Modulation of Toxicological Effect of Carrageenan-Induced Paw Oedema Using Sage Oil From *Salvia Officinalis* as Anti-Inflammatory and Antioxidant Drug

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ABSTRACT: The available anti-inflammatory drugs exert an extensive variety of side effects. The search for new anti-inflammatory agents (obtained from natural sources such as plant extracts) has been a priority of pharmaceutical industries. It is worth mentioning that, plants represent one of the most important sources of substances with biological activities. Moreover, plants continue to be an important source of new chemical substances with potential therapeutic effects. The aim of the present study was to assess the anti-inflammatory, antioxidant activities and histopathological changes of sage oil from *Salvia officinalis* in paw oedema model induced by carrageenan (Carr) in mice. Using carrageenan-induced paw oedema model, the experiment included induction of inflammation with injection of 2% carrageenan solution dissolved in 0.9% saline intraplantarily to the right hind paw. The examined pro-inflammatory parameters were nitric oxide (NO) level, interleukin IL-1ß in paw tissue infiltrate and plasma, total and differential leukocytes count and paw volume (thickness). Some antioxidant parametres were examined such as: catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities, in addition to, malondialdehyde (MDA) production in the paw tissue. The data indicated that, there were increase in the amount of nitric oxide level, interleukin IL-1ß in paw tissue infiltrate and plasma, total leukocytes count and that evidence of the occurrence of inflammation in this region. Furthermore, carrageenan administration caused increase in paw oedema volume. Also data indicated that there were decreases in the amount of CAT, SOD and GPx activities. On the other hand, an increase of MDA production in the paw tissue, these results demonstrated that there was an evidence of oxidative stress in the paw tissue after carrageenan induction. Pretreatment with sage oil extracted from the medicinal herb *Salvia officinalis* administered orally (20 ml/kg) body weight in a single dose 1 hr before injection with carrageenan solution resulted in significant increase of the antioxidant enzymes activity, in contrast, a significant decrease in the pro-inflammatory mediators; NO, IL-1ß and total leukocytes number compared to the treatment with diclofenac which used as a reference drug, administrated orally (50 mg/kg body wt.). of paw oedema sections stained with Harris haematoxylin and eosin (H&E) revealed a significant increase in the infiltration of ploymorphonuclear leukocytes (PMNs) mainly neutrophils, a common occurrence with inflammation. However, pretreatment with sage oil showed a significant reduction in the thickness of oedema and inflammatory cells infiltration compared to the carrageenan inflamed group. The current results denoted that, sage oil may have significant potential for the development of novel anti-inflammatory and antioxidant drug and could be used as pharmacological agent in the treatment of some inflammatory disorders in which free radical formation is a pathogenic factor.

Keywords: Inflammation, Sage oil, Carrageenan, Antioxidant enzymes, Pro-inflammatory mediators.

INTRODUCTION:

Natural plant products have been used since ancient times and their use is now increasing. Some essential oils are known to have various health benefit properties, especially anti-inflammatory and antioxidant activities. Since primitive ages, people have learned to use a variety of plants as medicines for different purposes. Among plants that are largely used are several species of *Salvia*. In Greece, Lebanon, Egypt and Palestine, several species of *Salvia* generally known as 'Habb el mariamiya' (Hala *et al.,* 2000). Moreover, medicinal plants continue to be an important source of new chemical substances with potential therapeutic effects (Mahdi, 2013; Elwy and Tabl, 2012 & 2013). Furthermore, *Salvia officinalis,* a plant endemic to the Mediterranean region is the most popular herbal remedy in the Middle East to treat common health complications (Gali-Muhtasib *et al.,* 2000; Elwy and Tabl, 2012 & 2013). Furthermore, they posses antibacterial (Sokovic *et al.,* 2010), anti-inflammatory (Baricevic *et al*., 2001; Elwy and Tabl, 2012 & 2013) and antioxidant activities (Kamatou *et al*., 2008). *Salvia* species are reported to have anti-inflammatory and antioxidant properties (Baricevic and Bartol, 2000; Zupko *et al.,* 2001; Capasso *et al.,* 2004; Ren *et al.,* 2004; Kamatou *et al.,* 2005, 2006 & 2008).

Inflammation is the first response of the immune system to infection or irritation. Additionally, inflammation is mediated by cytokines such as interleukin 1β (IL-1β) and prostaglandin E2 (PGE2) (Delgado *et al.,* 2003). Additionally, anti-inflammatory drugs are not free of side effects, thus the search for

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natural products with anti-inflammatory activity has increased markedly in recent years (Chung *et al.*, 2010).

Free radicals play a major role in the persistence of inflammation. During the process of inflammation, phagocytes secrete chemically reactive oxygen species (ROS), radicals, and electrophilic compounds that bring about the elimination of infectious agents (Krishnamoorthy and Honn, 2006). These inflammatory mediators can damage the surrounding host tissue (Coussens and Werb, 2002; Dedona and Tannenbaum, 2004). Antioxidants can protect against these radicals effects by forming an intricate network (Arts *et al.*, 2001). The antioxidants which can scavenge reactive oxygen species (ROS) are expected to improve the inflammatory disorders. Many drugs of plant origin having antioxidant activity have been reported to have anti-inflammatory activity (Surh, 2008). Thus, various plant extracts and essential oils have attracted interest as sources of natural antioxidant and anti-inflammatory drugs (Mahdi, E. J**.** 2013).

Carrageenan-induced rat paw oedema is a widely used test to determine the anti-inflammatory activity and it has been fully characterized in the past (Inmaculada *et al.*, 2004). Moreover, carrageenan-induced rat paw is a suitable experimental animal model for evaluating the anti-oedematous effect of natural products and is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation (Sawadogo *et al.*, 2006). Furthermore, mouse oedema has been increasingly used to test new anti-inflammatory drugs as well as to study the mechanisms involved in inflammation (Inmaculada *et al.*, 2004).

In general, the essential oils from some plants; sage oil from *Salvia officinalis*, are potentially useful mainly for their antibacterial, anti-inflammatory and antioxidant activities (Domaracky *et al.*, 2007).

The objective of the current study was to assess sage oil extracted from the natural herb *Salvia officinalis* as an anti-inflammatory and antioxidant drug using carrageenan-induced paw oedema model, compared to diclofenac as a reference drug.

MATERIALS AND METHODS:

Ethics statement

All animal experiments were approved and carried out according to the Guide for the Care and Use of Laboratory Animals, Animal Care Committee of Tanta University.

Experimental animals

The study was performed on pathogen-free 7 weeks-old adult male Swiss albino mice *Mus musculus* and weighing 25-30 g, purchased from the animal house of Theodor Bilhariz Research Institute, Al-Giza, Egypt. They were kept under standardized conditions, where diet and water were *ad libitum*. The animals housed in hygienic cages and maintained in a pathogen-free animal laboratory, the animals maintained at a constant temperature of 20-25°C with a photo cycle of 12-h light/dark.

Drugs and chemicals:

Carrageenan lambda (Irich Moss, Type IV) was purchased from Sigma-Aldrich Co., suspended in saline solution.

The non-steroidal anti-inflammatory drug (NSAID) diclofenac (Diclofenac sodium tablets) has been used as a reference anti-inflammatory drug. Diclofenac was purchased from El Nasr Pharmaceutical Chemicals Co. "ADWIC", Abu-Zaabal, Egypt.

