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Research Article

Anti-ulcer properties, cytokines, and apoptosis regulatory effects of *Olea europaea* leaves from Hail Province, Saudi Arabia

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Abstract

This study investigated the anti-ulcer properties of raw olive leaf powder (OLP) and its immunomodulatory potential through the cytokine network. The efficacy of OLP extract in treating stomach ulcers in rats in ethanol-induced models was examined using a single dosage (100, 200, 400 mg/kg) in groups 4, 5, and 6. The OLP demonstrated substantial anti-ulcer action even at 100 mg/kg. The activity was better at 400 mg/kg and almost equivalent to the conventional omeprazole treatment at 20 mg/kg in group 3. The cytokine network was studied in groups 1, 2, 3, and 6. The cytokine network was efficiently regulated by reducing the production of cytokines such as IL-1β, IL-2, IL-4, IL-6, IL-10, and TNF-α. The levels of caspase-3 and caspase-9 were also lowered in groups 3 and 4 considerably at $p < 0.05$. It is interesting to note that the expression of IFN was greater in animals treated with OLP in group 4, as compared to animals treated with omeprazole in group 3, as well as animals from the disease control group 2, when analyzed at a significance level

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of $p < 0.05$. The results revealed that OLP has intriguing potential for anti-ulcer action, and possesses immunomodulatory capabilities to control inflammatory cytokines and apoptotic markers.

Keywords: anti-ulcer activity; apoptosis markers; cytokine network; olive leaves; raw powder

Introduction

Olive is an excellent nutritional source, which contains several medicinally valuable compounds that can be used to prevent many diseases (Marta *et al*., 2022). Olives (*Olea europaea* L.) are small trees that have a rich cultural history and significant commercial influence around the Mediterranean areas (Faten *et al*., 2012; Arafa *et al*., 2021; Clodoveo *et al*., 2022; Food and Agriculture Organization, 2022). The leaves of olives are widely used by indigenous peoples for the treatment and prevention of various ailments (Hassen *et al*., 2015; Boss *et al*., 2016). In fact, worldwide, *Olea europaea* L. ranks amongst the most popular herbal medicines. *Olea europaea* L. is one of the most widely used botanical medications globally. The medicinal benefits of *O. europae*a have long been recognized in the traditional medical literature (Rahamat *et al.,* 2022). Olive leaf extract (OLE) contains various pharmacological qualities such as gastroprotective, neuroprotective, antibacterial, anticancer, anti-inflammatory, antinociceptive, and antioxidant effects. Moreover, studies have shown that OLE has cardioprotective, anti-diabetic, and weight-reducing effects (Debra, 2018; Al-Ruqaie *et al*., 2021). Millions of olive trees are grown in the Hail region of Saudi Arabia, which makes it a major global supplier of olive oil (Wafy., 2021). Through antioxidant, anti-ulcer, anti-tumor, and anti-microbial properties of olive and its contents demonstrate a crucial role in the management of diseases. Many bioactive metabolites are present in OLE, especially phenolic compounds, flavonoids, and oleuropein, which are particularly beneficial in reducing reactive oxygen species (ROS) in ulcers (Talhaoui *et al*., 2016). This study was carried out in continuation to our earlier study (Rahamat *et al.,* 2022), to conduct a more thorough investigation of the anti-ulcer properties of OLE.

Materials and Methods

Sourcing, taxonomy, and processing of Olea europaea L. leaves

Hail Province is in north-central Saudi Arabia and shares borders with the provinces of Madinah, Tabouk, Northern Border, Riyadh, and Qassim. It is the largest province in the country. Hail Province is located in the Waadi Hail region surrounding the Shammar mountain ranges, and serves as the country's provincial capital. The olive trees used in this study were grown at Al Khitah, 27.979363 N, 41.730706 E, situated 68.4 km from Hail city. Fresh leaves were collected from the trees and transported to the laboratory in plastic bags. After the removal of contaminants by rinsing under running water from a tap, the leaves were rinsed again with Millipore water, and then air-dried on a neat floor for 10 days. Washed branches of specimens from the olive trees were identified by a registered taxonomist and then deposited at the University of Hail Herbarium, under the reference number, UOHCOP002. The voucher specimen was also donated to the University Herbarium, where it is being preserved as a source of information for the future. The air-dried leaf samples were chopped into little bits and then finely ground into a powder using a blender. This finely-powdered leaf specimen was stored in a sealed vessel.

Materials

All chemicals and organic solvents were purchased from Sigma, USA. Ejadah Medical Supplies Est, Riyadh, Saudi Arabia, supplied all items for this study.

