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Gastroprotective Effect of *Salvia Palestina* against Indomethacin Induced Gastric Ulcer in Rats

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ABSTRACT

Salvia is the biggest genus of the mint family, comprising about 900 species distributed diversely throughout the world. Salvia plants are used commonly in folk medicine as a tonic, anti-rheumatoid and chronic pain killer. The present study used an experimental model to verify the possible ulcer healing effect of extract of *Salvia Palestina* plant. Indomethacin was used as an induction agent for gastric ulcer, while ranitidine was the standard treatment. The effect had been evaluated through PGE2, TNF-α, TBARS, and histopathological study. The results showed a significant improvement in the histopathological study with a significant reduction in TNF-α, TBARS, and a significant increase in PGE2 levels. The study concluded that extract of *Salvia Palestina* has ulcer healing properties possibly through enhancing gastric defense system and antioxidant with free radical scavenging along with anti-inflammatory properties.

1. INTRODUCTION

Peptic ulcer disease had an enormous effect on morbidity and mortality for decades until the last ten years of the 20th century, when epidemiological trends started to point to an outstanding fall in its incidence and that was due to the discovery of potent acid suppressants as well as the management of H. Pylori infection [1].

The multifactorial and complex pathogenesis of peptic ulcer has been studied for several decades, and results from an imbalance of aggressive gastric luminal (corrosive) factors acid and pepsin and defensive mucosal barrier function [2]. The using of the non-steroidal anti-inflammatory drugs (NSAID) increased the risk of complications of peptic ulcer disease by four folds and by two folds in aspirin users. Topical injury to the gastric mucosa by ion trapping and reduction of mucus gel hydrophobicity was thought to be an important mechanism of NSAID-induced gastric damage. Later on, NSAIDs were shown to cause damage to the stomach mainly by suppression of gastric prostaglandin synthesis [3], [4].

Herbal medicines are used generally in NSAIDs cases when they are indicated to be used chronically. Major work has been carried out on herbal medicine to identify their potential efficacy in peptic ulcer prevention and/or management. These drugs may be slow in action, but mostly augment the defensive elements and are reliable and safe [5]. The production of reactive oxygen specious (ROS) plays a major role in the initiation

and development of gastric pathologies, such as gastric adenocarcinoma, peptic ulcer, or gastritis. In this manner, gastric mucosal layers represent a dynamic barrier in counteracting the corrosive effects of noxious agents through a series of endogenous antioxidant defense mechanisms. Indeed, this increase of oxidative damages is known to be related to the destructive factors-induced gastric mucosal injury [6].

Salvia is the biggest genus of the mint family, comprising about 900 species distributed diversely throughout the world. Salvia plants are used commonly in folk medicine as a tonic, antirheumatoid and chronic pain killer and several studies have shown that the Salvia genus is a valuable source of the most potent antioxidants [7].

Till the discovery of the antibiotics, Salvia was a common constituent of herbal tea mixtures, recommended to patients with tuberculosis to prevent sedation, and was found to be an active ingredient in compound plant preparations for the treatment of chronic bronchitis. Several species are used in traditional medicine all around the world to treat microbial infections, cancer, malaria, inflammation, and disinfect homes after sickness [8].

2. Material and methods

Thirty-two male Wistar rats with weight range of 185-220 g were used in this study in the Biotechnology research center of the University of Al-Nahrain and the study procedure complied with the Iraqi Regulatory Board (IRB).

Fifty grams of *S. Palestina* were dried and finely powdered and then were put (separately) in the suxhlet with adding Ethanol as a solvent (absolute ethanol) by continuous flow method which was proved more efficient than the standard method. The procedure took a few hours and the final extract was left to evaporate the ethanol content and a final crude extract was collected [9].

The experiment was designed as four groups; induction group (indomethacin), negative control (sham), *S. Palestina*, and standard (ranitidine). All animals were deprived of food for 24 hours before the experiment but free to access water. Gastric ulcer was induced by a single dose of indomethacin (50 mg/kg) by oral gavage on empty stomach according to the method of Shahin and coworkers [10]. Gastric ulcer was confirmed grossly after 24 hours before proceeding with the experiment. The treatment started on the next day and continued for 7 days and then the animals were sacrificed by anesthesia overdose and their stomach was removed. Ranitidine (50 mg/kg) was used as a standard agent for the treatment of gastric ulcer [11].