Pure sage oil (the tested remedy) extracted from the medical plant *Salvia officinalis* (belongs to the family Lamiaceae) was purchased from El-Captain Company (Cap PHARM) For Extracting Natural Oils, Plants & Cosmetics, Cairo, Egypt.

Doses and route of administration:

Carrageenan was prepared into the concentration (2% W/V) using saline as a diluent. It was administered intraplantarily to the right hind paw in a dose volume of 0.05 ml of 2% carrageenan solution (Nahed *et al.*, 2008).

Diclofenac (Diclofenac sodium tablets) was dissolved in saline and used as a reference drug and administrated orally, as one single dose (50 mg/kg body wt.) 1 hr before injection with carrageenan solution (Mohamed *et al.*, 2013).

Sage oil dissolved in vehicle (0.2% Tween 80 in 0.9% saline solution), was administered orally in a single dose 1 hr before injection with carrageenan solution. The selected dose of sage oil was 20 ml/kg body wt. (the ideal dose selected experimentally).

Experimental protocol:

Five groups of mice were utilized (10 mice for each group).

Control group: received orally utilized vehicle (0.2% Tween 80 in 0.9% saline solution), also received intraplantarily 0.05 ml of 0.9% saline solution.

Sage oil group: received orally sage oil (20 ml/kg body wt.), also received interplantarily 0.05 ml of 0.9% saline solution.

Carrageenan group: received intraplantarily a dose volume of 0.05 ml of 2% carrageenan solution dissolved in saline.

Carrageenan and sage oil group: sage oil (20 ml/kg body wt.) dissolved in vehicle was administered orally 1 hr before the interplantarily injection with 2% carrageenan.

Carrageenan and diclofenac group: The reference drug diclofenac (Diclofenac sodium tablets, 50 mg/kg body wt.) was dissolved in 0.9% saline solution and administered orally 1 hr before the intrapalantar injection with 2% carrageenan.

Mouse model of carrageenan-induced paw oedema

The Carr-induced hind paw oedema model was used for determination of anti-inflammatory activity (Guan *et al.*, 2012). 0.05 ml of 2% carrageenan was injected in the plantar side of right hind paws of the mice. The paw volume was measured immediately before Carr injection and after Carr injection at 1, 3 and 6 hrs intervals (Posadas *et al.*, 2004; Sakaguchi *et al.*, 2006) using a gauge digital calliper (Ozaki Co., Tokyo, Japan). The degree of swelling (oedema) was evaluated by the difference in volumes (A-B), where A is the volume of the right hind paw after Carr injection, and B is the volume of the right hind paw before Carr injection. Six hours after carrageenan injection, blood

was withdrawn from the orbital sinus of each animal into separated tubes contain EDTA (5 mg/ml). The blood samples were mixed with EDTA and centrifuged for 10 min at 3000 r.p.m at 4°C, and stored in Eppindorff's tubes at -40°C untill used. The tissue infiltrates were withdrawn from the paw oedema and utilized for total white blood cells count, differential leucocytic count, as well as for the measurement of pro-inflammatory and antioxidant parameters.

Finally, the animals were sacrificed and all right hind paws were cut. Each of the right hind paws was homogenized in 2 ml saline and centrifuged at 3000 r.p.m for 10 min after which, exudates (supernatants) were collected. The collected tissue infiltrates and plasma samples were used for the measurements of pro-inflammatory mediators and antioxidant enzymes. Some paw tissues were stored in 10% formalin for the histopathological investigations.

Determination of the ideal dose of sage oil :

To estimate the ideal dose of sage oil used for treatment, the preliminary test was assayed as the following: sage oil with different doses (10, 20, 30, 40 and 50 ml/kg body wt.) was administered orally 1 hr before the interplantarily injection with 0.05 ml of 2% carrageenan. The total WBCs number and the total antioxidant activity were used as inflammatory and antioxidant markers, respectively to evaluate sage oil doses. Animals were observed for 24 hr for signs of toxicity and number of death.

It is worth noting from Fig.(1) that, the ideal dose of sage oil was 20 ml/kg body wt.

Fig. (2) showed that, the most effective dose of sage oil was also 20 ml/kg body wt.

The pro-inflammatory mediators and the antioxidant enzymes were quantified using routine laboratory methods using assay kits (Bio-diagnostics^{Tm}, Egypt).

Measurement of plasma and paw infiltrate nitrite content:

Nitrite concentration was used as an indication of nitric oxide (NO) production. The procedure for NO determination was based on the Griess reaction according to Wang and Mazza (2002).

Determination of IL-1ß in plasma and paw tissue infiltrate:

IL-1ß level was assayed in plasma and paw infiltrate using enzyme-linked immunoadsorbent assay kit (Mouse ELISA Kit) in accordance with the manufacturer's instructions (Biosource International, California, USA), read at 450 using ELISA reader (BioTEK. Instruments Inc., USA). The ELISA reader-controlling software (softmax) readily processes the digital data of raw absorbance value into a standard curve from which cytokine concentrations of unknown samples can be derived directly. Results were expressed as (pg/ml) (Nahed *et al.*, 2006).

Estimation of the total leukocytes count:

A drop of the diluted sample was used to load the Neubauer chamber and all cells within the chamber were enumerated. The dilution was 1:20; the number of enumerated cells for each sample was multiplied by 50 to obtain the absolute leukocyte count per mm³ of blood, (Coles, 1980).

Estimation of malondialdehyde (MDA) formation in the tissueL:

Lipid peroxidation product (malondialdehyde) concentrations were measured according to the method of Yoshioka *et al.* (1979).

Estimation of catalase (CAT) activity:

Catalase activity was measured according to a method described by Beers and Sizer (1952).

Estimation of superoxide Dismutase (SOD):

SOD activity was measured according to the method of Beauchamp and Fridovich (1971).

Glutathione peroxidase (GPx):

Glutathione peroxidase activity was determined by the method of Paglia and Valentine (1967).

Histopathological examinations:

Immediately after the animals were sacrificed, the mouse hind paw tissue (the skin+muscles) were immediately taken following 6 h treatment with the interplanetary injection of Carr. Pieces of the paw tissue were fixed in 10% neutral formalin for 24 hrs. The specimens were dehydrated in ascending grades of ethyl alcohol, cleared in xylene, embedded in paraffin wax and sectioned at 5µ thickness. Paraffin sections were deparaffinized with xylene and stained with Harris haematoxylin and eosin (H&E) for histopathology (Bancroft and Steven, 1996). All samples were examined by light microscopy (LM) and then photographed with BH-2 Olympus microscopy (contains a digital camera).

Statistical analysis:

The results obtained in the present investigation were statistically analyzed using one way ANOVA. Statistical presentation and analysis of the present study were expressed as Mean±SEM using column statistics with Newman-Keuls Multiple Comparison Test as a post test using the computer statistics Prism 3.0 package (GraphPad Software, Inc, San Diego, CA, USA). The minimum level of statistical significance was set at $p<0.05$.

RESULTS:

Anti-inflammatory activity of sage oil:

The anti-inflammatory effect of sage oil was evaluated in carrageenan-induced paw oedema in mice.