Animals

The study was initiated after obtaining approval from the Institutional Animal Ethical Committee. Healthy adult Sprague Dawley rats weighing 150±30 g were allowed to acclimatize to the laboratory environment for two weeks at 22±08 °C and relative humidity of 56±6%, which was attained with equal durations of light and darkness. Clean water and feed were provided *ad libitum*.

Gastric ulcer model

36 male rats were randomly divided into six groups, with five animals/group. The ulcer model was affected in line with a previously published procedure, but with slight modifications (Al-Wajeeh *et al*., 2016). The groups used and the treatments given are as follows:

Group 1: Normal control group: In this group, stomach ulcers were not produced, and so, the animals did not receive any treatment.

Group 2: Ulceration group: In overnight-fasted rats, ulceration was induced by the administration of 95% (v/v) ethanol [5 mL/kg body weight (bwt)].

Group 3: These animals received omeprazole (20 mg/kg bwt in distilled H_2O) in a single dose orally, two hours prior to ethanol administration.

Group 4: These animals received OLP (100 mg/kg bwt in distilled H_2O) in a single dose orally, two hours prior to the administration of 95% (v/v) ethanol.

Group 5: The animals received OLP (200 mg/kg bwt in distilled H_2O) in a single dose orally, two hours prior to the administration of 95% (v/v) ethanol.

Group 6: The animals received OLP (400 mg/kg bwt in distilled H_2O) in a single dose orally, two hours prior to the administration of 95% (v/v) ethanol.

One hour after ethanol administration, all the animals were sacrificed with an overdose of diethyl ether, and their stomachs were quickly excised. Blood samples were collected *via* cardiac puncture technique using a sterile 21-gauge needle. The serum samples obtained after centrifugation were stored in a refrigerator at 2–8 °C prior to use in the assay of cytokine levels.

Determination of ulcer index and % inhibition of ulcer

We measured the overall mucosal surface area as well as the total ulcerated surface area of the animals' stomachs. Ulcer index (U.I.) was determined as per the method used in a previously published work (Sabiu *et al*., 2015), by applying the following equation:

$$U.I. = \frac{Ulcerated\ area}{Total\ stomach\ area} \times 100$$

The % inhibition of ulceration was calculated as follows:

$$\%\ Inhibition = \frac{(Ulcer\ index\ of\ control) - (ulcer\ index\ of\ test)}{Ulcer\ index\ of\ control}$$

Macroscopic and biochemical gastric assessments

The contents of the stomach and tissues were extracted for macroscopic and pathological examination. Photographs were taken using a USB digital microscope with a magnification endoscope camera. The total ulcerated area was calculated using the standard method with minimal adjustments, and the percentage inhibition was calculated using a minor modification of the standard stated in an earlier report (Njar *et al*.,

1995). Acidity was determined using a pH meter and titration with sodium hydroxide solution, and the results were presented in milliequivalents per liter (Tan *et al*., 2002). A sensitive digital balance was used to determine mucus weight.

Determination of cytokine level in serum

Cytokine network was determined in four groups i.e., groups 1, 2, 3, and 6. Pro-inflammatory and anti-inflammatory cytokines and apoptosis markers were determined using an enzyme-linked immunosorbent assay, as follows:

Interleukin-1β (IL-1β)

The serum interleukin-1β (IL-1β) level was determined quantitatively using a Rat IL-1β ELISA kit in a sandwich assay (MyBioSources, USA). This assay utilized a sandwich enzyme immunoassay for the in vitro quantitative determination of IL-1β. The standard and samples (100 µL each) were placed separately in their respective wells of strips. The plates were covered using a plate sealer and incubated at 37 °C for one hour. After incubation, the liquid medium was removed from the wells and completely drained by inverting the plates on tissue towels for five minutes. Thereafter, 100 µL of reagent A was pipetted into each well, followed by incubation at 37 °C for one hour. Thereafter, the wells were washed three times with wash buffer using a microplate strip washer (Biotek ELX50, USA), after which 100 µL of reagent B was added into each of the wells and incubated at 37 °C for 30 minutes. The strips were washed five times with wash buffer using a microplate strip washer. Then, a substrate reagent (0.090 mL) was added, and after a 15-minute incubation, the optical density of the plate content was read at 450 nm. The concentration of IL-1β was obtained by extrapolating from a standard calibration graph.

