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Research article

Ceratonia siliqua leaves ethanol extracts exert anti-nociceptive and anti-inflammatory effects

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ABSTRACT

Background: *Ceratonia siliqua* L. (Leguminosae) has neuroprotective, mutagenic, hypotensive, anti-bacterial, hypoglycaemic, and anti-inflammatory effects through extracts from its leaves. Therefore, the aim of this study is to assess the anti-nociceptive activity of ethanol extracts of *Ceratonia siliqua* leaves.

Methods: Ethanol extract of *Ceratonia siliqua* leaves were studied using well-established animal models of inflammation and pain. A hot plate latency assay (55 °C) was used to assess the analgesic effect of 10, 31.6, 100, and 316 mg/kg doses of ethanol extracts in addition to paw licking time in early and late phase using a formalin-induced paw licking assay test. Paw oedema induction using carrageenan and cotton pellet granuloma assays were used to assess the anti-inflammatory effect of 10, 31.6, 100, and 316 mg/kg doses of ethanol extract.

Results: The ethanol extract of *Ceratonia siliqua* leaves reduces paw licking time in early and late phase after formalin injection. The same effect was also observed when the hotplate test was performed. Ethanol extract of *Ceratonia siliqua* leaves caused dose dependent inhibition in paw oedema after the injection of carrageenan and cotton pellet granuloma in mice. These effects were not antagonized when opioid receptors were blocked by naloxone (5 mg/kg). The preliminary phytochemical analysis of the ethanol extract of *Ceratonia siliqua* leaves showed the presence of tannins, alkaloids, flavonoids and terpenoids.

Conclusion: The present data indicate that ethanol extract of *Ceratonia siliqua* leaves might possess anti-inflammatory and anti-nociception properties and should be considered for further therapeutic research.

1. Introduction

Emotional and cognitive experiences induced by real or perceived tissue damage lead to an unpleasant sensation called pain which is realised through behavioural, psychological and autonomic reactions [1]. The two main classifications of pain are physical and psychological [2]. Pain in cancer patients is often described as existential pain, which is an example of psychological pain [3]. Physical pain can be classified as nociceptive, inflammatory, and neurogenic pain [4, 5]. Pain that arises from actual or threatened damage to non-neural tissue and which is due to the activation of nociceptors is known as nociceptive pain, which is the most common type of human pain [6]. Cytokines such as IL-1B, IL-6, and TNF-alpha, which activate NF-KB, peptides, lipids, excitatory amino acids, and protons are mediators that are secreted in response to tissue damage which activate nociceptors to induce pain [7, 8]. Moreover, the induction of inflammation by these chemical mediators lowers the threshold of nociceptors, which leads to neuronal membrane excitability that facilitates the activation of nociceptors and impulse transmission through nociceptor neurons [9]. The most common therapeutic agents for the treatment of nociceptive pain are non-steroidal anti-inflammatory drugs (NSAIDs), however, several significant side effects are associated with their long-term use, such as gastrointestinal lesions, bleeding, and peptic ulcers [10, 11]. Furthermore, severe pain is commonly treated using opioids, however, physical tolerance and the possibility of addiction limit their use

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[12]. Thus, plant-based medicine has received greater attention recently due to its effectiveness in pain management and fewer resulting side effects.

Food value is linked to its nutritional content and digestibility as well as the presence or absence of toxic ingredients [13]. Indeed, while the bionutrients which food offers are essential for life, food possesses other bioactive compounds that are important for health promotion and disease prevention [14]. The consumption of a healthy diet is strongly correlated with the reduced risk of several chronic diseases, such as cancer, diabetes, cardiovascular diseases and atherosclerosis, neurodegenerative disorders, and inflammation, along with associated complications [15]. These therapeutic properties of food have given rise to medicinal drugs made from certain kinds of food (plants in particular). Numerous medicinal plants have been widely used in the treatment of several diseases, such as cardiovascular diseases [16], cancer [17], diabetes [18], inflammation [19], and also in pain management [20]. Many medicinal plants have anti-inflammatory properties, such as curcuma longa Linn which blocks the JAK-STAT pathway, IL-6, and NF-KB inflammatory pathways [21]. Moreover, *Camellia sinensis* was found to have anti-inflammatory and anti-oxidant properties by altering certain inflammatory transducers, such as NF-KB, JAK/STAT, and COX-1, which is an effective agent for the treatment of atherosclerosis, colitis, and ulceration [22]. This demonstrates that medicinal plants can be an effective therapeutic approach to treat inflammation related disorders such as pain.

