Journal of Medicinal Plants and By-products (2023) 3: 267-274
https://doi.org/10.22092/jmpb.2022.356472.1421

Original Article

The Protective Effect of Hydro-alcoholic Extracts of Cactus Fruit (*Opuntia dillenii* (Ker Gawl.) Haw.) and Star Fruit (*Averrhoa carambola* L.) on Histological Changes Induced by Cadmium Chloride in Lungs of Male Wistar Rats

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Article History

Received: 25 October 2021
Accepted: 21 December 2021

Keywords
Alveoli
Blood Vessels
Bronchi and Bronchioles
Geavy Metal
Gastrointestinal Lumen

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ABSTRACT

Nowadays because of urban life, every vital resource has been polluted with heavy metals that are real prooxidants, Cactus fruit and Star fruit both have anti-oxidant activities and can protect animal tissues from oxidative stress. The purpose of this study was to evaluate the protective effect of Cactus Fruit and Star fruit extract on histological changes induced by cadmium in the lungs of Wistar rats. An experimental study was performed on 24 male Wistar rats over 16 days, Animals were randomly divided into four groups of six; negative control group, the positive control group that was poisoned at 2 mg/kg every 48 hours. Group 3 was the cadmium-poisoned group that was gavaged with cactus fruit extract at 200 mg/kg. Group 4 was the cadmium-poisoned group that was gavaged with star fruit extract at 200 mg/kg, administration of extracts was 90 minutes before the poisoning, After 16 days, the rats were euthanized by heart blood drainage under anesthesia. The main findings in the positive control group were the destruction of tissue architecture with the development of edema, hyperemia and congestion. Destruction of alveoli and air-space enlargement occurred. Vasculature structures were damaged and some degree of Inflammation and fibrosis happened, but the changes were much milder in the two other groups. Both fruits extract had high protective effects, each of them protected lung tissue against cadmium oxidative damage but cactus fruit extract seems to have more protection than star fruit extract.

INTRODUCTION

Cadmium is one of the heavy metals found in the earth's crust in combination with other elements such as Zinc, Copper and lead. The soluble forms of cadmium are Cadmium Chloride and Cadmium Sulfate. In the United States of America, it is derived as a metal beside the other metals. Industrial usage of cadmium is in making batteries, Paint and resin, Veneer, plastic and rubber industries. This element enters the vital resources by the way of mining, factories, usage of phosphate fertilizers, burning of fossil fuels, industrial waste disposal and also during the recovery process of worn-out batteries and after that it accumulates in the micro-organisms, plants and seeds. Although food and tobacco use are always a source of cadmium for the body, it also threatens air, water, and other sources of human life [1,2].

The epidemiological evidence of the last decade consistently identifies low-level environmental exposure to cadmium as a risk factor for total cancer and lung cancer [2]. 5 to 50% of cadmium is absorbed by the lungs and respiratory tract and 1 to 10% by the gastrointestinal tract, the metal in the gastrointestinal lumen competes with calcium, and in fact, consuming higher calcium foods results in less absorption. Cadmium goes into the kidneys and liver after it enters the body and stays there for

many years and only a small amount is excreted in the urine and feces. The human body is capable of converting cadmium into less harmful subunits, but today it accumulates in organs due to the high volume of overload it receives [3-6]. In general, the presence of cadmium in the body can damage the kidneys, liver, lungs, pancreas, testicles, placenta, bone and heart [7].

Lung tissue is among the first organs most vulnerable to exposure to cadmium, although acute exposure to low-dose cadmium aerosols has been reported to increase enzymatic activity [8]. But research on rats has shown that oral administration of cadmium over the medium term can cause emphysema and pulmonary fibrosis [9]. In addition, administration of this substance for 200 days in the drinking water of Sprague Dawley rats showed a potential reduction in static pulmonary complement and apparent pulmonary lesions, which were more frequent in zinc-deficient mice [10].

High levels of cadmium respiration can cause severe damage to the lung tissue and may even cause death, as well as breathing fewer volumes but over longer periods as mentioned, causing deposition of this substance in the body and irreparable damage to the body. The US Department of Health and Human Services (DHHS), the International Agency for Research on Cancer (IARC) and the US Environmental Protection Agency (EPA) have identified cadmium and its derivatives as potential carcinogens for humans and animals. And in a study by the IARC on workers with lung cancer, it was found that there was cadmium in these patients' respiratory air [3]. In vitro oncology studies performed on Wistar rats were administered cadmium aerosols for 18 months at specified doses, a high proportion of rats developed lung cancers [11]. Oxidative stress is the imbalance of oxygen free radicals and oxidative precursors that can damage cellular macromolecules including proteins, DNA, cell membrane fatty acids, etc. Increased Reactive oxygen species (ROS) also disrupts cellular signal transduction processes [12].