As illustrated in Table (1), the level of NO in the tissue infiltrate of the paw oedema recorded significant increases ($p<0.01$) in 2% Carr, Sage+2% Carr and Diclofenac+2% Carr groups as compared to the control group recording 106.40%, 44.82% and 36.76%, respectively and insignificant increase in Sage oil group recorded 4.43%. Moreover, the results showed significant increases ($p<0.01$) in 2% Carr, Sage+2% Carr and Diclofenac+2% Carr groups as compared to the sage oil group recording 97.63%, 38.67% and 30.95%, respectively. On the other hand, the data recorded significant decreases ($p<0.01$) in all studied groups compared to 2% Carr group recorded - 51.55%, - 49.40%, - 29.83% and - 33.74%. Also, the level of NO showed significant decreases ($p<0.01$) in both control and Sage oil groups recoding -30-95% and - 27.89% and a significant increase ($p<0.01$) in 2% Carr group recording 42.52% as compared to Sage+2% Carr

group. Furthermore, data revealed significant decreases (p<0.01) in the level of NO in the tissue infiltrate of the paw oedema in both control and Sage oil groups recording - 26.88% and - 23.64%, respectively and a significant increase (p<0.01) in 2% Carr group recording 50.92% as compared to Diclofenac+2% Carr group.

Regarding the level of NO in the plasma, Table (2), showed significant increases (p<0.01) in 2% Carr, Sage+2% Carr and Diclofenac+2% Carr groups as compared to the control group recording 95.34%, 37.91% and 27.93%, respectively and insignificant increase in Sage oil group recorded 1.96%. Moreover, the results showed significant increases (p<0.01) in 2% Carr, Sage+2% Carr and Diclofenac+2% Carr groups as compared to the sage oil group recording 91.52%, 35.21% and 25.43%, respectively. In contrast, the data showed significant decreases (p<0.01) in all studied groups compared to 2% Carr group recorded - 48.80%, - 47.79%, - 29.4% and - 34.51%. In addition, the level of NO in the plasma showed significant decreases (p<0.01) in both control and Sage oil groups recoding - 27.5% and - 26.04% and a significant increase (p<0.01) in 2% Carr group recording 41.64% as compared to Sage+2% Carr group. Furthermore, data revealed significant decreases (p<0.01) in the level of NO in the plasma in both control and Sage oil groups recording - 21.84% and - 20.28%, respectively and a significant increase (p<0.01) in 2% Carr group recording 52.69% as compared to Diclofenac+2% Carr group.

As illustrated in Table (3), the data showed that, there was significant increases (p<0.01) in the level of IL1ß in tissue infiltrate of the paw oedema of mice in 2% Carr, Sage+2% Carr and Diclofenac+2% Carr groups as compared to the control group recording 531.8%, 216.5% and 192.3%, respectively and insignificant increase in Sage oil group recorded 27.24%. Moreover, the results showed significant increases (p<0.01) in 2% Carr, Sage+2% Carr and Diclofenac+2% Carr groups as compared to the sage oil group recording 396.6%, 148.7% and 129.7%, respectively. In contrast, the data showed significant decreases (p<0.01) in all studied groups compared to 2% Carr group recorded - 84.2%, - 79.9%, - 49.9% and - 53.74%. In addition, the level of IL1ß in tissue infiltrate of the paw oedema showed significant decreases (p<0.01) in both control and Sage oil groups recoding - 68.4% and - 59.8% and a significant increase (p<0.01) in 2% Carr group recording 99.6% as compared to Sage+2% Carr group. Furthermore, data revealed significant decreases (p<0.01) in the level of IL1ß in tissue infiltrate of the paw oedema in both control and Sage oil groups recording - 65.8% and - 56.5%, respectively and a significant increase (p<0.01) in 2% Carr group recording 116.2% as compared to Diclofenac+2% Carr group.

Regarding the level of IL1ß in the plasma of mice, Table (4), showed significant increases (p<0.01) in the level of IL1ß in the plasma in 2% Carr, Sage+2% Carr and Diclofenac+2% Carr groups as compared to the control group recording 565.4%, 147.2% and 123.3%, respectively and insignificant increase in Sage oil group recorded 4.57%. Moreover, the results showed significant increases (p<0.01) in 2% Carr, Sage+2% Carr and Diclofenac+2% Carr groups as compared to the sage oil group recording 536.3%, 136.4% and 113.5%, respectively. In contrast, the data showed significant decreases (p<0.01) in all studied groups compared to 2% Carr group recorded - 84.97%, - 84.3%, - 62.85% and -.66.45%. In addition, the level of IL1ß in plasma showed significant decreases (p<0.01) in both control and Sage oil groups recoding - 59.54% and - 57.7% and a significant increase (p<0.01) in 2% Carr group recording 169.2% as compared to Sage+2% Carr group. Furthermore, data revealed significant decreases (p<0.01) in the level of IL1ß in plasma in both control and Sage oil groups recording - 55.2% and - 53.2%, respectively and a significant increase (p<0.01) in 2% Carr group recording 198.1% as compared to Diclofenac+2% Carr group.

Data recorded in Table (5) revealed that, there was significant decrease (p<0.01) in total WBCs mainly neutrophils percentage after treatment with sage oil compared to the carrageenan group. In this concern, sage oil appeared to suppress the activation of leukocytes and neutrophils infiltration which are of the most important mediators produced in the inflammatory response.

As illustrated in Table (6) the data showed that, there was significant decrease (p<0.01) in the paw oedema volume after treatment with sage oil compared to the inflamed carrageenan group. The maximum increase of the paw oedema thickness occured after three hours of carrageenan injection. On the other hand, the maximum decline recorded after six hours of sage oil administration.

Regarding the previous pro-inflammatory mediators, there were insignificant changes between the mice group treated with sage oil+2% carrageenan, and the group treated with the reference drug diclofenac+2% carrageenan. Moreover, there was insignificant difference between the results of both control and sage oil groups in all tested pro-inflammatory mediators.

The antioxidant activity of sage oil:

The oxidative stress biomarkers, malondialdehyde (MDA) and the antioxidant enzymes such as: catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured to assess the antioxidant activity of sage oil on carrageenan-induced paw oedema.

As illustrated in Table (7), the results showed that, there were significant increases (p<0.01) in MDA level in tissue of paw of mice in 2% Carr, Sage+2% Carr and Diclofenac+2% Carr groups as compared to the control group recording 286.8%, 98.1% and 86.7%, respectively and insignificant increase in Sage oil group recorded 24.7%. Moreover, the results showed significant increases (p<0.01) in 2% Carr, Sage+2% Carr and Diclofenac+2% Carr groups as compared to the sage oil group recording 210.2%, 58.9% and 49.8%, respectively. On the other hand, the data recorded significant decreases (p<0.01) in all studied groups compared to 2% Carr group recorded - 74.1%, - 67.8%, - 48.8% and - 51.7%. Also, MDA level in tissue of paw showed significant decreases (p<0.01) in both control and Sage oil groups recoding - 49.5% and

- 37.1% and a significant increase (p<0.01) in 2% Carr group recording 95.2% as compared to Sage+2% Carr group. Furthermore, data revealed significant decreases (p<0.01) in MDA level in tissue of paw in both control and Sage oil groups recording - 46.4% and - 33.2%, respectively and a significant increase (p<0.01) in 2% Carr group recording 107.1% as compared to Diclofenac+2% Carr group.

