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RESEARCH ARTICLE

Investigation of the Protective Effects of Black Garlic Extract an Experimental Gastric Ulcer Model in Rats

Serdar AKTAŞ[1*]

[1]Aydin Adnan Menderes University, Faculty of Veterinary Medicine, Aydın, Türkiye

ABSTRACT

This study aimed to evaluate the effects of black garlic extract in preventing gastric ulcers. For this purpose, twenty-eight 2-3 month-old *Spraque dawley* rats were randomly divided into four groups: the Control group (CG), Ulcer group (UG), Ulcer + pantoprazole treatment group (PTG), and Ulcer + Black garlic group (BGG). Stomach ulcers were induced by administering indomethacin at a dose of 25 mg.kg^{-1} to all groups except the control group. Then, pantoprazole (20 mg.kg^{-1}) and black garlic extract (275 mg. rat) were administered to the treatment groups. After the study, stomach samples were collected and macroscopic (ulcer scoring and ulcerative area mm^2) and histopathology examination (HE) were performed. In biochemical analysis, MDA (pg.ml^{-1}), SOD (ng.ml^{-1}) and CAT (ng.ml^{-1}) levels were determined in the tissue. According to the macroscopic results obtained; Statistically significant changes were found between BGG and UG ($p<0.0001$). PTG showed better effects than all groups. In histopathology; no lesion was found on CG. There was a similarity between PTG and CG. While there were intense bleeding and ulcer foci in UG, only limited focal ulcers were found in BGG. MDA, the highest values were seen in UG. There was no statistical significance between PTG and BCG. The lowest SOD activity was in UG, and there was a similarity in PTG and BGG. The highest CAT activity was determined in CG and the lowest in UG. In conclusion; black garlic extract applied in a single and high dose (5% w.v-1, 275 mg. rat) showed partial protective activity against stomach ulcers.

Keywords: Black garlic extract, pantoprazole, stomach ulcer, ulcer model.

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Siyah Sarımsak Ekstraktının Ratlarda Deneysel Mide Ülseri Modelinde Koruyucu Etkilerinin Araştırılması

ÖZ

Bu çalışmada gastrik ülserin önlenmesinde siyah sarımsak ekstraktının etkilerinin değerlendirilmesi amaçlandı. Bu amaçla 2-3 aylık 28 adet *Spraque dawley* ırkı rat rastgele olarak 4 gruba ayrıldı. Kontrol grubu (CG), Ülser grubu (UG), Ülser + pantoprazole tedavi grubu (PTG) ve Ülser + Siyah sarımsak grubu (BGG). Kontrol grubu hariç diğer gruplara 25 mg.kg^{-1} dozda indomethazin verilerek mide ülseri oluşturuldu. Daha sonra tedavi gruplarına Pantoprazol (20 mg.kg^{-1}) ve siyah sarımsak eksraktı (275 mg.rat) uygulandı. Çalışma sonrasında mide örnekleri alınarak makroskobik (ülser skorlaması ve ülseratif alan mm^2) ve histopatoloji incelemesi (HE boyaması) yapıldı. Biyokimyasal analizlerde oksidan/antioksidan parametre analizleri kapsamında malondialdehid (MDA, pg. ml^{-1}) seviyesi ile süperoksid dismutaz (SOD, ng. ml^{-1}) ve katalaz (CAT, ng. ml^{-1}) aktiviteleri değerlendirildi. Elde edilen makroskobik sonuçlara göre; BGG ve UG arasında istatisliksel anlamlı değişimler bulundu ($p<0.0001$). PTG tüm gruplara göre daha iyi etki gösterdi. Histopatolojide; CG'de herhangi bir lezyona rastlanmadı. PTG ile CG arasında benzerlik vardı. UG'de yoğun kanama ve ülser odakları mevcutken, BGG'de sadece sınırlı fokal ülser bulundu. MDA'da değerler en yüksel UG'de görüldü. PTG ve BCG arasında istatisliksel anlam yoktu. SOD aktivitesi en düşük UG olup, yine PTG ve BGG'de benzerlik vardı. CAT aktivitesi en yüksek CG'de en düşük ise UG'de belirlendi. Sonuç olarak; tek ve yüksek dozda (%5 w.v, 275 mg.rat) uygulanan Siyah sarımsak ekstraktı mide ülserine karşı kısmi koruyucu etkinlik göstermiştir.