Cadmium causes ROS production and release in living tissues of living organisms and Increased levels of superoxide anion, hydrogen peroxide and hydroxyl radicals form the basis of severe oxidative stress [13]. Antioxidants are compounds that are able to protect cell membranes against oxidative stress, the balance between Prooxidants and antioxidants allowing the cell to continue its normal physiological function [14,15].

Opuntia dillenii is a plant of the genus Cactaceae and belongs to the family Opuntia. The native of this plant is Equatorial and sub-equatorial regions such as Taiwan, also grown in Baluchistan Iran, the flowers of this plant are yellow and the fruit is red [16]. Various studies have investigated the anti-inflammatory and antioxidant effects of other fruits of this family. Opuntia ficus white fruit extract has been reported to have a high protective effect against lipid peroxidation due to the high level of betalains that has antioxidant activity [17-19]. Betalains has been introduced as a new class of oral antioxidants, they also attribute the anti-ulcer and antioxidant effects of *O. ficus* to the presence of high ascorbic acid, phenolics and the combination of betaxanthin and beta-cyanine pigments [16,20,21].

Carambola or star fruit is a plant of the genus Oxalidaceae and belongs to the family of the *Averhoa*. The main birthplace of the plant is Southeast Asia, it is also cultivated in parts of Pakistan and Baluchistan, Iran. Southeast Asian natives use the fruit to accelerate the healing of wounds and injuries, Compounds in this fruit are fatty acids, caramboxin, L-arabinose, lectin, apigenin, saponin, quercetin, a flavonoid. The antioxidant effect of this fruit on lead-induced acute liver injury in rats has been investigated and the result has been a reduction in the rate of injury [22, 23].

Due to the machine life of today's human societies, the growth of factories and mines and the excessive consumption of fossil fuels and etc Increasing heavy metal toxicity such as cadmium can cause serious tissue damage to vital organs, there is also a growing need to discover antioxidants. Since these fruits and their family of fruits have been studied in antioxidant and anti-inflammatory studies repeatedly, in this study we aimed to investigate the protective effect of these two fruits on cadmium-induced lung tissue changes.

MATERIALS AND METHODS

the fruit of Cactus (*O. Dillenii*) and Star (*Averhoa Carambola*) (Fig. 1) were prepared from the premises of Medicinal Plants Research Center of Zabol University of Medical Sciences and species were determined in the botanical laboratory.

The fruits were dried in the shade and then ground. Extraction was performed using hydro-alcoholic (double distilled water (30 percent)-alcoholic(70 percen)). The extracts were dried by rotary and oven and kept at 4 ℃ until used in refrigeration [18,19].
The present study was an experimental design performed on 24 male Wistar rats weighing approximately 200-250 grams.