As regards to the level of CAT activity in tissue infiltrate of the paw oedema of mice, the data in Table (8) indicated that, there were significant decreases (p<0.01) in the level of CAT activity in tissue infiltrate of the paw oedema in 2% Carr, Sage+2% Carr and Diclofenac+2% Carr groups as compared to the control group recording - 45.9%, - 19.9% and - 23.2%, respectively and insignificant decrease in Sage oil group recorded - 4.58%. Furthermore, the results showed significant decreases (p<0.01) in 2% Carr, Sage+2% Carr and Diclofenac+2% Carr groups as compared to the sage oil group recording - 43.4%, - 16.1% and - 19.5%, respectively. In contrast, the data recorded significant increases (p<0.01) in all studied groups compared to 2% Carr group recorded 85.02%, 76.5%, 48.1% and 42.03%. Moreover, the level of CAT activity in tissue infiltrate of the paw oedema showed significant increases (p<0.01) in both control and Sage oil groups recording 24.9% and 19.2% and a significant decrease (p<0.01) in 2% Carr group recording - 32.5% as compared to Sage+2% Carr group. In addition, data revealed significant increases (p<0.01) in the level of CAT activity in tissue infiltrate of the paw oedema in both control and Sage oil groups recording 30.3% and 24.3%, respectively and a significant decrease (p<0.01) in 2% Carr group recording - 29.65 as compared to Diclofenac+2% Carr group.

As illustrated in Table (9), the level of SOD activity in tissue infiltrate of the paw oedema of mice recorded significant decreases (p<0.01) in 2% Carr, Sage+2% Carr and Diclofenac+2% Carr groups as compared to the control group recording - 39.3%, - 16.1% and - 10.5%, respectively and insignificant decrease in Sage oil group recorded - 0.9%. Moreover, the results showed significant decreases (p<0.01) in 2% Carr, Sage+2% Carr and Diclofenac+2% Carr groups as compared to the sage oil group recording - 38.8%, - 15.4% and - 9.73%, respectively. On the other hand, the data recorded significant increases (p<0.01) in all studied groups compared to 2% Carr group recorded 64.81%, 63.3%, 38.3% and 47.4%. Also, the level of SOD activity in tissue infiltrate of the paw oedema showed significant increases (p<0.01) in both control and Sage oil groups recording 19.2% and 18.2% and a significant decrease (p<0.01) in 2% Carr group recording - 27.7% as compared to Sage+2% Carr group. Furthermore, data revealed significant increases (p<0.01) in the level of SOD activity in tissue infiltrate of the paw oedema in both control and Sage oil groups recording 11.8% and 10.8%, respectively and a significant decrease (p<0.01) in 2% Carr group recording - 32.2% as compared to Diclofenac+2% Carr group.

As illustrated in Table (10), the data showed significant decreases (p<0.01) in the level of GPx activity in tissue infiltrate of the paw oedema of mice in 2% Carr, Sage+2% Carr and Diclofenac+2% Carr groups as compared to the control group recording - 66.9%, - 22.2% and - 27.9%, respectively and insignificant decrease in Sage oil group recorded - 3.6%. Furthermore, the results showed significant decreases (p<0.01) in 2% Carr, Sage+2% Carr and Diclofenac+2% Carr groups as compared to the sage oil group recording - 65.7%, - 19.3% and - 25.2%, respectively. In contrast, the data recorded significant increases (p<0.01) in all studied groups compared to 2% Carr group recorded 202.2%, 191.3%, 135.1% and 117.9%. Moreover, the level of GPx activity in tissue infiltrate of the paw oedema showed significant increases (p<0.01) in both control and Sage oil groups recording 28.5% and 23.9% and a significant decrease (p<0.01) in 2% Carr group recording - 57.5% as compared to Sage+2% Carr group. In addition, data revealed significant increases (p<0.01) in the level of GPx activity in tissue infiltrate of the paw oedema in both control and Sage oil groups recording 38.7% and 33.7%, respectively and a significant decrease (p<0.01) in 2% Carr group recording - 54.1% as compared to Diclofenac+2% Carr group.

Concerning the previous oxidative stress biomarker MDA, and the antioxidant enzymes; CAT, SOD, GPx there were insignificant changes between the mice group treated with sage oil+2% carrageenan, and the group treated with the reference drug diclofenac+2% carrageenan. Moreover, there was insignificant difference between the results of both control and sage oil groups in all tested parameters.

Histopathological analysis :

The intraplantarily injection of 2% carrageenan into the mice right hind paw produced an intense oedema, characterized by epithelial and tissue blisters and infiltrates of inflammatory ploymorphonuclear leukocytes (PMNs), mainly neutrophils (Fig. C), as compared to the normal control and sage oil groups (Figs. A and B). In the carrageenan group pretreated with sage oil (Fig. D) or diclofenac (Fig. E) there were significant decrease (P<0.001) in the oedema as well as decrease in the inflammatory cells infiltration

Histopathological examination of paw oedema sections stained with Harris haematoxylin and eosin (H&E) revealed a significant increase (P<0.001) in infiltration damage by ploymorphonuclear leukocytes (PMNs), a common occurrence with inflammation. The injection of carrageenan into the paw of mice elicited an acute inflammatory response characterized by accumulation of fluid (oedema) and tissue injury that contained high level of PMNs.

Figs. (A and B): Control (Fig. A) and sage oil groups (Fig. B) showed the normal appearance of epidermis and dermis without any significant lesion. The results of the histopathological analysis were almost similar in both control and sage oil groups.

Fig. (C): This inflamed carrageenan group intraplantarily injected with carrageenan only. These sections showed a marked increase in the paw thickness with clear evidence of epidermal hyperplasia,

oedema, and substantial inflammatory cell infiltration in the dermis, associated with connective tissue disturbance (Fig. C). Moreover, the details of dermis layer showed the connective tissue with several irritated cells, enlargement of the interstitial space (vacuoles) and interstitial fluid caused by oedema.

Fig. (D): Based on the histological assessment, pre-treatment with sage oil to the intraplantarily injected-carrageenan sections (sage oil + 2% carrageenan group) showed a significant reduction ($P<0.001$) in the level of oedema thickness and the inflammatory cell infiltration compared to the inflamed carrageenan group (Fig. C).

Fig. (E): The pre-treament with diclofenac to the intraplantarily injected-carrageenan sections (diclofenac + 2% carrageenan group) significantly reduced ($P<0.001$) the level of oedema volume and the inflammatory cell infiltration compared to the inflamed carrageenan group (Fig. C).

DISCUSSION:

Nowadays, the search for new pharmacologically active agents obtained by screening natural sources such as plant extracts has led to the discovery of many clinically useful drugs for the treatment of many diseases (Kamatou *et al.*, 2005, 2006 &2008; Elwy and Tabl, 2012).

Plants represent one of the most important sources of substances with biological activities. Previous studies have evaluated the natural products as possible anti-inflammatory (Domaracky *et al.*, 2007; Johnson, 2011; Elwy and Tabl, 2012) and antioxidant agents (Kundu and Surh, 2005; Tabl and Elwy, 2013). Therefore, the present investigation was carried out to demonstrate the effect of sage oil as an anti-inflammatory and antioxidant drug in experimental model of acute inflammation using carrageenan-induced paw oedema in mice and diclofenac as a reference drug.

