Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.3.5

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (3): 284–289

ORIGINAL PAPER

Gastroprotective effects of *Prunus laurocerasus* L. fruit extracts against the oxidative stress induced by indomethacin in rats

Ozlem Aydin Berktas [1], Arif Keskin [2], Emine Gulceri Gulec Peker [1]

[1] Departmenf of Nursing, Faculty of Healthy Science, Giresun University, Giresun, Turkey
[2] Department of Anatomy, Faculty of Meram Medicine, Necmettin Erbakan University, Konya, Turkey

ABSTRACT

Introduction and aim. *Prunus laurocerasus* L. is a perennial plant belonging to the Rosaceae family and is grown on the shores of the Black Sea Region. In the current study, the effect of *P. laurocerasus* fruits was investigated in the ulcer model created by the application of indomethacin to rats.

Material and methods. Rats divided into five groups: fruit water extract (200 mg/kg), fruit ethanol-water extract (200 mg/kg), lansoprazole agent (LAN, 25 mg/kg), and indomethacin (IND, 25 mg/kg). All administrations were given to animals by oral gavage. At the end of the experiment, macroscopic and biochemical measurements were made in rats.

Results. The lipid peroxidation was quite high in rat stomach tissues given IND. The applied LAN and extracts reduced this increase to almost a healthy rate. On the other hand, the amount of glutathione, catalase, and superoxide dismutase activities were found very low in IND applied tissues. The LAN and fruit extracts treatments tried to show their protective feature by increasing this decreased antioxidant level in their own groups.

Conclusion. The data obtained determined that both enzyme and non-enzyme antioxidant markers measured in fruit extracts had a protective effect almost as strong as lansoprazole.

Keywords. indomethacin, lipid peroxidation, *Prunus laurocerasus,* ulcer

Introduction

Gastrointestinal system disorders have become an important public health problem in terms of morbidity and mortality, which affects many people.[1] Among them, the most common are; peptic ulcer, gastric and duodenal ulcers. Peptic ulcers are characterized by deep necrotic lesions and destruction of epithelial and connective tissue components, including fibroblasts, smooth muscle cells, blood vessels, and nerves in the gastric mucosa.[2] Gastric and duodenal ulcers occur in the upper part of the gastrointestinal tract (GIT) as a result of an imbalance between protective (e.g. mucus production) and aggressive (e.g. HCl release) factors at the gastric mucosa level. Mainly stomach ulcer; It occurs when the balance between more than one harmful factor (such as hydrochloric acid and pepsin secretion) and several gastroprotection's (such as prostaglandins, bicarbonate and mucus production, mucosal barrier and adequate blood flow) is disturbed.[3] Especially acid secretion, disorders in the protective mucosal barrier, in addition to these, genetic predisposition (heredity), stress, cortisone-type drugs, NSAIDs (Non-steroidal anti-inflammatory drugs) such as aspirin and indomethacin (IND), *Helicobacter pylori*, *Herpes simplex* virus (Type I, HSV-1) and variables such as smoking and alcohol use cause ulcers. Among these variables, regularly used NSAIDs constitute a very large part of gastric mucosal ulceration. It is known that anti-inflammatory drugs such as aspirin

Corresponding author: Ozlem Aydin Berktas, e-mail: ozlem.berktas@giresun.edu.tr

Received: 28.04.2022 / Revised: 16.05.2022 / Accepted: 18.05.2022 / Published: 30.09.2022

Berktas OA, Keskin A, Peker EGG. *Gastroprotective effects of Prunus laurocerasus L. fruit extracts against the oxidative stress induced by indomethacin in rats*. Eur J Clin Exp Med. 2022;20(3):284–289. doi: 10.15584/ejcem.2022.3.5.

