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Evaluation of the Antinociceptive and Antipyretic Effects of the Iraqi Olive Leaf Extract in Murine Animals

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Abstract

Intolerable acute or chronic pain is the main reason patients visit clinics to seek medical consultation. This is also applied to the pyrexia that can be fatal if not treated. Therefore, medicines such as opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) have been used for centuries as pain relievers and antipyretic anti-inflammatory medicines. Unfortunately, opioids and NSAIDs treatment accompanied with serious side effects. Thus, emerges the need to develop safe and effective medicinal or herbal therapies. The objective of the present study is to evaluate the antinociceptive and antipyretic effects of the Iraqi ethanolic olive leaf extract in view of the fact that this plant has been used to lessen pain in addition to its anti-inflammatory action in folk medicine. To accomplish this, analgesic activity of olive leaf extract was studied in mice using formalin and writhing tests. Moreover, the antipyretic activity of the extract was explored in rats by using Brewer's yeast-induced pyrexia model. The results indicate that the olive ethanolic extract is significantly effective in diminishing the number of writhing in mice in response to both formalin and acetic acid to a level that close to the indomethacin. In addition, the extract is effective as an antipyretic and anti-inflammatory therapy compared to the paracetamol drug. In conclusion, this study denotes the analgesic antinociceptive, and pyretic effects of the Iraqi ethanolic olive leaf extract, yet further studies are required to elucidate the plant main components and the mechanism(s) that underlying those effects.

Key words: Antinociceptive, antipyretic, olive, writhes, Analgesic

INTRODUCTION

Pain is a challenging term both to be defined and to be mitigated or relieved. According to the International Association for the Study of Pain, it is "Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage" (1). Opioids such as morphine have been used for centuries to alleviate pain by acting on the opioid receptors in the central nervous system (2). Unfortunately opioids surrounded by serious concerns about deleterious effects and addictive features that accompany the treatment (3). Other medicines that have been used to diminish pain and fever are the NSAIDs including a drug known as indomethacin which functions by inhibiting the synthesis of prostaglandins (4). Indeed, NSAIDs have earnest side effects including peptic ulcer, dyspepsia, and general irritation to the gastrointestinal tract (5). Moreover, according to the American Society of Health-System Pharmacists, paracetamol is the most common medicine that has been used to reduce pain and fever in all ages worldwide. Paracetamol is implicated in liver failure, kidney failure, and fatal skin reactions in some cases especially in response to overdoses (6). Thus, further approaches are needed to develop safer, less toxic, and equally effective therapy to relief pain and fever. Alternative medicines such as plant remedies are popular and have found to be less toxic than the manufactured drugs. Herein we seek to explore the effect of the Iraqi olive leaf extract to relief pain and reduce fever in experimental animals.

Olive plant, Olea europaea L. (O. europaea) belongs to the Oleaceae or the dicotyledons family (7). Olive plant has been used in folk medicine to cure many ailments and sober diseases such as diabetes, hypertension, gallstones (8), and inflammation (9). Boiled concentrates of dried olive leaves have been utilized to treat hypertension, respiratory, urinary, and gastrointestinal illnesses (10). Olive oil extracts have been extensively investigated during the recent decades for both nutritious and medicinal interests (11), but the other part of the plant grabbed less attention by researchers. Olive leaf infusion has been historically used as anti-inflammatory and antipyretic remedy (12). In addition, olive leaves have been used by Japanese people to treat gastrointestinal disturbances and liver pain (13). Of importance, neuroprotective effect of olive plant ingredients was also identified in a dose dependent manner in response to drug-induced toxicity, glutamate toxicity, and oxygen-glucose depletion (14). Olive leaf ingredients were also found to be effective in preventing fibrillization in Alzheimer's disease (15) and decreasing neuronal cell death in Parkinson disease (15).