Previous studies identified that, *Salvia* species is rich in polyphenols, flavonoides, the diterpenes such as carnosol, and carnosic acid and monoterpene ketones as thujone which is the most abundant constituent (La casa *et al.*, 2000; Miura *et al.*, 2002). In addition to their strong antioxidant character, carnosol and carnosic acid exert potent anti-inflammatory properties (Johnson, 2011). In the current study, the significant effect of the sage oil treatment as anti-inflammatory and antioxidant, suggests that such effect may be explained probably through its polyphenols and terpenoid contents especially carnosol, carnosic acid and thujone. Moreover, thujone reduces the production of pro-inflammatory cytokines such as interleukin IL-1ß (Siveen and Kuttan, 2011). This assumption is supported by the previous studies of La casa *et al.* (2000) and Johnson (2011).

The acute inflammatory response is characterized by an increase in vascular permeability and cellular infiltration leading to oedema formation, as a result of extravasation of fluid, proteins and accumulation of NO and leukocytes at the inflammatory site (Lukaces, 2001). Moreover, the carraageenan-induced inflammatory response has been linked to neutrophils infiltration and the production of neutrophils-derived free radicals as well as the release of other neutrophils-derived mediators (Maria *et al.*, 2001). The present results showed that, the treatment with sage oil effectively inhibited carrageenan-induced hind paw oedema in mice as a result of extravasation of fluid, proteins and accumulation of leukocytes at the inflammatory site. The present data support the assumption that, the inhibitory effect of sage oil on leukocytes migration contributes to its anti-inflammatory action. As well as, the anti-inflammatory activity could be related to its active constitues. This is in agreement with finding of Siveen and Kuttan (2011).

Nitric oxide is an important pro-inflammatory mediator involved in infiltration and leukocytes migration in the inflammatory process (Abramson, 2008). Moreover, NO also was found to activate COX-1 in the early phase of carrageenan inflammation and to up-regulate COX-2 expression in the late phase in the skin, resulting in production of PGE2 at the site of inflammation, Thus contributing to exacerbation of the inflammatory process (Toriyabe *et al.*, 2004). The present data demonstrated that, sage oil significantly decreased the level of NO produced by carrageenan induction. This anti-inflammatory effect of sage oil can be attributed to its inhibition of reactive oxygen species and decrease of PGE2 production which is a potent mediator of pain and inflammation. This is in accordance with Trebino *et al.* (2003) and Toriyabe *et al.* (2004).

Interleukin-1 (IL-1β) participate in the recruitment of leukocytes to inflammatory sites by inducing the expression of adhesion molecules on vascular endothelium and a number of chemokines that attract neutrophils, eosinophils, macrophages and lymphocytes to the sites of injury (Sanz *et al.*, 1995). In the present study, the exerted anti-inflammatory effects of sage oil appeared as decreasing IL-1β levels in both plasma and paw infiltrate and reducing activation and recruitment of leukocytes especially neutrophils, with a consequent decrease in the paw volume. This is in agreement with Siveen and Kuttan (2011).

Oxidative stress is a patho-physiological mechanism where there is an imbalance between concentrations of reactive oxygen species (ROS) and antioxidants. However, excessive ROS accumulation will lead to cellular injury, such as damage to DNA, proteins and lipid membranes. Because of their potential harmful effects, excessive ROS must be promptly eliminated from the cells by this variety of antioxidant defense mechanisms (Valko *et al.*, 2006). Inflammation is a complex process and ROS play an important role in the pathogenesis of inflammatory diseases (Surh, 2008). Thus antioxidants which can scavenge ROS are expected to improve the inflammatory disorders (Kris-Etherton *et al.*, 2004). Recently, various plant extracts have attracted interest as sources of natural antioxidant and sage (*Salvia officinalis*) is one of the most studied plants (Elwy and Tabl, 2012). Antioxidants can protect against these radicals by forming an intricate network (Arts *et al.*, 2001). As sage oil constituents were mainly monoterpene ketones characterized as thujone

which is potent antioxidant at very low concentration, believed to reflect their ability to scavenge endogenous ROS (Kundu, and Surh, 2005). The present results showed increase in MDA production after carrageenan administration. This may be attributed to the attack of the free-radicals on plasma membrane. Another possible explanation to the mechanism of inflammation injury may be correlated, in part, to release of reactive oxygen species (ROS) from activated neutrophils and macrophages. These overproduction lead to tissue injury by damaging macromolecules and lipid peroxidation of membranes. Malondialdehyde (MDA) levels indirectly reflect the extent of cellular damage by free radicals and are widely used as an index of free radical mediated lipid peroxidation (Mansour, 2000). In the present study, the significantly increased MDA levels suggests the enhanced lipid peroxidation leading to tissue damage due to failure of antioxidant defense mechanisms. This is in agreement with Amresh *et al.* (2007). Furthermore, the reactive oxygen species induce inflammation by stimulating release of cytokines such as interlukin 1ß which in turn causes recruitment of additional neutrophils and macrophages (Delaporte *et al*., 2002; Geronikaki and Gavalas, 2006), consequently, ROS may be considered as the main factor that provoking inflammatory process. Thus their neutralization by antioxidants and radical scavengers are very important to reduce inflammation process. On the other hand, CAT, SOD and GPx activities showed decline after carrageenan induction. These antioxidant enzymes play an important role against Carr-induced local inflammation (Chaturvedi, 2008). Also antioxidant enzymes can reduce MDA production (Guan *et al.,* 2012). The present findings revealed that the treatment with sage oil improved the disturbance of this antioxidant system caused by carrageenan induction. Thereby, these results suggested that the decrease of MDA production after sage oil treatment may be probably due to the increase of CAT, SOD and GPx activities. In addition, the anti-inflammatory and antioxidant mechanisms of sage oil may be related to the inhibition of the free radicles and therby the increase in the activites of antioxidant enzymes (CAT, SOD and GPx). These antioxidant enzymes posses the ability of free radicles scavenging mechanism which lead finally to anti-inflammatory and antioxidant activities of sage oil. The effect of diclofenac (the reference drug) showed similar effect as an anti-inflammatory and antioxidant drug to sage oil in both plasma and tissue.

The histopathological findings support the current physiological results and showed the difference between the severity (inflammtory) effect of carrageenan and the moderate inhibitory (anti-inflammatory) effect of sage oil on leukocytic migration mainly neutrophils. The current histopathological analysis were in agreement with the studies of Al-Reza *et al*. (2010) and Mohamed *et al.* (2013), their histological studies revealed that, Jujuba fruit oil inhibited the inflammatory responses of skin inflammation. The histopathological study provides an additional support to the concept of the anti-inflammatory effect of sage oil.

Conclusion: In summary, the current results suggested that the anti-inflammatory mechanism of sage oil may be related to the inhibition of locally-produced pro-inflammatory mediators such as NO, Interleukin-1ß and infiltration of leukocytes mainly neutrophil. This inhibition is associated with the increase in the activities of the antioxidant enzymes (CAT, SOD and GPx). In this concern, the antioxidant and anti-inflammatory activities of sage oil may related to the strongest active constituents of sage such as flavonoids, thujone, ursolic acid, carnsole, carnsolic acid, polyphenols, phenolic compounds and terpenes which have both antioxidant and anti-inflammatory effects. Additionally, sage oil could be used as pharmacological agent in the treatment of some inflammatory disorders in which free radicals formation is a pathogenic factor as illustrated in the present study. Moreover, because of the ameliorating effect of sage oil on inflammatory disorders, it could be used as natural source, safe and alternative to synthetic drug.

