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Evaluation the antinociceptive action of local olive extract in mice

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بسم الله الرحمن الرحيم

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سورة طه: من الآية ١١٤

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Dedication

TO:

My family and all my Friends

Acknowledgments

Firstly I would like to thank the Kind Merciful Allah for helping me in completing this work.

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Hewria

Abstract:

Olive and its products are an important part of the Mediterranean diet and olive leaf is the by-product of the olive oil industry. The leaves are rich in polyphenols, namely oleuropein, tyrosol, hydroxytyrosol, rutin, verbacoside, apigenin- 7-glucoside and luteolin-7-glucoside.The aim of the present study was to evaluate the pharmacological analgesic activity of the ethanolic extract of leaf of *Olea europaea* L. (olive plant) by using two different method for testing analgesia (formalin test & acetic acid induced writhing test) in experiment mice. The study was carried out using the dose 200 mg/kg BW given orally and compared their effect with reference drug (indomethacin 10 mg/kg).the pharmacological screening revealed that the extract had good analgesic activity as compared with positive control group in the relief of the pain in the two method used in the study.

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Introduction:

The medicinal uses of different parts of *Olea europaea* L. have been known for a long time in all the countries of the Mediterranean basin. Several species within the olive family, botanically known as *Olea europaea*, provide commercial products such as food, lumber, cosmetics and medicine. Olive oil is a component of the Mediterranean diet, containing variable amounts of triacylglycerols and small quantities of free fatty acids, glycerol, pigments, aroma compounds, sterols, tocopherols, phenols, unidentified resinous components and others (Kiritsakis , 1998). The pharmacological properties of olive oil, the olive fruit and its leaves have been recognized as important components of medicine and a healthy diet because of their phenolic content (Visioli *et al.*,2002). *O. europaea* has been widely used in folk medicine to treat inflammatory diseases such as rheumatoid arthritis and hemorrhoids, and as a vasodilator in vascular disorders (Vyas et al.,2007). So the present

study was aimed to evaluate the antinoceciptive effect of ethanolic extract of olive in experimental mice.

2-Literature review:

2-1-Historical introduction:

References to the olive tree date back to Biblical and Roman times and to Greek mythology .Historically, olive leaves are known as a symbol of peace, so it is not surprising that olive leaf tea provides a soothing, relaxing, gently mellow herbal brew that evokes a sense of wellbeing. Some lemon or honey enhances the flavour; it is excellent chilled and drunk over ice. Olive leaf tea leaves can be added to a marinade, especially for fish, or used in a spice rub to season a variety of dishes.The healing powers of Olive leaf were used in the 1880s to counteract malaria. In the early 1900s, scientists isolated Oleuropein from the Olive leaf. Leaves and drupes of Olive tree, It rich in Olive biophenols, such as oleuropein, verbascoside, ligstroside, tyrosol and hydroxytyrosol, which have exhibited antioxidant and antimicrobial,(Najafian *et al.*,(2009);Siddiquee *et al.*,2011) the leaves of Olive tree are the richest source of these compounds(Mustapa *et al.*,2009) Back in the early 1800s

it was used in liquid form as a very effective treatment for malarial infections. In the early 1900s, a useful compound was found in the Olive leaves named (Oleuropein). In 1962, an Italian researcher recorded that Oleuropein had the ability to lower blood pressure in animals. Other European researchers validated that claim and also found that use to increase blood flow in the coronary arteries, relieve arrhythmias and prevent
intestinal muscle spasms. The search began for the chemical agent within Oleuropein that would be the most important medically, Dutch researcher found that the chemical compounds were elenolic acids(Suganya and Renganathan,2012).

2-2-Olive plant:

Olea europaea L. belongs to the Tribe Oleae and family Oleaceae, comprising around 600 specious and some 25 genera, including *Olea* – which contains an economically important European olive tree known as *Olea europaea* L. The latter is one of the oldest known cultivated trees in the world with archaeological evidences back in 6000 BC in

the region corresponding to ancient Persia and Mesopotamia. The Phoenicians were the first who introduced it to the western regions, first Greek islands and later to the Mediterranean Basin (Spain, Italy *etc.*) through the colonies of Greeks and Romans. In the 15th century AD, *O. europaea* L. also reached a newly discovered America and today it is farmed all around the world except in the Antarctic (Kapellakis *et al.*, 2008; Wallander *et al.*, 2000).