2.1 TBARS, TNF-α, PGE2

Gastric tissue samples had been kept in cool phosphate buffer saline and homogenized and centrifuged by cool ultra-centrifugate and the supernatant had been collected and used in the ELISA assay kit. After preparing the number required of strips and wells, we add the standard and samples to the wells then add the anti (TBARS, TNF-α, PGE2 individually) to the sample wells, cover and incubate and then wash for 5 times and then add substrate A and B subsequently to each well, cover and incubate finally add stop solution and determine density at 450 nm [12].

2.2 Glutathione Immunohistochemistry Study

The immune-histochemistry technique has been used to determine glutathione expression in the sample. After serial sectioning for the paraffin blocks was done, paraffin slides were deparaffinized and rehydrated then hydrogen peroxide was added and washed in tris buffer saline. After incubation slides were washed one more time by Triss buffer saline and the primary antibody was added to the slides and incubated and then after washing, the secondary antibody was added and washed after incubation. Horseradish peroxidase was applied

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to the tissue and slides were incubated and washed by buffer and then treated with diluted liquid diaminobenzidine chromogen. Slides were washed and counterstaining was done by Myers hemotoxylin and tissues were mounted and coverslip and examined [13].

2.3 Histopathological examination

Tissues of all groups were harvested at the end of the experiment (day 8) and histopathological changes of each stomach tissue were evaluated and scored according to the method done by [14]; 0 score for normal mucosa, 1 score for Erosion of epithelial cells, 2 scores for Erosion of epithelial cells and lamina propria, 3 scores for Erosion until muscularis mucosa, 4 scores for Erosion until sub mucosa.

2.4 Statistical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). One-way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. Post hoc tests are an integral part of ANOVA. $P < 0.05$ is considered statistically significant.

3. Results

3.1 PGE2

The *S. Palestina* showed a significant rising in the PGE2 as compared with the induction group (Figure 1) The Indomethacin caused a significant reduction in PGE2 level compared to sham group in opposite to ranitidine which caused a significant elevation ($P<0.05$). The difference between *S. Palestina* and ranitidine was not significant.

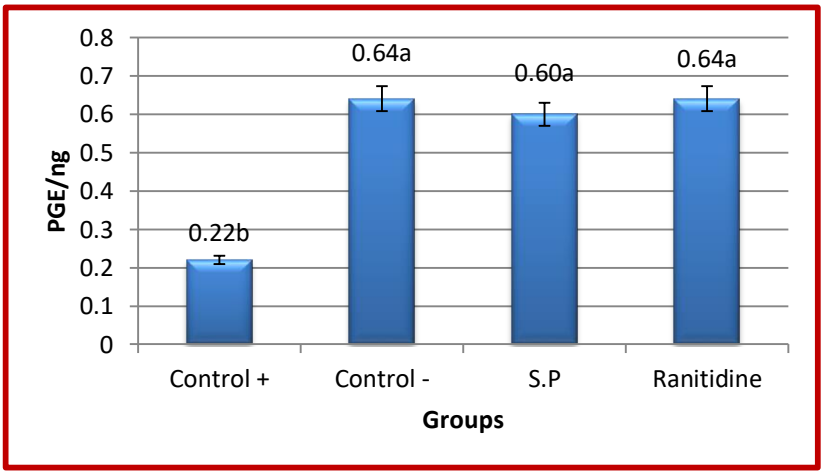

Figure (1): PGE2 level in gastric mucosa of groups

3.2 TBARS

The result showed that *S. Palestina* was reduced the TBARS level significantly (Figure 2) however it did not bring it to the normal level of sham group. TBARS level was significantly elevated in indomethacin group compared to sham group, whereas the ranitidine was reduced TBARS level significantly compared to indomethacin.

