The evaluation of the protective effect of propolis extract against acrylamide induced injury on the brain, lung, liver, and kidney of mice

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ABSTRACT

Propolis is a complex chemical compound, made by honeybees, and is known for its varied biological properties, including antitumor, antibacterial, immunomodulatory, antiviral, anti-parasitic, anti-inflammatory, and organ-protective effects. Acrylamide, a water-soluble chemical used in a variety of industrial products and created in hot foods, is a group 2A carcinogen with adverse effects on many body systems. The protective consequences of propolis extract on mice exposed to acrylamide are examined in this research. A total of 28 male C57BL/6 mice were randomly allocated into four treatment groups: control, acrylamide-treated alone, acrylamide plus propolis extract, and propolis-treated extract alone. A propolis extract is obtained by dissolving crude propolis in ethanol. Mice were administered 50 mg/kg acrylamide and 100 mg/kg propolis extract intraperitoneally for 11 days. Histopathological and biochemical analyses, focusing on oxidative stress markers (SOD, GPx, GSH, MDA, FRAP, and catalase), were performed on liver, kidney, lung, and brain tissues. In all tissues studied, acrylamide administration markedly increased oxidative stress and pathological lesions. The group that received propolis extract and acrylamide mitigated these effects, showing a notable decline in tissue lesions and improved oxidative stress parameters. Additionally, propolis extract injection reduced gliosis, edema, pneumonia, necrosis, and other tissue lesions. It also enhanced antioxidant enzyme activities and decreased MDA levels, indicating reduced lipid peroxidation. This research suggests propolis could act as a therapeutic agent to mitigate the harmful effects of acrylamide.

Introduction

Propolis, a resinous substance, is a complex chemical compound that honeybees gather from diverse plants (9). Due to its numerous biological properties like antitumor, antibacterial, and immunomodulatory effects, this substance has been used in traditional medicine. (7, 30, 40). Multiple studies have indicated that propolis offers antiviral (5), anti-parasitic (20), and anti-inflammatory (27) benefits, along with protecting the liver (7, 33), kidney (4, 41), and lung (30). This substance contains over 300 identified compounds, including fatty and phenolic acids, flavonoids, terpenes, esters, β-steroids, aromatic aldehydes, alcohols, sesquiterpenes, and naphthalene (21, 36). The phenolic compounds found in propolis, particularly flavonoids, are primarily responsible for its biological effects. Flavonoids demonstrate a broad spectrum of biological activities (37), including antibacterial (44), antiviral, anti-inflammatory, antiallergic, and vasodilatory properties (10). Additionally, they may also suppress lipid peroxidation, platelet aggregation, capillary permeability and fragility, and the action of enzymes like cyclooxygenase and lipoxygenase (26).

Acrylamide, formula C_3H_5NO, is colorless, odorless, and dissolves in water. It is a constituent unit found in

polyacrylamide and its related copolymers (18). Polyacrylamides and copolymers of acrylamide are utilized in many industrial processes such as paper manufacturing, plastic production, gel electrophoresis, soil softening, and purification processes for drinking water and wastewater treatment (48). Heating food also produces acrylamide through the Maillard reaction (24). A Schiff base results from the reaction between the carbonyl group in reducing sugars and the amine group in amino acids, notably free asparagine. This reaction leads to acrylamide production via subsequent decarboxylation. Acrylamide was classified by the International Agency for Research on Cancer in 1994 as a group 2A carcinogen to humans. Earlier findings indicated that acrylamide harms multiple bodily systems, including the genitourinary, gastrointestinal, pulmonary, and hepatobiliary systems, as well as the reproductive, cardiovascular, immune, and nervous systems (13, 22, 42, 52, 53). In addition, it has a carcinogenic effect (50). This work developed a mouse model exposed to acrylamide to examine the protective effects of propolis extract on the liver, kidney, lung, and brain tissues, aiming to establish an experimental base for clinical prevention and treatment of acrylamide toxicity.

Materials and Methods

Reagents: Acrylamide was purchased from Merck, Rahway, NJ, USA. The crude propolis used in this investigation was collected from the hive of *Apis mellifera* in rural areas of Mashhad, Khorasan Razavi, Iran. High-purity analytical reagents were used exclusively in the study.

Extraction of Propolis: For extraction, 100 ml of 70% ethanol alcohol was mixed with 20 grams of pure propolis in a dark brown bottle; the mixture was kept at 25ºC in the dark for 7 days. The container was shaken two to three times daily. The mixture was filtered through Whatman filter paper. This process is repeated twice. Finally, the alcohol was evaporated at 45ºC, and the resulting compound extract was weighed and stored in a dark storage container until use (23).

Animal Treatments: The experiment was carried out on 28 8-week-old male C57BL/6 mice (weighing 20–30 g), obtained from the Veterinary College, Ferdowsi University of Mashhad. Animals were housed in standard mice cages (290 × 220 × 140 mm; 7 mice per cage) under controlled temperature (21 ± 2°C) and 40–50% humidity conditions with a 12 h dark: 12 h light cycle. Food and water were provided ad libitum. After 7 days of acclimation, 28 mice were randomly divided into 4 groups of 7: [1] Controls, which received distilled normal saline intraperitoneally (IP) for 11 days; [2] mice that received 50 mg/kg of acrylamide (CAS No. 79-06-1, Merck) IP for 11 days; [3] mice simultaneously treated with acrylamide (50 mg/kg) and propolis extract (100 mg/kg) for 11 days; and [4] mice that received 100 mg/kg of propolis extract IP for 11 days (3, 31).

Histopathological Analyses: Following the experimental trial, all mice were euthanized and necropsied, and their lung, liver, brain, and kidney tissue samples were collected. After gross examination, tissues were fixed in 10% formaldehyde and embedded in paraffin for microscopic analysis. Then, 5 µm sections from paraffin blocks were H&E stained and analyzed microscopically; potential histological changes were graded as follows: Score 0: normal and without changes; Score 1: Microscopic tissue involvement is less than 25% of fields; Score 2: 25-50% tissue involvement; Score 3: 50-75% tissue involvement; Score 4: More than 75% tissue involvement (11). At least ten fields were scored per section to determine the median.