A leguminous evergreen tree grows throughout the Mediterranean region (in areas such as Morocco, Spain, Portugal, Italy and many Arabian countries) known as algarroba or *Ceratonia siliqua* L [23]. Popular beverages and confectionery are obtained from the fruit of this tree, and the powder of its pods can be used as a source of gum [24, 25]. Interestingly, this tree is also used for the treatment of abdominal pain, diarrhoea, diabetes, viral and bacterial infections, obesity, and fevers [26, 27, 28, 29, 30]. A bibliographical survey has shown that only a few phytochemical investigations have so far been carried out on this species. The isolation of (+)-catechin, (−)-epicatechin gallate esters, (−)-epicatechin gallate, (−)-epigallocatechin gallate and (-)-epigallocatechin or delphinidin, pelargonidin, and cyanidin from this species has also been reported [31]. Other compounds include quercetin glycosides, kaempferol, genistein, leuteolin and ellagic acid [32]. In vitro assays have demonstrated anti-oxidant activity in addition to scavenging activity for free radicals of the water extract of *Ceratonia siliqua* [33], as well as anti-proliferative activity in hepatocellular carcinoma [34]. Additionally, *Ceratonia siliqua* pulp contains high amounts of polyphenols and fibre. It is well known that fibre improves digestion, and reduces blood cholesterol and glucose [35, 36, 37]. Interestingly, a large body of evidence has revealed that *Ceratonia siliqua* extracts exert anti-inflammatory effects by inhibiting myeloperoxidase activity, human neutrophiles reactive oxygen species (ROS) production, and other inflammatory mediators [38, 39, 40], suggesting that *Ceratonia siliqua* might have anti-nociceptive properties. Therefore, the objective of our study is to assess the anti-nociceptive effect of *Ceratonia siliqua* ethanolic extract in mice using various analgesic and inflammatory models.

2. Methodology

2.1. Ceratonia siliqua leaves

Fresh leaves of *Ceratonia siliqua* were obtained from the campus of the Hashemite University. The plant was referred to a plant taxonomist at the herbarium of the Hashemite University for identification and taxonomic authentication. For reference in the future, a voucher specimen (voucher number: HU-357) was kept at the herbarium at the Hashemite University, Zarqa, Jordan.

2.2. Ceratonia siliqua leaves ethanol extract preparation

500 g of *Ceratonia siliqua* leaf powder was soaked in one liter of petroleum ether (60–80 °C) for 10 days. During the soaking period, 95% ethanol was used to extract the materials from the leaves of the plant two times daily. Cheesecloth was used to filtrate the plant material at room temperature. 30 g of gummy material was obtained after the evaporation of ethanol under reduced pressure [41].

2.3. Phytochemical analysis

A thin layer of chromatography was used to confirm the existence of tannins, alkaloids, flavonoids and terpenoids using the standard procedure [42].

2.4. Experimental animals

25–30 g male Swiss albino mice were used for performing the experiments. All animals were kept in the animal house under the proper conditions. One hour before conducting the experiments, the animals were moved to a testing area to adapt to the laboratory conditions and decrease stress. The Research Ethics Committee at the Hashemite University approved all experiments (approval number HU-5/4/2020/2019, date 06/04/2020).

2.5. LD_{50} assessment

The *Ceratonia siliqua* leaves extract was administered orally, using different doses (100, 500, 1000, 1500, and 2000 mg/kg) into a group of twenty mice to assess the value of LD50 and its 95% confidence limit [43]. 48 hours after treatment, the number of deaths were counted.

2.6. Ceratonia siliqua leaves extract doses

The largest doses were determined based on LD50 experiments and based on practical solubility. Smaller doses were calculated to be located at approximately 0.5 log units from each other on a log scale.

2.7. Formalin test

As described in [44], six groups of mice (n = 6) were treated as follows: group one received 5% DMSO as a vehicle control, groups two to four received 10, 31.6, 100, 316 mg/kg ethanol extracts of *Ceratonia siliqua* leaves, respectively, while the sixth group received 5 mg/kg morphine. All treatments were administered orally, and the doses were determined based on the preliminary LD50 experiment and the practical solubility of the extract. To induce pain at the sub-plantar region of the right hind paw, formalin (5%, 50 μl, purity ≥37%) was injected 60 min after treatments. Nociceptive response was recorded by measuring the duration each mouse licked the formalin injection site. Licking time was recorded at 0–5 min (early phase) and 15–30 min (late phase) after injection. The below formula was used to estimate the percent of licking inhibition:

Percent inhibition of licking (PIL) = [(Licking time (control)−Licking time (treatment))/Licking time (control)] × 100.