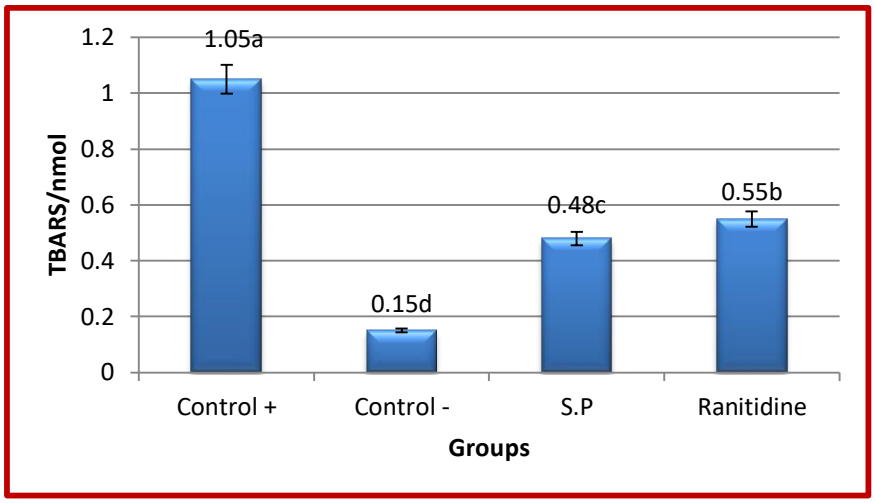

Figure (2) TBARS level in gastric mucosa of the study groups

3.3 TNF-α

The *S. Palestina* result was insignificantly different from ranitidine in reduction of TNF-α level in the rat gastric mucosa (Figure 3). Indomethacin caused a significant elevation in TNF-α level (P< 0.05) in comparisons to sham group, while rantitdine significantly reduced TNF-α level.

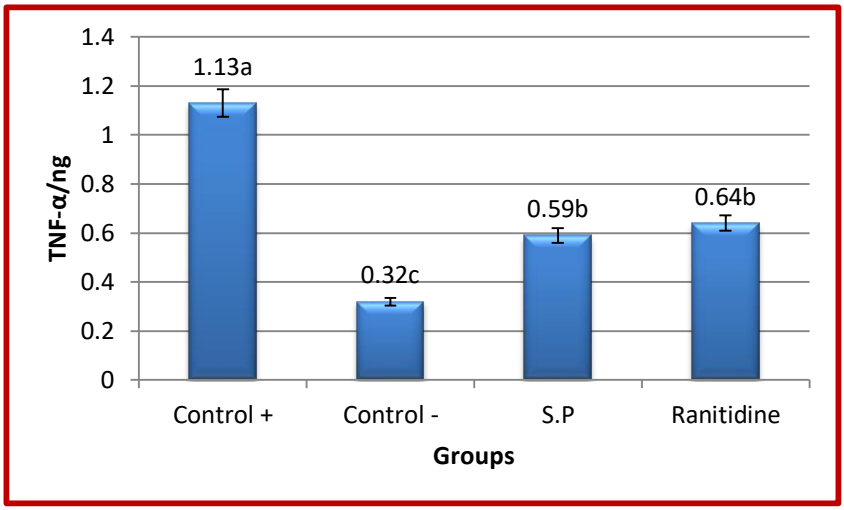

Figure (3) TNF-α level in gastric mucosa of the study groups

3.4 Glutathione

The results illustrated that the *S. Palestina* significantly increased glutathione level compared to the induction group (Figure 4). Indomethacin caused a significant decrease in tissue glutathione expression compared to sham group (which is expressed as color intensity as in figure 5), while ranitidine significantly increased glutathione level compared to induction group.