Biochemical Analyses: Frozen tissue samples were rapidly thawed and homogenized in chilled 0.05 M phosphate buffer (pH 7.4), which was freshly prepared by mixing equimolar solutions of sodium dihydrogen phosphate (NaH_2PO_4; DNAbiotech, Cat. No. DB9645-250) and disodium hydrogen phosphate (Na_2HPO_4; DNAbiotech, Cat. No. DB9644-500g) with the pH adjusted to 7.4 using a calibrated pH meter. Homogenization was performed at 10% w/v for 5 minutes. The homogenate was then centrifuged at 4°C and 4,000 × g for 15 minutes to remove debris. The supernatant was used for oxidative stress parameter measurements.

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were determined using diagnostic kits (Navand Salamat, Iran; Catalog Nos. NS-15082, NS-15083 for GPx and NS-15034 for SOD). The GPx and SOD results are reported as U/g tissue. Tissue catalase (CAT) activity was evaluated by measuring the reduction in absorbance at 240 nm resulting from the breakdown of hydrogen peroxide (H_2O_2) by catalase (25).

The measurement of glutathione (GSH) was performed using a diagnostic kit (Navand Salamat, Iran; Catalog Nos. NS-15086, NS-15087), based on its reaction with 5,5'-dithio-bis (2-nitrobenzoic acid) to form a yellow compound, measurable at 412 nm. The concentration of glutathione was expressed as µmol/g tissue.

The malondialdehyde (MDA) concentration was determined by its reaction to thiobarbituric acid, resulting in the formation of a pink compound, the absorbance of which was measured at 539 nm (49). The concentration of MDA was calculated utilizing an extinction coefficient value of 156,000 M^{-1} cm^{-1}, and the findings were expressed as nmol/g of tissue.

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The ferric-reducing antioxidant power (FRAP) was evaluated by the reduction of ferric tripyridyltriazine by antioxidants present in the sample (14). This reduction resulted in a deep blue color, the absorbance of which was calculable at 593 nm. FRAP values were determined using a standard curve of Fe^{2+} and are shown in µmol Fe^{2+}/g of tissue.

Statistical Analysis: In this research, we used SPSS version 27. Parametric and nonparametric data were presented as mean ± standard deviation and median ± interquartile range (IQR), respectively. One-way analysis of variance (ANOVA) with the Tukey-Kramer post hoc test for parametric data and the Kruskal-Wallis test and Mann–Whitney U test for nonparametric data were used to compare significant differences among treatment groups. A P-value < 0.05 was considered statistically significant.

Results

Histopathological Findings:

Brain: The acrylamide group showed significantly more severe lesions, including gliosis, edema, and hyperemia. Furthermore, the group receiving combined propolis extract experienced a considerable reduction in the severity of edema, ischemia, and gliosis (Figures 1, 2).

Lung: The outcomes demonstrated that acrylamide prescription significantly increased the incidence of lesions such as pneumonia, hyperemia, and emphysema compared with the control group. Compared with the group that received only acrylamide, the group treated with both propolis extract and acrylamide exhibited a substantial reduction in the incidence of pneumonia (Figures 3, 4)

Liver: Acrylamide administration was found to significantly increase tissue lesions, such as necrosis, congestion, and tissue degeneration, as compared with the control group. When comparing the group administered only acrylamide to the group receiving both acrylamide and propolis extract, a notable decrease in the severity of congestion was observed (Figures 5, 6).

Kidney: Acrylamide led to a considerable rise in tissue lesions such as cell swelling, necrosis, hyperemia, and hyaline cast compared with the control group. Compared with the acrylamide-only group, the group receiving both acrylamide and propolis extract experienced a notable reduction in lesion extent (Figures 7, 8).

Biochemical Findings:

SOD: Compared with controls, acrylamide significantly reduced SOD levels in liver and lung tissue. Administration of propolis extract and acrylamide in the third group caused the elevation of SOD levels to amounts that had no significant difference as compared with those of controls (Figure 9).

GPx: The administration of acrylamide led to a significant decrease in GPx levels of the liver and kidney from the second group of animals as compared with the control group. In group 3, combined propolis and acrylamide treatment increased kidney GPx levels, but not liver levels, without significant differences from controls (Figure 10).

GSH: In the liver as well as kidney tissues, administration of acrylamide caused a significant decrease in GSH concentration compared with the control group. Conversely, in group 3, propolis increased liver and kidney GSH concentrations to levels not significantly different from the control group (Figure 11).

MDA: Acrylamide administration in group 2 notably elevated MDA levels of all studied tissues in comparison with controls, although the difference was only significant for liver and kidney MDA values. Moreover, treatment with propolis during acrylamide injection declined MDA concentrations in the liver and kidney to amounts that had no significant difference from those of the control group (Figure 12).

FRAP: Figure 13 demonstrates that acrylamide treatment in group 2 significantly decreased FRAP in all tissues except the liver compared with controls. Tissue FRAP values remained largely unchanged in group 3 (propolis and acrylamide) compared with group 2 (Figure 13).

Catalase: As shown in Figure 14, hepatic catalase activity increased significantly in group 2 as compared with the control group. Acrylamide administration in group 2 caused a significant decrease in brain and kidney catalase activities as well as a non-significant decrease in lung catalase activities as compared with controls. Propolis treatment in the third group caused elevation of catalase activity in the lung, brain, and kidney of studied animals to the amounts that had no significant difference with those of the control group (Figure 14).