and IND show anti-inflammatory activity by inhibiting the cyclooxygenase (COX) enzyme system. However, these drugs cause gastric damage by inhibiting the COX enzyme system, suppressing prostaglandin (PG) biosynthesis, and resulting in disruption of the gastric mucosal barrier. Suppressing PG biosynthesis means inhibiting mucus secretion, which is a defense factor against gastrointestinal damage, and is manifested by some events such as decreased local blood flow, topical irritation, and inhibition of tissue repair.[4]

The current pharmacological treatment of peptic ulcers is carried out using drugs that act by two main mechanisms depending on these variables: the first is the inhibition of gastric acid secretion, and the second is the inhibition of gastric acid secretion. Accordingly, proton pump inhibitors such as omeprazole, lansoprazole; histamine H_2-receptor antagonists such as ranitidine, cytoprotective agents such as sucralfate, and drugs that increase the natural mechanisms of gastric protection such as misoprostol. However, as these drugs often bring with them serious side effects, it justifies the search for new alternatives for ulcer treatment.[5,6] Especially recently, natural products have started to be promising new sources of therapeutic agents. Intensive studies are continuing to produce more practical treatment solutions that provide easier healing. These therapeutic agents are especially selected from products with known high antioxidant properties and which can provide strong antioxidant support. As in many tissues damage of ulcer, it has been determined in many studies that they affect each other depending on the interaction of antioxidant enzymes, which are protective enzymes against reactive oxygen species.

Prunus laurocerasus L. (*Laurocerasus officinalis* Roem.) is a perennial plant belonging to the Rosaceae family and is grown on the shores of the Black Sea Region.[7] It is locally called Taflan, Karayemiş and Laz cherry in Turkey. While it is consumed as fresh fruit, especially in August, it is also suitable to be consumed as dried fruit, such as molasses, marmalade or jam.[8] In Turkey, both its fruit and seeds have been used for many years as an anti-diabetic, analgesic and diuretic agent, as well as being used for stomach ulcers, digestive system, bronchitis, eczema, and hemorrhoid complaints.[9] Although there are studies on the functional content, antioxidant capacity and anti-diabetic activity of seeds, fruits, molasses and leaves of *P. laurocerasus*, they are very limited.[10]

Aim

In the present study, it was aimed to investigate the gastroprotective effects of water and ethanol-water extracts of *P. laurocerasus* L. fruits on indomethacin-induced gastric ulcer.

Material and methods

Chemicals

All chemical products used in the experiments were obtained from Sigma Chemicals Company (Germany). Ketamine (80 mg/kg) and xylazine (10 mg/kg) used as anesthetics were purchased from a legitimate retailer.

Plant material and extraction of plant material

P. laurocerasus fruits were used as study material in the research and were obtained from a vendor selling dried fruit. These fruits, which are grown in the eastern cities of the Black Sea Region, are collected and dried in August.

Extraction method

P. laurocerasus fruits were dried in the shade after being collected in Giresun Province. Then, the dried fruits separated from their seeds were ground and powdered, and then mixed in a water-ethanol mixture (4:4). It was then kept in a shaking water bath at room temperature for 7 days. After 7 days, it was filtered through filter paper and then the solvents were evaporated under reduced pressure in the evaporator at 50°C to obtain fruit extract. Extraction was prepared by the methods used in previous studies.[9]

Experimental procedure

Animals and treatments

The experimental animals were obtained from Saki experimental animals in Ankara, which is a legal seller. Giresun University experimental animals were included in the experiment after they were approved by the ethics committee (2019/13).

First of all, experimental animals were divided into 5 different groups. (I) Control group, (II) IND group 25 mg/kg, (III) LAN group 25mg/kg, (VI) Prunus L. water extract group 200mg/kg and (V) Prunus L. ethanol-water extract group 200 mg/kg.

After the groups were determined, the groups separated as 6 animals in each cage were starved for 24 hours. The next day, all groups were administered at the determined doses (except for the control). After 10 minutes, IND, the agent that will cause gastric damage, was applied to all groups. The rats were sacrificed by giving ketamine (80 mg/kg) and xylazine (10 mg/kg) 6 hours after all applications were completed. Macroscopic and biochemical examinations were made in gastric tissues obtained after sacrification.