Interleukin-2 (IL-2)

The serum interleukin-2 (IL-2) level was determined quantitatively using a Rat IL-2 ELISA kit (ABCAM, USA) in a sandwich reaction. This assay utilized a captured antibody labeled with an affinity tag and a detection antibody linked to a reporter. The resultant complete conjugate was then fixed in the well by exploiting the immune-specificity of a coated anti-tag immunoglobulin. Thereafter, 50 µL of the antibody cocktail was added into each well, after which the wells were sealed and incubated under agitation in the laboratory for one hour. Then, the strips of the plate were washed three times with 1 x wash buffer, using a microplate strip washer (Biotek ELX50, USA). After washing the plates, TMB (0.10 mL) was pipetted into each well, after which they were placed on a plate agitator and incubated in darkness at laboratory temperature for 10 minutes. Finally, the reaction in each well was stopped by adding 100 µL stopping reagent. After vigorous shaking, the absorbance was read at 450 nm using an ELISA spectrophotometer (ELx800, USA). The concentration of IL-2 was obtained by extrapolating from a standard calibration graph.

Interleukin-4 (IL-4)

The serum interleukin-4 (IL-4) level was determined quantitatively using a Rat IL-4 ELISA kit (ABCAM, USA) in a sandwich reaction. This assay made use of an antibody specific for Rat IL-4 that has been impregnated on a 96-well plate, into which the samples and standards were added. The IL-4 in each specimen was attached to the fixed immunoglobulin, resulting in a positive result. The plate was closed with a lid and incubated for 2.5 hours. The plate strips were rinsed four times with 1 x wash buffer using a microplate strip washer (Biotek ELX50, USA), after which 0.10 mL of biotin-labeled IL-4 sensitive immunoglobulin was added to each well and incubated at laboratory temperature for 1 hour. Thereafter, the plate was rinsed four times and 0.10 mL of HRP-streptavidin solution (enzyme conjugate) was added into each well and incubated for 45 minutes with gentle shaking. This was followed by the addition of 100 µL of TMB 1-Step substrate reagent to all the wells and incubation for a minute in the dark. Then, the reaction in each well was stopped and

absorbance was read at 450 nm using an ELISA instrument (ELx800, USA). The concentration of IL-4 was determined by extrapolating from an IL-4 standard calibration graph.

Interleukin-6 (IL-6)

The serum interleukin-6 (IL-6) level was determined quantitatively using a Rat IL-6 ELISA kit (MyBioSource, USA). This assay employed the same double antibody sandwich technique that was used for IL-2 and IL-4. In essence, the standards and samples (0.10 mL each) in their respective wells of strips were kept at 37 °C for 1½ hours, prior to washing the ELISA plates two times with wash buffer. Then, 100 µL of biotinylated IL-6 antibody was added to each well. The plate was sealed and incubated at 37 °C for 60 minutes. The strips of the plate were washed thoroughly (three times) using a microplate strip washer (Biotek ELX50, USA), after which 0.10 mL of enzyme complex was added. Following ½ hour of incubation at 37 °C, the strips were rinsed using a microplate strip washer. Then, 100µL of the prepared color reagent was added to each well and incubated at 37 °C for 30 minutes. After 30 minutes, 0.10 mL of reagent C was added, and the content of each well was thoroughly mixed for one minute on a plate shaker, after which absorbance was measured at 450 nm using an ELISA spectrophotometer (ELx800, USA). The concentration of IL-6 was determined by extrapolating from a standard curve and expressed in pg / mL.

Interleukin-10 (IL-10)

The serum interleukin-10 (IL-10) level was determined quantitatively using a Rat IL-10 ELISA kit (Abcam, USA). This assay employed a captured antibody labeled with an affinity tag and a detection antibody coated with a reporter to immunocapture the sample analyte in the solution. The assay was performed in line with established protocol to determine the IL-2 in the serum. Then, IL-10 concentrations were determined by extrapolating from a standard calibration graph.

Interferon gamma (IFN – γ)

The *in vitro* Simple Step ELISA for Interferon (IFN) gamma was designed for determining the IFN gamma protein levels in the rat serum. In the assay, 50 µL of the standard or sample was placed in the appropriate well of strip, after which 0.050 mL of immunoglobulin was added. The well was then sealed and incubated (with agitation) for 60 minutes, and rinsed thrice with 1x wash buffer using a microplate strip washer (Biotek ELX50, USA). Then, after the addition of 0.10 mL of TMB substrate solution to the individual wells and incubation in darkness at room temperature for 10 minutes, the reaction was stopped using 0.10 mL of stopping reagent. The optical density of each well was then read at 450 nm using an ELISA instrument (ELx800, USA), and the concentrations of IFN–γ were determined by extrapolating from a standard calibration graph.