Anahtar kelimeler: Mide ülseri, pantoprazole, siyah sarımsak ekstraktı, ülser modeli

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ORCID ID; *SA: 0000-0003-3243-8093.*
*Corresponding author e-mail: saktas@adu.edu.tr

INTRODUCTION

The gastric ulcer has an important place among gastrointestinal problems and if left untreated, it can cause complications, stomach and duodenal cancers and even death. Many etiological factors can cause this problem. Nonsteroidal anti-inflammatory drugs, alcohol, stress, and Helibacterium pylori infections are the main ones (Sabiu et al. 2015). The size and number of ulcers formed in the stomach tissue may vary. Ranging between 1-5 mm in diameter, these ulcers may extend deeply from the mucosa to the muscular layer, resulting in a slow or incomplete healing process influenced by digestive fluids. Antacid drugs, H_2 receptor blockers and proton pump inhibitors are currently used in ulcer treatment. However, these drugs have side effects and long-term problems, such as absorption problems and the elimination of the initial barrier effect of the stomach.

In stomach ulcer research, the ulcer tissue must heal quickly by increasing the durability of the stomach tissue. For this purpose, many experimental models (non-steroids, alcohol and stress) and studies are available. Herbal extracts with antioxidant and antimicrobial effects against *Helibacterium pylori* are important in experimental ulcer studies. *Pistacia atlantica* essential oil has been reported to have a protective effect against gastric ulcers caused by ethanol and antibacterial activity on *Helicobacter pylori* (Memariani et al. 2017). The aqueous extract of *Carica papaya* seed exhibited anti-ulcerogenic and antioxidant effects (Oloyede et al. 2015), while *Xylocarpus granatum* fruit was found to be mucosal protective in rats (Lakshmi et al. 2010).

In recent years, many operations have been carried out to remove the unpleasant odour of garlic and increase its taste. Black garlic; It is a form of normal garlic (*Allium sativum*) with high antioxidant levels, formed as a result of heat treatments and fermentation steps. It has been stated that this form may be useful in preventing and reducing the effects of various diseases. Since the sulfur compounds and allicin it contains decrease during fermentation, its disturbing effects are no longer a problem. Black garlic extract; has been determined in previous studies to it could exhibit anti-inflammatory, anti-obesity, hepatoprotection, hypolipidemia, anti-cancer, anti-allergy, immunomodulation, nephroprotection, cardiovascular protection and neuroprotective effects (Tak et al. 2014; Jeong et al. 2016; Kim et al. 2017).

There are very few studies on the gastrointestinal effects of black garlic. This study aimed to determine the potential protective effects of single and high doses of black garlic extract on induced gastric ulcers in rats.

MATERIALS and METHODS

This study Animal Experiments Local Ethics Committee approval was taken. Twenty-eight 3-month-old *Spraque dawley* rats were randomly divided into four groups (n=7 rats per group). Group 1; Control group (CG), Group 2; Ulcer group (UG), Group 3; Ulcer +Standard treatment group (PTG), Group 4; Ulcer + Black garlic group (BGG). Indomethacin (Endol capsule/Deva Ilac/İstanbul/Türkiye) was administered orally by gavage at a dose of 25 mg.kg-1 to all rats except the control group, after a 12-hour fasting period. 10 minutes before indomethacin was administered to the treatment groups (Group 3); Pantoprazole (20 mg.kg^{-1}, Pulcet capsule, Nobel Ilac İstanbul/Türkiye) and black garlic (MDC Black Garlic, İstanbul-Türkiye) was administered to Group 4 by preparing its aqueous extract in powder form (5% w.v^{-1}, 275 mg. rat) was administered orally. 6 hours after drug administration, the rats were euthanized by cervical dislocation under ketamine (Keta control, Doga İlaç/İstanbul/Türkiye) and xylazine (Xylazınbio 2%, Bioveta) anaesthesia. After the stomach tissues were removed, they were washed in 0.9% NaCl solution.