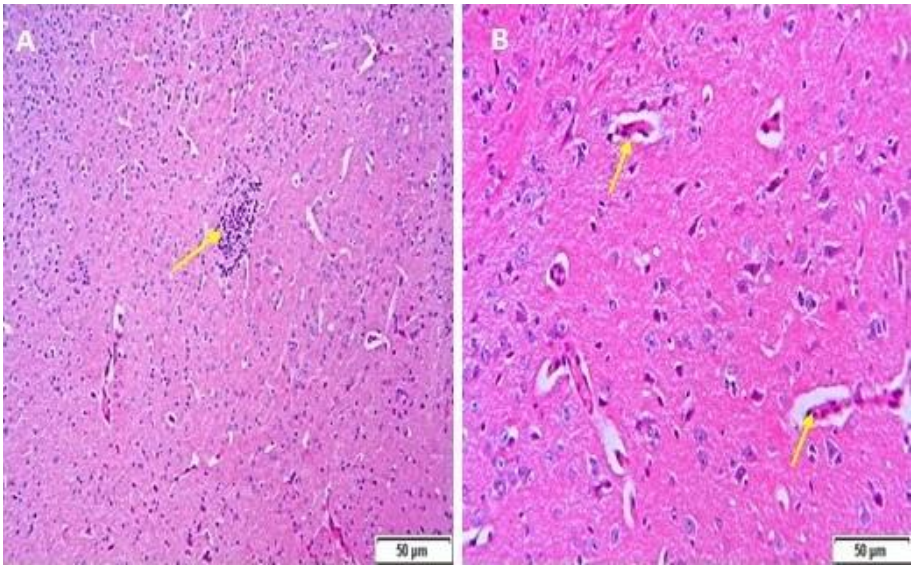

Figure 1. The histopathological changes of brain sections from different treatment groups were stained with H&E by light microscopy. (A) Induction of gliosis due to acrylamide administration in brain tissue (arrows). (B) Induction of hyperemia and edema due to acrylamide.

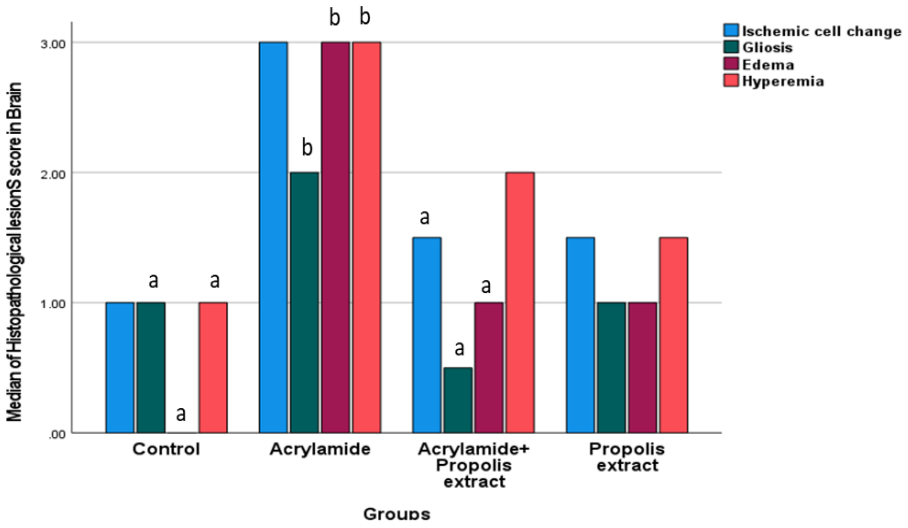

Figure 2. Histopathological lesion scores of brain tissue in different treatment groups. Median scores for ischemic cell change, gliosis, edema, and hyperemia were evaluated across all groups (n = 7 per group). Different letters (a, b) indicate statistically significant differences between groups (P<0.05).

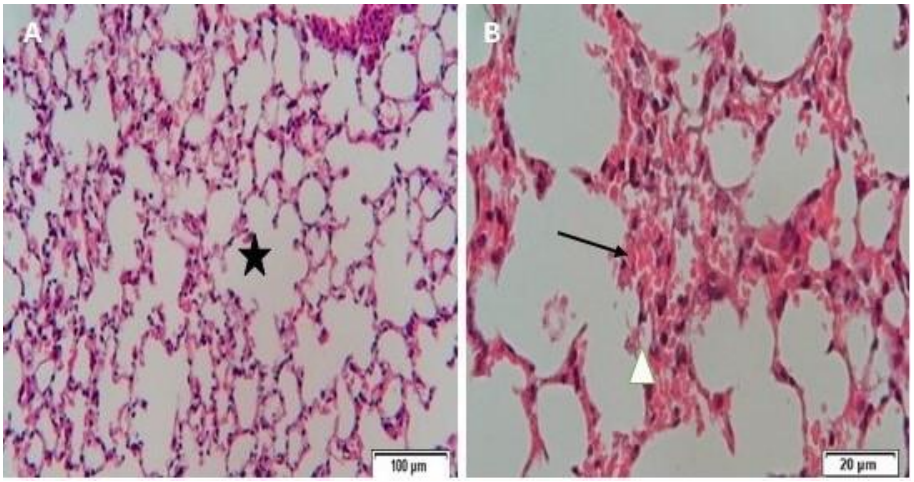

Figure 3. Changes in lung sections from different treatment groups stained with H&E by light microscopy. (A, B) Emphysema (asterisks), pneumonia (white arrowhead), and hyperemia (arrow) were induced in the group receiving acrylamide (× 100 and × 400 magnifications respectively).

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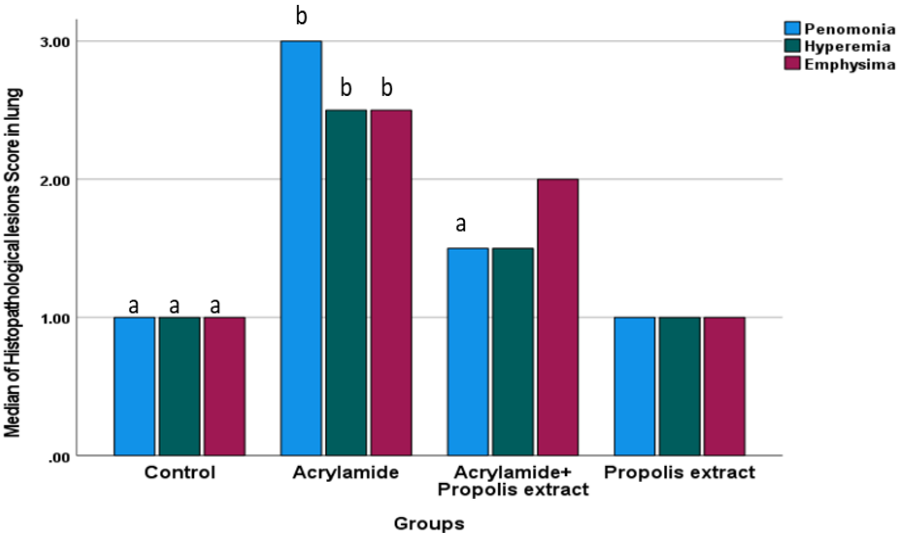

Figure 4. Histopathological lesion scores of lung tissue in different treatment groups. Median scores for pneumonia, hyperemia, and emphysema were evaluated across all groups (n = 7 per group). Different letters (a, b) indicate statistically significant differences between groups (P<0.05).