Tumor necrosis factor (TNF-α)

The serum Tumor necrosis factor (TNF-α) level was quantitatively evaluated using a Rat TNF-α ELISA kit (MyBioSource, USA). The kit utilizes a sandwich enzyme immunoassay for in vitro quantitative detection of TNF-α in rat serum. In this assay, 0.10 mL of the standard and 0.10 mL of the sample were added to the respective wells of strips. The plate was then sealed and left at 37 °C for 1½ hours, after which the medium in each well was replaced with 0.10 mL of biotinylated antibody, and then sealed and incubated for 1 hour. Then, after rinsing thrice with buffer using a microplate strip washer (Biotek ELX50, USA) and soaking for two minutes after each wash, 0.10 mL of HRP complex was added to each well of the washed plate. After sealing and incubating at 37 °C for ½ hour, rinsing was done thrice using a microplate strip washer, with soaking for two minutes after each wash. Then, 90µL of substrate solution was added to the individual wells, followed by incubation in the dark at 37 °C for 15 minutes. Finally, 50 µL of stopping reagent was added to every well, and

after mixing the contents, optical density was read immediately at 450 nm using an ELISA instrument (ELx800, USA). The concentrations of TNF-α were obtained by extrapolating from a standard calibration graph.

Determination of serum apoptosis markers Caspase-3 and 9

The serum levels of caspase-3 and caspase-9 were evaluated quantitatively using a Rat Caspase-3 and Caspase-9 ELISA kit (MyBioSource, USA). The kit utilizes a double antibody sandwich enzyme immunoassay for the in vitro quantitative determination of caspase-3 and caspase-9 levels in rat serum. In this method, 0.10 mL each of the sample and standard were added to the respective wells of strips. Then, the plate was sealed and incubated at 37 °C for 90 minutes, and then washed two times with 1x wash buffer using a microplate strip washer (Biotek ELX50, USA). After this, 0.10 mL of biotinylated antibody was added and incubated for 60 minutes at 37 °C. The plate was washed three times with 1x wash buffer using a microplate strip washer, after which 100 µL of enzyme complex was pipetted into each well, and then sealed and incubated at 37 °C for ½ hour. After rinsing the plate five times with buffer using a microplate strip rinser, 100µL of the color reagent prepared was pipetted into each well, followed by incubation in the dark at 37 °C for 30 minutes. After incubation, 100 µL of Color Reagent C was added to the individual wells, and after thorough mixing, absorbance was read at 450 nm using an ELISA instrument (ELx800, USA). The concentrations of caspase-3 and caspase-9 were determined by extrapolating from a standard calibration graph.

Statistical analysis

Data is presented as mean ± SD. Comparison amongst groups was done using ANOVA and Dunnett's multi-comparison test. Values of $p < 0.05$ indicated statistically significant differences. Statistical analysis was performed using GraphPad Prism 9 software, USA.

Results

Table 1 shows the impacts of OLP and omeprazole on the treatment of gastric ulcers in Wistar rats. Figure 1 shows the hemorrhagic lesions on the glandular part of the rat stomachs macroscopically. According to the results, the measured ulcer area of animals in the control group was 611±32 mm^2. When compared to the control group, the ulcer area of the groups subjected to treatment with OLP at a dose of 400 mg/kg bwt was significantly reduced, measuring 120 ± 9.4 mm^2, indicating 74 ± 1.9 % inhibition. On the other hand, omeprazole treatment reduced the ulcer area to 90 ± 3.5 mm^2, indicating a 79 ± 1.2 % healing rate. In the ulcer control group, the therapeutic efficacy of OLP at a dose of 400 mg/kg bwt was somewhat lower than that of omeprazole at a dose of 20 mg/kg bwt. Table 2 shows the levels of proinflammatory and anti-inflammatory cytokines. There were marked increases in IL-1β, IL-6, and TNF-α in group 2, after the induction of ulcers. However, the levels of these factors were decreased significantly in the treatment groups (groups 3 and 4; $p < 0.05$). These results are shown in Figure 2. Moreover, TNF-α was observed to have the highest percentage increase among the cytokines, followed by IL-1and IL-6. On the other hand, the percentage cytokine reductions achieved following treatment with OLP were in a different sequence: the most inhibitory effect was on TNF-α concentration, which had an 85.3 % reduction, followed by IL-1 concentration, which showed approximately 81% inhibition. Surprisingly, the percentage of inhibition of IL-6 was much lower, when compared to those of TNF- and IL-1 (Table 2).