Indomethacin-induced gastric damage

The protective effect of the experimental groups was determined by comparing with lansoprazole. In order for the IND to cause gastric damage, all animals were fasted for 24 hours and tested the next day. For administration, animals were administered orally by gavage with

200 mg/kg extracts and 25 mg/kg LAN. Then, IND was applied to all groups and the stomachs of the animals were removed at the end of 6 hours. The stomachs obtained were washed and counted macroscopically and ulcer areas were determined.

Biochemical investigation of stomach tissues
Stomach tissues extracted from animals were ground with liquid nitrogen for biochemical analysis. The ground stomach tissues were homogenized by treatment with appropriate homogenates and centrifuged at 4°C. The obtained supernatants were used for enzyme activities such as catalase (CAT), superoxide dismutase (SOD) and to determine the amount of glutathione (GSH) and lipid peroxidation level (LPO).

CAT activity
Decomposition of H_2O_2 in presence of catalase was at 240 nm.[11] Catalase activity was defined as the amount of enzyme required to decompose 1 nmol of H_2O_2 per minute, at 25°C and pH 7.8. Results were expressed as mmol/min/mg tissue.

SOD activity
SOD activity was measured according to the principle of superoxide radical formation of xanthine.[12] SOD activity was then measured at 560 nm by the degree of inhibition of this reaction.

GSH determination
The amount of GSH is determined according to the method of Sedlak et al. With homogenates compatible with the literature, glutathione in tissues is expressed as nmol/g.[13]

LPO determination
The level of LPO was determined with the homogenates prepared in accordance with Ohkawa's method. Data obtained were expressed as nmol/g tissue.[14]

Statistical analysis
The results were made using the appropriate SPSS program (IBM SPSS Statistics 20, Turkey). Statistical differences were determined by the ANOVA test. Multiple comparisons were expressed by Duncan. Significance was determined according to $p<0.05$.

Results

Gastroprotective effect of P. laurocerasus on indomethacin-induced gastric damage
The protective effects of the extracts on gastric damage caused by IND in rats are presented in Table 1 and Figure 1. While there was a very strong injury in the animals in the IND group, good protection was determined in water and ethanol-water extracts, as in the LAN group. When ulcer areas are compared; While the damage caused by IND was 32±0.6, it was seen that it was reduced by almost half in the treatment groups (16.5±2.6 and 9±1.6). We can even say that it was as effective as the LAN group, which is the positive control agent. In Figure 1, the damaged areas are clearly visible in the macroscopically counted tissues.

Table 1. Effects of different doses of species extracts and single dose of famotidine (FAM) on indomethacin (IND)-induced gastric damage in rats*

Treatment	n	Dose (mg/kg)	Ulcer Areas
Healthy	6	-	0 ± 0^a
IND	6	25	32 ± 0.6^d
LAN	6	25	6.5 ± 0.7^b
Prunus laurocerasus water extract	6	200	16.5 ± 2.6^c
Prunus laurocerasus ethanol-water extract	6	200	9 ± 1.6^b

*Means in the same column by the same letter are not significantly different to the Duncan test ($p<0.05$), results are means ± SE of three measurements, n – the number of rats

The comparison of enzyme activities in rat stomach tissues
Biochemical enzymes in rat stomach tissues were measured to express how the antioxidant defense system works. The results are shown in Table 2. According to the table; CAT and SOD enzyme activity is very low in IND applied tissues. In the LAN group, which is the positive control drug, it was increased to a level as high as healthy. Likewise, *P. laurocerasus* extracts have increased this level almost as much as LAN. Similarly, the GSH level in applied IND tissues is also very low. Again, the extracts and LAN tried to increase this decrease and determined their protective effects. Although IND increased the tissue damage considerably in the tissues where lipid peroxidation was measured, the treatment groups and LAN reduced this damage. Thus, enzymes with high damage indicators and LPO and GSH levels clearly showed their protection thanks to the extracts.