Olive tree is a polymorphous, medium-sized (up to 10 m) with a furrowed trunk. It has greyish-green leaves (5-6 cm long, 1-1.5 cm wide) with smooth edges and a short peduncle. The tree is well adapted to extreme environmental conditions, but requires high-intensity light and aerated soil. It is also known for alternating its fruit production . providing high yields in one, and low in the next year, causing major problems in olive industry. The fertilized fruit development begins with the appearance of floral buds, followed by pollination, fertilization, fruit bearing and ripening. The length and nature of each

phase depends on environmental conditions, but normally it begins in April and ends-up in November, when fruits attain their maximal weights and their colour change from green to brownish red and black (Ramírez-Tortosa *et al.*, 2006).

2-3-Cultivars :

The typical Mediterranean *O. europaea* L. includes the two main varieties, *sylvestris* (wild olive) and *macrocarpa* (domesticated olive), of which only the latter is being cultivated for the fruit production. There are approximately 2500 inventoried varieties, of which 250 are classified as commercial cultivars included in the World Catalogue of Olive Varieties published by International Olive Oil Council (IOOC). These olive cultivars are used for either olive oil or table fruits production, depending on the content of oil and the size of the fruit. The fruits used for olive oil production are medium in size, averaging less than 3.5 g in weight with a low pulp/stone ratio and a high oil yield (16-18%). By contrast, the table olives are medium to large, weighting 5-6 g (up to 17 g) with a high pulp/stone

ratio and a little oil content. In general, different cultivars are used for the oil and table fruits production, though a double-use cultivars are also known **(Mataix et al., 2006; Ryan et al., 1998).**

The differences between the thousands of varieties can be very subtle and the cultivars identification complicated due to all environmental factors influencing phenotype of the plant. The methods for a primary olive characterization follow a list adopted by the Conseil Oléicole Internationale (COI), which refers to the analysis of 32 different morphological characteristics including the tree, leaves, flowering, fruits and endocarp **(Pinheiro *et al.*, 2005).** Further identification at molecular level is based on genetic fingerprinting using different DNA markers like RAPDs, RFLPs, SSRs and others, allowing genetic differentiation among the cultivars **(Alba et al., 2009; Wünsch et al., 2002; Besnard et al., 2001)**

2-4-Some pharmacological activities of olive:

2-4-1-Anti-inflammatory activity

Anti-inflammatory agents are a substance that have ability to reduce inflammation. Anti-inflammatory drugs make up about half of analgesic, remedying pain by reducing inflammation, which affect the central nervous system and also cause other complications. Olive is a safe, inexpensive substitute in the reduction of inflammation and maintain or regulate the genes involve in this process. The mechanism by which Olive seed/leaf exerts have anti-inflammatory action appears to be through by inhibiting cyclooxygenase and lipoxygenase enzymes . Earlier studies have proven that, olive and their constituents have anti-inflammatory property. An important reports in the support of olive as anti-inflammatory reportedthat, Oleocanthal, phenolic compound of virgin olive oil shows similar anti-inflammatory properties to ibu-profen **(Lucas *et al.*,2011)** COX-1 and COX-2 convert arachidonic acid to prostaglandin, resu-lting in pain and in-flammation. None steroidal anti-inflammatory drugs, commonly prescribed to treat arthritis, work by inhibiting prostaglandins. NSAIDs, however, also cause gastrointestinal problemsulcers. Phenolic compounds

oleocanthal, an inhibitor of the COX-1 and COX-2 enzymes, possesses similar potency as the NSAID ibuprofen. Another enzyme play a vital role in inflammation is lipoxygenase. The constituents oleocanthal is not very effective in the control of lipoxygenase but others constituents shows a role in the inhibition of lipoxygenase. A report has shown that, oleuropein elicits anti-inflammatory effects by inhibiting lipoxygenase activity and the production of leukotriene B_4 (De La Puerta *et al.*,1999) Another supportive evidence of Olive leaf extract functions as anti-inflammatory properties and inhibition of platelet aggregation and thromboxane A2 production Nuclear factor kappa is a vital component in inflammation and also play role in pathogenesis of various diseases. Numerous medicinal plants play a role as anti-inflammatory and control the various diseases development and progression via inhibition of NF-κB and COX2. An interesting study demonstrated that, curcumin showed as anticancer, antioxidant and anti-inflammatory effects via the down-regulation of the transcription factors NF-κB, AP-1 and

