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Exploring the Antioxidant Defense: Super Oxide Dismutase and Glutathione Peroxidase as Key Biomarkers in *Juniperus Macrocarpa's* Antiulcer Effect on Rats Model

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Abstract

Background. *Juniperus macrocarpa* is a native North American species of juniper which is known as Rocky Mountain *juniper* which showed diverse array of biological activities, encompassing antioxidant, anti-inflammatory, analgesic, antibacterial, antifertility, and hypoglycemic properties. Chemicals such as phenolic and volatile that associated with J. macrocarpa exhibit significant biological potential, **Aim.** The present study aimed to explore the anti-ulcerative properties of *J. macrocarpa* against non-steroidal anti-inflammatory drug-induced peptic ulcers in albino rats, focusing on the histological changes and on the antioxidative enzymes Glutathione Peroxidase (GPX) and Superoxide Dismutase (SOD) level. **Methods.** Forty-two healthy male albino rats were used in this study, aged 6-12 months and weighing 160-250g, divided into 7 groups and subjected to peptic ulcer induced using indomethacin. The groups were treated with omeprazole as a reference drug, pretreated with *J. macrocarpa* extract, or treated post-induction with varying doses of the extract over 14 days. *Juniperus macrocarpa* was extracted using a modified Soxhlet extraction method, with a mixture of 80% ethanol and 20% distilled water at a temperature of 50 °C for 8 hours and the total phenolic compounds amount determined in the aqueous extract by Folin-Ciocalteu reagent while aluminum chloride colorimetric method used to determine the total *flavonoid* content in the extract. **Results.** The results demonstrated the impact of *Juniperus macrocarpa* treatments on oxidative stress biomarkers. Specifically, the mean levels of SOD and GPX across different groups which lead to conclude that *Juniperus macrocarpa* significantly enhance the antioxidative defense mechanisms by improving SOD and GPX activity in treated subjects.

Keywords: *Juniperus macrocarpa*, peptic ulcer, NSAIDs, Glutathione Peroxidase (GPX), Superoxide Dismutase (SOD).

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Introduction

Gastric ulcers, a subtype of peptic ulcer disease, are primarily caused by *Helicobacter pylori* infection(1), it affect about 10% of these infected individuals(2)and exacerbated using non-steroidal anti-inflammatory drugs (NSAIDs).The pathogenesis involves an imbalance between mucosal defensive factors and aggressive elements, leading to mucosal injury(3),although *H. pylori* stomach colonization can cause histologic gastritis in all infected patients, only minority show any apparent clinical signs of this colonization, patients who test positive for *H. pylori* have 20% lifetime risk of developing ulcer disease (4). Treatment strategies have evolved significantly, including eradicating *H. pylori* with antibiotic regimens by which considered the most crucial strategy for preventing gastric ulcer with the reducing of gastric acidity by proton pump inhibitors and H2-receptor antagonists(5-6)These approaches aim not only to alleviate symptoms but also to prevent recurrence and complications (6). Recent research has also explored the potential of phytochemicals in treating gastric ulcers. *Juniperus macrocarpa*, a plant known for its medicinal properties, has garnered attention in this context. Studies have highlighted its antioxidant, anti-inflammatory, and antimicrobial activities, suggesting its beneficial role in managing gastric ulcers (7-9). The phytochemical composition of *Juniperus macrocarpa*, including flavonoids and phenolic compounds, contributes to its therapeutic effects by modulating oxidative stress and inflammation, two key processes in the pathogenesis of gastric ulcers (7). Furthermore, the antimicrobial activity of *Juniperus macrocarpa* against *H. pylori* presents a promising avenue for adjunctive therapy in gastric ulcer treatment (8-10). The mechanism of action of *Juniperus macrocarpa* in gastric ulcer treatment involves inhibiting inflammatory pathways and reducing oxidative damage to the gastric mucosa, promoting healing and offering protection against further injury (11).While further clinical studies are required to understand its efficacy and safety fully, *Juniperus macrocarpa* represents a promising natural adjunct in the comprehensive management of gastric ulcers (7, 8, 12). This study evaluates the anti-ulcer potential of *Juniperus macrocarpa* against NSAID-induced ulcers in albino rats, leveraging the plant's rich bioactive compound profile comprising flavonoids, terpenoids, polyphenols, and essential oils. These compounds are postulated to offer combined antioxidant, anti-inflammatory, and mucosal protective actions that may contribute significantly to preventing and healing gastric ulcers (12-14). In conclusion, gastric ulcers represent a significant health burden globally, with existing treatments facing challenges such as side effects and recurrence rates. *Juniperus macrocarpa* emerges as a promising alternative, leveraging its medicinal properties. Further research is warranted to fully understand its efficacy and mechanism of action in the context of gastric ulcer treatment, potentially offering a safer and more natural therapeutic option.

