Research Article

Journal of Health and Biological Sciences

GJOHBS 2025;1(3):88-95. DOI: 10.65308/gjohbs.2025.013

The Effect of Tarantula Cubensis Alcoholic Extract on Advanced Oxidation Protein Products and Total Sulfhydryl Levels in The Stomach Tissue of Gastric Damaged Rats with Indomethacin

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Received Date: Oct, 2025; **Accepted Date:** Nov, 2025; **Publication Date:** Nov, 2025.

ABSTRACT

Purpose: There are many markers used to assess the oxidative stress state of the cell. Advanced oxidative protein products (AOPP), which are formed by the reaction of oxygen radicals with proteins in the cell, are one of these markers. Total sulfhydryl (t-SH) is accepted as an indicator of antioxidant status. Alcoholic extract of the spider *Tarantula Cubensis* (TCAE) is used as a homeopathic agent in the Veterinary medicine. TCAE has many therapeutic effects such as anti-inflammatory, antitumor, necrotizing action and wound healing. In this study, it was aimed to investigate the effect of TCAE on AOPP and t-SH levels in the stomach tissue of rats with indomethacin-induced gastric damage.
Methods: The rats were divided into 4 groups (n=7) as control, Indomethacin (25 mg/kg indomethacin), Indomethacin + Lansoprazole (25 mg/kg indomethacin +30 mg/kg lansoprazole) and Indomethacin +TCAE (25 mg/kg indomethacin + 200 µg/kg TCAE) group. Five minutes after lansoprazole and TCAE administration, all rats except the control group were given a single dose of indomethacin (25 mg/kg) orally, and six hours after indomethacin administration, the gastric tissue was removed for biochemical analysis under anesthesia. Spectrophotometric method was used to measure AOPP and t-SH levels in gastric tissue.
Results: In the study, the effects of TCAE were compared with the effects of lansoprazole used in the treatment of ulcers. A statistically significant ($p<0.05$) increase was found in the AOPP and t-SH levels in the indomethacin group compared to the control group. AOPP and t-SH levels were found to be lower in the Indomethacin + TCAE group compared to the Indomethacin and Indomethacin + Lansoprazole groups.
Conclusion: According to the results obtained in this study, it was determined that TCAE could prevent oxidative damage in gastric tissue by inhibiting the increase in AOPP and t- SH levels caused by indomethacin administration, and this effect was stronger than lansoprazole.

Keywords: *Tarantula cubensis* alcoholic extract, Indomethacin, Advanced oxidation protein products, Total sulfhydryl, Gastric damage.

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Introduction

Indomethacin, a drug prescribed for rheumatoid arthritis and joint diseases due to its anti-inflammatory properties, is used to create ulcer models because it has a higher ulcer-forming potential than other NSAIDs (1). The formation of free radicals due to the inhibition of prostaglandin synthesis by indomethacin has been recognised as a very important biochemical reaction in the pathophysiology of gastric ulcers (2). Studies have reported that reactive oxygen species and lipid peroxidation formation, GSH depletion, and increased gastric acid secretion play an important role in the pathogenesis of indomethacin-induced gastric ulcer, and that these changes are responsible for oxidative damage in gastric mucosal cells (3, 4).

The alcoholic extract of the *Tarantula cubensis* spider (TCAE) is used as a homeopathic medicine in the veterinary field. TCAE anti-inflammatory, anti-tumour, anti-flogistic, restrictive, necrotising effects and wound healing properties have been demonstrated in clinical studies. TCAE has also been reported to be effective in clinical veterinary medicine for the treatment of pododermatitis, cutaneous papillomatosis, endometritis, and foot and mouth lesions. It is effective in stopping the growth of canine tumours, reducing inflammation and tarsal bursitis volume, and also in stimulating epithelialisation in cutaneous wounds. In a study on farm animals, TCAE was found to be effective in wound healing and closure and to accelerate the healing process. It is thought that TCAE is preferred in the treatment of certain diseases because it separates diseased and normal cells and activates the immune system due to its very rapid regeneration properties (5).

