from 11% to 40%; the US Centers for Disease Control and Prevention (CDC) study estimated the point prevalence of chronic pain at 20·4% [5]. As stated in 2020 by the International Association for the Study of Pain (IASP). "An unpleasant emotional and sensory experience connected to, or resembling, potential or actual tissue damage is called pain" [6]. Pain is a visceral and emotional response to the potential for the tissue injury and is considered chronic if it is persists for more than three months. Beyond limiting one's

capacity to work and engage in social relationships, pain issues can lead to serious and worsening comorbidities including depression and thoughts of suicide [7]. It results in a considerable degree of disability that affects social and economic standing [8].

Anatomical location, bodily system, duration, severity, frequency, and cause have all been used to categorize pain [9]. The human body produces more reactive oxygen species (ROS) under these stressful circumstances, such as superoxide anion radicals (O-2), hydrogen peroxide (H_2O_2) and hydroxyl radicals (-OH) [10] compared to non-enzymatic antioxidants such the ascorbic acid ($C_6H_8O_6$), glutathione, alpha-tocopherol [vitamin E], carotenoids, flavonoids, and enzymatic antioxidants like glutathione peroxidase [GPx], superoxide dismutase [SOD], and catalase [11]. The processing of noxious stimuli by the CNS and PNS, such as temperature fluctuations and tissue injury, that activate nociceptors and related pathways, is known as nociception [12]. Nociceptors are desirable therapeutic targets for treating painful conditions [13]. An unpleasant and often pathogenic sensation is perceived in large part by the nociceptors [14]. NSAIDs are often prescribed to treat pain. They reduce pain and inflammation by inhibiting prostaglandin synthesis and decreasing the activity of the cyclo-oxygenase (COX) enzyme [15]. Although nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used, they may lead to conditions such as small bowel enteropathy, peptic ulcer disease, and symptoms related to foregut. Gastrointestinal haemorrhage and perforation can exacerbate such an iatrogenic damage [16]. Analgesics are classified into opioid and non-opioid categories. Opioid analgesics can lead to dependence and tolerance with continuous use, whereas non-opioid or peripheral analgesics do not [17];[18] . Chronic SAID or NSAID use may have detrimental effects on several organs, including bleeding in the gastrointestinal tract, deterioration of the liver, heart and kidney failure, and other symptoms [19].

Numerous plant species have been employed medicinally to treat and cure diseases [20]. Analgesics are drugs that reduce pain without making a person unconscious. Some plants, like basil (*Ocimum x africanum* L.), have been shown to have analgesic properties [21]. The study utilized sweet basil, also known as niazboo or *Ocimum basilicum* L., and identified numerous health benefits, including, antioxidant, insecticidal, antiparasitic, immunomodulatory, anti-osteoporotic, anti-inflammatory, antifungal/ hepatoprotective, , antimicrobial, cardioprotective, neuroprotective, and anti-cancer properties [22]. *O. basilicum* is a member of the Lamiaceae family. The plant may be found in (Chad, Eritrea, Algeria, Somalia, Ethiopia, Sudan, , Egypt, Libya, Tunisia and Mauritania) Africa, and in Asia [23].

Basil leaves contain flavonoids, phenols, saponins, and essential oils [24]. In addition, basil can function as an antibacterial [25] and hepatoprotector [26]. Flavonoids, in particular, inhibit cyclooxygenase and lipoxygenase enzymes, which are crucial in releasing pain mediators. Thus, inhibiting these enzymes can suppress pain stimuli.

O. basilicum is utilized by the pharmaceutical industry as a raw material. *O. basilicum* contains iron, carotene, calcium, potassium, phosphorus, magnesium, and vitamins A, B6, and C [27]. Basil leaves contain a wide range of aromatic chemicals, including methyl cinnamate, linalool, and estragole [28]. Since basil (*Origanum basilicum*) has been used for 1000 of years in traditional Indian and Asian medicines as a natural antibacterial, anti-inflammatory, analgesic, and diuretic, interest in its bioactive chemicals has grown in the modern era [29]; [30]. *O. basilicum's* flavonoid content can increase neutrophil phagocytic activity and immunostimulant effects [31].

The study aimed to enhance existing knowledge by offering a thorough assessment of the extraction process, as well as the anti-nociceptive and both *in vivo* and *in silico* activities of the ethanolic extract from *O. basilicum* seeds (EEOBS). These results may help guide future investigations and encourage the creation of fresh uses for sweet basil in the pharmaceutical sector.

Materials and Methodology

Ethical approval statement

All protocols for this research affirmed from Ethical Review Committee (ERC) by Approval number GCUF/ERC/372 dated 15Feb 2024, from Government College University, Faisalabad.

Collection of plant material and extract preparation

The seeds of *O. basilicum* were collected from Faisalabad (31°25′0″N 73°5′28″E), in September 2023. *O. basilicum* (Lamiaceae) plant was collected from the local market and identified by the authenticating team of the Department of Botany, GCUF with voucher no. 314-bot-22. Plant part was dried, according to the previously reported procedure [32] followed for plant extraction using 70 % ethanol as solvent and labelled as EEOBS (Ethanolic extract of *O. basilicum* seed).