This study aimed to assess *Juniperus macrocarpa's* effectiveness in treating NSAID-induced peptic ulcers in albino rats, focusing on histological improvements and the modulation of SOD and GPX enzyme activities. It explores the plant's potential as a complementary therapy, paving the way for its clinical application in ulcer management.

Figure 1: *Juniperus macrocarpa* (15).

Materials and Methods

Materials

In the present study, the treatments used which include Indomethacin capsules were obtained from Ajanta/ India and omeprazole capsule from Acino/Switzerland whereas Rat Superoxide dismutase (SOD) and Glutathione (GPX) ELISA kits were obtained from Cloud-Clone Corp./USA. *Juniperus macrocarpa* leaves were collected from Zakho (northern of Iraq) in April 2023. It was identified by Dr. Ibrahim Saleh Al Jubori at College of Pharmacy - Mustansiriyah University, Baghdad, Iraq.

Plant extraction and content determination

The extraction was carried out by placing 125 grams of *Juniperus macrocarpa* powder in an extraction thimble that was placed in the Soxhlet continuous extraction device, adding a mixture of 80% ethanol and 20% distilled water at a temperature of 50°C, and the extraction process continued for 8 hours. The solvent was evaporated using a rotary evaporator under vacuum pressure at a temperature of 45°C; 20 milliliters of the sample was in an oven at a temperature of 37 °C for 10 hours to obtain a dry powder, which was kept in the refrigerator until use (9). The preparation of the alcoholic extract of plant *Juniperus macrocarpa* necessitated the use of the method described by Desmukh and Borle,1975 (16) with some modifications as shown in Table 1.

The total amount of phenolic compounds was determined in the aqueous extract with a standard Folin-Ciocalteu reagent. The reaction mixture contained 0.5 ml of the extract, 0.5 ml of the Folin-Ciocalteu reagent (Merck, Germany) and 1.5 ml of 20% sodium carbonate. The sample was then mixed on a vortex mixer and diluted with distilled water to the final volume of 10 ml. After a two-hour reaction, the absorbance at 765 nm was determined and used to estimate the phenolic content using the calibration curve made with gallic acid (Sigma-Aldrich, Germany). The total amount of phenolic compounds was expressed in mg gallic acid equivalent (GAE) per g dry weight. (17)

The aluminum chloride colorimetric method determined the total flavonoid content of the crude extract. In brief, 0.25 ml of crude aqueous extract was made up to 1 ml with methanol, mixed with 4 ml of distilled water and then 0.3 ml of 5% $NaNO_2$ solution; 0.3 ml of 10% $AlCl_3$ solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 ml of 1 mol/l NaOH solution was added, and the final volume of the mixture was brought to 10 ml with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg rutin equivalent per g dry weight (18).

A sample, 2.5 ml of extracts were filtered and mixed with 1 ml of ferric chloride. A dark green solution indicates the presence of tannins. 1 ml of extract was added to two ml of sodium chloride (2%), filtered, and mixed with 5 ml of 1% gelatin solution. A precipitate indicates the presence of tannin. And measurement absorption at = 540 nm (19).

Table 1: Contents of *Juniperus macrocarpa* extract

Name	Concentration
Total phenolic content (mg Gallic/gm)	**298.0**
Total flavonoid content (mg Rutin / gm)	**166.9**

Phytochemical screening was performed to understand more about the chemical components found in the plant's crude extract. It is a simple, quick, and low-cost process that gives the researcher quick and accurate information about the many phytochemicals in plant crude extracts. It is an essential tool in the study of bioactive chemical compounds. Because crude extract contains diverse classes of chemical constituents with varying polarities, fractionation of the crude extract is recommended for full phytochemical profile screening for a given plant. This allows the main class of plant constituents to be isolated from each other based on differences in polarity and solubility.

Study design and experimental animals

Prospective, controlled, comparable experimental animal study was conducted on Forty-two healthy male albino rats (*Rattus norvegicus)* aged between 6 and 12 months, weighed 160-250 g acquired from the animal house of Mustansiriyah University's Research Center for Cancer and Medical Genetics and were housed there. They were placed in polyethylene cages with stainless steel covers and raised bases to prevent coprophagy. The rats were kept for acclimatization for one week before the experiment. They were maintained in normal laboratory conditions (232°F, 12-hour light-dark cycle) with free access to food from a chow pallet and tap water. They fasted for 24 hours before indomethacin administration and were allowed free access to water. The study started at the beginning of January 2024 and finished in May 2024. The Institute Review Board (IRB) of Baghdad University College of Medicine approved the current research.

Animals Grouping

Group 1. Healthy animal group control (no treatment) 0.5% carboxymethylcellulose (CMC) as vehicle only.

Group 2. Ulcer control received indomethacin 60mg/kg dissolved in the vehicle).

Group 3. Indomethacin 60mg/kg with Omeprazole treated 5mg /kg was dissolved in the vehicle and buffered with sodium bicarbonate.