In current oxidative stress studies, the focus has been on identifying specifically oxidised proteins (AOPP) in various human diseases (6). AOPP is defined as cross-linked protein products containing dithirozine. On the other hand, it has been reported that AOPP structurally resembles advanced glycation end products (7). AOPP is a sensitive marker for determining the degree of protein oxidation. Protein oxidation is defined as the covalent modification of proteins resulting directly from reactions with reactive oxygen metabolites or indirectly from secondary products of oxidative stress (8). The oxidative modification of proteins by reactive derivatives plays a role in the aetiology or progression of a number of disorders and diseases (6). In humans, AOPP is associated with chronic renal failure (7), diabetes mellitus (9), diabetic nephropathy (10), coronary artery disease (11), and obesity (12). The chronic accumulation of AOPP has been shown to trigger inflammatory events in the diabetic kidney (10) and in chronic renal failure (7) and may be a by-product of neutrophil activation during infection.

All aerobic cells possess protective mechanisms that minimise the formation of free radicals or rapidly break them down as soon as they are formed. These antioxidant protective mechanisms also include compounds containing sulphhydryl groups (13, 14). Free radicals cause oxidative protein damage by leading to the oxidation of thiol (-SH) groups in proteins (15-18). The thiol groups in proteins exhibit antioxidant function by trapping oxidants that initiate peroxidation. Thus, lipids are protected from the oxidative attack of free radicals (18). The earliest observable sign of radical-induced protein damage is the conversion of thiol groups to disulphides and oxy acids. Biological thiols such as glutathione and cysteine prevent oxidant-induced protein inactivation either by reacting directly with radicals or indirectly by forming reversible bonds with free thiol groups in proteins (16). Therefore, the total sulphhydryl (t-SH) level is an important indicator of oxidative stress (19, 20).

This study aimed to determine the effect of TCAE, which has been reported to have a multifaceted positive effect, on AOPP and t-SH levels in the gastric tissue of rats with gastric damage induced by indomethacin. As the effect of TCAE on AOPP and t-SH levels has not been determined in previous studies, we believe this study will make significant contributions to the literature.

Materials and Method

Experimental Animals

In this study, 28 male Wistar albino rats weighing 180-230 grams and aged 5 months, bred at the Experimental Research Unit of the Faculty of Medicine, Van Yüzüncü Yıl University, were used. The study was conducted with the permission of the Local Ethics Committee for Laboratory Animals, dated 02.05.2019, No. 2019/04. It was also supported by the Scientific

Research Projects Unit of Van Yüzüncü Yıl University under project number TDK-2019-8534. Before starting the study, the rats were allowed to adapt to the environment for 2 weeks. The rats were kept in cages at a room temperature of 22-25°C, with 12 hours of light and 12 hours of darkness. After the adaptation period, the animals were weighed and randomly divided into seven equal groups with similar weights. All groups were fasted for 24 hours before the start of the experiment. However, there were no restrictions on water intake. The current practices regarding the care of experimental animals were carefully followed.

Gastric Ulceration and Collection of Gastric Samples

This study, conducted to investigate the effects of TCAE on indomethacin-induced gastric damaged tissue, was based on the method of Guidobono et al. (21).

Table 1. Experimental groups and drugs

Groups	Drugs
Control	Only 1ml saline solution (n:7)
İndomethacin (IND)	25 mg/kg İndomethacin (n:7)
Indomethacin + Lansoprazole (IND+LAN)	25mg/kg Indomethacin + 30 mg/kg lansoprazole (n:7)
İndomethacin + TCAE (IND+TCAE)	200 µl/kg TCAE + 25mg/kg indomethacin (n:7)

All experimental groups except the control group were fasted for 24 hours prior to drug administration. Five minutes after oral administration of lansoprazole [lansoprazole neutralizes stomach acid, which is crucial for the treatment of stomach-related diseases such as stomach ulcers and gastroesophageal reflux. The use of proton pump inhibitors in the treatment of these diseases has revolutionized treatment. These drugs suppress stomach acid strongly and specifically. This eliminated acidic environment allows for rapid healing of lesions (22). Therefore, lansoprazole (Lansor®, Sanovel, İstanbul, Turkey) was used to compare the AOPP and t-SH activity of TCAE in gastric injury] at a dose of 30 mg/kg (22-24) and subcutaneous administration of TCAE (Theranekron® 50 mg, Richter Pharma, Wels, Austria) at a dose of 200 µl/kg (25), to induce gastric damage, indomethacin (Endol®, oral capsule, DEVA Pharmaceutics, İstanbul, Turkey) was administered at a dose of 25 mg/kg (26-28) to all rats except the control group. Prior to the animal experiments, all rats in the experimental groups were euthanised by intracardiac blood collection from the heart using 50 mg/kg ketamine (Alfamine®, 100mg/ml, Alfasan, Holland) and 5 mg/kg xylazine (Alfazyne®, 20mg/ml, Alfasan, Holland) administered 6 hours after drug administration. Their stomachs were then removed. 0.5 g tissue sample was weighed and washed with 10% 150 mM phosphate buffer (pH 7.4). After drying, the tissue sample was homogenised again with phosphate buffer (10%) in a homogeniser at 2000 rpm for 1 minute. The homogenates were centrifuged at 12,000 rpm at +4 °C for 10 minutes, and the supernatants were stored at -80 °C until analysis. AOPP and t-SH levels were determined in these supernatants obtained from stomach tissue homogenates. The AOPP level was studied according to the method of Witko et al. (29) and the AOPP value was expressed as µmol/g tissue. The t-SH level was determined according to the Ellman method modified by Hu (19).