Macroscopic evaluation

Stomach tissues were placed on a clean surface and ulcer areas were visualized. Ulcer areas (mm^2) on the stomach surface were determined using millimetric paper. In ulcer scoring, the ulcer level (0-5 scale) was determined and recorded separately for each animal, as stated in previous studies (Cantarella et al. 2005).

Histopathological evaluation

After the experimental study, the stomach tissues removed after euthanasia were divided into 2 equal parts. Gastric tissue samples taken for histopathology were stored in 10% formaldehyde solution. 4 μm sections were taken from the tissue samples taken into paraffin blocks and examined by Hematoxylin-Eosin (HE) tissue staining. The severity of the ulcer and the level of healing were determined by 3 experts in pathology.

Oxidant-antioxidant balance parameters

Other stomach tissue samples taken from each rat were homogenized in an ice cube in PBS (Phosphate buffer saline) for 1 minute in a tube with 2000 rotations per minute. The resulting homogenates were centrifuged at 7000 rpm for 10 min at 4 °C in a refrigerated centrifuge, and the supernatants were stored at −80°C until subsequent analysis. Total protein (TP, Abcam Company ab113463) levels and malondialdehyde (MDA Assay Kit, ab238537), glutathione (GSH Colorimetric ab102530), superoxide dismutase (SOD Abcam Company, ab285309), and catalase (CAT, BT Lab E0869Ra) activities in the

stomach tissue were measured from the prepared samples.

Statistical evaluation

All numerical values obtained will be evaluated by one-way analysis of variance (ANOVA) if appropriate, or by Kruskal Wallis H-Test if not appropriate. SPSS 22 and GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA, USA) were used in the study. In the study, $p<0.5$ was considered significant.

RESULTS

Stomach ulcer areas are considered in macroscopic evaluation; No lesion was found in the control group (CG). While the highest ulcer area (mm^2) was seen in UG, the lowest amount was seen in the PTG group. Statistical significance was found between PTG and UG. (Figures 1 and 2, $p<0.0001$).

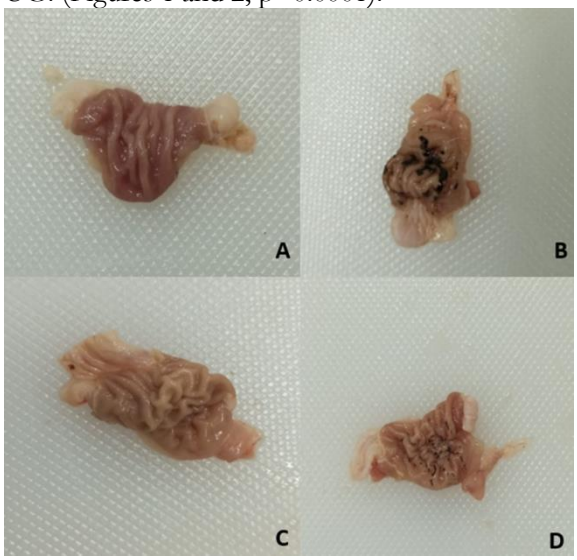

Figure 1: Macroscopic appearance of the stomach tissues; A: Control group (CG), B; Ulcer group to which indomethacin was applied (UG), C; Pantoprazole treatment group (PTG), D; Black garlic treatment group(BGG).

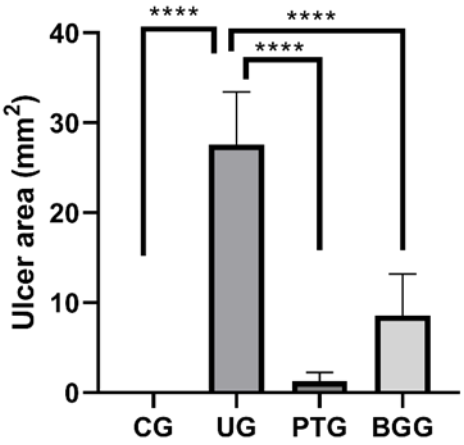

Figure 2: Ulcer areas (mm^2) formed after indomethacin application in gastric tissue samples. Control group (CG), Ulcer group (UG), Ulcer + Pantoprazole treatment group (PTG) and Black garlic group (BGG). Statistically significant (*$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$).