Table 1. A comparative study on the effects of Olive leaves and omeprazole in the treatment of ulcer in Wistar rats

Groups	Treatment	Ulcer area (mm^2)	% of inhibition	Mucus weight	pH
1	Normal control	0.00	0.00	2.8 ± 0.11	3.61 ± 0.09
2	Ulcer Control	611±32	NA	0.95 ± 0.2	3.61 ± 0.21
3	Omeprazole	90±3.5	79±1.2	1.45 ± 0.3	6.54 ± 0.09
4	OLP (100 mg/Kg)	302±20.5	59±3.6	1.4 ± 0.22	5.2 ± 0.081
5	OLP (200 mg/Kg)	213.4±2.5	68.2±2.2	2.0 ± 0.18	5.6 ± 0.065
6	OLP (400 mg/Kg)	120±9.4	74±1.9	2.9 ± 0.09	6.1 ± 0.19

OLP: Olive leaves Powder

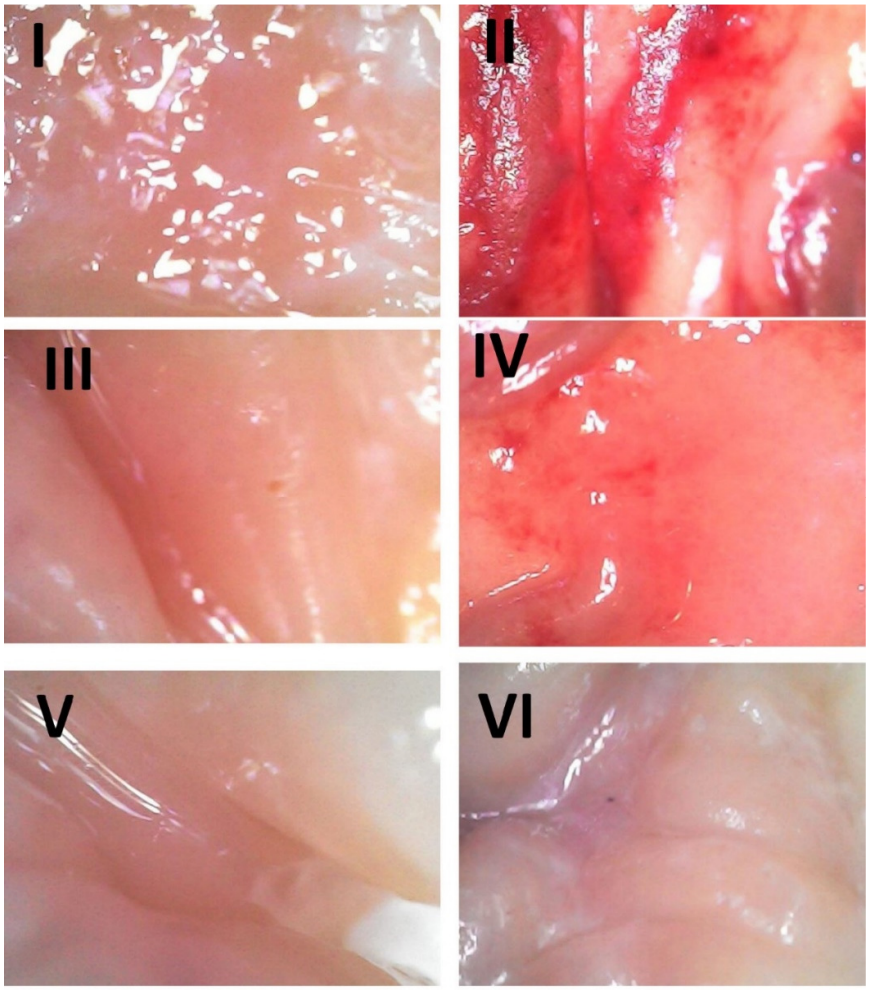

Figure 1. Examination of hemorrhagic lesions macroscopically on the glandular part of the rat stomach
I, II, III, IV, V and VI are representative photos from groups 1, 2, 3, 4, 5 and 6 respectively. Photo II showed the most severe hemorrhagic lesions on the glandular part of the stomach obtained from Group 2.

Table 2. The Cytokines level before and after treatment

Type of cytokines	Cytokine level (pg/mL)						
	Group 1	Group 2	% Increase	Group 3	% Decrease	Group 4	% Increase / Decrease
IL-1β	40.14 ± 5.91	506. 27 ± 23.99	1144	79.98 ± 6.4	84.2	39.9 ± 4.7	92.11 (D)
IL-6	48.93 ± 3.3	424.76 ± 10.7	768	201.9 ± 39	52.5	161.6 ± 9.8	61.96 (D)
IL-2	0.4 ± 0.15	24.13 ± 1.4	5932	3.51 ± 0.66	85.45	1.533 ± 0.44	93.64 (D)
IL-4	0.75 ± 0.04	3.834 ± 0.336	724.8	2.03 ± 0.21	47	2.48 ± 0.612	35.31(D)
IL-10	41.13 ± 1.3	226.44 ± 25.82	450.54	69.98 ± 6.3	69	74.75 ± 8.8	66.98 (D)
IFN – γ	4.7 ± 2.25	26.3 ± 1.9	459.57	24.8 ± 1.82	5.08	35.6 ± 1.11	36.24 (I)
TNF-α	23.69 ± 4.89	899.95 ± 62	3698	131.95 ± 7.56	85.3	84.48 ± 6.12	90.61 (D)