Statistical Analyses

Descriptive statistics for the characteristics under consideration are expressed as the median, mean, standard deviation, minimum and maximum values. For these characteristics, the Kruskal-Wallis test was used to compare groups. In the calculations, the statistical significance level was set at 5%, and the SPSS statistical package programme (version 21) was used for the calculations.

Results

Findings related to AOPP and t-SH levels in gastric tissue are shown in Tables 2 and 3. When the results obtained in terms of AOPP level in gastric tissue were evaluated statistically, the difference between the Control and IND + TCAE group and IND group was found to be statistically significant ($p<0.05$). There was a

statistically significant increase in AOPP levels in the IND group compared to the Control group, but there was no statistically significant increase in the IND + LAN group compared to the Control group.

In this study, the difference in t-SH levels between the Control and IND + TCAE groups and the IND group was found to be statistically significant ($p<0.05$). While t-SH levels were higher in the IND group compared to the other groups, t-SH levels were lower in the IND + TCAE group compared to the other groups.

Discussion

In this study, aimed to determine the effect of TCAE on AOPP and t-SH levels in the gastric tissue of rats with gastric damage induced by indomethacin, it was determined that AOPP levels increased in the IND and IND+LAN groups as a result of gastric damage in the rat stomach, but remained unchanged in the IND+TCAE group (Table 2, Figure 1).

Table 2. AOPP levels (µmol/g tissue) of groups (mean ± standard deviation)

Group	n	$\bar{x} \pm SD$	Minimum	Maximum
Control	7	8.36 ± 4.12^{b}	4.15	14.00
İndomethacin	7	37.60 ± 23.08^{a}	9.89	65.60
İndomethacin + Lansoprazole	7	23.16 ± 16.97^{ab}	4.50	46.97
İndomethacin + TCAE	7	6.65 ± 5.94^{b}	3.91	14.33