Discussion

NSAIDs, which are widely used clinically as anti-inflammatory and analgesic agents, are drugs with strong side effects in many systems. It manifests itself with ulcerative lesions, especially in the gastrointestinal tract. There are restrictions on its use as an anti-inflammatory due to these side effects.[15] In eliminating this damage caused by NSAIDs; Inhibition of prostaglandin synthesis and inhibition of epithelial cell proliferation occurring around the ulcer is suggested.[16,17] IND is one of these drugs that are not corticosteroid and cause stomach damage. It contains lipids with good affinity for the lipophilic parts of cell membranes. With this advantage,

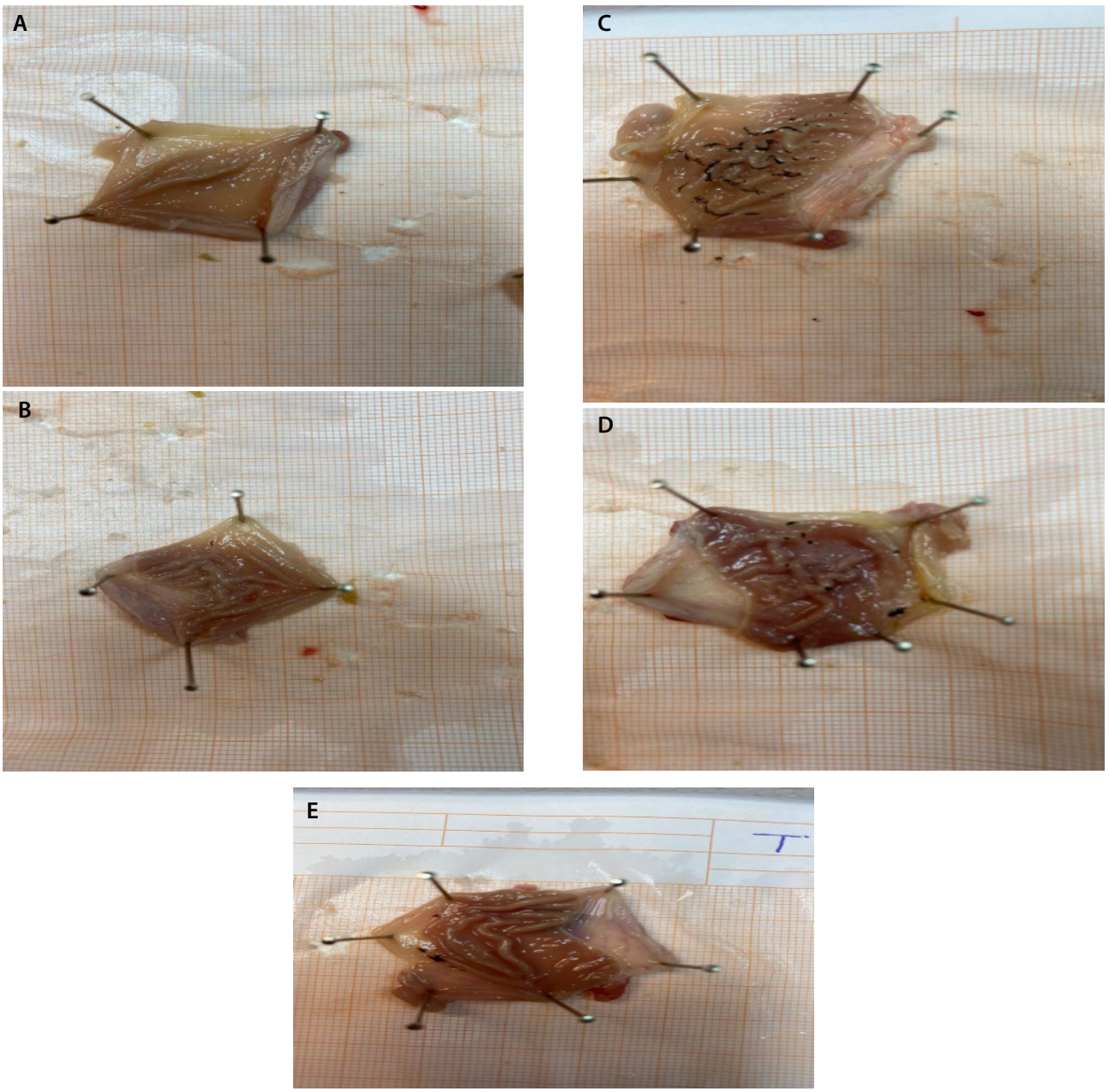

Fig. 1. The stomach samples taken from gastric damaged tissues induced by IND (25 mg/kg); (A) healthy, (B) LAN, (C) IND, (D) *P. laurocerasus* water extract, (E) *P. laurocerasus* ethanol-water extract

Table 2. Effects of *P. laurocerasus* extracts treatments on changes in activities of catalase (CAT), superoxide dismutase (SOD) and with levels of lipid peroxidation (LPO) and total glutathione (GSH) in rat's indomethacin (IND)-induced gastric tissue*

Treatment	n	Dose (mg/kg)	CAT activity (mmol/min/mg tissue)	LPO (nmol/g tissue)	SOD activity (mmol/min/mg tissue)	GSH (nmol/g tissue)
Healthy	6	-	19.11 ± 1^{b}	34.75 ± 1.4^{a}	4.13 ± 0.3^{c}	3.8 ± 0.2^{c}
IND	6	25	14.95 ± 1^{a}	90.24 ± 1^{d}	1.75 ± 0.2^{a}	0.7 ± 0.1^{a}
LAN	6	25	18.57 ± 0.4^{b}	34.5 ± 1.9^{a}	3.95 ± 0.1^{c}	3.1 ± 0.2^{b}
Prunus laurocerasus water extract	6	200	17.57 ± 0.5^{b}	62.25 ± 1.04^{c}	2.2 ± 0.1^{a}	3.3 ± 0.3^{b}
Prunus laurocerasus ethanol-water extract	6	200	19.32 ± 0.2^{b}	53.8 ± 1.7^{b}	3.03 ± 0.1^{b}	3.2 ± 0.4^{b}