Group 4. Indomethacin +Treatment *Juniperus macrocarpa* plant 500 mg

Group 5. Indomethacin +Treatment *Juniperus macrocarpa* plant 300 mg

Group 6. Pretreatment *Juniperus macrocarpa* plant 100 mg + indomethacin

Group 7. pretreatment *Juniperus macrocarpa* plant 200 mg + indomethacin

All the groups received indomethacin to induce peptic ulcers, except for the first group. The second group and all the other groups presented peptic ulcers. The third group was treated with omeprazole (the reference drug), while the sixth and seventh groups were pretreated with the medicinal plant extract to evaluate its protective effect. After induction, the remaining groups received the *Juniperus macrocarpa* extract in different doses to evaluate its therapeutic effect for 14 days.

Acute toxicity study

The acute toxicity study was conducting on 24 rats that divided into 4 groups (6 in each group). During the 14-day observation period in the acute toxicity study, The test animals fasted firstly for 12 hours and had unlimited access to water. The control group was given 5% CMC, while the other three groups received a single oral dose of the medicinal plant crude extract 1000, 3000, and 5000 mg/kg that dissolved in 5% CMC solution. There were no significant changes in body weight gains. As a result, *Juniperus macrocarpa* extract (JME) 's approximate lethal dose that amount of the extract given all at once, that causes the death of 50% of test rats (LD50) was estimated to be greater than 5000 mg/kg.

Dose Selection

For the assessment of the activity of *Juniperus macrocarpa* crude extract, the high dose selected in a way that it was approximately one-tenth of the maximum dose during acute toxicity studies 300, 500mg/kg (20), While the protective doses (100,200) mg/kg were calculated according to a previous studies used a different species of the same plant (21, 22). **Assessment of oxidative stress biomarkers in the rat's serum.**

The principle of the Rat SOD Elisa kit was Quantitative Sandwich enzyme immunoassay. This kit is used for the determination of SOD level in undiluted original Rat serum, the kit is intended for in vitro and research use only according to instruction of manufacturers. The principle of the Rat GPX1 kit is the double-sandwich ELISA technique and the assay procedure performed according to the manufacturer instructions.

Statistical analysis

Statistical analysis was performed using SPSS version 25.0 with a p value of < 0.05 is considered to be statistically significant. Continuous variables are presented as mean ±SD while nominal and ordinal variables are presented as categories. Bonferroni test was used as a post-hoc test to find out the exact location of the association, if present, for all the variables tested using one-way ANOVA.

Results Fractionation

Preliminary phytochemical screening of the tested fractions showed the presence of flavonoids, phenols, alkaloids, steroids, saponin, tannin, glycoside and terpenoids as demonstrated in Table 2.

Table 2. The phytochemical analysis of Some Active Groups in *Juniperus macrocarpa* Plant Extract

Chemical Groups	Type of Reagent	Detection Result
Glycosides	Benedict's reagent	+ Red precipitate
Alkaloids	Mayer's reagent	+++ White precipitate
Tannins	1% Ferric Chloride	+++ Blue-green colour appearance
Resins	Ethyl alcohol in distilled boiling water + HCL acid	++ Turbidity
Saponins	1- Shaking the extract 2- Mercuric chloride	+++ Dense foam White color appearance
Coumarins	Sodium hydroxide + filter paper + UV source	-
Phenols	1% Ferric Chloride	+++ Blue-green colour
Flavonoids	Ethyl alcohol + Potassium hydroxide	+++ Yellow colour
Terpenes	Chloroform + Glacial acetic acid + Concentrated sulfuric acid	+++ Brown colour appearance
Steroids	Chloroform + Glacial acetic acid + Concentrated sulfuric acid	-

Key: + Available, ++ Abundantly available, +++ Highly abundantly available, - Not available.

The association between the treatment with *Juniperus macrocarpa* and the biomarkers of peptic ulceration

Superoxide dismutase (SOD)

The mean of SOD level (ng/mL) among the control group, which include rats have not been exposed to indomethacin as an inducer of gastric damage, and don't receive any drug as a treatment for the development of peptic ulcer was 7.25 (± 0.079). While the mean of SOD among the second group, which received indomethacin to induce the gastric injury, with no other drugs used for treatment was 6.068 (± 0.514). As it can be seen that the observed mean level of SOD was lower among the group of rats which haven't been treated. The means of this marker (SOD) among the following 3 groups which have been treated (after exposure to indomethacin) with either omeprazole for the 3rd group, 500 mg of *Juniperus macrocarpa* for the 4th group or 300 mg of *Juniperus macrocarpa* for the 5th group were 10.914 (± 1.853), 8.139 ±0.040) and 6.663 (± 0.064) respectively as illustrated in Table 3.

Table 3. ANOVA test for the difference between SOD mean among the 5 groups.