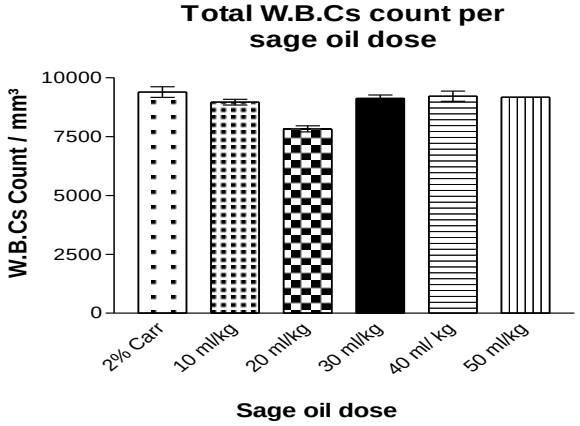

Fig. (1): The total WBCs number (count)/mm³ in the tissue infiltrate of the paw oedema of mice in the different doses of sage oil.

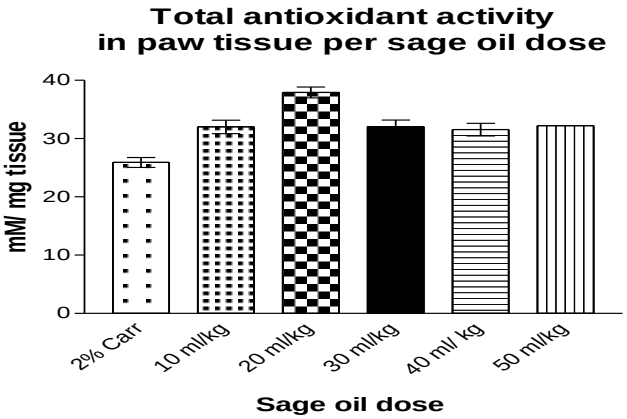

Fig. (2): The level of total antioxidant in the tissue (mM/mg tissue) of the paw oedema of mice in the different doses of sage oil.

Table (1): Level of nitric oxide (NO) in tissue infiltrate (nm/mg protein) of the paw oedema of mice.

NO in tissue infiltrate (nm/mg protein)	Control	Sage oil	2% Carr	Sage+ 2% Carr	Diclofenac+ 2% Carr
Range	20.50 - 28.00	21.30- 28.00	36.80 – 53.30	25.80 – 37.00	27.50 – 35.00
Mean ± SEM	23.45 ± 0.68	24.49 ± 0.66	48.40 ± 1.44	33.96 ± 0.98	32.07 ± 0.84
Percentage difference (*p*<vs. Control)	-	4.43%	**106.40%	**44.82%	**36.76%
Percentage difference (*p*<vs. Sage oil)	-4.25%	-	**97.63%	**38.67%	**30.95%
Percentage difference (*p*<vs. 2% Carr)	**-51.55%	**-49.40%	-	**-29.83%	**-33.74%
Percentage difference (*p*<vs.Sage+2% Carr)	**-30.95%	**-27.89%	**42.52%	-	-5.57%
Percentage difference (*p*<vs. Diclofenac+ 2% Carr)	**-26.88%	**-23.64%	**50.92%	5.89%	-

Data expressed as mean ± SEM. Number of mice per group (n) = 10 mice. The significant difference between different groups using ANOVA, *: significant at $p<0.05$ and **: significant at $p<0.01$.

Table (2): Level of nitric oxide (NO) in plasma (nm/mg protein) of mice.

NO in plasma (nm/ mg protein)	Control	Sage oil	2%Car	Sage+ 2% Carr	Diclofenac+ 2% Carr
Range	15.60 -20.10	14.50 – 19.80	29.50 –40.20	20.80 – 27.20	20.80 – 26.30
Mean ± SEM	18.04 ± 0.45	18.40 ± 0.53	35.24 ± 1.04	24.88 ± 0.68	23.08 ± 0.58
Percentage difference (*p*<vs. Control)	-	1.96%	**95.34%	**37.91%	**27.93%
Percentage difference (*p*<vs. Sage oil)	-1.96%	-	**91.52%	**35.21%	**25.43%
Percentage difference (*p*<vs. 2% Carr)	-**48.80%	-**47.79%	-	-**29.4%	-**34.51%
Percentage difference (*p*<vs.Sage+2% Carr)	-**27.5%	-**26.04%	**41.64%	-	- 7.23%
Percentage difference (*p*<vs. Diclofenac+ 2% Carr)	-**21.84%	-**20.28%	**52.69%	7.81%	-

Data expressed as mean ± SEM. Number of mice per group (n) = 10 mice. The significant difference between different groups using ANOVA, *: significant at $p<0.05$ and **: significant at $p<0.01$.

Table (3): Effect of sage oil treatment on interleukin-1ß (IL1ß) production (Pg/ml) in tissue infiltrate of the paw oedema of mice.

Level of IL1ß in tissue infiltrate (Pg/ml)	Control	Sage oil	2%Carr	Sage+ 2% Carr	Diclofenac+ 2% Carr
Range	237.0 - 455.0	290.0 - 608.0	2004 –2325	955.0 –1233	885.0 - 1174
Mean ± SEM	342.5 ± 25.94	435.8 ±36.58	2164 ±40.57	1084 ±40.27	1001 ± 35.20
Percentage difference (*p*<vs. Control)	-	27.24%	**531.8%	**216.5%	**192.3%
Percentage difference (*p*<vs. Sage oil)	- 21.4%	-	**396.6%	**148.7%	**129.7%
Percentage difference (*p*<vs. 2% Carr)	-**84.2%	-**79.9%	-	-**49.9%	-**53.74%
Percentage difference (*p*<vs.Sage+2% Carr)	-**68.4%	-**59.8%	**99.6%	-	- 7.66%
Percentage difference (*p*<vs. Diclofenac+ 2% Carr)	-**65.8%	-**56.5%	**116.2%	8.3%	-

Data expressed as mean ± SEM. Number of mice per group (n) = 10 mice. The significant difference between different groups using ANOVA, *: significant at $p<0.05$ and **: significant at $p<0.01$.

Table (4): Effect of sage oil treatment on interleukin-1ß (IL1ß) production (Pg/ml) in plasma of mice.

Level of IL1ß in plasma (Pg/ml)	Control	Sage oil	2%Carr	Sage+ 2% Carr	Diclofenac+ 2% Carr
Range	2.950 - 5.650	3.300 -6.300	25.40 -31.50	8.500 12.50	8.300 - 11.20
Mean ± SEM	4.244 ± 0.3132	4.438 ± 0.3280	28.24 ±0.7373	10.49±0.5149	9.475 ± 0.352
Percentage difference (*p*<vs. Control)	-	4.57%	**565.4%	**147.2%	**123.3%
Percentage difference (*p*<vs. Sage oil)	- 4.37%	-	**536.3%	**136.4%	**113.5%
Percentage difference (*p*<vs. 2% Carr)	-**84.97%	-**84.3%	-	-**62.85%	-**66.45%
Percentage difference (*p*<vs.Sage+2% Carr)	-**59.54%	-**57.7%	**169.2%	-	- 9.68%
Percentage difference (*p*<vs. Diclofenac+ 2% Carr)	-**55.2%	-**53.2%	**198.1%	10.7%	-

Data expressed as mean ± SEM. Number of mice per group (n) = 10 mice. The significant difference between different groups using ANOVA, *: significant at $p<0.05$ and **: significant at $p<0.01$.