A statistical difference was found between BGG, UG and CG in ulcer index (0-5 scale) values. The highest ulcer index was seen in UG, while the lowest level was seen in PTG. There was statistical significance between PTG and BGG (Figure 3, $p<0.01$).

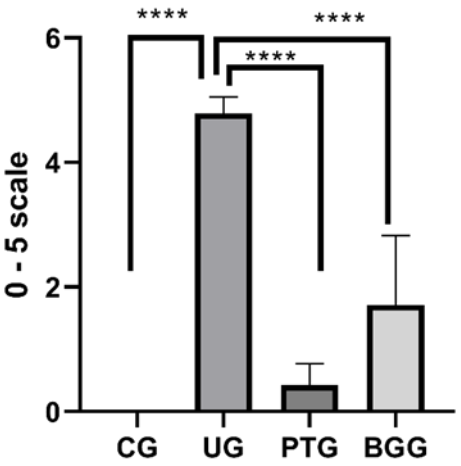

Figure 3: Ulcer scale (0-5) all groups. Control group (CG), Ulcer group (UG), Ulcer + Pantoprazole treatment group (PTG) and Black garlic group (BGG). Statistically significant (*$p< 0.05$, **$p< 0.01$, ***$p< 0.001$, ****$p < 0.0001$).

In histopathological evaluation; In the material examined with serial sections on CG, mucous columnar epithelium in the stomach and gastric glands in the submucosa were observed as normal. Multiple bleeding ulcerative foci and erosions were observed in the UG. In the PTG group, there was an appearance close to normal tissue. Mucosa and glands were normal. No ulcer was detected. In BGG, there were a few focal mucosal damages and ulcers. An increase in mucus cells and mucus was observed. The multilayered cutaneous stomach section was normal in all groups. The fact that fewer ulcer areas were observed in BGG than in UG showed that black garlic has incomplete partial protective effects.

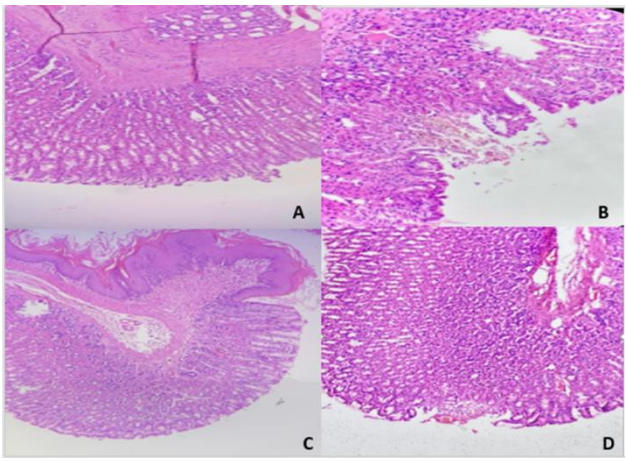

Figure 4: Evaluation of gastric tissue samples by Hematoxylin and Eosin staining (HEx20 Magnification). **(A):** Control group; normal stomach tissue. **(B):** Ulcer group; mucosal damage, cell infiltrates, multiple ulcers and haemorrhage. **(C):** Ulcer + Pantoprazole treatment group; Near normal mucosa, **(D):** Black garlic group; Very low rate of focal mucosal damage.

When examined in terms of oxidative stress parameters, MDA levels (pg. ml-1) were highest in UG and lowest in CG. A statistically significant decrease was observed in BGG compared to UG ($p<0.01$). No statistical significance was found between PTG and BGG (Figure 5).