Groups	Mean (ng/mL)	Standard Deviation	One-Way ANOVA
			Sig. (p-value)
Control	7.250	0.079	<0.001
Exposure only	6.068	0.540	
Omeprazole treatment	10.914	1.853	
Juniperus macrocarpa 500mg	8.139	0.040	
Juniperus macrocarpa 300mg	6.663	0.064	

The results of Bonferroni test have demonstrated that the mean of SOD among the 3rd group (which is treated with omeprazole after exposure to indomethacin) was significantly higher than the mean of SOD among all other groups. The P-values for the difference between the SOD mean among the 3rd group and the 1st, 2nd, 4th and 5th groups were 0.005, 0.001, 0.043 and 0.001 respectively as shown in Table 4

. **Table 4. Bonferroni test for the association between the 3rd group and the other groups regarding SOD.**

Bonferroni test		Mean difference	Standard error	Sig. (p-value)
3rd group (Omeprazole Rx after exposure)	1st group (control)	3.663	0.901	0.005
	2nd group (No Rx after exposure)	4.846	0.987	0.001
	4th group (*Juniperus macrocarpa* 500mg)	2.775	0.875	0.043
	5th group (*Juniperus macrocarpa* 300mg)	4.251	0.875	0.001

Glutathione peroxidase (GPX)

Among the 1st group of rats (control group), the mean of GPX level was 39.569 (± 0.939) ng/mL. While the mean of GPX among the second group's rats, which have received indomethacin and haven't been treated with any substances was 29.092 (± 6.691). The mean of GPX among the 3rd group which has been treated (after exposure to indomethacin) with omeprazole was 75.711 (±17.525). While among the last 2 groups, which have been treated with the studied plant (*Juniperus macrocarpa*) with doses of 500 mg and 300 mg (after being exposed to indomethacin), the mean of GPX level was 60.065 (± 11.066) and 31.199 (± 0.615) for the 4th and 5th groups respectively. The means of GPX among the 3rd group, which has been treated with omeprazole, and the 4th group, which has been treated with *Juniperus macrocarpa* plant, are obviously higher than the means of the 1st 2nd, and 5th groups as illustrated in Table 5 which also showed the difference between the means of GPX among the 5 groups under study by One-way ANOVA test which revealed a statistically significant difference between the 5 means with a p-value of less than 0.001.

Table 5. ANOVA test for the difference between GPX mean among the 5 groups.

Groups	Mean (ng/mL)	Standard Deviation	One-Way ANOVA
			Sig. (p-value)
Control	39.569	0.939	<0.001
Exposure only	29.092	6.691	
Omeprazole treatment	75.711	17.525	
Juniperus macrocarpa 500mg	60.065	11.066	
Juniperus macrocarpa 300mg	31.199	0.6156	

The results of Bonferroni test

That illustrated in Table 6 demonstrated that the mean of GPX of the **3rd** group (which is treated with omeprazole after exposure to indomethacin) was significantly higher than the mean of GPX of **1st**, **2nd**, and **5th** groups with *p*-values of less than 0.001 for all these three associations whereas non-significantly differ from that of 4th group (p = 0.096).

Table 6. Bonferroni test for the association between the 3rd group and the other groups regarding GPX.

Bonferroni test		Mean difference	Standard error	Sig. (p-value)
3rd group (Omeprazole Rx after exposure)	1st group (control)	36.141	5.705	<0.001
	2nd group (No Rx after exposure)	46.618	6.250	<0.001
	4th group *(Juniperus macrocarpa* 500mg)	15.645	5.540	0.096
	5th group (*Juniperus macrocarpa* 300mg)	44.511	5.540	<0.001

Similarly, the Bonferroni test results showed that the mean of GPX level of the 4^{th} group, which include rats that have been exposed to indomethacin and treated with 500 mg of *Juniperus macrocarpa* were significantly higher than the means of the 1^{st}, the 2^{nd} and the 5th groups. The P-values for these 3 associations were 0.004, > 0.001 and > 0.001, respectively as shown in Table 7.

Table 7. Bonferroni test for the association between the 4th group and the other groups regarding GPX.

Bonferroni test		Mean difference	Standard error	Sig. (p-value)
4th group (*Juniperus macrocarpa* 500mg)	1st group (control)	20.496	4.917	0.004
	2nd group (No Rx after exposure)	30.973	5.540	<0.001
	5th group *(Juniperus macrocarpa* 300mg)	28.865	4.724	<0.001

The association between the use of the plant *Juniperus macrocarpa* for prevention of peptic ulcer and the biomarkers of peptic ulceration

Superoxide dismutase (SOD)

Results postulated in Table 8 showed that the mean level of SOD in the 1^{st} group was 7.250 (± 0.194). While the means of SOD of the 6th groups which received 100 mg *Juniperus macrocarpa* were 10.284 (±3.712) and 14.106 (± 4.642) for the 7^{th} group (200 mg). Lastly the mean of SOD among the 2^{th} group (which has been exposed to indomethacin without protection) was 6.068 (±0.514). The 7^{th} group has obviously higher mean of SOD than all other 3 groups (the 1^{st} group: control group and the 2^{nd} group: the induction group and the 6^{th} group: that received 100 mg of the plant extract) and these higher results when tested using the One-way ANOVA has showed that there was a statistically significant difference between these 4 means with a p-value of 0.004.