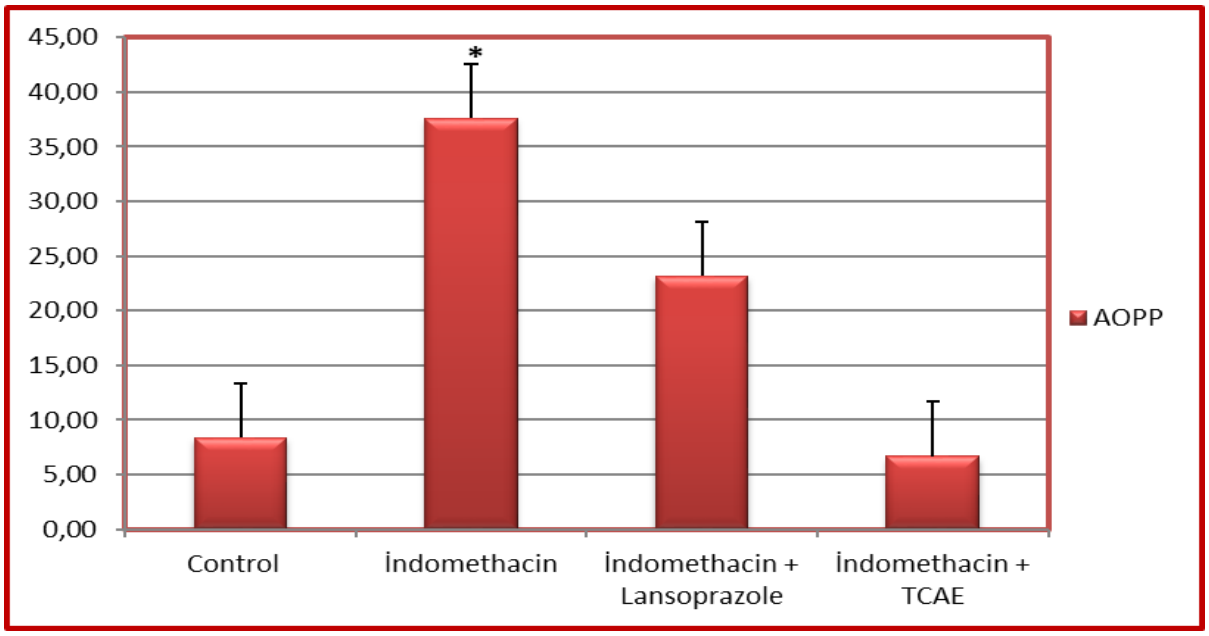

Figure 1. Graphical representation of AOPP (µmol/g tissue) levels of groups
*The difference compared to the control group is significant ($p > 0,05$)

In recent years, there has been an upward trend in the number of studies investigating the increase in protein oxidation in various diseases in humans (30, 31) and experimental studies in animals (8, 32). AOPP is produced by chloramines and hypochlorous acid generated by myeloperoxidase in activated neutrophils during oxidative stress. AOPP is considered a reliable marker for estimating the degree of protein oxidation. AOPP is thought to be closely related to inflammation. Furthermore, a strong relationship has been reported between the progression of disease and protein oxidation (29, 33). However, no studies have been found regarding AOPP levels in studies conducted by inducing gastric damage. In this study, it is thought that increased free radicals due to inflammatory events caused by gastric damage in the IND and IND+LAN groups initiated protein oxidation and caused an increase in

AOPP levels due to the shift of the oxidant/antioxidant balance towards oxidative damage, but TCAE prevented this increase with its antioxidant effect. Some substances that exert a protective effect by participating in the antioxidant system within cells (such as silymarin, lycopene, and selenium) can be used for gastroprotective purposes in ulcer treatments. Homeopathy is an alternative treatment method used in the treatment of many diseases (34). There are studies indicating that TCAE, a homeopathic medicine, has antioxidant activity. In a study similar to ours, Cankara et al. (35) investigated the gastroprotective effect of TCAE in a rat model of indomethacin-induced peptic ulcer and determined that pretreatment with TCAE reduced the total oxidative status (TAS/TOS) and that TCAE treatment improved indomethacin-induced peptic ulcers through antioxidant and anti-inflammatory effects. Eren and Aksit (36) found that the increase in MDA levels and decrease in SOD levels resulting from gentamicin nephrotoxicity showed the opposite change with TCAE administration. Karabacak et al. (25) reported that MDA levels increased in the kidney tissue of rats administered aflatoxin, while TCAE administration decreased MDA levels.

Table 3. t-SH levels (µmol/g tissue) of groups (mean ± standard deviation)

Group	n	$\bar{x} \pm SD$	Minimum	Maximum
Control	7	0.093 ± 0.11^{b}	0.079	0.106
İndomethacin	7	0.320 ± 0.14^{a}	0.134	0.479
İndomethacin + Lansoprazole	7	0.247 ± 0.20^{ab}	0.093	0.588
İndomethacin + TCAE	7	0.090 ± 0.08^{b}	0.063	0.140

↓ a, b, c: Differences between groups with different lowercase letters in the same column are significant ($p<0.05$).