Egr-1 (Han *et al*.,2002) Black seed also shows an anti-inflammatory effect. Earlier report showed that mechanism of action via inhibition of TNF-α gene expression and NO synthases and provides the down modulation of various cytokines and chemokines **(Richard *et al*.,2011).**

2-4-2-Anti-diabetic activity

Diabetes mellitus is metabolic disorder and also a major problem in both sexes worldwide. Diabetes mellitus are responsible for renal failure, blindness or diabetic cataract , poor metabolic control, and increased risk of cardiovascular disease including atherosclerosis and advanced glycation end products (Yokozawa *et al.*,2004) Natural products as medicinal plants and their constituents are good substitute to treat the diabetes and its complication. Ayurvedic and Unani medicine used various plants and their products to treat the diabetes and their complication including diabetic retinopathy. Earlier reports showed that, based on *in-vivo* and *in-vitro* studies olive leaves, seed and oil shows a vital effect in the management of diabetes.

Hypoglycemic activities of olive and their constituents have been observed in various studies. An important reports showed that, hypoglycemic effects of the leaves of Olea europaea **(Jouad *et al.*,2001).** The eventual mechanism responsible of the hypoglycemic activity of oleuropein and hydroxytyrosol may result from a potentiating of glucose-induced insulin release or increasedperipheral uptake of glucose .Another findings also shows that oleuropein has role in diabetes via hypolipidemic effects in diabetic rats **(Somova *et al.*,2003).**

2-4-3-Neuroprotector activity

Olive and their constituents show a role in neu-roprotector but the exact mechanism behind this is not fully known. It might be due to the phenolic compound present in olive shows neuroprotective effect. Olive oil phenols have various protective role in brain hypoxia-reoxygenation cerebral ischemia (Mohagheghi *et al.*,2010) brain damage after hypoxia reoxygenation in diabetic rats and ageing.An important study showed that Myelo

Peroxidases activity reduced significantly in Olive Extract treated rats when compared with non-treated rats (Khalatbary *et al.*,2011). Another study (Bazoti *et al.*,2006) has reported that, oleuropein decreases or even prevents Aβ aggregation, which is inherent to Alzheimer's disease (AD). Another report showed that OLE-induced ischemic tolerance in rats is partly associated with changes in brain lipids level (Rabiei *et al.*,2015).

2-4-3-Anti-aging activity

Aging is a multi-factorial process that depends on diverse molecular and cellular mechanisms, such as protein availability, genome maintenance and inflammation Various factors are responsible for aging phenomenon including reactive oxygen species. Olive and their constituents show antioxidant activity and finally play a role in anti-aging process. Earlier studies explore the molecular mechanisms of EVOO by which may influence longevity and this activity are due to the antioxidant potential of its phenolic compounds and free-radical scavengers, such as vitamin E (Trichopoulou and Critselis

,2004) A report (Canuelo *et al.*,2012) suggesting that tyrosol, a phenol present in EVOO, may increase lifespan and stress resistance in *Caenorhabditis elegans*, via activation of hormetic mechanisms. Other constituents (oleuropein-treated cultures) exhibit a delay or slowdown in the appearance of senescence morphology, and their life span is extended by 15% approximately .