Experimental Design and animals

The present study used adult, male and female forty Swiss albino mice were kept from the animal house for *In vivo* activities. A 12-hour light-dark cycle was used to maintain the temperature at 22 ± 2°C. The mice were employed for this study after a period of 7 days during which they were acclimated to the laboratory environment. In compliance with the NIH Guide for the Care and Use of Laboratory Animals, the study was carried out. Treatments were administered via Intraperitoneal injections, and the animals were randomly assigned to five groups.

Assessment of anti-nociceptive activity

Acetic acid-induced writhing test in mice

The writhing test was used for visceral pain following the protocol described [33] with slight modification. Mice were individually weighed and randomly assigned to groups (number of groups, n=5). There were forty mice total, eight mice (four male and four female) in each group: control group 1 (distilled water), standard group 2 (diclofenac sodium 50 mg/kg body weight), and test groups 3, 4, and 5 received (50, 100, and 200 mg/kg body weight) of EEOBS repectively. An intraperitoneal injection of 1% acetic acid (0.1 ml per 10 g body weight) was given at intervals of 60 minutes to cause writhing in the abdomen. One minute after the acetic acid injection and every five minutes thereafter, the total number of writhing was recorded. The following formula was used to get the % inhibition of analgesic activity.

$$\%\ inhibition\ = \frac{(Wc - Wt)}{(Wc)} \times 100$$

Formalin Test

The formalin test as described [34] was used to evaluate neurogenic nociceptive systems. Mice were individually weighed and randomly assigned to groups (number of groups, n=5). There were forty mice total, eight mice (four male and four female) in each group: control group 1 (distilled water), standard group 2 (diclofenac sodium 50 mg/kg body weight), and test groups 3, 4, and 5 received (50, 100, and 200 mg/kg body weight) of EEOBS respectively. A sub-plantar injection of 10 µL of 5% formalin was administered to the right paw's dorsal side sixty minutes after the therapy. Mice were then placed in an acrylic cylinder surrounded by mirrors to count the number of flinches in the injected paw. The formalin test has two phases: the early phase (0 to 5 minutes) and the late phase (15 to 30 minutes) [33]; [35].

Tail Flick Test

The tail-flick test was conducted according to the previously published technique by [36]. After an overnight fast, mice were randomly divided up into five groups. There were forty mice total, eight mice (four male and four female) in each group: control group 1 (distilled water), standard group 2 (diclofenac sodium 50 mg/kg body weight), and test groups 3, 4, and 5 received (50, 100, and 200 mg/kg body weight) of EEOBS respectively. Heat stimulus was delivered to the distal portion of the tail after 60 minutes, and the tail-flick latency was recorded at the beginning, 30, 60, and 90 minutes. Twenty seconds was chosen as the cutoff period to avoid tail damage[36]; [37]. The response time, defined as the amount of time needed to remove or flick the tail out of the water, was recorded and calculated as follows:

$$\%\ MPE\ = \frac{(Post\ \ drug\ latency - pre\ drug\ latency)}{(Cut\ off\ period - Pre\ \ drug\ latency)} \times 100$$

Hot plate test

This activity was evaluated using, with minor modifications, the methodology of Eddy et al. [39]. were forty mice in total, eight mice (four male and four female) in each group: control group 1 (distilled water), standard group 2 (diclofenac sodium 50 mg/kg body weight), and test groups 3, 4, and 5 received (50, 100, and 200 mg/kg body weight) of EEOBS respectively. After 1 hour of oral administration, mice were tested on the hot plate (55 ± 2.0°C). A 20-second threshold was established to avoid the damage to the paw [40]. Three readings were noted 30 minutes apart following the therapy. The analgesic effects of the extracts were examined using the hot plate test and expressed using the maximum possible effect (%MPE) formula [35]:

$$\%\ MPE\ = \frac{(Post\ \ drug\ latency - pre\ drug\ latency)}{(Cut\ off\ period - Pre\ \ drug\ latency)} \times 100$$

Mechanistic studies

In the mouse model of the acetic acid-induced writhing mentioned earlier, efforts were made to investigate the role of noradrenergic, opioidergic, cholinergic system , and potassium channel blockers in the analgesic effects of the EEOBS [38].

Assessment of the potential involvement of the opioidergic pathway

Mice in groups 1-3 were given 50 ml/kg distilled water, 50 mg/kg Diclofenac sodium, and 200 mg/kg EEOBS, respectively; groups 4 and 5 were given nalbin (2 mg/kg) as a pretreatment to activate opioid receptors prior to receiving 200 mg/kg EEOBS and 50 mg/kg DS. Groups 4 and 5 were administered oral dosages of Diclofenac sodium and EEOBS, Correspondingly, after a 15-minute interval. After the one hour, writhing responses were seen in each group.

Assessment of the potential involvement of the cholinergic system

EEOBS (200 mg/kg), and Diclofenac sodium (50 mg/kg) were given orally to the mice in groups 1-3, respectively after (50 ml/kg) distilled water and atropine (1 mg/kg, i.p.) was given 15 minutes prior to the oral administration of EEOBS (200 mg/kg) and diclofenac sodium (50 mg/kg) to groups 4-5, respectively, a writhing response was induced by acetic acid and recorded in all groups.

Assessment of the potential involvement of the KATP channel blocker system

In groups 1-3, the mice received treatments of distilled water (50 ml/kg), Diclofenac sodium (50 mg/kg) and EEOBS (200 mg/kg) respectively. Groups 4-5 were given Glibenclamide (5 mg/kg) as a pretreatment before receiving EEOBS (200 mg/kg) and diclofenac sodium (50 mg/kg). All treatment mouse groups were evaluated using the acetic acid-induced writhing test after one hour.