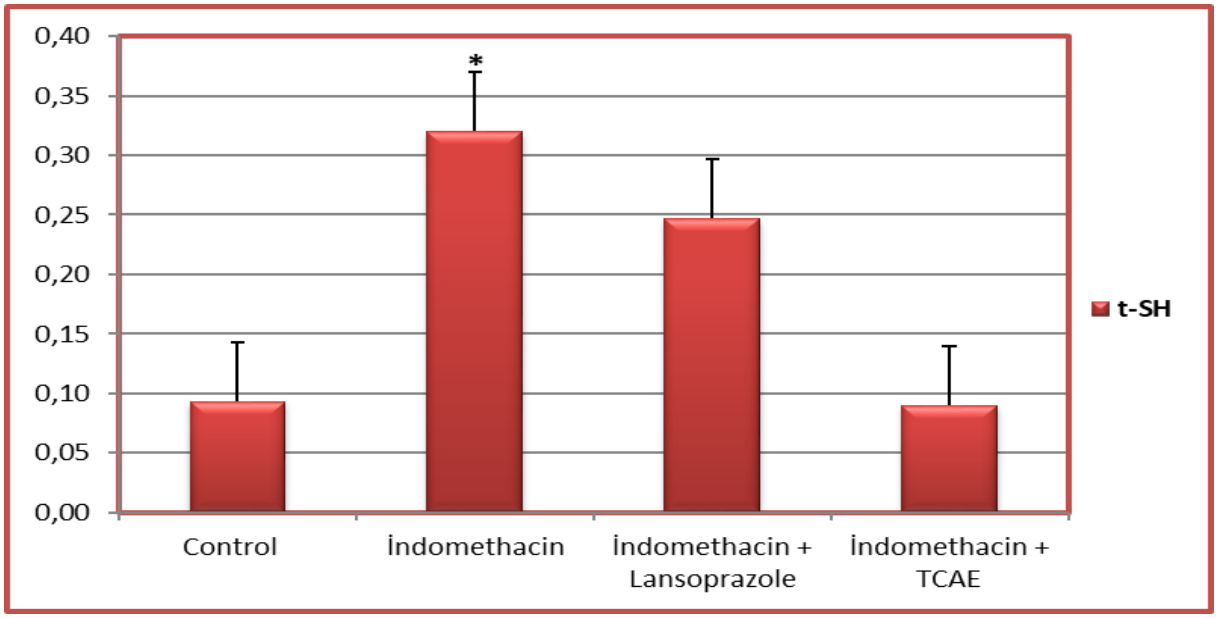

Figure 2. Graphical representation of t-SH (µmol/g tissue) levels of groups
*: The difference compared to the control group is significant (p > 0,05)

A study by Şebin et al. (37) found that TCAE reduced the effects of oxidative stress and inflammation associated with ischaemic perfusion in renal ischaemic perfusion.

t-SH groups are part of the antioxidant system and are also referred to as thiols. When reactive nitrogen species and reactive oxygen species increase, t-SH can be oxidised, thereby reducing the antioxidant defence pool.

Therefore, t-SH levels reflect the overall redox status of the body. Their decrease is indicative of high oxidative stress, and conversely, increased levels are attributed to the detoxification of the harmful effects of ROS/RNS and cell repair (38). According to the findings obtained in this study, it was determined that t-SH levels increased in rats in the IND and IND+LAN groups, but remained unchanged in the IND+TCAE group (Table 3, Figure 2). As a result of the study, a positive relationship was found between AOPP and t-SH levels. Therefore, since the increase in AOPP, an oxidation product, was prevented by TCAE administration, it was concluded that there was no increase in t-SH levels, an indicator of antioxidant status.

Conclusion

According to the results obtained in this study, it was determined that TCAE could prevent oxidative damage in gastric tissue by inhibiting the increase in AOPP and t-SH levels caused by indomethacin administration, and this effect was stronger than lansoprazole.

Declaration

Ethics Committee Approval

This study was accepted by the Van Yuzuncu Yil University Animal Experiments Local Ethics Committee with its decision dated May 2, 2019, and numbered 2019/04.

Author Contribution Statement

Concept - UMY; Design - UMY; Supervision - UMY; Resources - UMY; Materials - UMY; Data Collection and/or Processing - UMY; Analysis and/or Interpretation - UMY; Literature Search - UMY; Article Writing - UMY; Critical Review - UMY; Other – UMY.

Fund Statement

The author declared that this study received no financial support.

Conflict of Interest

The author declare no conflicts of interest.

Acknowledgements

I would like to thank Assoc. Prof. Dr. Ahmet Ufuk KÖMÜROĞLU for his support during the biochemical analyses and Prof. Dr. Sıddık KESKİN for his assistance with the statistical analyses. This study was presented as an oral presentation at the International Black Sea Modern Scientific Research Congress held in Rize between 29 September-02 October 2022.

Declaration on the Use of AI

The author confirm that AI tools were used solely for auxiliary purposes such as language support, grammar and spelling corrections, translation, or limited data analysis; and declare that all original ideas, interpretations, and academic content are solely the work of the author.

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