Table (5): Total and differential leukocyte counts in tissue infiltrate.

Groups	Total WBCs	Differential WBCs				
		% Neutrophil	% Lymphocyte	% Monocyte	% Eosinophil	% Basophil
Control (Saline)	4603 ± 198.5	43 ± 245.	54 ± 2.32	2 ± 0.43	1 ± 0.36	0
Sage oil	4918 ± 145.4	47 ± 2.8	48± 1.66	3 ± 0.56	2 ± 0.48	0
2 % Carr	9388 ± 2253.	56 ± 2.33	36 ± 1.87	5 ± 0.66	1 ± 0.28	2 ± 0.52
Sage oil + 2 % Carr	7880 ± 166.4	39 ± 1.76	44 ± 2.45	4 ± 0.86	3± 0.49	0
Diclofenac + 2% Carr	7427 ± 180.7	45 ± 2.53	49 ± 1.70	3 ± 0.36	3± 0.65	0

Total WBCs in the Table expressed as Mean±SEM (count/mm^3), however, Differential WBCs count expressed as percentage (%) of Mean±SEM for each type.

Table (6): Effect of sage oil treatment on carrageenan-induced paw oedema volume.

Level of paw oedema (Paw volume 'mm')	Control	Sage oil	2% Carr	Sage oil+ 2% Carr	Diclofenac+ 2% Carr
Level of paw oedema after 1 hr	0.2650 ± 0.0311	0.2850 ±0.0263	1.460 ± 0.0846	1.200 ± 0.0614	1.108 ± 0.0682
Level of paw oedema after 3 hrs	0.2767 ± 0.0202	0.2450 ± 0.0176	2.123 ± 0.0731	1.112 ± 0.0659	0.9733 ± 0.0729
Level of paw oedema after 6 hrs	0.2717 ± 0.0216	0.2617 ± 0.0240	1.683 ± 0.0607	0.8750 ±0.0531	0.7483 ± 0.0556

Data expressed as mean ± SEM. Number of mice per group (n) =10 mice.

Table (7): Effect of sage oil treatment on malondialdehyde (MDA) level in tissue of paw (n mol/gm tissue) of mice.

MDA in tissue (n mol/gm tissue)	Control	Sage oil	2%Carr	Sage+ 2% Carr	Diclofenac+ 2% Carr
Range	3.250 –4.360	3.900 -5.600	12.50 -15.86	6.430- 8.500	6.540 – 7.550
Mean ± SEM	3.736 ±0.1334	4.658 ± 0.1709	14.45 ±0.3767	7.401± 0.1766	6.976 ± 0.1049
Percentage difference (*p*<vs. Control)	-	24.7%	**286.8%	**98.1%	**86.7%
Percentage difference (*p*<vs. Sage oil)	- 19.8%	-	**210.2%	**58.9%	**49.8%
Percentage difference (*p*<vs. 2% Carr)	-**74.1%	-**67.8%	-	-**48.8%	-**51.7%
Percentage difference (*p*<vs.Sage+2% Carr)	-**49.5%	-**37.1%	**95.2%	-	- 5.74%
Percentage difference (*p*<vs. Diclofenac+ 2% Carr)	-**46.4%	-**33.2%	**107.1%	6.12%	-

Data expressed as mean ± SEM. Number of mice per group (n) = 10 mice. The significant difference between different groups using ANOVA, *: significant at $p<0.05$ and **: significant at $p<0.01$.

Table (8): Effect of sage oil treatment on catalase (CAT) activity in tissue infiltrate (n mol/min/mL) of the paw oedema of mice.

CAT in tissue infiltrate (n mol/min /ml)	Control	Sage oil	2%Carr	Sage+ 2% Carr	Diclofenac+ 2% Carr
Range	26.80 - 30.80	22.50 - 31.50	12.30 -18.10	18.50 - 26.50	19.50 - 25.80
Mean ± SEM	28.79 ± 0.399	27.47 ± 0.815	15.56 ± 0.574	23.04 ± 0.758	22.10 ± 0.596
Percentage difference (*p*<vs. Control)	-	- 4.58%	-**45.9%	-**19.9%	-**23.2%
Percentage difference (*p*<vs. Sage oil)	4.8%	-	-**43.4%	-**16.1%	-**19.5%
Percentage difference (*p*<vs. 2% Carr)	**85.02%	**76.5%	-	**48.1%	**42.03%
Percentage difference (*p*<vs.Sage+2% Carr)	**24.9%	**19.2%	-**32.5%	-	- 4.1%
Percentage difference (*p*<vs. Diclofenac+2% Carr)	**30.3%	**24.3%	-**29.65	4.3%	-

Data expressed as mean ± SEM. Number of mice per group (n) = 10 mice. The significant difference between different groups using ANOVA, *: significant at $p<0.05$ and **: significant at $p<0.01$.

Table (9): Effect of sage oil treatment on superoxide dismutase (SOD) activity in tissue infiltrate (unit/ g protein) of the paw oedema of mice.

SOD in tissue infiltrate (unit/g protein)	Control	Sage oil	2%Carr	Sage+ 2% Carr	Diclofenac+ 2% Carr
Range	22.80 - 28.00	22.70 - 28.00	13.40 - 18.00	18.40 - 25.00	18.70 - 26.20
Mean ± SEM	25.81 ± 0.560	25.58 ± 0.501	15.66 ± 0.480	21.65 ± 0.749	23.09 - 0.760
Percentage difference (*p*<vs. Control)	-	- 0.9%	-**39.3%	-**16.1%	-*10.5%
Percentage difference (*p*<vs. Sage oil)	0.9%	-	-**38.8%	-**15.4%	-*9.73%
Percentage difference (*p*<vs. 2% Carr)	**64.81%	**63.3%	-	**38.3%	**47.4%
Percentage difference (*p*<vs.Sage+2% Carr)	**19.2%	**18.2%	-**27.7%	-	6.7%
Percentage difference (*p*<vs. Diclofenac+ 2% Carr)	*11.8%	*10.8%	-**32.2%	- 6.2%	-

Data expressed as mean ± SEM. Number of mice per group (n) = 10 mice. The significant difference between different groups using ANOVA, *: significant at $p<0.05$ and **: significant at $p<0.01$.

Table (10): Effect of sage oil treatment on glutathione peroxidase (GPx) activity in tissue infiltrate (n mol/min/ml) of the paw oedema of mice.

GPx in tissue infiltrate (n mol/min / ml)	Control	Sage oil	2%Carr	Sage+ 2% Carr	Diclofenac+ 2% Carr
Range	141.0 -161.0	126.0 - 163.0	37.00 - 65.00	108.0 -128.0	96.00 - 127.0
Mean ± SEM	152.3 ± 2.176	146.8 ±3.750	50.40 ± 3.243	118.5 ± 2.094	109.8 ± 3.116
Percentage difference (*p*<vs. Control)	-	- 3.6%	-**66.9%	-**22.2%	-**27.9%
Percentage difference (*p*<vs. Sage oil)	3.7%	-	-**65.7%	-*19.3%	-**25.2%
Percentage difference (*p*<vs. 2% Carr)	**202.2%	**191.3%	-	**135.1%	**117.9%
Percentage difference (*p*<vs.Sage+2% Carr)	**28.5%	**23.9%	-**57.5%	-	- 7.3%
Percentage difference (*p*<vs. Diclofenac+ 2% Carr)	**38.7%	**33.7%	-**54.1%	7.9%	-

Data expressed as mean ± SEM. Number of mice per group (n) = 10 mice. The significant difference between different groups using ANOVA, *: significant at $p<0.05$ and **: significant at $p<0.01$.