2-4-4-Antioxidant ctivity :

Various plant and their constituents such as turmeric, black seed, dates and ginger play a major role in control of diseases via antioxidant activity. Phenol compounds play a role in trapping the free radicals directly or scavenge them via a series of coupled reactions with antioxidant enzymes **(Reynolds & Dweck ,1999)** Olive fruits, oil and leaves play a salient role in the management of various diseases due to the presence of simple phenol (hydroxytyrosol, tyrosol), polyphenols (oleuropein glucoside); and other constituents secoiridoids (SID), the dialdehydic form of oleuropein (SID-1). Earlier investigators have shown that *in vitro* and *ex vivo* models;

olive oil phenolic have shown to have antioxidant properties, higher than that of vitamin E, on lipids and DNA oxidation **(Masella *et al.*,2004).**

2-4-5-Antimicrobial activity.

Earlier reports based on in-vitro and in-vivo studies have shown that olive leaf, seed extract shows a vital role in the inhibition of bacterial activity.

An important study based on olive leaf activity against bacteria such as *Campylobacter jejuni, Helicobacter pylori* and methicillin-resistant *Staphylococcus aureus* (MRSA) showed anti-microbial activity.

Other report in the support of olive leaf extract act as antimicrobial where all tested bacteria were killed within three hours with the dose/concentration of 0.6% (w/v) water extract but Dermatophytes were inhibited via other dose with 1.25% (w/v) plant extract a 3-day exposure **(Markin *et al.*,2003)** An important finding showed that Virgin olive oil as protective effect against foodborne pathogens and olive oil also reduced the count of

inoculated *Salmonella enteritidis* and *Listeria monocytogenes* by approximately 3 log CFU/g in salad and mannose .*Helicobacter pylori* is a main culprit in the stomach cancer and also responsible for gastric cancer **(Romero *et al.*,2007)** An interesting study showed the antimicrobial activity of olive oil against the Gram-negative bacteria *Helicobacter pylori* . Another study reported that, different constituents of olive play a significant role as inhibitory effect or slowdown the growth of various types of bacteria and fungi **(Bisignano *et al.*,1999)** In the support of olive as antimicrobial has shown that, olive hydroxytyrosol might be considered as a promising antimicrobial agent for treating human infections.

Olive oil vegetation water act as toxic for both phyto-pathogenic *Pseudomonas syringae* (Gram-negative) and *Corynebacterium michiganense* (Gram-positive) bacteria **(Soni *et al.*,2006))** Olive and their constituents play a role against virus and US patent declare that Oleuropein contains a powerful antiviral activities against some virus such as herpes mononucleosis, hepatitis virus, rotavirus,

bovine rhinovirus, canine parvovirus, and feline leukemia virus

3-Materials & methods

3-1-Plant material and preparation of extracts

Fresh leaves of *Olea europaea* L. (*olive plant)* were collected from the adult plants in local gardens in Al-Diwanyia city. The leaves were washed and dried under shade for one week, grounded and 200g of plant material were extracted with ethanol alcohol (95% v/v) in a soxhlet apparatus by continuous heat extraction. The extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50°C. The alcohol extract was prepared in distilled water containing 2% v/v Tween 80 (as a suspending agent) for experimental purpose.

3-2-Animals:

Swiss albino mice weighing 18-25 g of either sex were used for the study. The animals were procured and housed in the animal house in college of veterinary medicine / Al-Qadissyia university , maintained under standard hygienic conditions, at 20 ± 2o C, humidity (60 ± 10%) with 12 hour day and night cycle, with food and water *ad libitum*.

3-3-Analgesic activity

Analgesic activity of ethanolic extract of *Olea europaea* L. was studied by two different method : formalin test and writhing test and.

3-3-1-Formalin test

To determine the anti-nociceptive activity of the ethanolic extract of the leaves of *Olea europaea* L. formalin test was used . Male swiss albino mice (5 per group) were fasted for 24 h before the experiment, but with free access to water. Twenty µL of 5% formalin in distilled water was then injected subcutaneously into the right hind paw of mice to cause pain. Mice were administered orally with 200mg/Kg BW ethanolic extract of olive before 60 minute of formalin administration and the same volume of distilled water by oral administration as the vehicle control. Indomethacin (10 mg/kg, i.p) was administered 30 min before formalin administration. These 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 the index of pain and was recorded separately from 0 to 5 min as early phase or neurogenic pain and from 20 to 30 min as late phase or inflammatory pain (Mitchell *et al.*,1973)