2.8. Test using hot plate

In this test, six groups of mice (n = 6) were treated as follows: group one received 5% DMSO as the vehicle control. 10, 31.6, 100, 316 mg/kg ethanol extract of *Ceratonia siliqua* leaves was administered to groups two to five, respectively. Morphine (Sigma-Aldrich, purity ≥98.5%, 5 mg/kg) was administered to group six. All treatments were orally administered 60 min before exposure to testing with the analgesiometer hot plate (55 ± 5 °C). The time between when the animal was placed on the hotplate until the beginning of paw licking, along with their reaction times, were measured 60 min before and after treatment [45]. Results were represented as the percent increase in the baseline according to the following formula:

Increase in baseline = ((A−B)/B) × 100

A; reaction time after treatment, B; reaction time before treatment.

2.9. The involvement of opioidergic system

In order to assess the involvement of the opioidergic system [46], five groups of mice (n = 6) were tested as follows: group one received 5% DMSO as the vehicle control, group two received 316 mg/kg ethanol extract of *Ceratonia siliqua* leaves, group three received 316 mg/kg ethanol extract of *Ceratonia siliqua* leaves and nonselective opioid receptor antagonist, naloxone hydrochloride (Sigma-Aldrich, purity ≥98%, 5 mg/kg), while group four received 5 mg/kg morphine, and group five received 5 mg/kg morphine and 5 mg/kg naloxone. Then, the hotplate and formalin tests were performed as previously described.

2.10. Paw oedema induction using carrageenan

Acute inflammation was assessed using paw oedema induced by carrageenan [47]. Six groups of mice (n = 6) were fasted 24 h before performing the experiment. The first group received 5% DMSO orally as a vehicle control, groups 2–5 received 10, 31.6, 100, 316 mg/kg ethanol extract of *Ceratonia siliqua* leaves, respectively, whilst group 6 received 10 mg/kg indomethacin as a positive control. Acute paw oedema was induced by plantar injection of 0.1 ml of 1% freshly prepared carrageenan suspension in normal saline into the right hind paw of each mouse 1 h after treatment administration. A plethysmometer (type 7140 Ugo Basile, Italy) was used to measure the oedema formed in the paw by the water displacement method. The oedema was measured before (0 h) and at 1, 2, 3, and 5 h after carrageenan injection [48, 49, 50]. Inflammation percent was calculated by using the below formula:

Inflammation percent = (paw final volume−initial volume/initial volume) × 100.

2.11. Cotton pellet granuloma in mice

As previously described in Mossa et al., 2008, 30 mg of sterilized cotton pellet was inserted subcutaneously into the region of mice [51]. Six groups of mice (n = 6) were treated orally as follows: group one received 10 ml/kg 5% DMSO as a vehicle control, ethanol extract of *Ceratonia siliqua* leaves of 10, 31.6, 100, 316 mg/kg were administered to groups two to five, respectively, daily for 4 days. The sixth group received indomethacin (10 mg/kg). On the fifth day, the mice were exposed to an overdose of ether to sacrifice them before the pellets and the surrounding granuloma tissue were obtained. The constant weight of the pellets and the surrounding granuloma were obtained by drying off at 60 °C. The percentage inhibition was measured by comparing the mean weight in the test groups with the mean weight in the control group.

2.12. Statistical analysis

All analysed parameters were tested for normality of the data using the Kolmogorov-Smirnov test. Data were represented as mean ± SEM. One-way analysis of variance (ANOVA) or two-way ANOVA followed by Tukey post-hoc tests were used to perform the comparison between groups. A P value of less than 0.05 was taken as the cut off value for statistical significance. Statistical analysis was performed using Prism 5 software (GraphPad software, USA).

3. Results

3.1. Acute toxicity studies

Up to a dose of 2000 mg/kg of *Ceratonia siliqua* leaves ethanol extract is not lethal according to the acute toxicity test, and no toxicity signs were noticed in the treated group. No signs of toxicity, such as diarrhoea, motor impairment, ataxia, hyperactivity or alterations on respiratory frequency or piloerection, were noted in the control or experimental animals at lower doses. Also, no gastric ulcerogenic effect was observed in either the control or treated animals. Severe depression, abnormal gait, ataxia, increased respiration and decreased activity were observed at doses higher than 2000 mg/kg.