Each value is the mean of five samples with standard deviation. *(D) Decrease (I) Increase

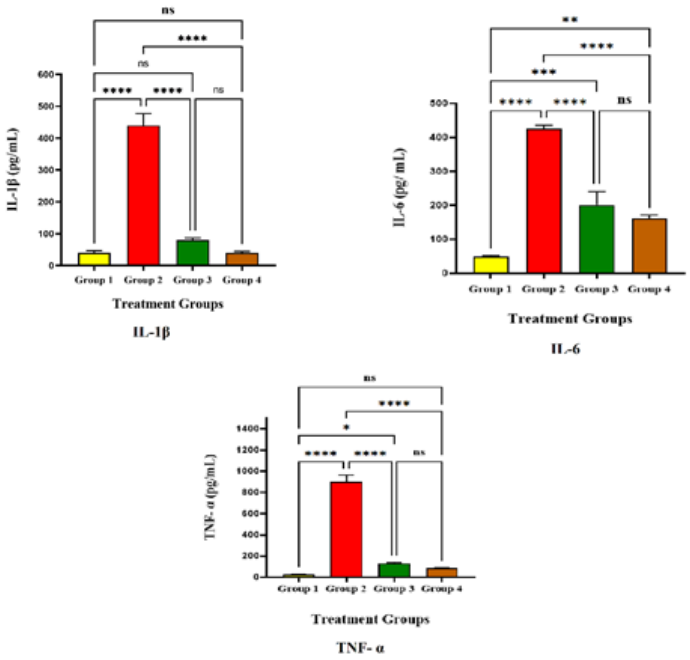

Figure 2. Study on Proinflammatory cytokines

**** The values are very high significant at $p< 0.05$ level. Highly significant at $p< 0.05$ level. *** The values are high significant at $p< 0.05$ level. Highly significant at $p< 0.05$ level, ** significant at $p< 0.05$ level, * significant at $p< 0.05$ level, ns: non-significant at $p< 0.05$ level

Figure 3 presents the anti-inflammatory cytokine levels before and after the treatments. The figure depicts a non-significant decrease in IL-2 in group 4 ulcer animals treated with OLP, when compared to group 3 ulcer animals treated with omeprazole. However, the IL-4 level was decreased significantly in group 4 ulcer animals treated with OLP, when compared to group 3 ulcer animals treated with omeprazole ($p < 0.05$). On the other hand, the levels of IL-10 and IFN-γ were increased in group 4 ulcer animals, relative to those in group 3. Interestingly, there was no marked increase in IL-10 in group 4 ulcer animals that were given OLP, when compared to group 3 ulcer animals treated with omeprazole. In comparison with other anti-inflammatory cytokines, the level of IL-2 was substantially elevated. IL-2 had the highest percentage rise, followed by IL-4, IFN-γ, and IL-10. However, following treatment with OLP, IL-2 levels increased by 85.45 %, IL-10 by 69 %, and IL-4 by 47 %. However, IFN–γ was reduced by only 5.08 % due to the OLP treatment (Table 2). The apoptosis marker caspases-3 and -9 in group 2 showed a marked increase after the induction of ulcers (Table 3). The results showed that both caspase-3 and caspase-9 were significantly reduced when compared to the disease group 2 (Figure 4). However, the decrease in the levels of caspases-3 and -9 was not significant in the

OLP-treated animals of group 4, when compared to the omeprazole-treated animals in group 3 ($p < 0.05$). From this study, it is evident that OLP exerted immunomodulatory properties, which led to the cure of ulcers.