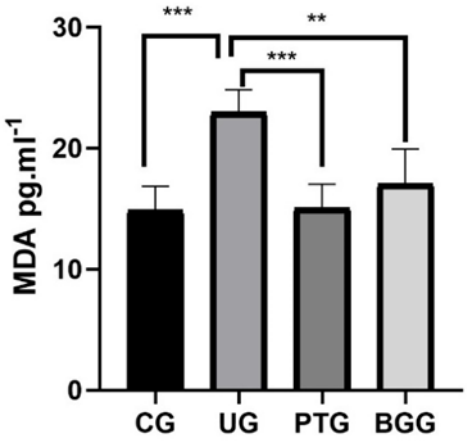

Figure 5: MDA (pg.ml-1) levels in samples obtained from gastric tissue homogenates. Control group (CG), Ulcer group (UG), Ulcer + Pantoprazole treatment group (PTG) and Black garlic group (BGG). Statistically significant (*p<0.05, **p<0.01, ***p<0.001).

The highest SOD activity (ng. ml-1) was seen in CG and the lowest level was seen in UG. There was a statistical difference between BGG and UG ($p<0.5$). There was no statistical difference between BGG and PTG (Figure 6).

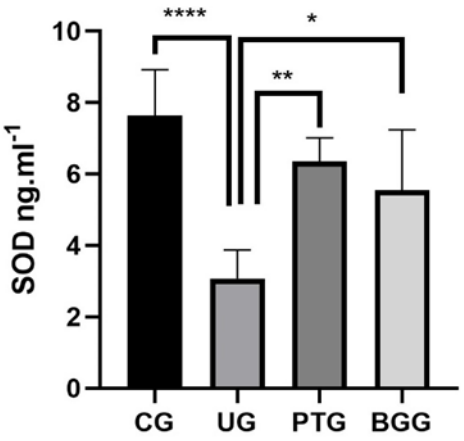

Figure 6: SOD (ng.ml-1) activities in samples. Control group (CG), Ulcer group (UG), Ulcer + Pantoprazole treatment group (PTG) and Black garlic group (BGG). Statistically significant (*p< 0.05, **p<0.01, ***p<0.001, ****p<0.0001).

While there was a statistical difference between PTG and UG in CAT activity (ng. ml-1, $p<0.5$), no significance was found between UG and BGG (Figure 7).

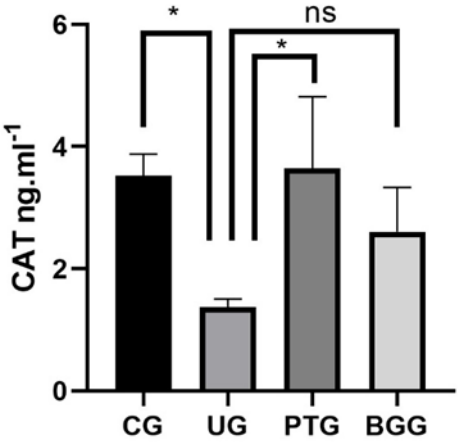

Figure 7: CAT (ng.ml-1) activities in samples. Control group (CG), Ulcer group (UG), Ulcer + Pantoprazole treatment group (PTG) and Black garlic group (BGG). Statistically significant (*p < 0.05, **p <0.01). Ns: Not significant. No statistical significance was found between UG and BGG values.

DISCUSSION

Stomach ulcers are one of the gastrointestinal problems that significantly reduce the quality of life, which may have different etiological reasons. Non-steroids are one of the factors playing a role in aetiology. Indomethacin is a non-steroidal drug and has many side effects. They have side effects on the stomach (especially ulcers) when used in normal doses and sometimes unconsciously in high amounts (Suleyman et al. 2010). There is a search for new

treatments for ulcer treatments due to the negative effects of currently used drugs, such as long-term absorption disorders and eliminating the barrier effect of the stomach. Black garlic extract is one of them. The amount of total phenolic substances and flavonoids is much higher than normal garlic (Ahmed and Wang 2021). Antioxidant compounds such as lycopene show protection by showing antioxidant effects in gastric ulcer models (Boyacioglu et al. 2016). In another study, it was stated that there was a higher level of protection at the 200 $mg.kg^{-1}$ dose in the stomach ulcer model created by applying 100 and 200 $mg.kg^{-1}$ doses for 30 days (Badr et al. 2014).