3.2. Phytochemical screening

Alkaloids, tannins, flavonoids, and terpenoids were found to be present in the *Ceratonia siliqua* leaves ethanol extract after performing the phytochemical screening. According to the existing literature, phenolic acids, condensed tannins, and flavonoids have been detected in *Ceratonia siliqua* leaf extract. 4-hydroxy-benzoic acid and myricitrin were the main phenolic compounds recognised. Furthermore, gallic acid, syringic acid, chlorogenic acid, 4-hydroxy-cumaric acid, caffeic acid, and ferulic acid were the other phenolic acids identified. Moreover, epigallocatechin, catechin, epigallocatechin gallate, and epicatechin gallate were the condensed tannins identified. Additionally, flavonols, namely rutin, Myricetin, luteolin and quercitrin were assigned in in *Ceratonia siliqua* leaf extract [40, 52, 53].

3.3. Anti-nociceptive effect of Ceratonia siliqua leaves ethanol extract

Following formalin injection, the licking time was reduced after the administration of *Ceratonia siliqua* leaf ethanol extract. This reduction was observed in the early phase (10 mg/kg 56.5 ± 2.9, 31.6 mg/kg 43.2 ± 2.9, 100 mg/kg 37.2 ± 2.5, and 316 mg/kg 23 ± 2.4) as well as in the late phases (10 mg/kg 124.3 ± 2.7, 31.6 mg/kg 85.2 ± 4.4, 100 mg/kg 73.2 ± 4.4, and 316 mg/kg 55.6 ± 4.4) compared to the control (Early phase 71.8 ± 4.5, late phase 151 ± 6.9) (Figure 1A and B, 10 mg/kg, $p < 0.05$: 31.6, 100, and 316 mg/kg, $p < 0.001$, n = 6). A dose-dependency effect was revealed in the reduction in licking time in both phases, with similar pattern. In addition, morphine, the reference drug, reduced the paw licking time significantly following formalin injection compared to the control in both phases (Figure 1A and B, $p < 0.001$, n = 6). The same pattern was observed when the hot plate test was performed. Furthermore, a significant increase in the time between when the animal was placed on the hotplate until the beginning of paw licking (latency time which was represented by an increase in baseline) was observed in the groups treated with ethanol extract of *Ceratonia siliqua* leaves (10 mg/kg 25.5 ± 2.06, 31.6 mg/kg 35.5 ± 2.52, 100 mg/kg 56.8 ± 2.57, 316 mg/kg 77.66 ± 2.23) compared to the control (4.98 ± 1.39) (Figure 2, 10 mg/kg, $p < 0.05$; 31.6, 100, and 316 mg/kg, $p < 0.001$, n = 6) and this increase in baseline was found to be dose-dependent. The latency time also increased in the group treated with morphine, the reference drug, compared to the control, and was higher compared to ethanol extract of *Ceratonia siliqua* leaves (Figure 2, $p < 0.001$, n = 6).

Naloxone was administered to mice before administering ethanol extract of *Ceratonia siliqua* leaves (316 mg/kg) or morphine. Then, paw

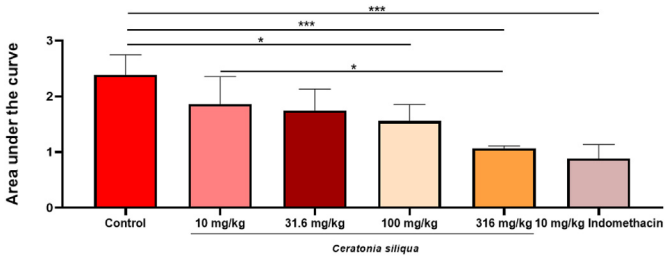

Figure 1. *Ceratonia siliqua* leaves ethanol extract activity on the non-inflammatory (A) phase and the inflammatory (B) phase in formalin test. In comparison to the control group, *Ceratonia siliqua* leaves ethanol extract significantly reduced the time of paw licking. One-way ANOVA was performed followed by Tukey posthoc (*<0.05, ***<0.001, n = 6).