Assessment of the potential involvement of the noradrenergic system

Mice were divided into three groups: 50 ml/kg of distilled water 200 mg/kg of EEOBS, and 50 mg/kg of Diclofenac sodium solution for the first, second, and third groups, respectively. Groups 4 and 5 received pretreatment of terazosin (1 mg/kg, i.p.) before the administration of EEOBS (200 mg/kg) and diclofenac sodium (50 mg/kg). After an hour, writhing was seen in all mouse groups.

***In silico* analysis for the analgesic activity**

Selection of the phytocompounds

Many isolated chemicals from various areas of the investigated plant were found after a thorough examination of the literature. Nonetheless, the study's chosen chemicals are those that were separated from the seeds. The most prevalent compounds were Octadecatrienoic acid (Pub chem ID

5312493), Octadecanoic acid (Pub chem ID 5281), Eicosenoic acid (Pub chem ID 5460988) and n-Hexadecanoic acid (Pubchem ID 985) were downloaded from PubChem database.

Ligand (compound) preparation

The PubChem website (https://pubchem.ncbi.nlm.nih.gov) provided access to O. basilicum phytocompounds via their 2D structures. Chem 3D pro and Chemdraw ultra-12.0 were then used for ligand energy minimization and optimization.

Preparation of receptor and grid generation

Protein structures with the highest resolution were retrieved from the Protein Databank (PDB) and subsequently used for molecular docking with Pyrex software. During the protein preparation process, in primary stage, bond order was assigned, hydrogen atoms were added, solvent water molecules were removed, bond orders were determined, disulfide bonds were developed, and the protein's protonation state was adjusted to PH 7.4 [39].

Predicting pharmacokinetic parameters by SwissADME

The identification of these substances as possible therapeutic candidates is largely dependent on their molecular characteristics. SwissADME, an internet resource (http://www.swiss.adme.ch/), was used to predict the pharmacokinetic features of the substances. Lipinski's rule of five (RO5) was followed to screen the compounds to determine their suitability as drug candidates. The criteria included (i) Hydrogen bond acceptor less than 5 (ii) hydrogen bond donor less than 5 (iii) low molecular weight (favourable range: <500); (iv) highest lipophilicity (expressed as log Po/w, favourable range: <5); and (v) appropriate molar refractivity (acceptable range: between 40 and 130). This procedure made use of the canonical SMILES that were obtained from PubChem using the swiss ADME online database [40]; [41].

Statistical analysis

A $P<0.05$ was considered statistically significant. GraphPad Prism was used to carry out a one-way ANOVA (San Diego, CA, USA).

Results

In vivo* Acetic Acid Induced Writhing Response and Analgesic Effect of Ethanolic Seed Extract of *O. basilicum

The EEOBS had potent peripheral analgesic effects, as demonstrated by a statistical analysis of the collected data. The EEOBS at 200 mg/kg body weight dose had the highest peripheral analgesic effectiveness (57.44% writhing inhibition) among all test groups, in comparison to standard (68.78% writhing inhibition). The outcome further showed that modest to **%** moderate peripheral analgesic action is also present at lower dosages (Figure 1).

Formalin test

Using the Formalin-Induced Test Method, four groups of mice were used to test the analgesic activity. Early in formalin-induced pain, the dose-dependent antinociceptive association was noted. Significant differences were seen between the 200 and 50 mg/kg D.F. doses as compared to the control group (79.33 and 52.83, respectively). At a dosage of 200 mg/kg, the pretreatment significantly reduced the duration of biting and the late-phase subcutaneous injection of formalin-induced licking (30.43, $p < 0.05$). During the late phase, D.F. demonstrated noteworthy anti-nociceptive activity (24.36, $p < 0.05$). The obtained test results are displayed in Figure 2.

Hot-plate test

The latency duration of heat-induced nociception increased in the experimental animals in a dose-dependent manner, which was comparable to the effects of prescribed drug (diclofenac sodium) (Figure 3). Following the injection of EEOBS at dosages of 50 , 100 , and 200 mg/kg body weight, the latency times increased at all doses. Furthermore, the pain inhibition at 30 minutes at a dose of

200. mg/kg was even more potent than the conventional medication D.F. The delay time and MPE percentage in the 60 minutes likewise showed significant differences from the control group. The 90-minute observations matched those from prior research periods in that they demonstrated an increase in response to dosage increases. The low dose of extract showed the least amount of effect out of all the levels.

Figure 3. Anti-nociceptive qualities of EEOBS in a hot plate test. Data values are displayed as mean ± SEM. ($P < 0.05$) indicating a statistically significant when compared to the control ("*") and standard groups ("#") (Dunnett's test in a one-way ANOVA with post hoc analysis).

Tail flick test

When hot water was administered in the tail-flick test, EEOBS and D.F considerably decreased pain perception at all doses. This is an interesting illustration of dose-dependent anti-nociceptive activity. (Figure 4).

Action mechanism determining the potential rivals of the opioidergic pathway

The mean number of abdominal writhes was considerably ($p < 0.05$) decreased by the seed extract (200 mg/kg) and D.F (10 mg/kg) when compared to the group administered with distilled water. Nevertheless, by raising the average frequency of abdominal writhes, naloxone considerably ($p < 0.05$) counteracted the effects of the 200 mg/kg extract and D.F. 50 mg/kg (Figure 5).