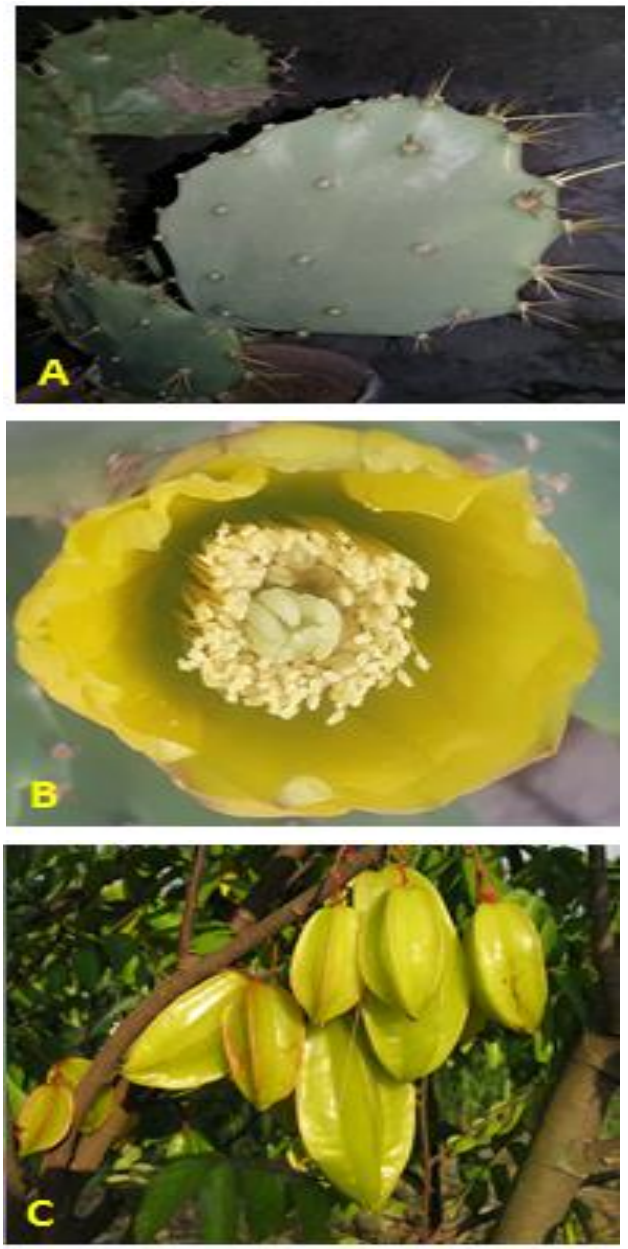

Fig. 1 the characterisitci of Cactus Fruit (*O. Dillenii* (Ker Gawl.) Haw.) (A, B) and Star fruit (*A. Carambola* L,) (C)

The rats were maintained in standard conditions and had access to plated water and food. Animals were randomly divided into four groups of six as follows: The first group consisted of healthy rats that were considered as a negative control. The second group consisted of cadmium chloride poisoned rats at 2 mg/kg every 48 hours, which were considered as a positive control [24]. The third group was the cadmium-poisoned group that were gavaged the cactus fruit extract at 200 mg/kg 90 minutes before the poisoning. The fourth group was the cadmium-poisoned group that was gavaged the star fruit extract at 200 mg/kg 90 minutes before the poisoning [22]. The experiment lasted 16 days and the extracts and cadmium gavage were repeated every 48 hours. At the end of 16 days, the rats were anesthetized with ether and were euthanized by direct blood drainage. Then lungs were sent to the laboratory for tissue sections. Method of Extraction: The fruits were obtained from the local market and were identified by a botanical expert, first the fruits were exposed to air for one month in the shade and dried. 50 g of the powders were dissolved in 500 ml of a solution containing 50% ethanol 96% and 50% water and placed on the shaker for 24 hours. The solution was then coated with aluminum foil and stored in the refrigerator. The solution was passed through the filter paper and the filtered solution was placed in a rotary evaporator at 45 °C for evaporation of the solvent. Finally, 5.5 mg dried powder of cactus fruit and 6.9 dried powder of Star fruit were obtained.

RESULTS

The microscopic cross-section of healthy lung tissue in the negative control group is shown in H&E and PAS staining in Figure 2; In the H&E staining, normal lung tissue and the pleura, bronchi and bronchioles, airway, blood vessels and alveoli are seen with a natural appearance and there are no abnormal findings. In the PAS stain, the lung tissue was similar to the usual stain, in which the basement membrane, blood vessels, bronchi and bronchioles, airway and alveoli were easily visible and had no abnormal findings. Microscopic section of lung tissue of cadmium chloride poisoned group (positive control group) is shown in H&E and PAS staining in Figure 3. In H&E staining in lung tissue, congestion and severe bleeding can be seen in all its internal structures including bronchi and bronchioles, airway, alveoli, and blood vessels. The walls of blood vessels, bronchi and bronchioles, airways and alveoli have been destroyed. Irregularities are observed in these structures, destruction of the basement membrane of blood vessels and alveoli, vacuolization in the lumen of blood vessels and some cells of the alveoli are

visible. The increase in lung macrophages (Dust cells) within the alveoli indicates an inflammatory process. In PAS staining, the basement membrane changes in lung tissue structures are seen.