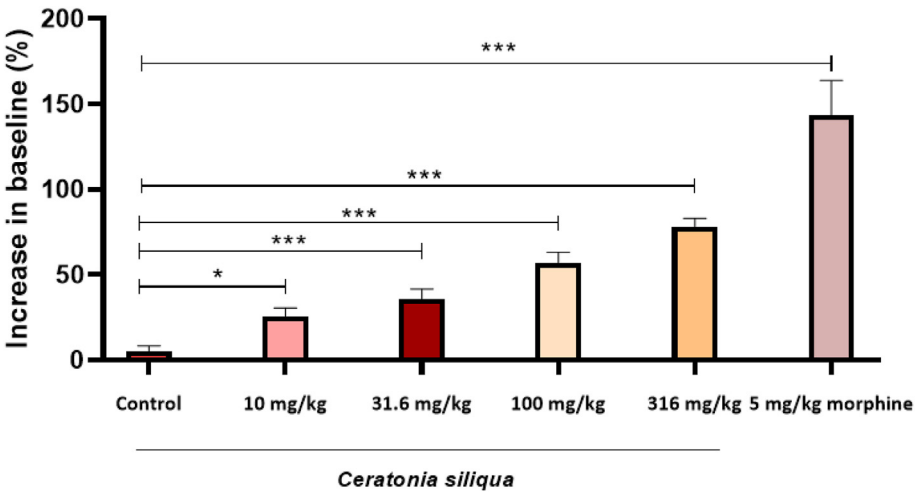

Figure 2. *Ceratonia siliqua* leaves ethanol extract activity on baseline percent of mice subjected to hotplate test. Baseline percent was increased in the groups received *Ceratonia siliqua* leaves ethanol extract in comparison to control group. One-way ANOVA test was performed followed by Tukey posthoc (*<0.05, ***<0.001, n = 6).

licking was induced by performing the formalin test and the hot plate test. Mice paw licking time was not affected when naloxone was administered with ethanol extract of *Ceratonia siliqua* leaves compared to ethanol extract of *Ceratonia siliqua* leaves alone after formalin injection (Table 1, 47 ± 3.5, 50 ± 5.3, respectively). Moreover, the same results were observed when the hot plate test was performed, which showed that the latency time was not affected when naloxone was administered with ethanol extract of *Ceratonia siliqua* leaves compared to ethanol extract of *Ceratonia siliqua* leaves alone (Table 1, 59 ± 3.8, 60 ± 6.9, respectively), suggesting a different anti-nociceptive mechanism of ethanol extract of *Ceratonia siliqua* leaves other than the opioidergic system.

3.4. *Ceratonia siliqua* leaves ethanol extract anti-inflammatory effect

Our results demonstrated that ethanol extract of *Ceratonia siliqua* leaves did not reduce the swelling volume induced by carrageenan injection after 1 h compared to the control (Table 2, n = 6, control 1.26 ± 0.04, 10 mg/kg 1.24 ± 0.05, 31.6 mg/kg 1.25 ± 0.07, 100 mg/kg 1.24 ± 0.02, 316 mg/kg 1.25 ± 0.05). However, ethanol extract of *Ceratonia siliqua* leaves demonstrated a dose-dependency pattern of significant reduction in swelling volume after 2 (10 mg/kg 2.20 ± 0.22, 31.6 mg/kg 2.12 ± 0.05, 100 mg/kg 1.87 ± 0.10, 316 mg/kg 1.56 ± 0.11), 3 (10 mg/kg 2.69 ± 0.06, 31.6 mg/kg 2.55 ± 0.11, 100 mg/kg 2.46 ± 0.05, 316 mg/kg 2.23 ± 0.03), and 5 (10 mg/kg 1.82 ± 0.10, 31.6 mg/kg 1.75 ± 0.08, 100 mg/kg 1.71 ± 0.03, 316 mg/kg 1.59 ± 0.12) hours compared to the control (2 h 2.31 ± 0.12, 3 h 2.91 ± 0.13, 5 h 1.91 ± 0.04) (Table 2, $p < 0.05$, n = 6). The area under the curve indicated a significant reduction in swelling volume at a dose of 316 mg/kg compared to 10 mg/kg dose and the control, respectively (Figure 3, n = 6, $p < 0.05$, <0.001, respectively) suggesting that ethanol extract of *Ceratonia siliqua* leaves might have an ant-inflammatory effect. Indomethacin, a reference drug, reduced the swelling volume after 1, 2, 3, and 5 h compared to the control (Table 2, $p < 0.05$, n = 6). In addition, the area under the curve was significantly lower in the indomethacin group compared to the control group (Figure 3, n = 6, $p < 0.001$). A similar observation was found when the cotton pellet granuloma test was performed. The cotton pellet and its surrounding granuloma mean weight was significantly reduced in the groups treated with ethanol extract of *Ceratonia siliqua* leaves (Table 3, $p < 0.05$, n = 6, control 59.1 ± 1.9, 10 mg/kg 54.3 ± 2.1, 31.6 mg/kg 43.6 ± 1.1, 100 mg/kg 37.5 ± 3.1, 316 mg/kg 27.2 ± 4.2), and this reduction was higher when the dose of the extract was increased. Indomethacin, a reference drug, also reduced the pellet weight significantly compared to the control (Table 3, $p < 0.05$, n = 6). This suggests that ethanol extract of *Ceratonia siliqua* leaves might have an anti-inflammatory effect.