To detect the possible antinociceptive properties of Iraqi olive leaf extract, formalin and acetic acid-induced writhing tests were undertaken. To identify the antipyretic activity of the plant extract, Brewer's yeast-induced pyrexia test was conducted in this study. We compare between the action of the Iraqi olive leave extract and indomethacin as antinociceptive agents. Also we compare the Iraqi olive leave extract and paracetamol as antipyretic and anti-inflammatory therapies. In details, formalin test is very efficient and accurate antinociception test and is highly responsive for many analgesics (16). Injection of formalin subcutaneously induces the central nervous system to express biphasic antinociceptive response (17). Thus the formalin test is used popularly in researches to elucidate and explore drug induced-antinociceptive mechanism of action (18). It was shown that the first antinociceptive phase of formalin induction represents the earlier effects of formalin on nociceptors. While the second phase represents an inflammatory pain response that is ascribed to production of prostaglandins (19). In the present study, we chose to conduct the formalin test over other tests due to the fact that formalin test can evaluate the response to a long-lasting nociceptive inducers that resembles the clinical pain (20). In addition, writhing test was also conducted here to measure the antinociceptive activity of the olive leaf extract in mice. Intraperitoneal injection of acetic acid causes what is known in the literature as acetic acid-induced writhing. Acetic acid-induced writhing is attributed to the rise levels of prostaglandins; PGE2 and PGF2α (21). Stimulation of other chemicals is also implicated in this pain response such as sympathomimetic amines, TNFα, Il1β, and Il8 (22). It was also found that the acetic acid-induced writhing might be related to stimulation of receptors in the peritoneum (23). Intraperitoneal injection of acetic acid triggers continuous inescapable pain that is reflected by contractions of the abdomen, twisting of the dorsoabdominal muscles, and decreasing motor activities what all confirm the visceral pain (24).

In the current study, the antipyretic effects of olive leaf extract were examined by conducting Brewer's yeast-induced pyrexia model using rats. This model is less sensitive to the NSAIDs (25), and so we chose to use paracetamol as an antipyretic and anti-inflammatory agent to compare to the olive leaf extract. In this model, edema is resulted from vascular dilation that increased the permeability of the local blood vessels (26). The overarching hypothesis of our study is that the Iraqi olive leaf extract have analgesic and antipyretic effects in response to pain and pyrexia inducing agents. The reason to mention the source of the olive is ascribed to what was raised by

researchers about the importance of the olive cultivated area in its medicinal utilities. Those researchers grabbed the attention to the fact that different environments can substantially affect the plant components (27). This was another trigger to specifically studying the Iraqi olive throughout this study.

MATERIALS AND METHODS

Plant material and preparation of extracts

Fresh leaves of *O. europaea* L. were collected from mature plants grew in local gardens in Al-Diwanyia city. The leaves were washed and dried under shade for one week. Then they were powdered, and 200g of the powder was applied for extraction using ethanol alcohol (95% v/v) in a Soxhlet apparatus with continuous heat extraction. The extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50ºC. The alcohol extract was mixed with distilled water containing 2% v/v Tween 80 (as a suspending agent) for experimental purpose.

Animals

Swiss albino mice weighing 18-25 gm of both sexes & Wister albino rats weighing 150-200 gm of both sexes were used in the present study. The animals were procured and housed in the animal house that belongs to the college of Veterinary Medicine/ University of Al-Qadisiyah. They were maintained under standard hygienic conditions of 20 ± 2 ^{o}C, humidity (60 ± 10%) with 12 hour day: night cycle, and ad libitum access to food and water unless otherwise stated.

Analgesic activity

Analgesic activity of ethanolic extract of *O. europaea* L. was studied in mice by two different methods; formalin test and writhing test.