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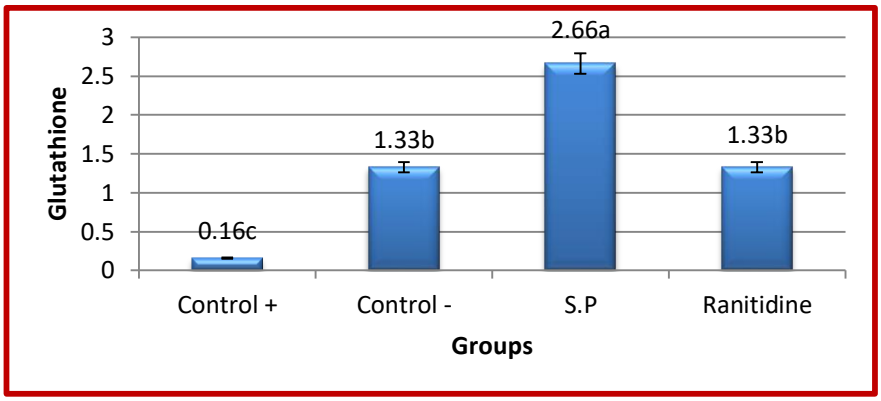

Figure (4): Glutathione scoring in gastric tissue among study groups

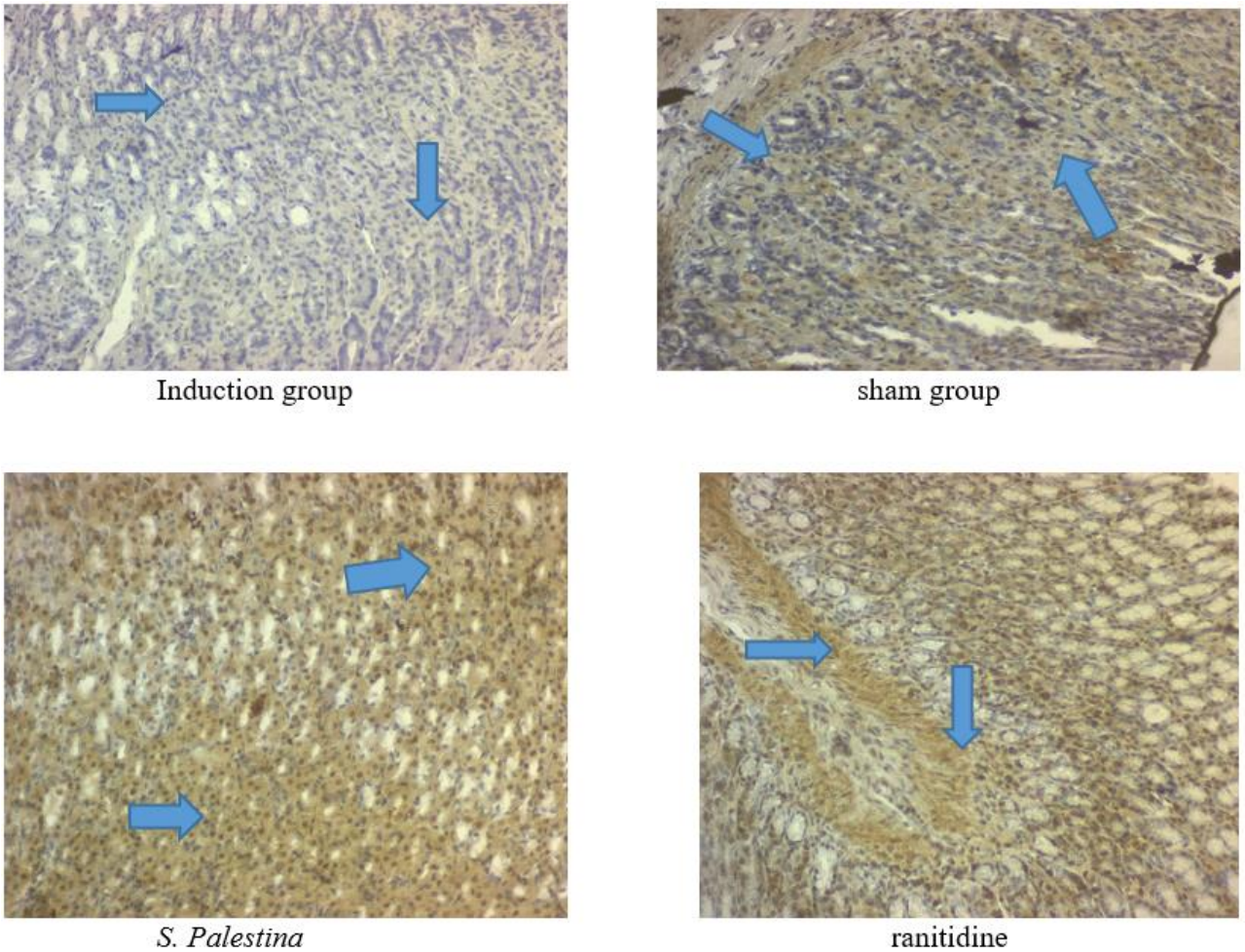

Figure (5) glutathione immunohistochemistry expression in study groups

3.5 Histopathological examination

Histopathological study showed a significant damage in induction group compared to sham group (figure 7) while the control group (ranitidine), *S. Palestina* treatment groups showed an improvement in histopathological scoring compared to induction group ($P < 0.5$). Gastric ulcer induced by indomethacin was evidenced by the histopathological scoring and the gastric tissue obtained from the indomethacin only group (induction group) which showed evidence of mucosal injury, loss of epithelial layers, decreased mucosal thickness with the distortion of mucosa and its glands and inflammatory cells infiltration. In *S. Palestina* case

the damage were superficial (mostly erosions) with preserving integrity of gastric pits and secreting glands (figure 6).