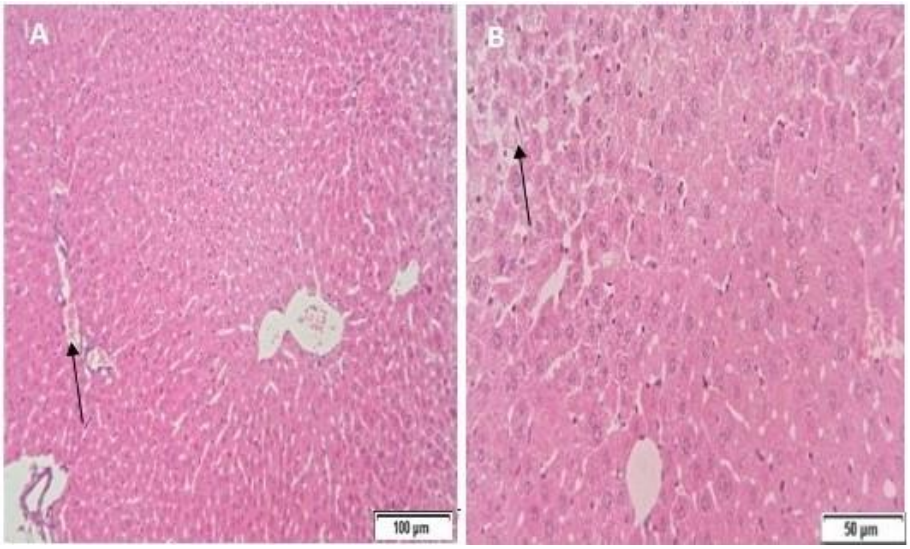

Figure 5. Changes in liver sections from different treatment groups stained with hematoxylin and eosin (H&E) by light microscopy. (A) Hyperemia and congestion in the liver tissue because of receiving acrylamide (x100 magnification). (B) Degeneration due to receiving acrylamide shown by arrow (x200 magnification).

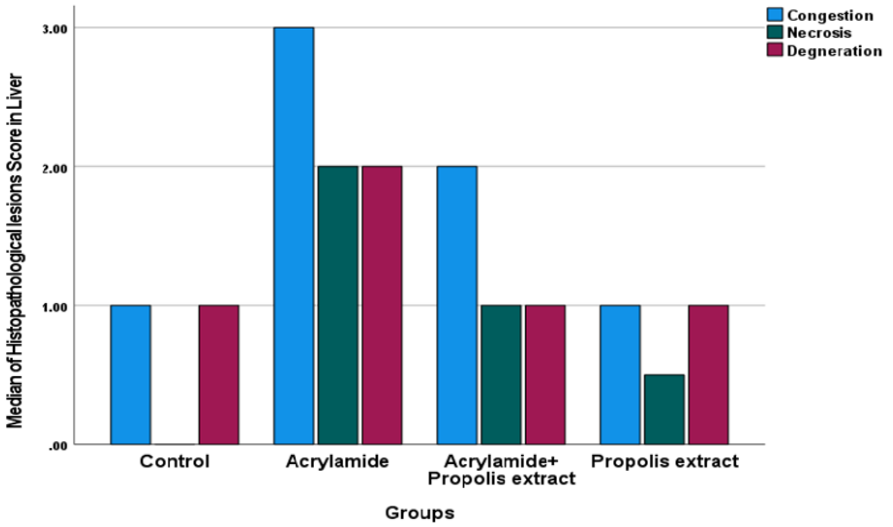

Figure 6. Histopathological lesions score of liver tissue in different treatment groups. Median scores for necrosis, congestion, and degeneration were evaluated across all groups (n = 7 per group). Different letters (a, b) indicate statistically significant differences between groups (P<0.05).

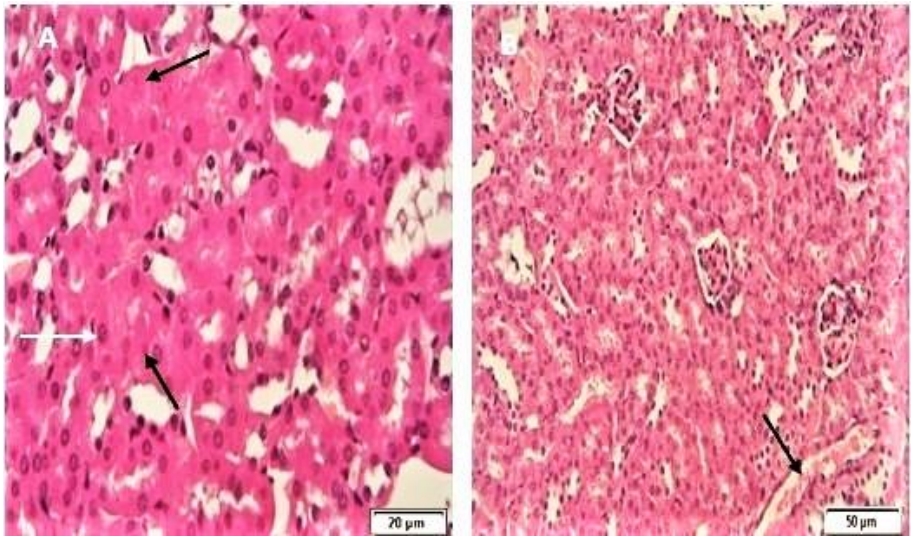

Figure 7. The histopathological changes of kidney sections from different treatment groups were stained with hematoxylin and eosin (H&E) by Light microscopy. (A) The presence of cell swelling (white arrow) and necrosis (black arrow) due to the administration of acrylamide in the kidney tissue (x400 magnification). (B) hyperemia and hemorrhage were induced in the group receiving acrylamide as shown by the black arrow (x200 magnification).