*Means in the same column by the same letter are not significantly different to the Duncan test ($p<0.05$), results are means ± SE of three measurements, n – the number of rats

it easily adheres to structural phospholipids and causes disruption of the cell membrane structure and hydrophobic structures in the mucosal structure. With this loss, it paves the way for lipid peroxidation and also allows the entry of water-soluble agents into the cell that will cause injury. Thus, damage occurs with the entry of substances such as pepsin, acid, bile salts, which easily pass through the cell membrane.[16,17]

The initiation of the ulcer process by IND administration is attributed to several processes, including production of reactive oxygen species, initiation of lipid peroxidation, infiltration of leukocytes, induction of apoptosis, and inhibition of prostaglandin E2.[17] In the current study, when the macroscopic findings were examined, the degree of ulceration was found to be quite high in the stomach tissues of the IND applied rat, due to the combination of these reasons (32±0.6) This damage in the ulcer areas is also clearly seen in the macroscopic examination of the excised stomach tissues. The decrease in the ulcer areas extracted by the count made on the millimetric paper is expressed in figure 1. The findings in IND-induced ulcer experiments, which were carried out in some experimental studies before, are also parallel.[18-20] The applied treatment groups and the positive control ulcer drug showed their protective effects by significantly reducing this damage (*P. laurocerasus water extract;* 16.5±2.6, *P. laurocerasus ethanol-water extract;* 9±1.6, LAN; 6.5±0.7).