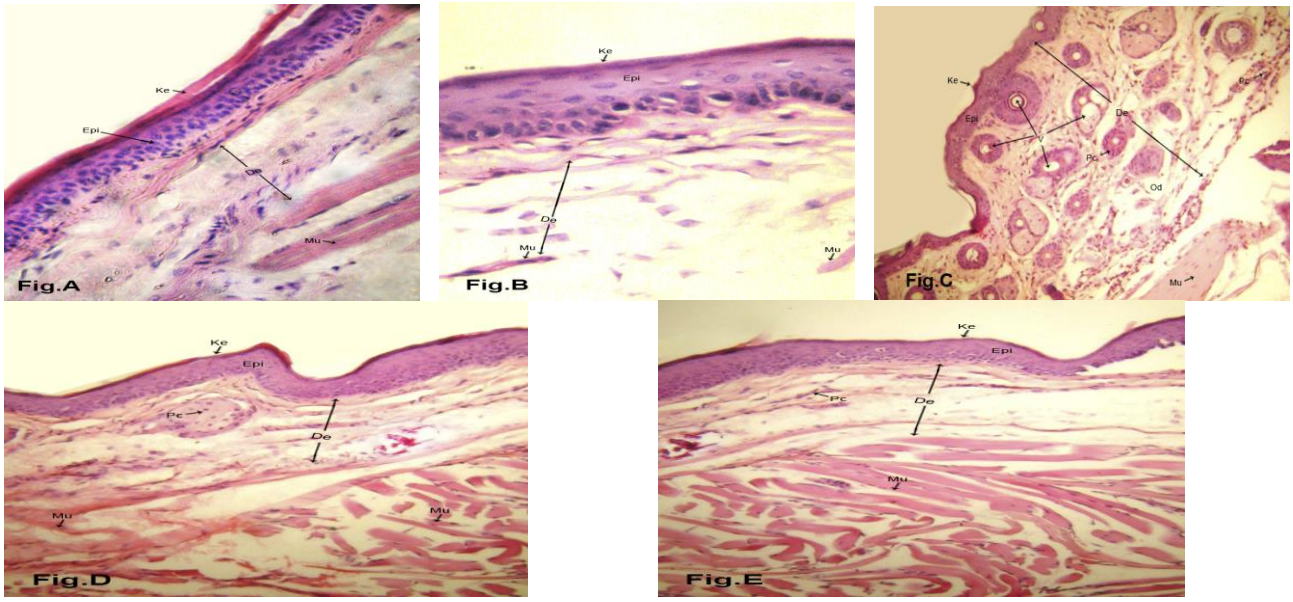

Fig. 3: Histological examination of paw sections of mice. (A) Control (normal saline only), (B) Sage oil only, (C) 2% Carrageenan only, (D) Treated group with sage oil (sage oil + 2% Carrageenan) and (E) Treated group with diclofenac (Diclofenac + 2% Carrageenan). Ke: keratin; Ep: epidermal layer; De: dermal layer; Mu: muscle; Od: oedema; Pc: polymorphonuclear cell infiltration (H&E stain, magnification x200).

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تعديل التاثيرات السمية لمادة الكاراجينان المحدثة لأوديما إخمص القدم بإستخدام زيت المريمية المستخلص من نبات سالفيا أوفيسينالس كعلاج مضاد للإلتهاب ومضاد للأكسدة

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العقاقير المضادة للإلتهابات تسبب مجموعة واسعة من الآثار الجانبية، ومن هنا كان البحث عن مضادات جديدة للإلتهابات له أولوية في الصناعات الدوائية.

دراسة تقييم الأنشطة المضادة للإلتهابات والمضادة للأكسدة من زيت المريمية المستخلص من نبات السالفيا أوفيسيناليس علي الجرذان وذلك بإستخدام نموذج أوديما الكف المستحثة بمادة الكاراجينان.

بإستخدام نموذج أوديما الكف فقد تم تقسيم التجربة الي خمس مجموعات: المجموعة الاولي هي المجموعة الضابطة حييث يتم حقنها بمحلول الملح، والمجموعة الثانية تم حقنها بزيت المريمية فقط لنري تأثيره منفردا، المجموعة الثالثة وهي المجموعة التي يتم حقنها بتركيز 2% من الكاراجينان التي تحدث إلتهاب في بطن قدم الجرذ محدثة نوعا من الاوديما، وعن المجموعة الرابعة فقد تم معالجتها بزيت المريمية بحقن بتركيز 20 مل/كجم من وزن الجسم بعد حقنها بمادة الكاراجينان، أما المجموعة الخامسة والأخيرة فقد تم معالجتها بعقار مضاد للإلتهاب وهو الديكلوفيناك بتركيز 50 مج/كجم من وزن الجسم كعقار مرجعي. بعد عملية الحقن والمعالجة فقد تم أخذ عينات من نسيج الاوديما لعمل إختبارات فسيولوجية وهستولوجية.

تم تقييم درجة الإلتهاب عن طريق قياس العدد الكلي لكرات الدم البيضاء ومستوى أكسيد النيتريك، وانترلوكين IL-1ß في البلازما والأنسجة.

كما تم قياس النشاط الانزيمي لبعض الانزيمات المضادة االتأكسد مثل: أنشطة الديسميتاز(SOD) ، الكاتلاز (CAT) والجلوتاثيون بيروكسيديز (GPX)، بالإضافة إلى المالنوالدهيد (MDA) أيضا في البلازما وأنسجة بطن القدم.

وقد أظهرت النتائج أن هناك مؤشرات إيجابية للمعالجة بزيت المريمية، حيث أدت المعالجة المسبقة بزيت المريمية المستخرج من عشبة السالفيا عن طريق الفم قبل الحقن بمحلول الكاراجينان بساعة في إرتفاع نشاط الإنزيمات المضادة للأكسدة، وإنخفاض كبير في مستوي وسطاء للإلتهابات مثل IL-1ß، NO والعدد الكلي لكريات الدم البيضاء مقارنة مع المجموعات الأخري، وقد تم تأييد هذا بالصور الهستولوجية التي أوضحت إنخفاض في هجرة خلايا الدم البيضاء في المجموعات المعالجة.

من النتائج السابقة يتضح أن زيت المريمية المستخدم له تأثير واضح في تقليل الإلتهابات من خلال خفض نسبة وسطاء الإلتهاب مثل انترلوكين IL-1ß، NO والعدد الكلي لكريات الدم البيضاء، كما أنه ذو نشاط مضاد للأكسدة عن طريق زيادة نسبة الإنزيمات المضادة للأكسدة مثل SOD، CAT، GPx مقارنة بعقار الديكلوفيناك المستخدم كمرجع. وترجع الخواص المضادة للإلتهاب والمضادة للأكسدة لزيت المريمية الي مكوناته وهي: الفلافونويد، الثيوجون، كارنوسول، بوليفينول وكذلك التيربينات.