Table 8. ANOVA test for the difference between SOD means among the 4 groups.

Groups	Mean	Standard Deviation	One-Way ANOVA
			Sig. (p-value)
Control	7.250	0.194	0.004
Juniperus macrocarpa 100 mg	10.284	3.712	
Juniperus macrocarpa 200 mg	14.106	4.642	
No pretreatment	6.068	0.540	

When Bonferroni test was used to assess the extent of this association between the plant under study and prevention of gastric injury after indomethacin exposure, The 7th group of rats (the one pretreated with 200 mg of *Juniperus macrocarpa*) has a significantly higher mean of SOD than the 1st and the 2th groups (the control group and the exposure group) while there was non-statistically significant difference between the means of SOD among the 6th and 7th groups (those who have been pretreated with 100 mg of *Juniperus macrocarpa* and 200 mg of *Juniperus macrocarpa* respectively) as illustrated in Table 9.

Table 9. Bonferroni test for the association between the 7th group and the other groups regarding SOD.

Bonferroni test		Mean difference	Standard error	Sig. (p-value)
7th group (Juniperus macrocarpa 200 mg)	1st group (control)	6.855	1.858	0.009
	6th group *(Juniperus macrocarpa* 100 mg)	3.821	1.738	0.239
	2th group (No pretreatment)	4.846	0.987	0.001

Glutathione peroxidase (GPX)

On the other hand, and according to results tabulated in Table 10, the mean level of GPX for the 1st group included in this study was 39.569 (± 0.939). While the means of GPX of the 6th and 7th groups which have received *Juniperus macrocarpa* in different doses (100 mg and 200 mg respectively) were 68.142 (± 22.369) for the 6th group and 89.555 (±9.257) the 7th group. Lastly the mean of GPX among the 2th group (which has been exposed to indomethacin without pretreatment) was 29.092 (±6.691). The second group has an observed mean that is lower than the means of the 3 other groups. These differences between the means of GPX among the 4 groups when tested using the One-way ANOVA test has been found to be statistically significant with a p- value of the test of 0.001.

Table 10. ANOVA test for the difference between GPX means among the 4 groups.

	Mean	Standard Deviation	One-Way ANOVA
			Sig. (p-value)
Control	39.569	0.939	0.001
***Juniperus macrocarpa* 100 mg**	68.142	22.369	
***Juniperus macrocarpa* 200 mg**	89.555	9.257	
No pretreatment	29.092	6.691	

Histological Results

Group 1 (G1)- Control group

The histopathological features of gastric mucosa that illustrated in Figure 1 showed normal surface epithelial cells, gastric glands and muscularis mucosa, the gastric glands revealed normal appearance of surface epithelial cells, normal appearance of parietal cells and chief cells with normal mucous secreting cells.

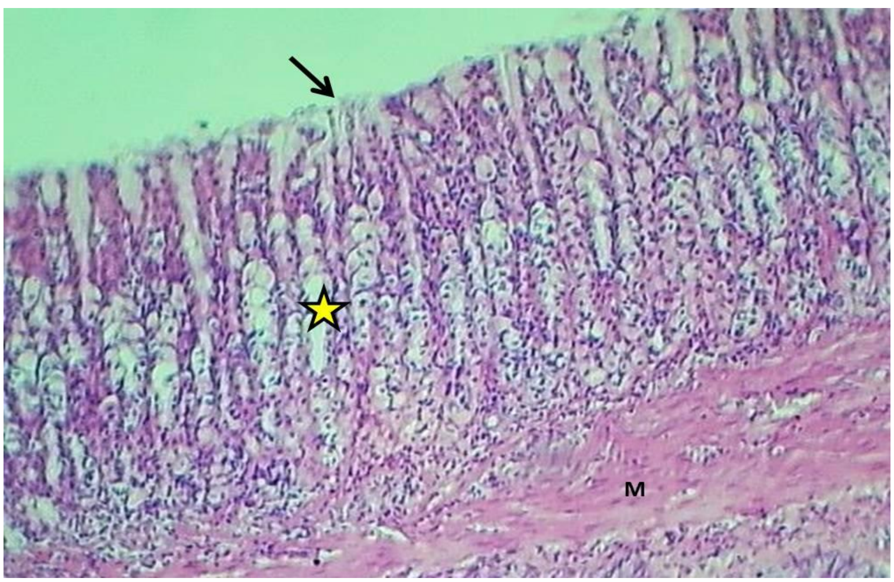

Figure 1. Section of gastric mucosa (G1), H&E.40x

Group 2 (G2)

The histopathological results of gastric mucosa which demonstrated in Figure 2 revealed irregular surface epithelial cells with epithelial sloughing, deterioration of gastric glands with marked vascular dilation of muscularis mucosa, shallow gastric mucosa with depletion of gastric glands cells that associated with degeneration of gastric glands cells and submucosal edema.