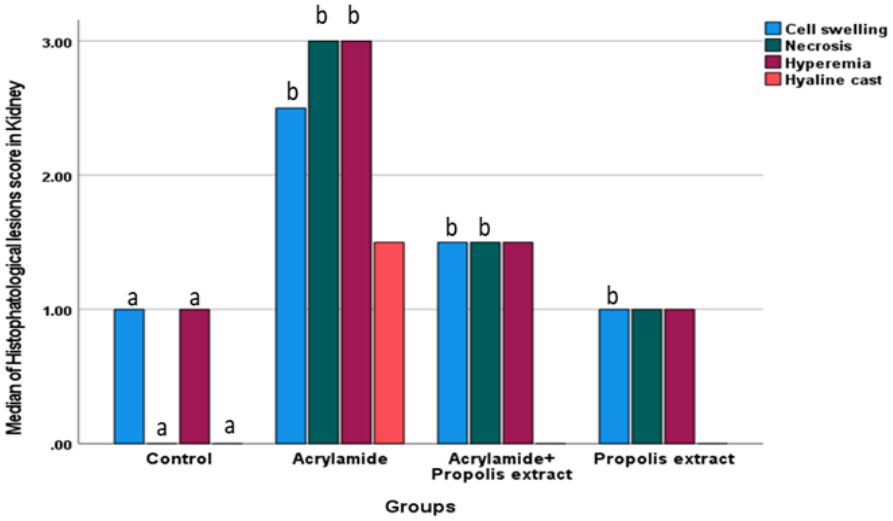

Figure 8. Histopathological lesions score of kidney tissue in different treatment groups. Median scores for necrosis, cell swelling, hyperemia, and hyaline casts were evaluated across all groups (n = 7 per group). Different letters (a, b) indicate statistically significant differences between groups (P<0.05).

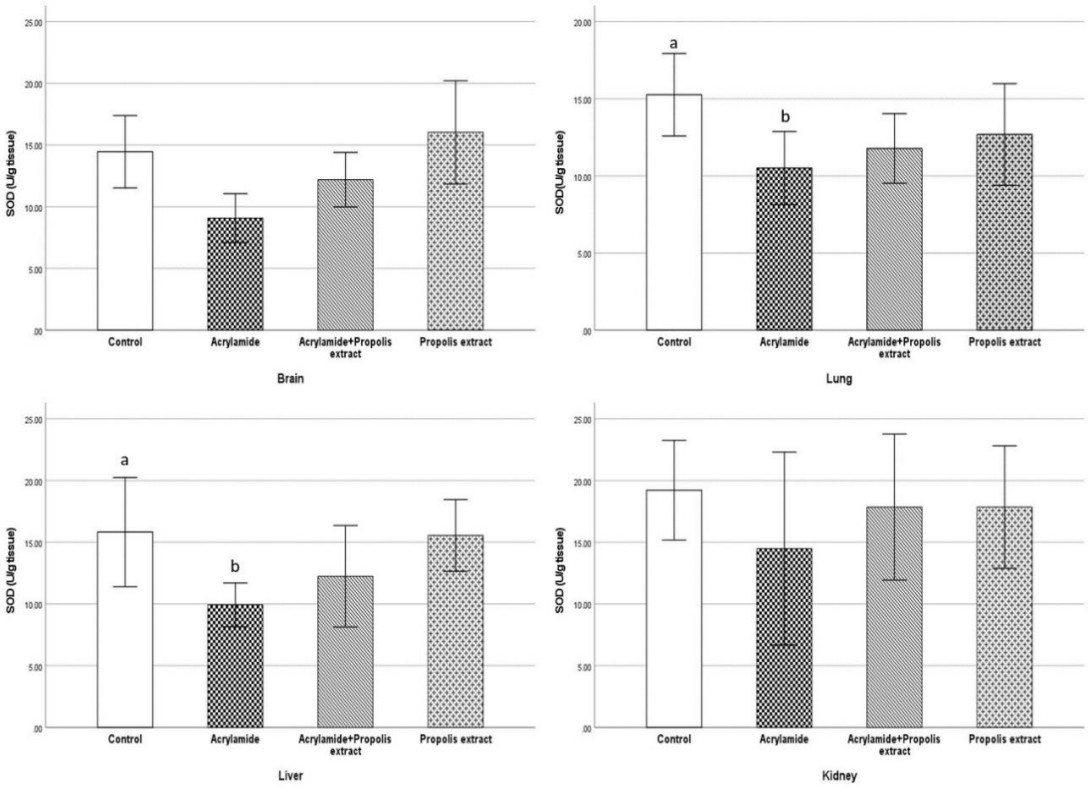

Figure 9. The SOD activity levels (U/g) in the examined groups (n= 7 samples in each group) are presented as the Mean ± Standard Deviation. Non-similar letter indicating significant difference P<0.05.