4. Discussion

The anti-nociceptive and anti-inflammatory effects of ethanol extracts of *Ceratonia siliqua* leaves were evaluated in this study to justify its future therapeutic use. To assess the peripheral and central inhibition of nociceptive activity of *Ceratonia siliqua* ethanol extract, paw licking induced

Table 1. Naloxone effect on *Ceratonia siliqua* leaves extracts' anti-nociceptive activity.

Type of treatment	Percent of baseline (hotplate test)	formalin-induced late time (Late phase)
Vehicle	10 ± 0.79	147 ± 11.9
Ceratonia siliqua (ethanol) (316 mg/kg)	60 ± 6.9***	50 ± 5.3***
Ceratonia siliqua (ethanol) (316 mg/kg) + Naloxone (5 mg/kg)	59 ± 3.8***	47 ± 3.5***
Morphine 5 mg/kg	130 ± 10.2***	33 ± 7.1***
Morphine + *Naloxone*	13 ± 1.4$$$	133 ± 14.2$$$

Results are represented as mean ± SEM. The % of inhibition was calculated comparing with vehicle group. *: compared to control, $ compared to morphine.

Table 2. *Ceratonia siliqua* leaves ethanol extract activity on paw oedema induced by carrageenan in mice.

Swelling volume (ml) (mean ± SEM)					
Treatment	Dose (mg/kg)	1 h	2 h	3 h	5 h
Control	———	1.26 ± 0.04	2.31 ± 0.12	2.91 ± 0.13	1.91 ± 0.04
Ceratonia siliqua	10	1.24 ± 0.05	2.20* ± 0.22	2.69* ± 0.06	1.82* ± 0.10
Ceratonia siliqua	31.6	1.25 ± 0.07	2.12* ± 0.05	2.55* ± 0.11	1.75* ± 0.08
Ceratonia siliqua	100	1.24 ± 0.02	1.87* ± 0.10	2.46* ± 0.05	1.71* ± 0.03
Ceratonia siliqua	316	1.25 ± 0.05	1.56* ± 0.11	2.23* ± 0.03	1.59* ± 0.12
Indomethacin	10	0.71* ± 0.08	1.45* ± 0.17	1.35* ± 0.10	0.92* ± 0.11

Dataset are represented as mean ± SEM (n = 6). Two-way ANOVA followed by Tukey post hoc. * Denote a significant difference compared to control ($P < 0.05$).

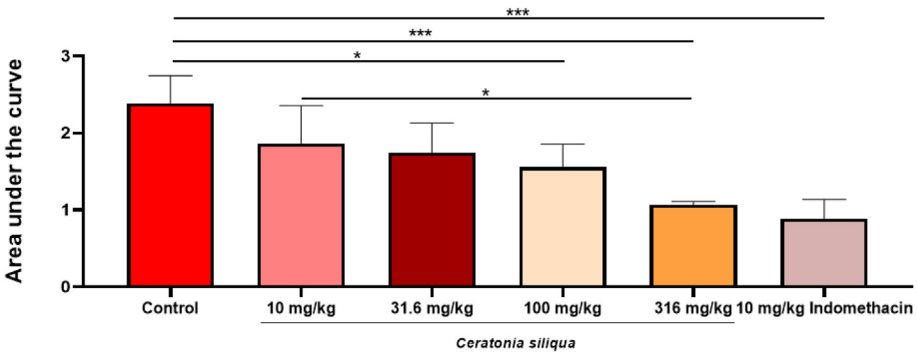

Figure 3. *Ceratonia siliqua* leaves ethanol extract activity on paw oedema induced by carrageenan in mice. Paw oedema was reduced in mice received 316 mg/kg *Ceratonia siliqua* leaves ethanol extract as indicated by area under the curve compared to control. One-way ANOVA test was performed followed by Tukey posthoc (*<0.05, ***<0.001, n = 6).