Formalin test

To determine the anti-nociceptive activity of the ethanolic extract of the leaves of *O. europaea* L. formalin test was used. Swiss albino mice (5 per group) were fasted for 24 h before the experiment, but with free access to water. Those mice were administered orally with 200mg/Kg BW of the plant extract dissolved in distilled water. Thirty minutes later, the mice were administered intraperitoneally with indomethacin (10 mg/kg, i.p). Thirty minutes later, twenty µL of 5% formalin was injected subcutaneously into the right hind paw of the mice to cause pain. Then, the mice were individually placed in a transparent Plexiglas cage (25 × 15 × 15 cm). The time spent licking and biting the injected paw was used as index of pain and was recorded for each mouse separately from 0 to 5 min as early phase or neurogenic pain and from 20 to 30 min as late phase or inflammatory pain (28).

Acetic Acid-induced Writhing Test

To determine the anti-nociceptive activity of the *O. europaea* L. leaves ethanolic extract, acetic acid induced writhing test was used. Swiss albino mice (5 per group) were fasted for 24 h before the beginning of the experiment, but with free access to water. The mice were administered orally with 200mg/Kg BW of the plant extract dissolved in distilled water. Thirty minutes later, the mice were injected with indomethacin (10 mg/kg, i.p). Thirty minutes later, he mice were injected with 1% acetic acid (0.1 mL/10 g BW i.p). The, the mice were placed in observation boxes separately and the number of writhing responses was counted through 20 minutes (29).

Antipyretic activity

Anti-pyretic activity of ethanolic extract of *O. europaea* L. leaves was studied by using Brewer's yeast-induced pyrexia model (30). Rats were randomly divided into four groups of 5 animals per group. The normal body temperatures of the rats were taken by inserting a digital thermometer into their anal cavities for about 2 min. The steady temperature readings obtained were recorded as the pre-temperatures. Pyrexia was induced in the rats by administering 10 mg/kg BW of 15% aqueous suspension of Brewer's yeast in normal saline subcutaneously into the animal's dorsum region and 18 h later of yeast administration, the rectal temperatures were measured again.

After 18 hours of yeast injection, the normal saline, standard drug, and test plant extract were given to different groups of rats. Twenty rats selected were grouped into four groups and treated as follows: Normal saline 10 ml/kg BW were administered to group I as negative control, while group II left without treatment as positive control, group III were treated with Paracetamol 100mg/kg/BW. Group IV was treated with ethanolic extract of leaves of *O. europaea* L. (200 mg /kg BW). All the treatments were administered orally. The rectal temperature was then recorded after every sixty minutes interval of drug administration for each rat up to 4 hours.

Statistical analysis

All the values ware statistically analyzed by one-way analysis of variance (ANOVA) and two-way analysis of variance followed by least significant differences (LSD). Comparison between control and plant & drug treated groups were considered to be significant. All values are expressed as mean ± SEM.

RESULTS

Analgesic activity

Formalin test

Our results showed that formalin injection provoked a typical biphasic nociceptive response as shown in Table 1. Treatment with ethanolic olive leaf extract at dose 200 mg/kg body weight led to a marked reduction ($P<0.05$) in the nociceptive response represented by the number of writhes during 20 minutes compared to the positive group. Treatment with indomethacin at a dose of 10 mg/kg BW produced even more reduction in the nociceptive response during 20 minutes compared to the positive group. The maximal inhibition of the nociceptive activity was achieved with indomethacin treatment. Although the treatment with the ethanolic olive leaf extract showed less effect than the indomethacin, it is still very effective as an analgesic supplement.

Acetic acid-induced writhing test

Our results revealed that acetic acid injection evoked a nociceptive response as shown in Table 2. Treatment with ethanolic olive leaf extract initiated marked decrease ($P<0.05$) in the nociceptive response represented by the number of writhes during 20 minutes compared to the positive group. Treatment with indomethacin significantly ($P<0.05$) lowered the nociceptive response even more than the olive treatment. Similar to the formalin test, the maximal inhibition of the nociceptive activity was achieved with indomethacin treatment. Despite the fact that the indomethacin treatment was more effective than the ethanolic olive leaf extract, it is still very effective as an analgesic supplement indeed.