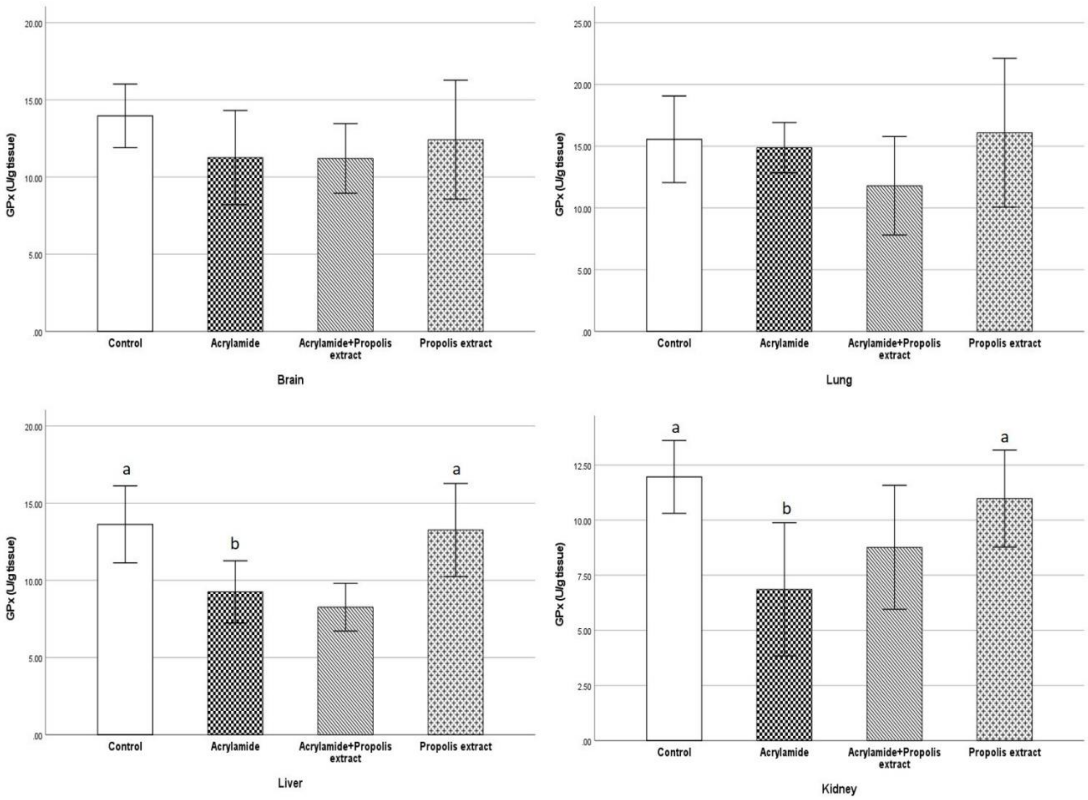

Figure 10. The GPx activity levels (U/g) in the examined groups (n= 7 samples in each group) are presented as Mean ± Standard Deviation. Non-similar letter indicating significant difference P<0.05.

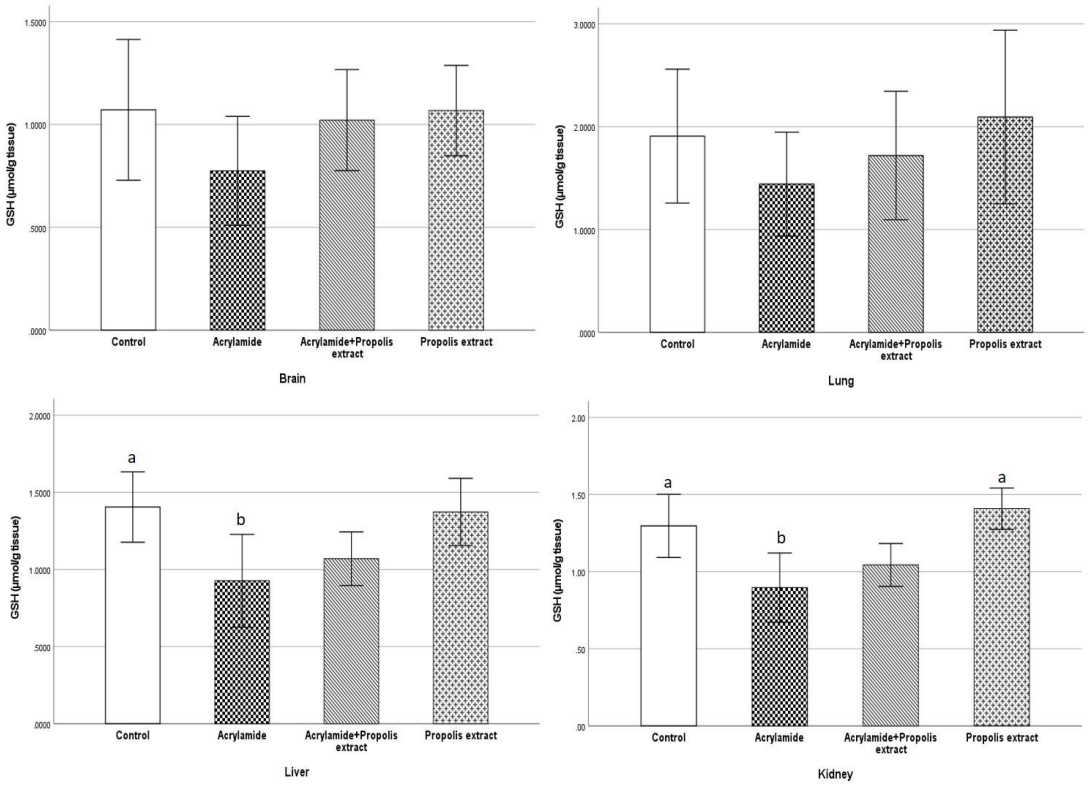

Figure 11. The GSH levels (µmol/g) in the examined groups (n= 7 samples in each group) are presented as the Mean ± Standard Deviation. Non-similar letter indicating significant difference P<0.05.