Determination of the possible participation of the cholinergic pathway

When mice were administered 200 mg/kg of EEOBS and 50 mg/kg of D.F , the average number of abdominal writhes was significantly ($p < 0.05$) decreased by atropine pretreatment (Figure 6).

Determination of the potential involvement of KATP channel blocker pathway

Compared to the group that received distilled water, the animals treated with EEOBS (200 mg/kg) and D.F. (50 mg/kg) exhibited significantly less mean abdominal writhes. The extract's decrease in the quantity of abdominal writhes was reversed with glibenclamide. However, in mice having D.F., glibenclamide pre-treatment had no appreciable impact on the average number of abdominal writhes (Figure. 7).

Determination of the possible participation of the noradrenergic system

The group treated with distilled water had a substantially higher mean number of abdominal writhes ($p < 0.05$) than the mice treated with EEOBS 200 mg/kg, D.F. 50 mg/kg, extract pre-treated with terazosin, and D.F. pre-treated with terazosin (α1 and α2 adrenergic antagonist). However, in mice administered 200 mg/kg of EEOBS, the average number of abdominal writhes was not significantly affected by terazosin pretreatment (Figure 8).

Computational analysis *(In silico)*

Physicochemical Properties

The way a medication is metabolized in the body has a big impact on its physicochemical characteristics. Every chemical followed the guidelines of Lipinski (Table 1). Each compound contains less than 10 rotatable bonds and their molar ratios are within the acceptable range of 40 to 130. Another important component of medication bioavailability is topological polar surface area (TPSA). Based on TPSA values ranging from 40 to 140Å, Table 1 indicates that the 15 compounds selected are polar. High solubility enhances oral drug absorption, while low solubility can prevent its gastrointestinal absorption. For guaranteed optimal oral circulation, the water content of these medications should be assessed, with a Log-S value between 1 and 8.

Pharmacokinetics Properties of Phytocompounds

The blood-brain barrier (BBB) illustrates the relationship between drugs targeting and those affecting brain tissue. Compounds must function as P-gp inhibitors to interact with phospholipoids

and the phenyl ring. The CYP enzyme family is important for the medication clearance through metabolic biotransformation. Table 2 showed that all compounds' log-Kp values ranges between -8.0 and 1.0.

Lipophilicity and Drug-likeness of phytocompounds

Table 3 displays the log-p values for each of the suggested compounds. These values are all within the permissible range of -0.7 to +7.0, indicating favorable oral permeability and absorption. The drug-likeness of the molecule, based on its bioavailability, is then assessed to determine its potential as an oral medication candidate (Table 3).

Five Lipinski's rules for phytocompounds selection

According to Lipinski's rule, a compound is likely to be developed as an oral medication if none or fewer than one of the following four conditions are violated. Nearly all the selected compounds passed the ADME screening, except one compound had four violations. This indicates that the series has potential for developing drug-like substances

Medicinal Chemistry

Compounds can produce misleading reactions affecting life significantly regardless of the protein receptor involved. Table 3 indicates that PAINS were recovered. The synthetic accessibility score (SA) was found to be below 5, suggesting good performance. From 1 to 10 (basic easy to strong connection), the SA is rated and less than 4 is associated with the highest synthetic accessibility. Table 3 indicates that all compounds are suitable for biological optimization. Additionally, the radar scheme demonstrates that 15 compounds, which do not undergo degradation, also fulfil the requirements for oral bioavailability. According to the Swiss ADME prediction, these 15 phytocompounds exhibit the most favourable balance of all factors, making them strong candidates for effective chemotherapeutic potential.

Computationally Molecular Docking Analysis

The molecular interactions of the ligand and receptor protein were investigated by docking analysis; chem-draw ultra pro 12.0 and chem draw 3D were utilized for ligand analysis and energy minimization in the pyrex [45]. The receptor ID of the protein data bank is (PDB ID: 1SG1) then incrementally imported into Pyrex at a resolution of 2.70 to complete the docking process, which is a vital pathogenic component, to evaluate the efficacy of receptor protein. The substance that interacts with the receptor protein the best is pyrex. The best medication was selected based on its docking score and binding fitness of the ligand that had the greatest connecting score to the receptor protein. The results were assessed by evaluating binding compatibility.

Once the Swiss ADME 4 compounds were screened, we met the requirements and docked the compounds with the receptor protein 1SG1 (Human COX-1). Of the compounds, n-Hexadecenoic acid, octadecanoic acid, eicosenoic acid, and octadecatrienoic acid demonstrated the best interaction, with docking scores of -6.4, -4.5, -4.9, and -4.8, respectively. The best poses from the discovery studio are shown in 2D to show the interactions between proteins and ligands. The docking score prediction reveals that these four compounds exhibit the most favourable balance of all factors, indicating their potential as effective chemotherapeutic agents. Figure 9 presents the top poses from Discovery Studio, highlighting their protein-ligand interactions in 2D."