Antipyretic activity

The antipyretic response results were depicted in Table 3. First, the results showed that all the animals had similar temperature values before the yeast injection. Second, the Brewer's yeast increased the body temperature significantly ($P<0.05$) in all the injected animals compared to the negative control. Then, there was a significant drop in the recorded temperature in the olive leaf extract or paracetamol administered groups during 4 hours after the injection. The results indicated that there was no difference between the olive leaf extract and the paracetamol activity in curing the pyrexia.

Table 1: the analgesic effect of ethanolic extract of olive compared with indomethacin on mice with formalin test.

Group	Dose(mg/kg)	Number of mice	Number of writhing in 20 minute	Inhibition %
Negative control	2ml/kg	5	0[a]	---------
Positive control	------	5	32.58±1.2[b]	--------
Indomethacin	10	5	13.26±0.68[c]	59.3%
Olive Ethanolic extract	200	5	19.75±0.96[d]	39.37%

Different letters denote to the significant difference at p<0.05

Table 2: the analgesic effect of ethanolic extract of olive compared with indomethacin on acetic acid induced writhing response in mice.

Group	Dose(mg/kg)	Number of mice	Number of writhing in 20 minute	Inhibition %
Negative control	2ml/kg	5	0[a]	-------
Positive control	------	5	82.12±1.2[b]	------
Indomethacin	10	5	38.18±0.92[c]	53.5%
Olive Ethanolic extract	200	5	51.67±1.32[d]	37.07%

Different letters denote to the significant difference at p<0.05

Table 3: the antipyretic effect of ethanolic extract of olive compared with paracetamol on Wister albino rats.

Group	Dose(mg/kg)	Number of mice	Temp. (C°)	Temp. after induction of pyrexia(C°)	Temp. after drugs administration (C°) at different hours			
					a	2hrs	3hrs	4hrs
Negative control	2ml/kg	5	37.2±0.12^{Aa}	37.3±0.06^{Aa}	37.1±0.1^{Aa}	37.5±0.06^{ACa}	37.6±0.07^{Aa}	37.1±0.04^{Aa}
Positive control	------	5	37.5±0.08^{Aa}	39±0.04^{Bb}	39.7±0.11^{Bb}	39.2±0.08^{Bb}	38.7±0.02^{Bb}	39.12±0.08^{Bb}
Paracetamol	100	5	37.1±0.16^{Aa}	38.8±0.08^{Bb}	37.7±0.09^{Cc}	37.2±0.04^{Aa}	37.2±0.03^{Aa}	37.12±0.06^{Aa}
Olive Ethanolic extract	200	5	37.5±0.1^{Aa}	39.2±0.12^{Bb}	37.6±0.05^{Ca}	37.8±0.04^{Ca}	37.6±0.08^{Aa}	37.6±0.02^{Ca}

Different letters denote to the significant difference at p<0.05
Capital letters denote to the vertical statistical reading
Small letters denote to the horizontal statistical reading