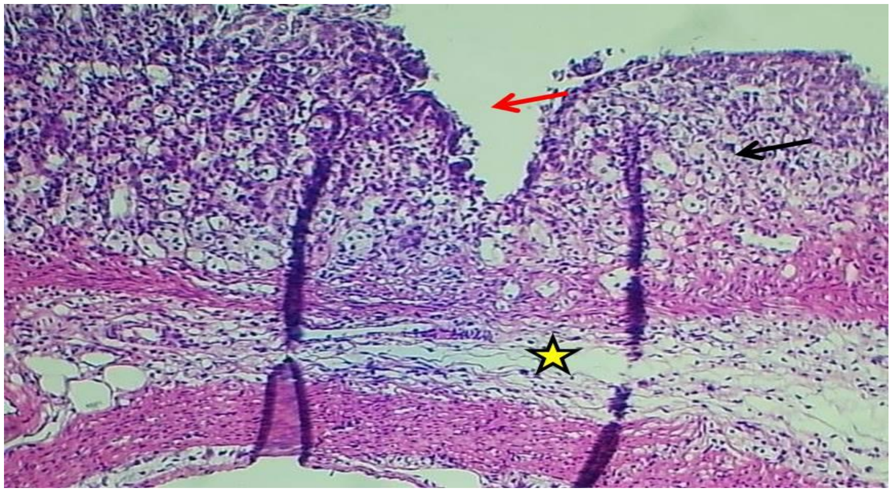

Figure 2 Section of the gastric mucosa (G2), H&E.100x.

Group 3 (G3)

According to findings illustrated in Figure 3, gastric mucosa revealed mild mucosal thickening with markedly secretory activities of mucous cells, dilation of gastric glands, there was no gastritis or gastric ulcers with mild sloughing of surface epithelial cells with secretory activities of mucous cells, cyst of gastric glands and mild submucosal edema without gastritis or gastric ulcers.

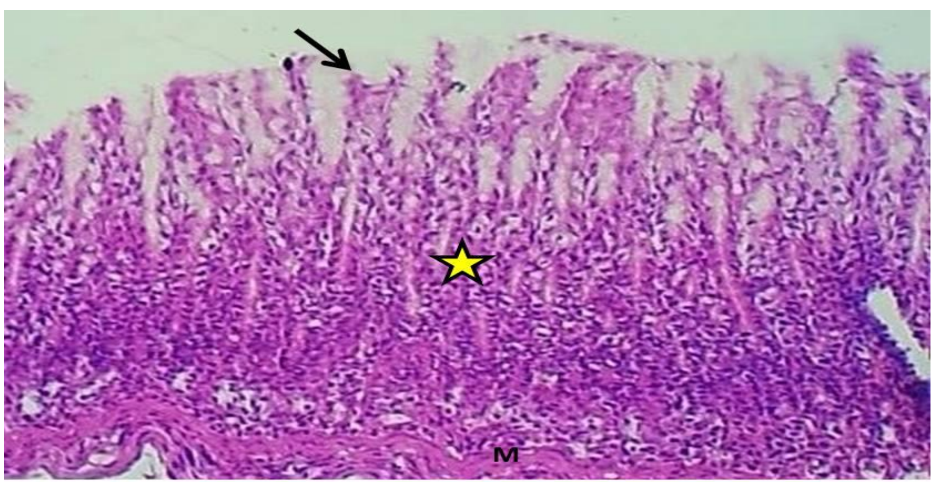

Figure 3. Section of gastric mucosa (G3), H&E.100x

Group 4 (G4)

Figure 4 shows marked gastric ulcer that characterized with complete loss of surface epithelial cells which replaced with thick area of depressed necrotic tissue followed by inflammatory zone and shallow atrophied gastric glands with mild depressed mucosal surface with normal surrounding gastric glands and normal submucosa tissue.

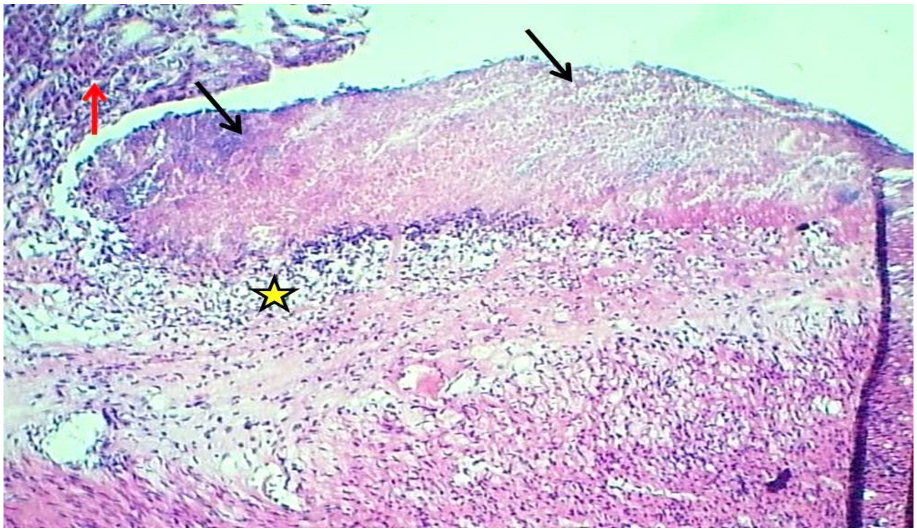

Figure 4. Section of gastric mucosa (G4), H&E.40x.