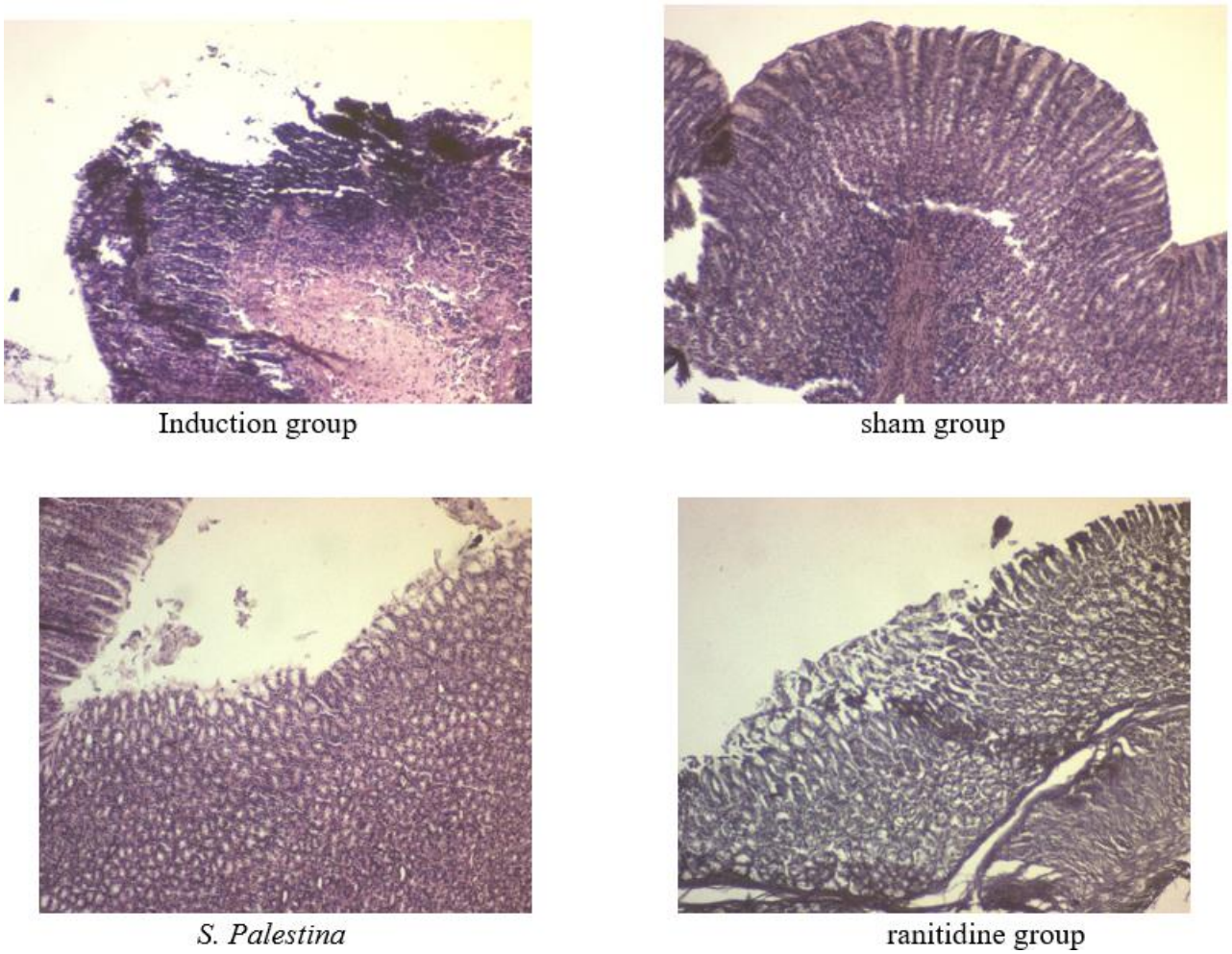

Figure (6): Hematoxylin and eosin staining of study groups

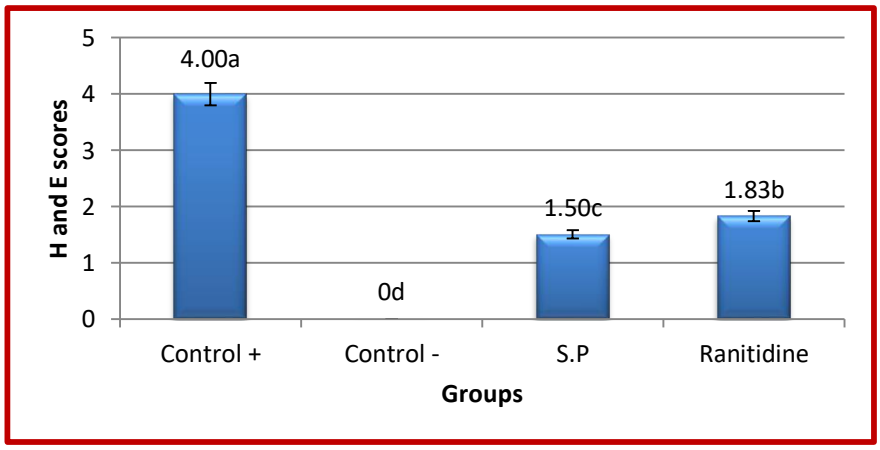

Figure (7) H & E scoring of gastric ulcers among groups

4. Discussion

In this study, we used indomethacin as an induction model for gastric ulcer and it resulted in gross morphological injuries in the stomach as well as histopathological changes to the gastric mucosa [15]. Among

all NSAIDs, indomethacin is the most ulcerogenic that exerts its damage through induction of a range of proinflammatory mediators, inhibition of gastroprotective cox 1, and angiogenesis [16]. Many studies proposed a link between leucocyte infiltration (induced by indomethacin) and the generation of free radicals. Impairment of leukocyte recruitment and depletion resulted in a reduction in more than half of ulcer cases related to indomethacin-induced ROS formation [17].

The study results showed a significant reduction in PGE2 level in the indomethacin only (induction) group as compared to the normal group, many studies showed that indomethacin causes gastric ulcers by inhibiting COX1 in addition to the fact that administration of exogenous PGE2 analogs (misoprostol) prevented ulcers due to indomethacin use. PGE2 is believed to improve gastric protection by increasing mucin production, improving blood flow and decreasing gastric acid secretions [18]. However, there are some data suggesting no direct parallelism between indomethacin (NSAIDs) induced gastric damage degree and COX enzyme and/or PGE2 level (lansoprazole is an anti-ulcer with no effect on PGE2 level) [19]. Ranitidine treatment significantly increased PGE2 level compared to the indomethacin group, this agrees with previously done studies which proved that treatment with ranitidine significantly increased mucin and PGE2 levels and reduced ulcer size and concluded that combination of ranitidine with citrulline afford better gastroprotection than either alone [20]. *S. Palestina* showed a significant increase in PGE2 level compared to the indomethacin group. Results of [21], in his studies which was consistent with several previous studies, gives the evidence that phenolic compounds present in the medicinal plant extracts suppress mucosal lesions through regulation of NO and PGE2 production thereby preventing the accumulation of the inflammatory cells and enhancing the antioxidant enzyme activity.