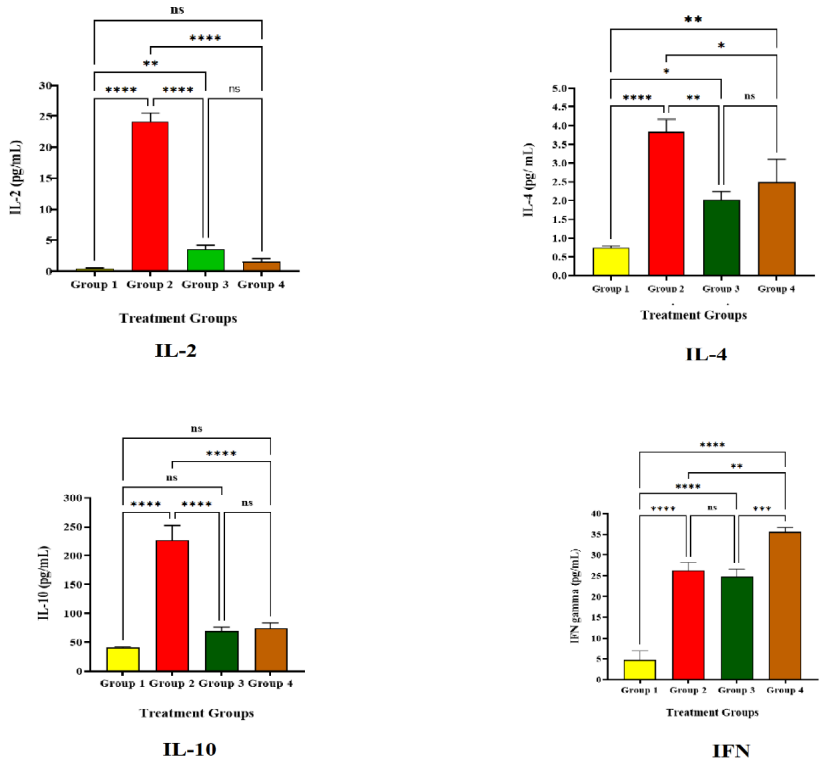

Figure 3. Study on Anti-inflammatory cytokines

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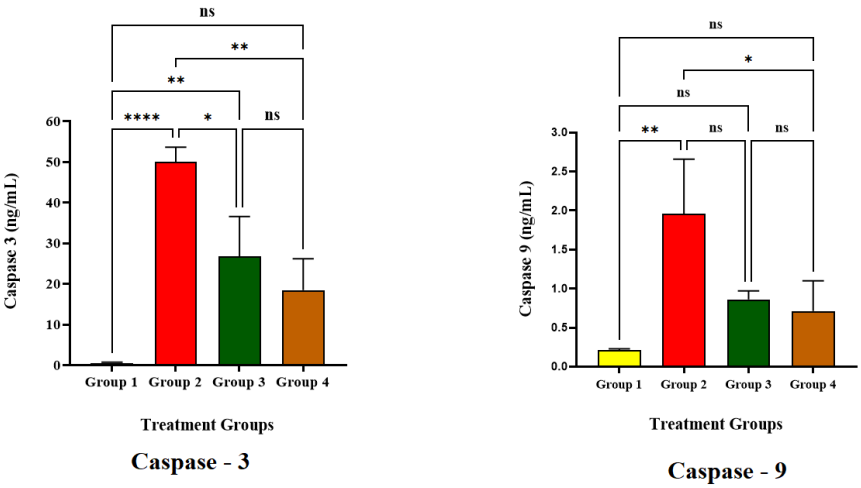

Figure 4. Study on apoptosis markers

**** The values are very high significant at $p < 0.05$ level. Highly significant at $p < 0.05$ level. *** The values are high significant at $p < 0.05$ level. Highly significant at $p < 0.05$ level, ** significant at $p < 0.05$ level, ns: non-significant at $p < 0.05$ level

Table 3. Apoptosis markers level

Type of cytokines	Apoptosis markers level (ng/mL)						
	Group 1	Group 2	% Increase	Group 3	% Decrease	Group 4	% Decrease
Caspase 3	0.55 ± 0.15	49.99 ± 3.61	8989	26.78 ± 9.8	46.4	18.45 ± 7.71	63.09
Caspase 9	0.214 ± 0.014	1.95 ± 0.69	5277	0.86 ± 0.107	55.89	0.713 ± 0.38	63.4