Group 5 (G5)

Most histopathological findings that shown in Figure 5 demonstrated that the gastric mucosa of G5 were similar to those of control group with marked secretory activities of mucous cells of gastric glands with mild glandular dilation.

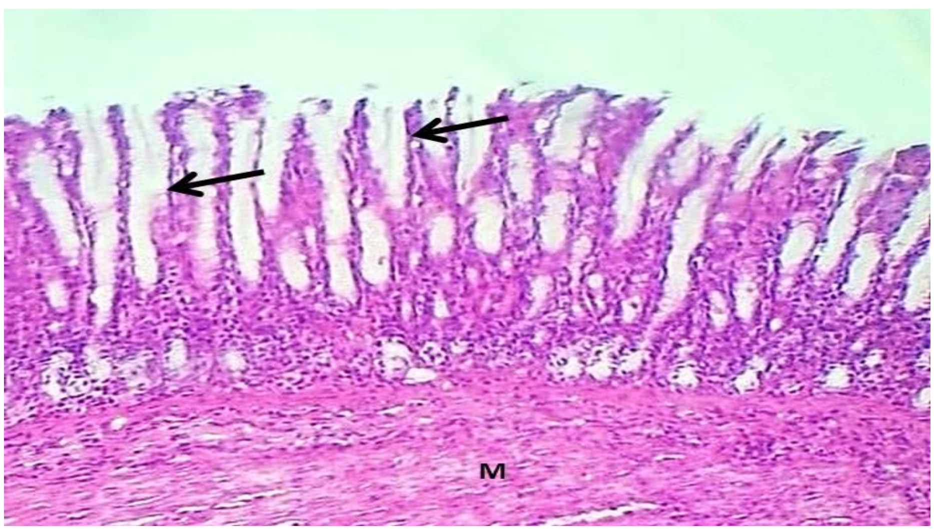

Figure 5. Section of the gastric mucosa (G5), H&E.100x.

Groups 6 and 7 (G6 and G7)

All histopathological features illustrated in Figures 6 and 7 shows that the gastric mucosa of these groups was too similar those of control group.