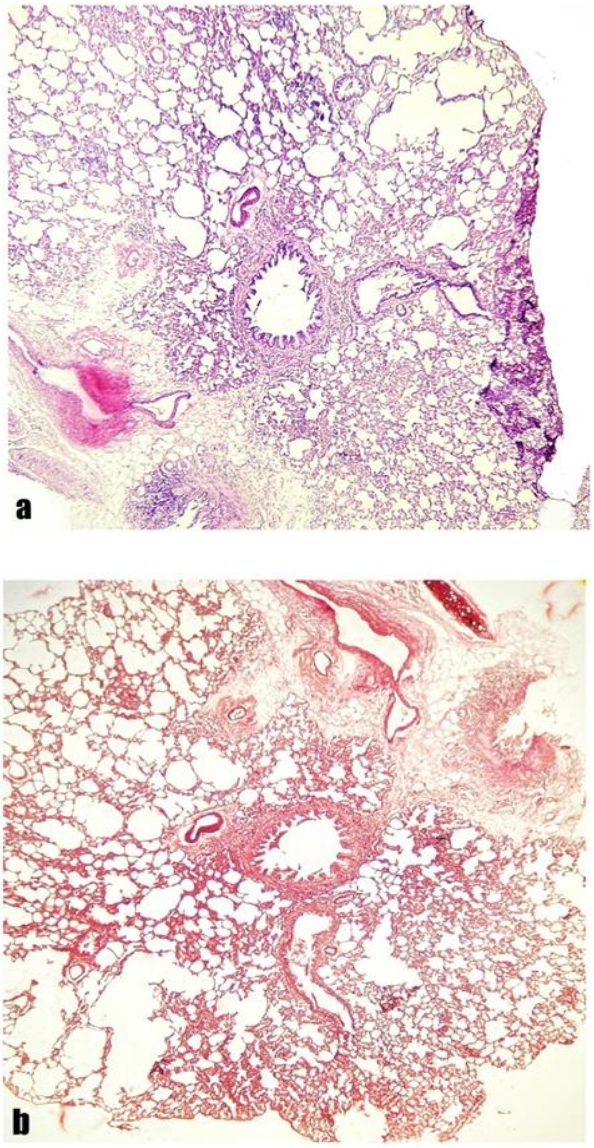

Fig. 2 Lung tissue section of the negative control group in both H&E (a) and PAS (b) staining with 40x magnification, intact lung tissue architecture, with no effect of tissue changes.

Microscopic section of lung of the cadmium-poisoned group treated with cactus fruit extract, shown in H&E and PAS stain in Figure 4. Congestion and bleeding in various components such as bronchi and bronchioles, airways and alveoli have decreased and alveolar and airway irregularities have been reduced. Vacuolization and lymphatic follicles are observed in the connective tissue less intensively below the epithelial cells. Changes in the PAS stain are similar, and the restored basement membrane is evident.

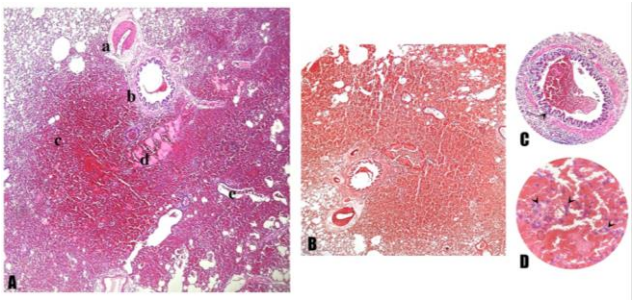

Fig. 3 Lung tissue sections of the positive control group in both H&E (A) and PAS (B) in 40x magnification. A) Lung tissue architecture is generally disrupted and congestion, bleeding and edema are observed(c). destruction of alveoli and interstitial alveolar septa occurred. Necrosis and fibrosis can be seen in some areas(d). Bronchial damage and intra-bronchial hemorrhage occurred(b). Arterial wall injury (a) and venous wall injury (e). B) Tissue cross-section of the lung with PAS staining. C) Bronchial cross-section with 100x magnification, macrophage infiltration and epithelial cell proliferation, epithelial detachment from the basement membrane and loss of basement membrane. D) Infiltration of lymphocytes and macrophages (Arrowhead) and destruction of alveoli and septa and tissue hemorrhage at 400x magnification.

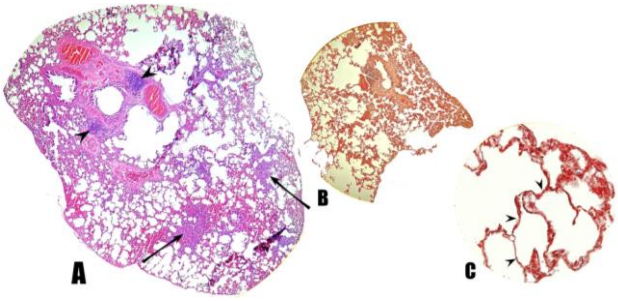

Fig. 4 Lung tissue sections of rats receiving Cactus fruit extract in H&E (A) and PAS (B) staining with 40x magnification. A) Lung tissue view with 40X magnification and H&E staining. In general, lung tissue structure and structures are preserved. Air-space enlargement can be seen. The majority of the alveoli are preserved and the alveolar septa remain stable. In some areas, pulmonary lesions are seen (Arrows). Vascular Walls and bronchioles are protected from damage. Congested Blood Vessels. Some pre-bronchial lymphoid tissue is seen (arrowhead). B) Lung tissue section in PAS Staining with 40x magnification. C) Tissue cross-section of alveoli with magnification 400x. The alveolar septa are well preserved.