Indomethacin only group showed a significant increase in TBARS compared to the negative group which comes consistent with earlier reports of indomethacin-induced ulcer that showed load and clear evidence of increasing TBARS levels after oral indomethacin use which was attributed to phospholipid oxidation [22]. Ranitidine treated group reduced the level of TBARS as it prevented lipid peroxidation for its anti-secretory and its anti-oxidant effects that prevented further tissue damage [23]. *S. Palestina* showed a significant reduction in TBARS level a result came consistent with Glutathione results which proves the anti-oxidant effect that protected against lipid peroxidation. Results agree with the concept of anti-oxidant prevent lipid peroxidation [24].

The induction group showed significant elevation in TNF-α ($P < 0.05$) when compared to the sham group (negative control) which was expected as indomethacin was shown to cause elevated levels of TNF-α which is known to play roles in gastric injury and necrosis [25].

Inas and co-workers found in 2011 that indomethacin causes up-regulation of TNF-α biosynthesis and they attributed this effect to the fact that indomethacin inhibition of prostaglandin E2 is responsible for TNF rise, and inhibition of TNF-α will ultimately inhibit tissue destruction [26].

Ranitidine treated group (standard group) showed a significant reduction of TNF-α than the positive control group ($P < 0.05$) and that is consistent with previously results obtained by [27] who showed the protective effect of ranitidine of gastric ulcer and its reductive effect on TNF-α compared to indomethacin only group. *S. Palestina* caused a significant reduction to TNF-α when compared to indomethacin-only group ($P < 0.05$) and this effect may be caused by the anti-inflammatory effect which was well documented by previous studies [28]. [29] found in a study on six salvia species (including *S. Palestina*) that the extract of *S. Palestina* has an anti-inflammatory effect in a dose-dependent manner, the effect was evaluated on their preventative effect on ear oedema suggesting anti-TNF-α effect. In another study, *Salvia sclarea* (a member of luminacea family)

extract produced an anti-inflammatory effect on induced peritonitis in rats which was attributed to inhibition of TNF-α, IL-6, and IL-1β. Rosmaric acid was predominant in the extract [30].

It's well known that reactive oxygen species (ROS) has a role in the pathogenesis of gastric lesions induced by indomethacin since its cause a significant decrease in glutathione level due to depletion for the interaction of the free radicals which are produced from infiltration of neutrophils [31], and this agrees with the results of our study that showed a significant reduction of glutathione in gastric tissue from indomethacin only group. The groups treated with ranitidine, *S. palestine* showed a significant increase in glutathione level compared to indomethacin-only group, this result came consistent with TBARS results which all prove the free radical scavenging efficacy [32]. Both the enzymatic and non-enzymatic antioxidant systems are necessary for cellular reaction with oxidative stress under normal physiological conditions. Glutathione is a very important intracellular antioxidant element which is a direct scavenger to remove reactive radicals and protect the cells from possible oxidative damage [33]. [34] found that the alcoholic extract of different salvia plants (including *S. Palestina*) have high antioxidant activity with good correlation with the total phenolic content of the extract and therefore he concluded that the antioxidant efficacy of *S. Palestina* can be used to treatment of prevention of oxidative stress related disease.

5. Conclusion

The study concluded that extract of *Salvia Palestina* has ulcer healing properties possibly through enhancing gastric defense system with antioxidant and free radical scavenging effect along with anti-inflammatory properties that reduced neutrophil infiltration and free radical generation.

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