Discussion

The most popular technique for examining the peripheral analgesic impact of any plant portion is the writing test. In this test, acetic-acid is the main inducer of pain in an animal model [42]. It has been proposed that prostaglandin pathways and peritoneal mast cells trigger the reaction [43]; [44]. Substances of P, the bradykinin, histamine, serotonin and the prostaglandins are among the inflammatory mediators whose release is enhanced when acetic acid is injected intraperitoneally. Inflammatory mediators are released, which results in more tightness of the abdomen or pain perception [45]. [46] Additionally clarified the increased prostaglandin quantity in the peritoneal

exudates following acetic acid administration intraperitoneally. It can be deduced that the EEOBS's peripheral analgesic effect might be due to the inhibition of these mediators' synthesis or release.
There formalin test reaction consists of 2 distinct stages. Within the first five minutes following the formalin injection, the first or early phase (neurogenic nociceptive phase) takes place. After formalin injection, the inflammatory nociceptive response (2^{nd} or late phase) begins 15 to 30 minutes later. According to the research and studies, analgesic medications or drugs (such as opioids or centrally-acting analgesics) seem to decrease pain behaviours during both stages of the pain cycle. Conversely, NSAIDs only work during the second stage of the condition [47]. Similar to what Onasanwo and Elegbe observed, our results indicated that the extract decreased both phases when compared to the control [48]. Based on the suppression observed in the 1^{st} and 2^{nd} stage of the formalin test, the EEOBS showed the possibility of central and peripheral anti-nociceptive activity.
In animal models where thermal stimuli are used to induce pain in the hot plate and tail immersion experiments are two basic techniques to evaluate central analgesic activity. These techniques emphasize changes occurring above the level of the spinal cord, offering a valuable example of centrally mediated antinociceptive responses [49]. Because of the techniques' excellent selectivity for opioid-derived analgesics, they are preferred [50]. In both models, the *Ocimum basilicum* seed extracts exhibited a strong dose-dependent anti-nociceptive effect. The extracts' primary anti-nociceptive activity is demonstrated by the hot plate method's significant dose-dependent increase in latency time [51];[52]. A potential suppression or alteration of pain induction via this route is indicated by the inhibition of nociceptive activity. Furthermore, the tail immersion test results corroborated the hot plate method's findings. This approach targets just central analgesia because peripherally acting medications are ineffective against these kinds of heat stimulation [53]. Spinal reflexes, measurable through the tail immersion method, are a mechanism by which the opioid receptors that contribute to the nociception [51];[52]. This method is also demonstrated a dose-dependent enhancement in the nociceptive techniques
The anti-nociceptive properties of EEOBS appear to be mediated by spinal and supra-spinal receptors, based on the results of both approaches.

Generally speaking, the way that opioid analgesics work is that they attach to opiate receptors, which causes the descending brain stem inhibitory systems to become active and suppress spinal neuron responses to painful inputs [50]. Nalbin is a non-selective opioid antagonist that inhibit the opioid supraspinal receptors in the central nervous system, it was possible to ascertain the role of the opioidergic route in the analgesic effect of EEOBS analgesic impact of EEOBS; atropine was administered before to treatment. Acetylcholine and other choline esters' muscarinic effects are countered with atropine, a non-selective anticholinergic or antimuscarinic drug [54]. In this study, the analgesic effect of EEOBS was reversed by pre-treatment with atropine. This suggested that the extract's analgesic effect was mediated through cholinergic pathways.
Neuronal excitability, which governs the production of several pain signals in the human nervous system, is that mostly dependent on ion channels [55, 56]. By lowering neuronal excitability and preventing the release of several neurotransmitters in the spinal cord, K^+ channel opening causes analgesia. This study also demonstrated glibenclamide's capacity to block the analgesic effect of morphine, as previously reported [56]. Consequently, the likelihood that ATP-sensitive K+ channels are involved in EEOBS's analgesic activity is raised by the analgesic effect's reversal after glibenclamide pre-treatment. At the spinal and supraspinal levels, the noradrenergic system contributes to nociception mainly via activating α-adrenoceptors and descending inhibitory pathways [57]. These suggest that the α1 and α2-adrenoceptor pathways are unrelated to the analgesic effect of the EEOBS [58].
Next, to ascertain the molecular level nociceptive techniques of the plant and the effectiveness of the receptors, Table 4 displays the binding fitness and molecular docking score of the isolated compounds from *O. basilicum* that have the receptor protein PDB ID: 1SG1, which is a key for the Common Neurotrophin receptor. Although selective cox-2 inhibitors are a superior option to block the pain-stimulating function, they are linked to a variety of negative effects. Acute pain is primarily caused by the cycloxygenase-2 enzyme [56]. The four compounds have good binding affinities in

our investigation. Consequently, *in silico* molecular docking supported the effectiveness of *O. basilicum* as an analgesic.

Conclusion

According to the study's observed data, *O. basilicum* seeds have a significant potential for peripheral and central anti-nociceptive effects in a variety of *in vivo* models. The outcome of these in vivo experiments motivates us to assess the animals' motor function further to determine whether or not they have the potential for both central and peripheral anti-nociceptive activities. Additionally, the study has shown that opening ATP-sensitive K+ channels and activating opioidergic and cholinergic receptors may be involved in the analgesic effects of EEOBS. Additionally, the chemicals extracted from this plant's seeds demonstrated a binding affinity for the protein 1SG1 in the molecular docking investigation. As a result, the compounds that showed promise in the in-silico test may provide a solid basis for the creation of novel anti-nociceptive medications. These results align with the experimental results, indicating the need for further comprehensive research to determine the exact molecular mechanism of action of these medications in the animal models.