3-3-2-Acetic Acid-Induced Writhing Test

To determine the anti-nociceptive activity of the ethanolic extract of the leaves of *Olea europaea* L. acetic acid induced writhing test was used .Male swiss albino mice (5 per group) were fasted for 24 h before the beginning of experiment, but with free access to water. The writhes were induced by an intraperitoneal injection of 1% acetic acid in distilled water (0.1 mL/10 g body weight). Mice were administered orally with 200 mg/Kg BW ethanolic extract of olive before 60 minute to acetic acid injection and the same volume of distilled water by oral administration as the vehicle control. Indomethacin (10 mg/kg, i.p) was administered 30 min prior to acetic acid administration. Mice were placed in an observation box

separately and the number of writhing responses was counted over 20 minutes.(Chiu *et al.*,2012).

3-4-Statistical analysis

All the values ware statistically analyzed by one-way analysis of variance (ANOVA) 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.

4-Results :

4-1-Results of formalin test:

The effect of the ethanolic leaf extract of *Olea europaea* L. *compared with indomethacin drug* on irritating response induced by injection of formalin substance in mice showed In table (1) . The results revealed that the ethanolic extract of *Olea europaea* L. at 200mg/kg caused 39.37% inhibition, on the lacking response induced by injection of the formalin compared with 59.3% induced by reference drug (indomethacin) The statistical analysis reveals that there are significant difference recorded among different groups of animals in response to the pain effect induced by injection of formalin at ($p<0.05$)

4-2-Results of acetic acid induced writhing test.

The effect of the ethanolic leaf extract of *Olea europaea* L. *compared with indomethacin drug* on writhing response induced by acetic acid in mice showed In table (1) . The results revealed that the ethanolic extract of *Olea europaea* L. at 200mg/kg caused 37.07% inhibition, on the writhing response induced by acetic acid compared with

53.5% induced by reference drug (indomethacin). The statistical analysis reveals that there are significant difference recorded among different groups of animals in response to the pain effect induced by injection of acetic acids intrapeartonally at ($p<0.05$)

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 $p<0.05$

5-Discussion:

Olea europaea L. (olive plant) is a medicinal plant of good repute in the *Ayurveda* and amongst its multifarious therapeutic uses, mention may be made of its use as analgesic. The fruit of *Olea europaea* L. (olive) are mentioned as countering poison, anti-inflammatory and analgesic medicine in *Ayurveda*.

Analgesic effect of the extracts was demonstrated in the experimental models using formalin test and acetic acid induced writhing test.

The formalin test is a valid and reliable model of nociception, which is predominantly used for detection on the pain protection in rats and mice.The response to the formalin as a noxious stimulus are favoring, lifting, licking, and shaking of the injured paw by animals.

In the present study demonstrated that ethanolic extract of the olive has good analgesic effect, but the mechanisms of extract-induced analgesia and which compounds are responsible of this activity, were not obvious.

Haloui and their colleagues (2011) suggested that hydroxytyrosol, as well as oleuropein,which is present in the olive extract contribute in a large part to the analgesic effect of the plant.The olive leaves extract also seems to appear a notable antinociceptive effects in the acetic acid-induced writhing test in the mice. It is reported that acetic acid causes an increase in the peritoneal fluid levels of prostaglandins (PGEs), involving in part peritoneal receptors (Deraedt *et al.,*1980) Prostaglandins may be involved in the antinociceptive action of the olive leaves extracts. The presence of iridoids and flavonoids in the organic extracts of the olive could be responsible for the anti-inflammatory and antinociceptive effects (Marzouk *et al.*,2002 ; Mesia-Vela *et al.,*2004).

6-Conclusions & recommendation :

6-1-Conclusions:

1- The leaves of the olive plant has good analgesic activity in the relief of the pain induced experimentally in the mice.

2- The indomethacin activity overcomes on the activity of the ethanolic olive extract in the analgesic property in mice.

6-2-Recommendations:

1- It is important to attempt to isolate and purificate active ingredients in olive extracts which consider active area for further research on the analgesic activity

2- Use the other methods for evaluation the analgesic activity of plant and research on its mechanism of action in this aspect.