Possible protective efficacy can be easily observed and measured macroscopically in gastric ulcer models created with indomethacin. Calculating ulcer areas and ulcer scores is one of them. In a previous study, it was reported that there was a statistical decrease in ulcer areas in the stomach tissue as a result of the application of silymarin at different doses (25, 50 and 100 $mg.kg^{-1}$) in the stomach ulcer model created in rats (Boyacioglu 2019). In this study, there was a statistically significant decrease in ulcer scores and areas (mm^2) in the BGG group compared to the UG group ($p<0.0001$). However, the use of higher doses of black garlic extract compared to previous studies, did not achieve higher levels of protection.

In histopathological evaluation, while no ulcers and mucosal damage were found in CG and PTG, a high rate of haemorrhage and ulcer foci were observed in UG. In BGG, fewer focal ulcer foci were detected (Figure 4). This showed that the protective effectiveness of black garlic remained at a partial level. In a previous study, it was determined that black garlic applied to rats at a dose of 200 $mg.kg^{-1}$ for 10 days had protective effects on stomach ulcers but was lower than omeprazole application (El-Ashmawy et al. 2016). In the same study, it was stated that omeprazole showed 94.5 % anti-ulcerative effects, while black garlic extract showed a protective effect of 83.4 %. In this study, black garlic extract used in higher doses compared to previous studies did not show protection reaching higher levels. This showed that increasing doses were not effective in protection.

Some researchers have stated that black garlic increases the inflammatory response due to its high sugar content compared to fresh garlic and that it is only more effective in ROS-related issues (Kim et al. 2017). They found that the anti-inflammatory activity of black garlic fermented with *Lactobacillus rhamnosus* was dependent on the activation of NF-ϰB, inhibition of cytokine production, and expression of iNOS and COX-2 (Tak et al. 2014). In previous studies, black garlic has been shown to exert protective effects by reducing oxidative stress parameters in rats subjected to nephrotoxicity (Maldonado et al. 2003). In another study, it was reported that it similarly reduced 8-hydroxydeoxyguanosine and MDA levels and regulated TGF-β1, SOD, CAT, and GSH levels in a colistin-induced renal failure model (Lee et al. 2019). In this study, black garlic application statistically significantly reduced MDA levels in BGG compared to UG ($p<0.01$). There is no significance between BGG and PTG for the same values. These values showed that black garlic has significant effects on reducing MDA levels. Considering the SOD activity, it is seen that there is statistical significance between BGG and UG ($p<0.05$), but there is no significance between BGG and PTG. It has been observed that black garlic has beneficial effects in maintaining antioxidant enzyme levels. Considering the activities of another antioxidant, CAT, it was seen that the levels were higher in the PTG group. It has been determined that black garlic has limited effectiveness in protecting CAT activities. Additionally, due to the oxidation of γ-glutamyl cysteine, black garlic does not produce irritating and soluble sulfur-containing compounds, thus it does not irritate the digestive system. It has also been determined that it can help reduce constipation because it causes increased motility in the digestive system. Aged black garlic extract induces gastric cancer cell growth inhibition in vitro and in vivo (Wang et al. 2012). It also induces apoptosis in the HT29 colon cancer cell line (Dong et al. 2014).

CONCLUSION

It was found that the application of black garlic extract in a single and high dose (275 mg. rat) showed partial gastroprotective effects in the gastric ulcer model induced by a nonsteroidal anti-inflammatory drug (indomethacin). Although its protective effects are not as strong as pantoprazole, it is thought to have the potential for use in prophylaxis and treatment. Since black garlic has compounds that dissolve only in oil, it is thought that it may be beneficial to conduct this study in oil and different solvents (such as carboxy methyl cellulose).

Ethical Approval: Aydin Adnan Menderes University Animal Experiments Local Ethics Committee (Approval No: 64583101/2023/156)

Funding: Self-funded.

Conflict of Interest: There is no conflict of interest with any person or institution in this study.

Limitations of the Study: In this study, determination levels of TNF-α, IL-2, PGE1 and PGE2 could not be performed due to budget constraints.

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