Abbrevations

EEOBS Ethanolic extract of seeds from *Ocimum basilicum*
D.W Distilled water
NAL Nalbin
Tzs Terazosin
D.F Diclofenac sodium
GLI Glibenclamide
Atr Atropine

Data Availablity

The data underlying this article will be shared on reasonable request to the corresponding author.

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Author contributions

NZ and AA performed the lab work and collected data. NZ and SN organized the literature, analyzed the data, and drafted the manuscript. AA and AR conceptualized the study, edited the final version, and obtained funding for the study. All authors have approved the final version of the manuscript for submission.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References:

1. Nicholas, M., Vlaeyen, J. W., Rief, W., Barke, A., Aziz, Q., Benoliel, R., & Treede, R. D. (2019). The IASP classification of chronic pain for ICD-11: chronic primary pain. *Pain*, *160*(1), 28-37.
2. Mills, S. E., Nicolson, K. P., & Smith, B. H. (2019). Chronic pain: a review of its epidemiology and associated factors in population-based studies. *British journal of anaesthesia*, *123*(2), e273-e283.
3. Alalawi, A., Luque-Suarez, A., Fernandez-Sanchez, M., Tejada-Villalba, R., Navarro-Martin, R., Devecchi, V., ... & Falla, D. (2022). Perceived pain and disability but not fear of movement are associated with altered cervical kinematics in people with acute neck pain following a whiplash injury. *Musculoskeletal Science and Practice*, *62*, 102633.

4. Esu, K. D., Bakare, A. O., & Owoyele, B. V. (2022). Effects of co-administration of vitamin E and lithium chloride on chronic constriction injury-induced neuropathy in male Wistar rats: Focus on antioxidant and anti-inflammatory mechanisms. *Pain Practice*, *22*(2), 148-158.
5. Cohen, S. P., Vase, L., & Hooten, W. M. (2021). Chronic pain: an update on burden, best practices, and new advances. *The Lancet*, *397*(10289), 2082-2097.
6. Benzon, H., Rathmell, J. P., Wu, C. L., Turk, D., Argoff, C. E., & Hurley, R. W. (2022). *Practical Management of Pain E-Book*. Elsevier Health Sciences.
7. Haspert, V. (2023). *Improving acute pain management with emotion regulation strategies: A comparison of acceptance, distraction, and reappraisal* (Doctoral dissertation, Universität Würzburg).
8. Rege, M., Størksen, I., Solli, I. F., Kalil, A., McClelland, M. M., Ten Braak, D., ... & Hundeland, P. S. (2024). The effects of a structured curriculum on preschool effectiveness: A field experiment. *Journal of Human Resources*, *59*(2), 576-603.
9. Kasman, S. A., & Duruöz, M. T. (2019). Pain and hand function. *Hand Function: A Practical Guide to Assessment*, 57-72.
10. Mohammadi, M. (2019). Oxidative stress and polycystic ovary syndrome: a brief review. *International journal of preventive medicine*, *10*(1), 86.
11. Strycharz-Dudziak, M., Fołtyn, S., Dworzański, J., Kiełczykowska, M., Malm, M., Drop, B., & Polz-Dacewicz, M. (2020). Glutathione peroxidase (GPx) and superoxide dismutase (SOD) in oropharyngeal cancer associated with EBV and HPV coinfection. *Viruses*, *12*(9), 1008.
12. Kendroud, S., Fitzgerald, L. A., Murray, I. V., & Hanna, A. (2022). Physiology, nociceptive pathways. In *StatPearls [Internet]*. StatPearls Publishing.
13. Middleton, S. J., Barry, A. M., Comini, M., Li, Y., Ray, P. R., Shiers, S., ... & Bennett, D. L. (2021). Studying human nociceptors: from fundamentals to clinic. *Brain*, *144*(5), 1312-1335.
14. Nikolenko, V. N., Shelomentseva, E. M., Tsvetkova, M. M., Abdeeva, E. I., Giller, D. B., Babayeva, J. V., ... & Sinelnikov, M. Y. (2022). Nociceptors: their role in body's defenses, tissue specific variations and anatomical update. *Journal of pain research*, 867-877.
15. Machado, G. C., Abdel-Shaheed, C., Underwood, M., & Day, R. O. (2021). Non-steroidal anti-inflammatory drugs (NSAIDs) for musculoskeletal pain. *Bmj*, *372*.
16. Tai, F. W. D., & McAlindon, M. E. (2021). Non-steroidal anti-inflammatory drugs and the gastrointestinal tract. *Clinical Medicine*, *21*(2), 131-134.
17. Mehuys, E., Crombez, G., Paemeleire, K., Adriaens, E., Van Hees, T., Demarche, S., ... & Boussery, K. (2019). Self-medication with over-the-counter analgesics: a survey of patient characteristics and concerns about pain medication. *The journal of pain*, *20*(2), 215-223.
18. Milani, D. A. Q., & Davis, D. D. (2023). Pain management medications. In *StatPearls [Internet]*. StatPearls Publishing.
19. Bindu, S., Mazumder, S., & Bandyopadhyay, U. (2020). Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. *Biochemical pharmacology*, *180*, 114147.
20. Brandão, D. O., Barata, L. E. S., & Nobre, C. A. (2022). The effects of environmental changes on plant species and forest dependent communities in the Amazon region. *Forests*, *13*(3), 466.
21. Sarimanah, U., & Angin, M. P. (2023). Evaluation of analgesic activity of ethanol and ethyl acetate extracts from basil leaves (Ocimum x africanum L.) in acetic acid-induced rat models. *Pharmacy Reports*, *3*(1), 51-51.
22. Dhama, K., Sharun, K., Gugjoo, M. B., Tiwari, R., Alagawany, M., Iqbal Yatoo, M., ... & Farag, M. R. (2023). A comprehensive review on chemical profile and pharmacological activities of Ocimum basilicum. *Food Reviews International*, *39*(1), 119-147.
23. Al-Snafi, A. E. (2021). Chemical constituents and pharmacological effects of Ocimum basilicum-A review. *International Journal of Pharmaceutical Research*, *13*(2), 2997-3013.
24. Kumalasari, M. L. F., & Andiarna, F. (2020). Uji fitokimia ekstrak etanol daun kemangi (Ocimum basilicum L). *Indonesian Journal for Health Sciences*, *4*(1), 39-44.
25. Hidayati, A. N. A., & Bahar, Y. (2019). Efek daun kemangi (Ocimum basilicum L.) terhadap bakteri Staphylococcus epidermidis. *Sainteks*, *15*(1).