Discussion

Alcohol use can cause stomach ulcers; excessive drinking raises the risk of gastric mucosal injury. Ethanol induces gastric ulcers by reducing the levels of protective factors in the stomach mucosa (Franke *et al.,* 2005; Choi *et al.,* 2009). The present study focused on establishing the healing effect of OLP on gastric ulcers and the involvement of cytokine network modulation in the process. The results revealed that the efficacy of OLP was better in modulating inflammatory cytokine levels, when compared to the standard omeprazole treatment. The presence of α-linoleic acid, palmitic acid, oleamide, palmitic acid β -monoglyceride, squalene, coumarins, and vitamin E in OLP has been reported earlier (Rahamat *et al*., 2022). IL-1β is a vital cytokine that controls inflammation-linked responses in the gastrointestinal mucosa. By inhibiting the migration of neutrophils produced by chemotaxis, IL-1β plays a crucial role in regulating some gastric functions and preserving the integrity of the gastrointestinal mucosa. In the current research, IL-1β level was very high in the ulcer animals in group 2. An earlier study reported that IL-1β levels were increased in patients with alcoholic liver disease in response to inflammatory stimuli and endogenous danger-associated molecules (Petrasek *et al*., 2012). IL-6 has pleiotropic activity, meaning it can operate as either a pro-inflammatory cytokine or an anti-inflammatory myokine. In reaction to tissue damage, IL-6 is produced almost instantly but only for a short time (Tanaka *et al*., 2014). TNF- α is an inflammatory cytokine produced by macrophages/monocytes during acute inflammation. Up-regulated levels of pro-inflammatory cytokines in gastric ulcers have been reported by earlier studies (Lanas *et al*., 2001; Gyulai *et al*., 2004; Dipanjana *et al*., 2011; Wen *et al*., 2011)

IL-2 is an important immune response regulator that has a significant impact on both pro-inflammatory and anti-inflammatory responses (Ng *et al*., 2003). The expression of IL-2 in gastric ulcers was reported in an earlier study (Sugimoto *et al*., 2010). A protein that performs a significant role in the regulation of anti-inflammatory responses is IL-4; it functions as a growth factor for B and T cells. IFN-γ activates macrophages and natural killer cells. A previous study had reported a high expression of IFN-γ in gastric ulcers (Obonyo *et al*., 2002). Increased IL-10 has also been reported earlier in a gastric ulcer model (Jesús *et al*., 2020). Earlier studies suggested that the immunomodulatory effect of OLP might be due to the presence of α-linoleic acid and palmitic acid. Zhao *et al*. (2007) reported that the dietary intake of α-linoleic acid can inhibit pro-inflammatory cytokine networks. On the other hand, palmitic acid triggers NF-kB-dependent mechanisms, which lead to the generation of proinflammatory factors such as IL-6, Il-1β, and TNF. However, palmitic acid also inhibits inflammation-related factors through the inhibition of expressions of macrophages (Lee *et al*., 2010). In contrast, this research showed that macrophage activator IFN-γ was markedly increased in the ulcer animals; it was not reduced in the OLP-treated ulcer animals when compared to those treated with omeprazole. From the study, it is obvious that OLP created a balance between pro-inflammatory and anti-inflammatory activities. On the other hand, α-linoleic acid caused a decrease in IL-6 and an increase in IL-10 in Wistar rats, resulting in the inducing of an anti-inflammatory effect (Figueiredo *et al*., 2011). In the present study, the levels of IL-10 and IL-4 were reduced significantly, when compared to ulcer animals. However, their levels were more than those in omeprazole-treated animals. Caspase-3 is an essential protein that is a part of the apoptosis mechanism. It cleaves many cellular proteins, and as a result, triggers the removal of cells that have become old, damaged, or autoreactive. Caspase-9 is a protease that is involved in the apoptotic process, and has been related

to mitochondrial damage. An earlier study suggested that aqueous OLE exhibited anti-apoptosis markers by reducing the levels of caspases 3 and 9 due to the presence of oleuropein and hydroxytyrosol (Morandi *et al*., 2021). Yaguchi *et al*. (2010) reported the inhibitory effect of linoleic acid on caspases 3 and 9. Thus, OLP created a balance between proinflammatory and anti-inflammatory activities, which was reflected in the anti-ulcer effect.

Conclusions

Medicinal plants have historically been the principal method of treating many diseases worldwide and are necessary for maintaining optimum health**.** Consumption of vegetables and fruits is advantageous for the maintenance of good health. The demand for the development of novel pharmaceuticals is rising gradually and consistently. Since the time immortal, plant resources have been outstanding resources for the development of therapeutic substances. Olives are rich in numerous pharmacologically important biochemicals that aid in the prevention of a wide range of illnesses. This study has proved the significance of raw olive leaves in the prevention of stomach ulcers. Intriguingly, the leaves were capable of altering both pro-inflammatory and anti-inflammatory cytokines, in addition to apoptotic markers.

Authors' Contributions

RUS: Principal investigator, Conceptualization, funding resources, proofing, editing; SMS: Conceptualization, experimentation, investigation, processing of results, data analysis, writing; HB, SSA, SSA, TKMA, MHA, ASAL, HEH, ASA: Plant collection, processing, data analysis; SIA, MT: Experimentation

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

The study has been properly approved by the Institutional Animal Ethical Committee before commencing the experiment. MRC/JU/1443/SA1.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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