Microscopic section of lung of the cadmium-poisoned group treated with star fruit extract, shown in H&E and PAS Stain in Figure 5. In H&E staining in the lung tissue, congestion and bleeding in the

blood vessels, airways, alveoli and bronchi are much less, the walls of these structures are less damaged and irregularities in the airways and alveoli are also limited. Vacuolization is also negligible.The lymphatic follicles are increased in the connective tissue below the epithelial cells. The basement membrane of the alveolar epithelial cells, the airways, the blood vessels and the bronchioles have no appreciable changes. In the PAS Staining, the changes were similar to those of normal Stain, the degradation and alteration of the basement membrane of the aforementioned structures is also less pronounced.

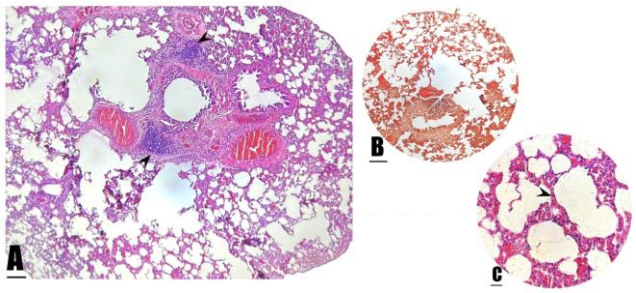

Fig. 5 Lung tissue sections of rats receiving Star fruit extract in H&E (A) and PAS (B) staining with 40x magnification. A. Lung tissue view with 40X magnification and H&E staining. In general, lung tissue structure and structures are preserved. Air-space enlargement can be seen. The majority of the alveoli are preserved and the alveolar septa remain stable. Vascular Walls and bronchioles are protected from damage, Some pre-bronchial lymphoid tissue is seen (arrowhead). Congested Blood Vessels. Lymphocytic infiltration and macrophage infiltration are greater than the Cactus fruit Group (Figure 4). B. Lung tissue section in PAS Staining with 40x magnification. C. Tissue cross-section of alveoli with magnification 400x. The alveolar septa are preserved. The proliferation of macrophages and pneumocytes.

DISCUSSION

In the past, many studies have been performed on acute and chronic tissue damage of cadmium in animal models. The majority of them obtained almost similar results to each other in examining the acute and chronic damage caused by different doses of cadmium. The results cited in their reports wide-ranged damages from inflammatory processes, proliferation of pulmonary macrophages, peribronchial lymphoid tissue congestion, Hemoconcentration and edema to the destruction of pulmonary tissue architecture and necrosis then fibrosis [25-32].

In the results of the present study in tissue samples from the lungs of the negative control group as seen in Figure 2, tissue architecture is normal and various components including alveoli and alveolar septa, bronchi and bronchioles, arterioles and venules all have a normal and uniform appearance and no damage is seen. However, in the samples obtained from the lungs of the positive control group as shown in Figure 3, Tissue architecture is disrupted and generalized loss of alveoli, severe congestion and heme-concentration and edema are observed. In some areas, cell necrosis and even fibrosis can be seen. these are similar to the study done by Pearson et al. that investigated the effect of cadmium on E-cadherin and VE-cadherin with the administration of 65 nmol cadmium dose to the mice. They found that this dose caused the alveolar septa to loosen and disappear [27]. On the other hand, in the positive control group, the intense proliferation of pulmonary macrophages and pneumocytes occurred. This result was similar to that study of McKenna et al. that had been done to compare the inflammatory lung responses in Wistar rats and C57 and DBA Mice, they administered 1mg/m3 cadmium and the results were included the severe proliferation of Dust Cells, Type II pneumocytes, and bronchial epithelial cells [26]. The results of the present study revealed the loss of connections between the layers of the bronchial epithelium and the basement membrane, which was not mentioned in previous studies.