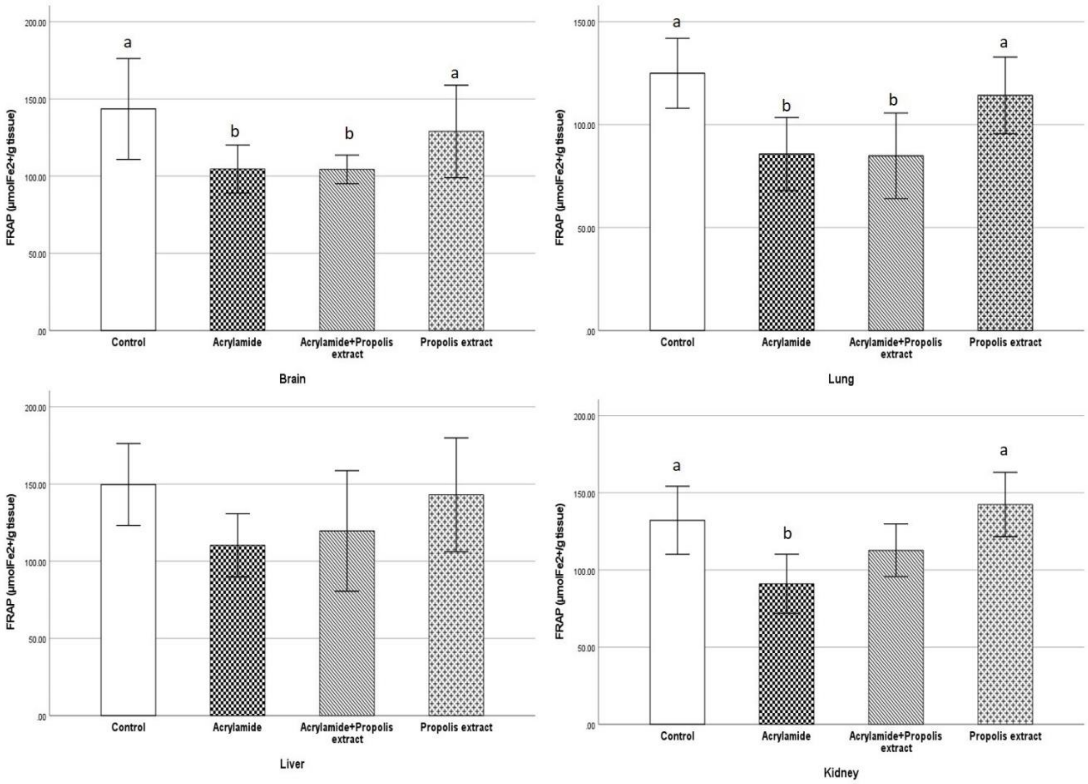

Figure 12. The MDA levels (nmol/mg) in the examined groups (n= 7 samples in each group) are presented as the Mean ± Standard Deviation. Non-similar letter indicating significant difference P<0.05

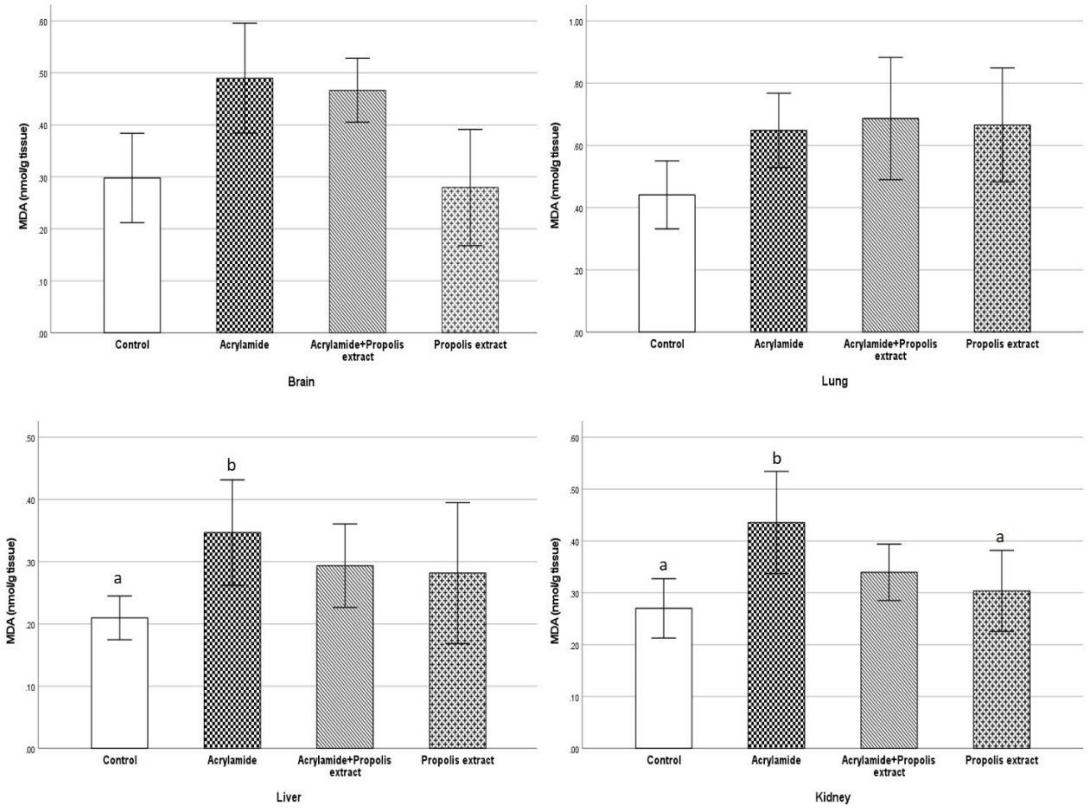

Figure 13. The FRAP levels (µmolFe2+/g) in the examined groups (n= 7 samples in each group) are presented as the Mean ± Standard Deviation. Non-similar letter indicating significant difference P<0.05.

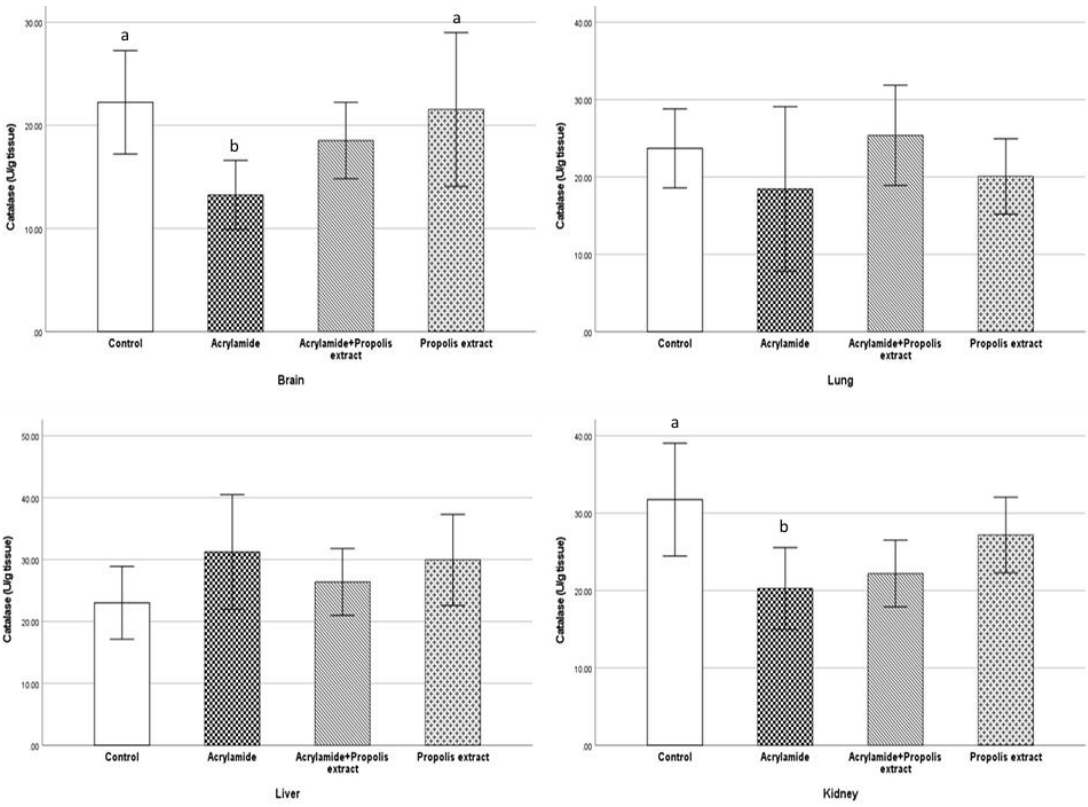

Figure 14. The Catalase activity levels (U/g) in the examined groups (n= 7 samples in each group) are presented as the Mean ± Standard Deviation. Non-similar letter indicating significant difference P<0.05.