DISCUSSION

Olive or *O. europaea* L. has been very well known as source of food and in alternative medicine field for centuries all over the universe. The plant has many therapeutic uses such as in diabetes, cholesterolemia, hypertension, inflammation, urinary diseases, gastrointestinal disturbances, respiratory ailments, and many more (8,9,13). The various components of olive also participated in its widespread utilities (11,13). Olive is one of the plants that have been heavily investigated recently to confirm its historical uses as detailed in reviews (31). In this study we validated the antinociceptive and antipyretic effects that belong to the *O. europaea* L. leaf extract. The study showed that the ethanolic olive leaf extract play fundamental role in mitigating the undesirable effects of pain and fever reactions initiators. The extract supplementation lowered the numbers of writhes that reflected by licking, biting, and shaking hind paw resulted from formalin injection. In early phase or neurogenic pain, formalin triggers the nociceptors resulting in C fibers stimulation (32). This resulted in releasing of bradekinin and prostanoids that are known as pronociceptive substances that lead to the characteristic writhing response or what is known as nociception behavior (33). The olive leaf extract ameliorated the nociception response by acting directly on the central and peripheral nervous system to block the nociceptors, and this effect was also found when treating mice with extraversion olive oil (27). Similarly, olive leaf extract worked to mitigate the inflammatory pain or late phase of formalin injection. This effect might be related to inhibition of prostaglandin production by blocking the release of cyclooxygenases enzymes that are required to synthesize prostaglandins (34). This analgesic and anti-inflammatory effects of olive is attributed to some of the olive ingredients such as phenols (importantly oleocanthal), triterpenes, α-tocopherol, flavonoids, and oleuropein aglycone (35). This effect was also proven for Russian olive water extract compared to indomethacin, the effect that was connected mostly to the flavonoid content (36). In the same regard, the acetic acid-induced writhing test revealed the analgesic effect of the Iraqi olive leaves. Intraperitoneal injection of acetic acid leads to increase prostaglandins, sympathomimetic amines, TNFα, Il1β, and Il8, and other substances that resulted in a typical abdominal writhing (37). The effect of the olive extract is attributed to the blocking of prostaglandins production or functions (38) since the latter play a fundamental role in the acetic acid-induced nociceptive mechanism (39). It was also found that bioflavonoids can prevent the bradykinin, substance P, and arachidonic acid synthesis at the neuronal ends to control continuous pain (40). Moreover, the alkaloids such as harman and harmaline content of the plant act centrally to diminish the pain by inhibiting the release of mono-amino oxidase enzyme and increasing serotonin and noradrenaline secretion (41). Strikingly, olive leaf extract was found to be as powerful as opioids to function as analgesic but through different receptors (42).

Olive leaf is traditionally known to function as antipyretic and anti-inflammatory agent. To investigate this practically, studies were done on Russian and Moroccan olives for examples (42), but the Iraqi cultivated plant has never been investigated regarding these issues. To accomplish this goal, we injected laboratory rats with Brewer's yeast to induce pyrexia and then treated these animals with the Iraqi olive leaf extract. The results of the current study showed that this extract worked to lower the body

temperature significantly and cure fever in the treated rats. This effect might be related to the fact that olive plant was found to contain linoleic acid that was reported to repress the gene expression of inflammatory cytokines that suppresses inflammation (43). Olive was also found to inhibit cyclooxygenases I and II synthesis that inhibit the inflammatory reactions (42). In a similar study, Osman et al., 2017 found that treating animals with Extra version olive oil reduced the pyrexia that was provoked by the Brewer's yeast injection in mice (44). The authors stated that the olive oil might function through an analogous mechanism to that of the NSAIDs to diminish fever and inflammation. In more details, the researchers attributed this action to the effect of olive ingredients in inhibiting synthesis and release of prostaglandins centrally in the hypothalamus (44). The antipyretic effect of olive oil was also ascribed to the presence of caffeic acid and oleuropein that is also available in the olive leaves, which function to block the Il1β. In addition to the kaempferol compound that reduces the prostaglandins endogenous pyrogenic agent (45). So far, most studies connect the antipyretic and anti-inflammatory effects of olive plant to the ability of its components to work centrally and peripherally to curb prostaglandins as stated previously.

Recently, many studies were tackled to surrogate the undesirable side effects of synthetic medicines. In this regard, fresh plant, plant decoction, and plant infusion for example emerged as valuable players. This is related to the fact that plant components have fewer side effects, less toxic, and less restrictions of safety. Therefore, this study aimed at exploring the antinociceptive and antipyretic effects of olive leaf extract as an alternative medicine. This study is the first to indicate the pain and fever relief effects of the Iraqi olive leaf extract. Further studies are required to discover the main components of olive plant that are responsible for these effects to figure the exact mechanisms that are involved in the analgesic and anti-inflammatory effects of Iraqi Olea europaea.

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