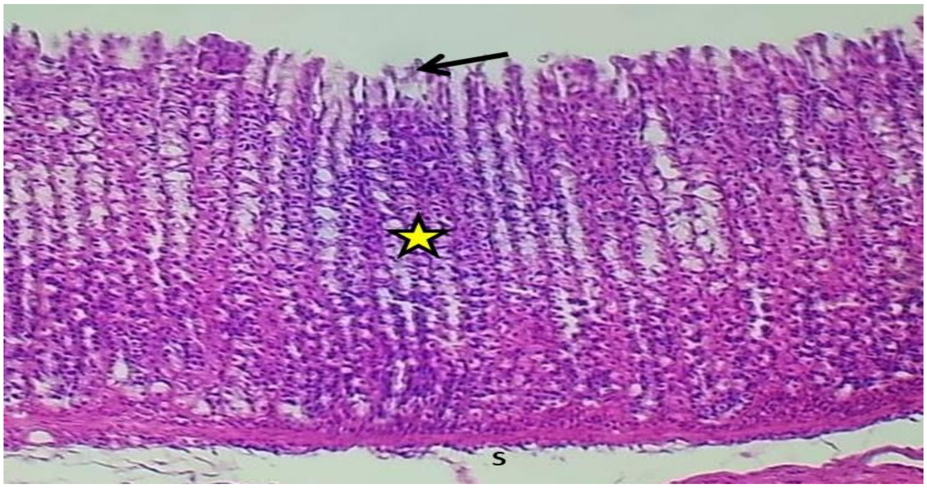

Figure 6. Section of the gastric mucosa (G6), H&E.100x

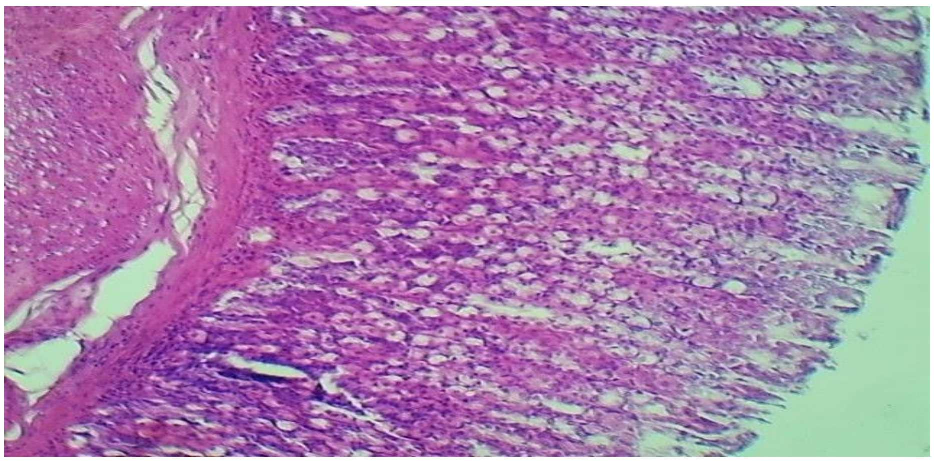

Figure 7. Section of gastric mucosa (G7), H&E.100x

Discussion

The present study sheds light on the antioxidative and gastroprotective potential of *Juniperus macrocarpa* extract against NSAID-induced peptic ulcers in albino rats, emphasizing the pivotal roles of Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPX) as biomarkers of oxidative stress. Our findings reveal that the groups treated with *Juniperus macrocarpa* exhibited a significant increase in SOD and GPX levels compared to the control and ulcer-induced groups, suggesting the extract's efficacy in enhancing the body's antioxidative defense mechanisms. This study corroborates existing literature on the antioxidative role of natural compounds in peptic ulcer management. Salah Eddine *et al.* (2017) and Sofiane *et al*. (2017) have documented the detrimental effects of oxidative stress in the pathogenesis of peptic ulcers and the protective benefits of antioxidants (17, 18, 23-25). The antioxidant activity of *Juniperus macrocarpa*, as reflected in the elevated levels of SOD and GPX, is likely attributable to its high phenolic and flavonoid content, supporting previous assertions about the therapeutic potential of phytochemicals in oxidative stress modulation (26, 27).

In comparing the effects of *Juniperus macrocarpa* extract with omeprazole, a standard treatment for peptic ulcers, our study offers valuable insights into the plant extract's relative efficacy. While omeprazole is known for its ability to alleviate ulcer symptoms and reduce associated oxidative stress and inflammation effectively, our findings suggest that *Juniperus macrocarpa*, especially as a pretreatment, might provide a comparable, if not synergistic, protective effect. This aligns with the growing body of research advocating for integrating natural compounds into conventional treatment paradigms for enhanced therapeutic outcomes (18, 28). Moreover, the distinct antioxidative response observed across different treatment groups highlights the nuanced interplay between natural extracts and pharmacological agents in managing peptic ulcers. The significant variance in SOD and GPX levels, particularly in groups pretreated with *Juniperus macrocarpa*, underscores the extract's potential to bolster the antioxidant defense system preemptively, offering a protective barrier against the oxidative stress induced by NSAIDs. The therapeutic implications of these findings are profound. They suggest that *Juniperus macrocarpa* extract could serve as a complementary therapy for peptic ulcer disease, leveraging its antioxidative and anti-inflammatory properties. The anti-oxidant and anti-inflammatory effect of *Juniperus macrocarpa* were reported in several previous studies which is attributed to the presence of the aforementioned phenolics and terpenes, compounds known for their antioxidant effects (7, 29, 30). The effect of *Juniperus macrocarpa* against gastric ulcer that elucidated by its antioxidant effect was also supported by the histological findings that revealed a significant improvement in the gastric tissue in rats received *Juniperus macrocarpa* extract either as a treatment or as protective remedy. The histological effect of *Juniperus macrocarpa* can be considered as a novel outcome of the current study which is not reported previously. The reduction in the ulceration that obtained with the use of plant extract can be attributed to the previously mentioned anti-oxidant and anti-inflammatory effect that also reported in previous study which assessed the protective histological effect of *Juniperus macrocarpa* on thioacetamide (TAA)-induced nephrotoxicity in male mice who demonstrated that these extract and their chemical constituents are effective in modulation of oxidative stress induced by thioacetamide(TAA) administration (31) which is also aligned with the results obtained in the current study. However, validating these experimental results through clinical trials is imperative to ascertain the extract's safety, efficacy, and optimal therapeutic dosages in human subjects. Such studies would confirm the pharmacological benefits of *Juniperus macrocarpa* and elucidate the molecular mechanisms underpinning its gastroprotective effects. Future studies should focus on validating *Juniperus macrocarpa's* efficacy in human clinical trials for peptic ulcer treatment, exploring its anti-inflammatory mechanisms, and comparing its effects with existing therapies. Developing pharmaceutical formulations and investigating synergies with other treatments are essential for enhancing its therapeutic potential in ulcer management.

Conclusion

In conclusion, *Juniperus macrocarpa* extract demonstrates significant protective and therapeutic effects against indomethacin-induced peptic ulcers in albino rats. Its ability to enhance antioxidative defense mechanisms and reduce inflammatory responses positions it as a promising complementary therapy for peptic ulcer disease. Further research is warranted to explore its clinical applications and understand the molecular basis of its pharmacological actions.

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