in comparison of the results of rats receiving Cactus fruit extract lung tissue sections (Fig. 4) with those of the positive control group (Fig. 3), The most obvious finding is that the architecture of the lung remained stable. The congestion rate was much lower and there is no longer any hemoconcentration or hyperemia and the amount of edema is much lower, in contrast to the positive control rats. This is maybe due to the protection of the cactus fruit extract from vascular structures. the alveolar septa were also well maintained in the rats receiving Cactus fruit extract compared to the positive control group as seen in Figure 4. Pulmonary alveoli have also been able to maintain their structure to a great extent, and Air-Space Enlargement can be found only in some areas of the lung. In these tissue sections, fibrosis is no longer seen and pulmonary lesions exist only in some areas of the lung. overall, these findings indicate the protective effect of cactus

fruit extract against oxidative damages, which have been reported in previous studies. Butera et al reported that the fruits of the Opuntia family have high antioxidant properties due to their high levels of betalains, betanin, indicaxanthin and vitamin C [17]. Lymphocytic and macrophage infiltration were also lower in tissue samples from the lungs of rats receiving Cactus fruit extract and peri-bronchial lymphoid tissue can only be found in some areas of the sample. This result may also prove to be an anti-inflammatory activity of the Opuntia family fruits that have been reported in various studies [33].

In general, cactus fruits have anti-inflammatory and anti-cancer properties due to their high levels of antioxidants, which have been mentioned in various articles [16, 17, 33-37]. Li et al. In a study of cactus polysaccharides found that these polysaccharides have an anti-tumor effect on lung Squamous Carcinoma Cells [34]. Feugang *et al*. Also found that cactus fruit extract could induce apoptosis in ovarian cancer cells [35].

In tissue sections obtained from the lungs of rats receiving carambola extract as shown in Figure 5, generally, lung tissue architecture is well preserved compared to the positive control group and the extent of damage and disruption of structures is less. But it is more irregular compared to the recipient group of the cactus fruit extract, suggesting a better protective effect of cactus than carambola. Pulmonary alveoli and interstitial alveolar septa have also retained their structure and several areas of Air-space Enlargement can be seen. The rate of congestion and hyperemia was significantly lower than the positive control group, a finding that indicates the vascular protection of carambola extract. both fruits had similar effects in this respect. These findings indicate the antioxidant and protective effects of star fruit that have been reported in various studies [22, 38-43]. Zainudin. et al Reported that carambola fruit has antioxidant properties due to its high levels of phenolic and carotenoid contents and is able to protect tissue against oxidants [43]. Shirazinia et al., Also conducted a study to investigate the protective effect of carambola hydro-alcoholic extract on acute liver injury induced by lead in Wistar rats and found that star fruit extract improved liver injury [22].

In the comparison of the carambola extract recipient group tissue sections and the positive control group, lymphocyte and macrophage infiltration rates were also significantly reduced, and there was no evidence of necrosis or fibrosis. This finding also confirms the anti-inflammatory effects of this fruit, which has been mentioned in various articles, Sripanidkulchai. et al reported that the anti-inflammatory property of carambola at a dose of 300 mg/kg was comparable to acetylsalicylic acid [39,44-46].

CONCLUSION

In general, both fruits had high protective effects, each of them protected the lung tissue against cadmium oxidative damage but cactus fruit extract seems to have more protection than star fruit extract. Due to the expansion of urban life and the progress of industry and the exposure of vital resources to heavy metals, there is always a need for more antioxidants.

The family of these fruits contains high levels of antioxidants and can be used to achieve this goal, but further research is needed.

The present study did not measure ACE enzyme activity due to a lack of Laboratory facilities that the researchers could investigate.

Appendix

Abbreviation

ROS: Reactive oxygen species

IARC: International Agency for Research on Cancer

EPA: Environmental Protection Agency

Compliance with Ethical Guidelines

This Study is performed under all ethical considerations important in animal studies. We used the minimum number of animals, they got access to appropriate water and food during the experiment and were maintained in appropriate facilities. All of the work has been approved by the regional research ethics committee. Approval ID: IR.ZBMU.REC.1398.162.

Funding

This research was funded by Zabol University of Medical Sciences.

Author’s Contributions

All authors contributed to this research.

CONFLIC OF INTEREST

The authors declared no conflict of interest.

ACKNOWLEDGEMENTS

Many thanks to the Biotechnology Center and Faculty of Veterinary Medicine of the University of Zabol for providing the facilities.

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