There are also in vivo experimental studies to eliminate the damage caused by the ulcer. These studies are particularly against IND-induced reactive oxygen species (ROS) at the tissue level.[21] These agents act as oxidants in cells and contribute to the production of ROS. Against this, organisms activate a series of enzymatic and non-enzymatic defense mechanisms. Antioxidant enzymes such as SOD, CAT, GPx, MPx, LPO and GSH play an important role in the elimination of free oxygen radicals and lipid hydroperoxides in gastric mucosal cells.[15,21] With the application of IND, a decrease in antioxidants in the rat gastric mucosa, deterioration in cell permeability and accordingly the oxidation of phospholipids in the cell membrane and their conversion to peroxide derivatives initiate lipid peroxidation. There is an increase in the level of malondialdehyde, the parent compound, indicating the onset of lipid peroxidation. This compound is a very harmful substance and affects the permeability negatively by causing ion exchange in the membrane.[22] LPO, which is found at a very high rate in the tissues together with the IND applied in the current study, is an indicator of these reasons. However, the extracts and LAN in the treatment groups decreased this increase positively and activated the antioxidant defense system. Also, in another study, Abdallah et al.[22], it has been observed that IND-induced gastric ulceration is accompanied by a severe oxidative stress in gastric tissue that damages essential biomolecules such as lipids. Again, in some studies, it has been shown that LPO causes an increase in reactive oxygen species.[23]

Another antioxidant molecule that tries to eliminate the oxidative damage caused by NSAIDs in tissues is GSH. It plays a role in neutralizing hydrogen peroxide, one of the reactive oxygen species, and stimulating prostaglandin synthesis. It shows the protective feature by transferring electrons to free radicals. As in the studies of Halici and Kaplan, the protective effects of fruit extracts were determined in this study.[24,25] Another enzyme that neutralizes hydrogen peroxide, one of the reactive oxygen species in the environment, is the SOD enzyme. It reduces superoxide, which is a highly reactive oxygen radical, to hydrogen peroxide. The enzyme catalase reagents, which convert this H_2O_2 into molecular water and oxygen, can destroy it. Therefore, since a high rate of reactive oxygen is produced in IND applied tissues, there will be no transformation in the environment and the antioxidant level will remain low. Likewise, the CAT activity that will convert the H_2O_2 in the environment to water will also be very low. The data obtained in the study also show this. The treatment groups, on the other hand, increased both SOD and CAT activities in parallel with the applied positive group, thereby removing superoxide radicals and hydrogen peroxide from the environment.[23,26-28] The enhancing antioxidant status could be one of the mechanisms behind *P. laurocerasus* gastroprotective effects.

Conclusion

In recent studies, gastric damage has occurred in IND-induced ulcer models, and antioxidant enzymes have been activated with some treatment groups. Some recent research is mostly on new natural therapeutic agents. It is to be used as antioxidant support due to positive reasons such as being easily available, inexpensive, easy healing and strong effect. We can say that *Prunus laurocerasus* fruit extracts used in the current research can be used easily by determining their protective effects. In addition, its protectiveness can be proven in previous studies. Its protection can be proven and its use can be expanded both in diseases such as diabetes, diuretic, bronchitis, eczema, and in digestive system disorders.

Declarations

Funding

Authors have no commercial interest and financial interest. The costs of the research were covered by the researchers.

Author contributions

Conceptualization, O.A.B.; Methodology, A.K and G.P; Software, A.K and G.P; Validation, O.A.B; Formal Analysis, O.A.B A.K and G.P; Investigation, A.K and G.P; Resources, A.K and G.P; Data Curation, A.K and G.P;

Writing – Original Draft Preparation, O.A.B; Writing – Review & Editing, O.A.B. and G.P; Visualization, O.A.B; Supervision, A.K; Project Administration, O.A.B; Funding Acquisition, G.P. and O.A.B.

Conflicts of interest

All authors declare that there are no conflicts of interest.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

The ethical approval was obtained from Giresun University Animal Experiments Local Ethics Committee for the applications (2019/13).

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