26. Panggabean, R., & Ulfa, A. M. (2021). The Hepatoprotective Effects of Basil Leaf (Ocimum sanctum L.) Extract on Paracetamol Induced Liver Damage in Male Rat. *Biomedical Journal of Indonesia*, *7*(2), 444-450.
27. Khater, E. S., Bahnasawy, A., Abass, W., Morsy, O., El-Ghobashy, H., Shaban, Y., & Egela, M. (2021). Production of basil (Ocimum basilicum L.) under different soilless cultures. *Scientific Reports*, *11*(1), 12754.
28. Vázquez-Fresno, R., Rosana, A. R. R., Sajed, T., Onookome-Okome, T., Wishart, N. A., & Wishart, D. S. (2019). Herbs and spices-biomarkers of intake based on human intervention studies–a systematic review. *Genes & nutrition*, *14*, 1-27.
29. Srivastava, S., Cahill, D. M., Conlan, X. A., & Adholeya, A. (2014). A novel in vitro whole plant system for analysis of polyphenolics and their antioxidant potential in cultivars of Ocimum basilicum. *Journal of agricultural and food chemistry*, *62*(41), 10064-10075.
30. Al Abbasy, D. W., Pathare, N., Al-Sabahi, J. N., & Khan, S. A. (2015). Chemical composition and antibacterial activity of essential oil isolated from Omani basil (Ocimum basilicum Linn.). *Asian Pacific Journal of Tropical Disease*, *5*(8), 645-649.
31. Jermini, M., Dubois, J., Rodondi, P. Y., Zaman, K., Buclin, T., Csajka, C., ... & E. Rothuizen, L. (2019). Complementary medicine use during cancer treatment and potential herb-drug interactions from a cross-sectional study in an academic centre. *Scientific reports*, *9*(1), 5078.
32. Obese, E., Biney, R. P., Henneh, I. T., Anokwah, D., Adakudugu, E. A., Woode, E., & Ameyaw, E. O. (2021). Antinociceptive effect of the hydroethanolic leaf extract of Calotropis procera (Ait) R. Br.(Apocynaceae): possible involvement of glutamatergic, cytokines, opioidergic and adenosinergic pathways. *Journal of Ethnopharmacology*, *278*, 114261.
33. Uddin, M. M. N., Kabir, M. S. H., Hasan, M., Al Mahmud, Z., Alam Bhuiya, N. M., Ahmed, F., ... & Alam, M. S. (2018). Assessment of the antioxidant, thrombolytic, analgesic, anti-inflammatory, antidepressant and anxiolytic activities of leaf extracts and fractions of Tetracera sarmentosa (L.) Vahl. *Journal of Basic and Clinical Physiology and Pharmacology*, *29*(1), 81-93.
34. Bhuiyan, M. M. R., Bhuiya, N. M. A., Hasan, M. N., & Nahar, U. J. (2020). In vivo and in silico evaluation of antinociceptive activities of seed extract from the Holarrhena antidysenterica plant. *Heliyon*, *6*(5).
35. Bor, Z., Arslan, R., Bektaş, N., Pirildar, S., & Dönmez, A. A. (2012). Antinociceptive, antiinflammatory, and antioxidant activities of the ethanol extract of Crataegus orientalis leaves. *Turkish Journal of Medical Sciences*, *42*(2), 315-324.
36. D'Amour, F. E., & Smith, D. L. (1941). A method for determining loss of pain sensation. *J Pharmacol Exp Ther*, *72*(1), 74-79.
37. Mansouri, M. T., Naghizadeh, B., & Ghorbanzadeh, B. (2014). Involvement of opioid receptors in the systemic and peripheral antinociceptive actions of ellagic acid in the rat formalin test. *Pharmacology Biochemistry and Behavior*, *120*, 43-49.
38. Rangel, R. A., Marinho, B. G., Fernandes, P. D., de Moura, R. S., & Lessa, M. A. (2014). Pharmacological mechanisms involved in the antinociceptive effects of dexmedetomidine in mice. *Fundamental & clinical pharmacology*, *28*(1), 104-113.
39. Andleeb, R., Ijaz, M. U., Rafique, A., Ashraf, A., Bano, N., Zafar, N., ... & Ahmedah, H. T. (2021). Biological activities of methanolic extract of aegle marmelos against HN protein of newcastle disease virus. *Agronomy*, *11*(9), 1784.
40. Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific reports*, *7*(1), 42717.
41. Hasan, M. N., Bhuiya, N. M. M. A., & Hossain, M. K. (2019). In silico molecular docking, PASS prediction, and ADME/T analysis for finding novel COX-2 inhibitor from Heliotropium indicum. *J. Comp. Med. Res*, *10*, 142-154.
42. De Souza, M. M., Pereira, M. A., Ardenghi, J. V., Mora, T. C., Bresciani, L. F., Yunes, R. A., ... & Cechinel-Filho, V. (2009). Filicene obtained from Adiantum cuneatum interacts with the