Discussion and Conclusion

Research extensively documents the positive effects of natural compounds in combating toxins and harmful agents. Honeybees produce propolis, a substance found at hive entrances, which offers broad protection against many chemical toxins. The antioxidant properties lessen acrylamide's damaging effects considerably. Histological and biochemical analyses assessed how propolis extract reduces acrylamide-caused lesions in the liver, kidneys, brain, and lungs in this study. The acrylamide group exhibited a marked increase in pathological lesions across all studied tissues (liver, kidney, brain, and lung) when compared with the control group. However, the lesions in the propolis/acrylamide group showed a decrease.

Administering propolis extract to the acrylamide group reduced pathological lesions, consistent with research showing propolis protects against gentamicin-induced kidney damage (4) and carbon tetrachloride-induced liver and kidney damage (16). In those studies, propolis showed protective effects against gentamicin-induced kidney lesions and reduced transaminase, alkaline phosphatase, and lactate dehydrogenase levels elevated by carbon tetrachloride. It also decreased oxidative stress and organ damage in the liver and kidneys, aligning with this study's findings. Another investigation assessed propolis extract's protective role in the rat cerebellum exposed to aluminum silicate, revealing Purkinje cell damage, swollen lysosomes, mitochondrial impairment, and reduced collagen. Propolis treatment diminished these cerebellar and brain lesions, suggesting its protective potential (40).

In terms of histology and biochemistry, propolis shows preventive properties against free radical formation, which is attributed to its polyphenolic and flavonoid constituents. In addition, propolis effectively inhibits lipid peroxidation (26, 43). Its ability to decline tissue lesions, as shown by lower MDA levels, is noteworthy (39). Furthermore, propolis contains caffeic acid phenethyl ester (CAPE), a component that effectively reduces oxidative stress (1, 29). The other probable mechanism that can be suggested for the preventive effects of propolis extract against lesions, especially in the kidney, is decreasing the level of blood glucose (17). Propolis also has the capacity to activate matrix metalloproteinases (MMPs) types 8 and 9 to protect kidney tissue by inhibiting cell membrane thrombocytopenia and mesenchymal matrix expansion (45). Additionally, propolis reduces inflammation by decreasing the production of inflammatory cytokines, such as IL-1β, TNF-α, and IL-6, and by inhibiting the activation of pathways like NF-κB (35). In the brain, propolis enhances factors crucial for neuronal survival and function, such as BDNF and Arc, through pathways like the PI3K/Akt pathway, while also influencing processes like autophagy and microRNA regulation (6, 38). In the lungs, it moderates inflammation via the Jak2/STAT3 pathway and demonstrates antiviral properties by hindering viruses from binding to ACE2 receptors (15).

Findings from this and other studies deem acrylamide a harmful substance. Several factors, including dose, exposure duration, and frequency, affect the severity and extent of the pathological lesions (8, 12, 32, 54). Experiments explored the effect of acrylamide on hemoglobin using different administration techniques, including inhalation, ingestion, and intraperitoneal injection (47).

Our bodies possess inherent defenses against harm caused by unstable molecules known as free radicals. Cells and systems neutralize free radicals through antioxidant mechanisms. This protection relies on several antioxidants that play important roles. Key antioxidant enzymes include superoxide dismutase, glutathione, glutathione peroxidase, and catalase. They act as the first line of defense, protecting cells from harmful molecules and toxins (34). Specifically, superoxide dismutase initially transforms superoxide anions into hydrogen peroxide. Following this, hydrogen peroxide is further broken down by catalase and glutathione. Moreover, by oxidizing lipid peroxides and hydrogen peroxides, glutathione peroxidase regenerates glutathione, enabling the cycle to continue. This network's collaborative function protects cells from the damaging effects of oxidative reactions (5). This study's findings of decreased glutathione, glutathione peroxidase, and superoxide dismutase in acrylamide-exposed mice tissues, compared with controls, corroborate earlier research (46). A decrease in antioxidant activity may stem from a combination of factors, including overproduction of acrylamide or free radical metabolites and a build-up of reactive oxygen species in the tissues (2). This study, consistent with prior research, showed acrylamide treatment significantly reduced glutathione peroxidase in the liver and kidneys (28, 46). The amount of intracellular glutathione peroxidase is inversely correlated with the severity of infection (2, 34). FRAP analysis showed a significant drop in total antioxidant capacity in the kidney, lung, and brain tissues of the acrylamide group compared with controls. Previous studies also linked acrylamide exposure to increased malondialdehyde levels (29). This investigation shows propolis extract directly impacts antioxidant activity, potentially lessening acrylamide-induced lesions and tissue lipid peroxidation (51). Likewise, earlier research using propolis extract demonstrated enhanced antioxidant enzyme activity and positive effects against oxidative stress (19).

This research shows propolis extract might alleviate the tissue lesions in the liver, brain, kidney, and lungs caused by acrylamide. Additionally, propolis extract's

antioxidant effects seem to mitigate oxidative stress changes from acrylamide in most tissues studied. Therefore, propolis extract shows promise as a therapeutic agent to lessen the harmful biochemical and pathological consequences of acrylamide.

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Ethical Statement

The study was approved by the Ethics Committee of Ferdowsi University of Mashhad (IR.UM.REC.1401.112).

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author Contributions

MJH performed the experiments. ZM and HB supervised the research and experiments. ZM and HB contributed to data analysis of histopathology and biochemistry respectively.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Animal Welfare

The authors confirm that they have adhered to the ARRIVE Guidelines to protect animals used for scientific purposes.

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