Table 3. *Ceratonia siliqua* leaves ethanol extract effect against cotton pellet granuloma weight in mice.

Treatment	Extract dose (mg/kg)	Pellet weight (mg)	Percent of inhibition
Control	———	59.1 ± 1.9	
Ceratonia siliqua	10	54.3* ± 2.1	8.1
Ceratonia siliqua	31.6	43.6* ± 1.1	26.2
Ceratonia siliqua	100	37.5* ± 3.1	36.5
Ceratonia siliqua	316	27.2* ± 4.2	54
Indomethacin	10	23.1* ± 1.6	61

Dataset are represented as mean ± SEM (n = 6). * Denote a significant difference compared to control ($P < 0.05$).

by the formalin test was used. Early activation of nociceptors by formalin injection can induce the non-inflammatory/neurogenic (early) phase, which lasts for about 5 min. However, the inflammatory (late) phase occurs 15–30 min after formalin injection in response to the release of several mediators for inflammation, such as bradykinin, prostaglandins, as well as histamines [54, 55, 56]. It is well known that centrally acting analgesic agents, such as opioids, inhibit both the non-inflammatory (early) and inflammatory (late) nociceptive phase, whereas peripherally-acting analgesic agents such as NSAIDs inhibit only the inflammatory (late) nociceptive phase [44, 57]. Interestingly, our results demonstrated that *Ceratonia siliqua* ethanol extract has anti-nociceptive properties in the non-inflammatory and inflammatory phases, which suggest that the ethanol extract of *Ceratonia siliqua* leaves could have a centrally and peripherally acting analgesic activity.

To further assess the anti-nociceptive effect of ethanol extract of *Ceratonia siliqua* leaves, a hot plate test was performed. In this test, paw licking and jumping behaviours in the animals are responses produced at the supraspinal level without the involvement of the peripheral level due to the high and constant temperature on the hot plate, which is measured as the time between the instance when the animal placed on the plate and the beginning of these behaviours [58, 59, 60]. Since centrally acting opioid-like analgesic agents are the only agents that can increase the animal latency time in response to high temperature, therefore, any substance or drug that may increase this latency time must have centrally-mediated activity like opioids [61, 62, 63]. Our results show that the latency time significantly increases with administration of ethanol extract of *Ceratonia siliqua* leaves, suggesting that this extract has a centrally mediated anti-nociceptive activity. Taking into consideration the anti-nociceptive activity of ethanol extract after formalin injection, *Ceratonia siliqua* leaves ethanol extract might have peripheral and central anti-nociceptive activity.

Moreover, tested animals also received naloxone, which is an antagonist for opioid receptors, to study the anti-nociceptive mechanism of *Ceratonia siliqua* leaves ethanol extract. As expected, naloxone inhibited the morphine anti-nociceptive effect, however, this effect was not noticed in the groups that received ethanol extract of *Ceratonia siliqua* leaves, which suggests that ethanol extract of *Ceratonia siliqua* leaves' anti-nociceptive effect is mediated by mechanisms other than the opioidergic system, such as adrenergic α1 and α2, cholinergic, serotoninergic, nitrergic, adenosine, dopaminergic, glutamatergic, and vanilloid nociceptive modulation routes, which should be investigated in the future.

Carrageenan-induced oedema is an important technique in assessing the ability of chemicals or substances to reduce inflammatory responses *in vivo*, which was firstly described by Winter [64]. Immediately after the injection of carrageenan subcutaneously, signs of acute, nonimmune inflammation appear, such as oedema, hyperplasia, and erythema, due to the secretion of pro-inflammatory mediators such as nitric oxide, histamine, bradykinin, and tachykinins, which are produced either at insult site or by infiltrating inflammatory cells. Moreover, the inflammatory process can be exacerbated by neutrophiles and the production of reactive oxygen species at the inflammation site. The oedema size of the paw, which lasts for around 5 h, is considered the quantification process for the inflammatory response [65, 66, 67]. Our results show that *Ceratonia siliqua* leaves significantly reduced the oedema size of the animal paw. Interestingly, this reduction was similar to the reduction observed in the group that received indomethacin, the reference drug. To elucidate the ability of *Ceratonia siliqua* leaves ethanol extract to reduce inflammatory responses, cotton pellet granuloma assay was used, which was first described by Meier [68] to study the anti-proliferative effect of chemicals or substances during inflammatory responses [69]. In this test, an inflammatory response is evoked, which involves macrophages, neutrophiles, and fibroblast proliferation, as well as the formation of small blood vessels to form granuloma [70]. This assay demonstrated that *Ceratonia siliqua* leaves ethanol extracts reduced the cotton pellet surrounded by granuloma weight compared to the control. This was also comparable to indomethacin, which indicated anti-proliferative activity in *Ceratonia siliqua* leaves ethanol extract during the inflammatory phase. Taken together, ethanol extract of *Ceratonia siliqua* leaves could have an anti-inflammatory role, which needs to be evaluated further in future studies.