cholinergic, dopaminergic, glutamatergic, GABAergic, and tachykinergic systems to exert antinociceptive effect in mice. *Pharmacology Biochemistry and Behavior*, *93*(1), 40-46.
43. Ribeiro, R. A., Vale, M. L., Thomazzi, S. M., Paschoalato, A. B., Poole, S., Ferreira, S. H., & Cunha, F. Q. (2000). Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. *European journal of pharmacology*, *387*(1), 111-118.
44. Vogel, H. G., & Vogel, W. H. (1998). Drug discovery and evaluation. *Biomedicine & Pharmacotherapy*, *52*(1), 47-47.
45. Ikeda, Y., Ueno, A., Naraba, H., & Oh-Ishi, S. (2001). Involvement of vanilloid receptor VR1 and prostanoids in the acid-induced writhing responses of mice. *Life sciences*, *69*(24), 2911-2919.
46. Deraedt, R., Jouquey, S., Delevallée, F., & Flahaut, M. (1980). Release of prostaglandins E and F in an algogenic reaction and its inhibition. *European journal of pharmacology*, *61*(1), 17-24.
47. Reeve, A. J., & Dickenson, A. H. (1995). The roles of spinal adenosine receptors in the control of acute and more persistent nociceptive responses of dorsal horn neurones in the anaesthetized rat. *British journal of pharmacology*, *116*(4), 2221-2228.
48. Onasanwo, S. A., & Elegbe, R. A. (2006). Anti-nociceptive and anti-inflammatory properties of the leaf extracts of Hedranthera barteri in rats and mice. *African Journal of Biomedical Research*, *9*(2).
49. Wigdor, S. E. T. H., & Wilcox, G. L. (1987). Central and systemic morphine-induced antinociception in mice: contribution of descending serotonergic and noradrenergic pathways. *Journal of pharmacology and Experimental Therapeutics*, *242*(1), 90-95.
50. Abbott, F. V., Melzack, R., & Samuel, C. (1982). Morphine analgesia in the tail-flick and formalin pain tests is mediated by different neural systems. *Experimental neurology*, *75*(3), 644-651.
51. Chapman, C. R., Casey, K. L., Dubner, R., Foley, K. M., Gracely, R. H., & Reading, A. E. (1985). Pain measurement: an overview. *Pain*, *22*(1), 1-31.
52. Jinsmaa, Y., Fujita, Y., Shiotani, K., Miyazaki, A., Li, T., Tsuda, Y., ... & Lazarus, L. H. (2005). Differentiation of opioid receptor preference by [Dmt1] endomorphin-2-mediated antinociception in the mouse. *European Journal of Pharmacology*, *509*(1), 37-42.
53. Srinivasan, K., Muruganandan, S., Lal, J., Chandra, S., Tandan, S. K., Raviprakash, V., & Kumar, D. (2003). Antinociceptive and antipyretic activities of Pongamia pinnata leaves. *Phytotherapy Research*, *17*(3), 259-264.
54. Howland, R. H., Rush, A. J., Wisniewski, S. R., Trivedi, M. H., Warden, D., Fava, M., ... & Berman, S. R. (2009). Concurrent anxiety and substance use disorders among outpatients with major depression: clinical features and effect on treatment outcome. *Drug and alcohol dependence*, *99*(1-3), 248-260.
55. Longhi-Balbinot, D. T., Martins, D. F., Lanznaster, D., Silva, M. D., Facundo, V. A., & Santos, A. R. (2011). Further analyses of mechanisms underlying the antinociceptive effect of the triterpene 3β, 6β, 16β-trihydroxylup-20 (29)-ene in mice. *European Journal of Pharmacology*, *653*(1-3), 32-40.
56. Zakaria, Z. A., Abdul Rahim, M. H., Roosli, R. A. J., Mohd Sani, M. H., Omar, M. H., Mohd. Tohid, S. F., ... & Abdul Kadir, A. (2018). Antinociceptive activity of methanolic extract of Clinacanthus nutans leaves: Possible mechanisms of action involved. *Pain Research and Management*, *2018*(1), 9536406.
57. Millan, M. J. (2002). Descending control of pain. *Progress in neurobiology*, *66*(6), 355-474..
58. Roczniak, W., Wróbel, J., Dolczak, L., & Nowak, P. (2013). Influence of central noradrenergic system lesion on the serotoninergic 5-HT3 receptor mediated analgesia in rats. *Advances in Clinical and Experimental Medicine*, *22*(5), 629-638.