It should be noted that the observed anti-inflammatory or anti-nociceptive activities of *Ceratonia siliqua* leaf extract might be as a result of its chemical compositions. Rutin has demonstrated an anti-inflammatory effect through the suppression of high mobility group box (HMGB1) protein, a late mediator of severe vascular inflammatory condition, TNF-α, IL-6, and NF- κB in vivo and in vitro [71]. In another study, a docking simulation demonstrated that rutin interacted strongly with cyclooxygenase, suggesting that it might have anti-nociception activity [72]. In addition, quercetin was able to reduce eosinophil and neutrophil counts and IL-5 levels in animal models of asthma, and attenuate nociception scores in the chronic phase of formalin tests in

diabetic rats [73, 74]. Moreover, myricetin has shown an ability to reduce inflammation in in vivo models of acute and chronic inflammation, such as xylene-induced ear oedema, acetic acid-induced vascular permeability, carrageenan-induced paw oedema, and cotton pellet granuloma models. Myricetin also decreased the levels of malonaldehyde (MDA) and increased the levels of super oxide dismutase, suggesting that myricetin has anti-oxidant properties [75]. Moreover, myricetin demonstrated an anti-nociception activity through the peripheral inhibition of nitric oxide synthesis [76]. Catechin demonstrated anti-inflammatory activity by downregulating the expression of TNF- α, IL-1β, and IL-4, as well as myeloperoxidase activity in an in vivo model of allergic contact dermatitis [77]. Luteolin also exerts anti-oxidant and anti-inflammatory activities through the inhibition of STAT3 pathway and the suppression of several cytokines and chemokines [78, 79]. Luteolin exhibited anti-nociceptive activity via the use of a hot plate test and formalin test [80]. Furthermore, caffeic acid, furolic acid and coumaric acid revealed anti-inflammatory activity through decreasing NF-κB, IL-6, TNF-α and IL-1β expression in vivo and in vitro [81, 82, 83]. Caffeic acid and furolic acid also showed an anti-nociception activity using tail-flick assay and chronic construction injury-induced neuropathic pain model [84, 85]. Taken together, the observed anti-inflammatory and anti-nociception activities of *Ceratonia siliqua* leaf extract in our study could be the result of the activity of the chemical components. However, it is also possible that the activity of one compound is modulated by other compounds, which should be investigated further in future research.

Despite the significant findings in the present study, there are some limitations which are worth mentioning. Variation in the neurobiology of nociceptive systems between species limits the ability to extrapolate our findings from animal research to humans. Furthermore, the labour-intensive nature of the formalin test could be a limitation regarding the time required to train the observers to carry out the test in a reliable way [86]. Moreover, only the opioid system was evaluated in this study.

Natural products are successful sources of potential leads in the drug discovery process and play a major role in human therapy, representing a huge reservoir of bioactive chemical diversity. In addition, ethnopharmacological studies are of great importance in the development of herbal medicine, through the scientific reporting of the use of medicinal plants in particular therapeutic purposes [87]. Our results in this study demonstrated, for the first time, that *Ceratonia siliqua* leaves extract might have anti-inflammatory and anti-nociception properties, which will enrich the ethnopharmacological research and present new findings that will impact the future pain management and inflammation research.

In summary, our findings indicate that *Ceratonia siliqua* leaves ethanol extract might have an anti-nociceptive effect within the peripheral and central nervous system, in addition to the ability to reduce inflammatory responses using two in vivo inflammatory models, suggesting that *Ceratonia siliqua* leaves extract could be used in pain and inflammation management.

Declarations

Author contribution statement

Abdelrahim Alqudah; Esam Y. Qnais; Mohammed A. Wedyan: Conceived and designed the experiments; Wrote the paper.

Muna Oqal; Rawan AbuDalo: Performed the experiments.

Mohammed Alqudah: Analyzed and interpreted the data.

Nabil AL-Hashimi: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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