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Preventive effect of an ethanolic avocado on apoptosis induced via oxidative damage in albino rats tissues

Fatimah Qasim Mohammed Al-Hayyali* **and Aljuboury Maha Khalaf**

Biology Department, Science of college, Mosul university, Mosul, Iraq. *Author for correspondence. E-mail: fatsbio25@uomosul.edu.iq

ABSTRACT. Oxidative stress is the result of an imbalance between the production of oxidant precursors and the capacity of antioxidant defense. Oxygen free radicals play an important role in causing diseases. In this study, the protective effect of ethanolic avocado on apoptosis caused by oxidative damage in the tissue of albino rats was investigated. 24 male albino rats of the Faculty of Veterinary Medicine in Mosul, Iraq, which were kept in standard conditions for at least 10 days before and through the experimental work, were examined. Four groups of rats include the control group (healthy group), the group of male rats with ethanolic avocado consumption; The third group of male rats that were treated with 0.5% of hydrogen peroxide H_2O_2; and the fourth group of male rats that were treated with both 0.5% H_2O_2 and avocado ethanolic extract (50 mg kg^{-1} BW) for four weeks. After fixing the tissues of the liver, kidney, lung, spleen and testis in 10% buffered formalin, they were stained with hematoxylin. TUNEL assay was performed using the TUNEL cell death assay kit to detect apoptotic cells. In this investigation, the histology results in four groups of rats showed that in the rats that were treated with avocado, there were minor tissue changes in their liver, kidney, and intestine, and the tissues of these organs were healthy. In TUNEL staining, it was also shown that there are no apoptotic cells in the liver, kidney and testis cells in avocado-treated rats. The results showed that ethanolic Avocado is useful against oxidative stress damage and it may be used to protect tissues against oxidative stress.

Keywords: oxidative stress; persea americana; avocado seeds; apoptosis; rats.

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Introduction

At present, interest in studies of oxidative stress has increased because it is of great importance in the emergence of many organic physical diseases, degenerative diseases, and cancers (Sharma, 2014; Sies, 2015).

The occurrence of a disproportion between the development of free radicals and their elimination is once again referred to as a state of oxidative stress (Pizzino et al., 2017).

Avocado is (Persea americana) coceder one of the fruits known to be rich in antioxidants, flavonoids, carotene, and vitamin A (Krumreich et al., 2018; Jiménez et al., 2021).

Many studies appear that it is possible to use ethanol alcohol to produce the alcoholic extract of this fruit, which is one of the substances that has shown a protective antioxidant capacity and prevents programmed cell death (Tremocoldi et al., 2018).

A study showed that caspases 3 and 9 are responsible for the consequences of programmed death, and plant extracts of some antioxidants can stabilize them (Ding et al., 2007). As proven

plant extract of the avocado stimulates PI3K/Akt molecular pathway, which has a role in keeping the cell alive (Fulgoni et al., 2013).

It was found that the use of avocado seeds has medical importance in reducing oxidative stress as well as reducing inflammation and genetic effects associated with immune functions in the body, according to what was found in the results of the study conducted by Elmoslemany et al. (2021), when using treatment with avocado seeds against cyclosporine-induced nephrotoxicity (Elmoslemany et al., 2021).

The use of diethylnitrosamine, which is toxic to liver cells, can be prevented by using the alcoholic extract of avocado, as it reduces oxidative stress, enhances immunity, and reduces the occurrence of programmed cell death (Hammouda, 2015; Tremocoldi et al., 2018).

There are some researches related to studying the beneficial effect of the alcoholic extract of avocado in reducing programmed cell death, there is a lack of knowledge regarding the amount of doses used and the length of time of use in animals, the aim of our study is to found and investigate the preventive effect of ethanolic avocado on apoptosis caused by oxidative damage in the tissue of albino rats.

Materials and methods

Chemicals

Hydrogen peroxide (H2O2) was purchased from Hopkins and Willman's, Germany. TUNEL Kit (Elabscience CK-A331).

Plant materials

Presea americana Mill (avocado plant) was supplied from local supermarkets, in Mosul city, Iraq.

Preparation of ethanolic avocado extract

The avocado seeds were removed and cleaned, washed with plain water, then with distilled water, and then dried well. After that, the seeds cut to the small pieces, then placed in the oven at a temperature of (40ºC) for six hours in order to get rid of moisture (Imafidon, 2023). Then it was dried at room temperature. The dried seeds were ground using a coffee grinding machine to obtain a fine powder, then placed in plastic bags and then placed in the freezer until use. Then, flavonoids and phenolic compounds were extracted from avocado seeds using Continuous Soxhlet Apparatus (Kupnik et al., 2010). Then extract was filtered and concentrating use a vacuum evaporator. Finally, the crude extract was obtained, which was placed in dark bottles. It was placed in the refrigerator until use (Harborne, 1998) .

Experimental animal design

24 male rats (albino) weighing 170-250g housed the animal house, Veterinary College, Mosul University, Mosul, Iraq, In temperature 24±2ºC at a 10 days before experimenting work, The standard diet was provided water ad libitum. rats divided to 4 groups:

-1st group (positive control) the healthy group fed ad-libitum for four weeks.

-2nd group: male rats dosed orally with avocado ethanolic only (extract 50mg/kg BW) for four weeks in which positive control group.

-3rd group: treated 0.5% H2O2 for 4 weeks

-4th group: treated orally both 0.5% H2O2 and the avocado ethanolic extract 50mg/kg for 4weeks.

Collect of Samples

At the end of experiment, all rats sacrificed via neck dislocation, and the liver, kidney, spleen, lung, and testis of each rat were rapidly excised and washed in isotonic saline; then cut, and fixed in neutral buffer formalin solution 10% for histological and immunohistochemical analysis.

Immunohistochemical analysis

TUNEL Assay for Cell Apoptosis

TUNEL assay was performing use the cell death detect Kit, for apoptotic detection. According to the TUNEL assay, slides washed by the PBS for 30 min. slide washed by PBS\ 5 min\ × 3. Slide and incubating in the Blocking Buffer at RT. After pass 10 min of incubation, slide permeabilized on the ice bath. slides e washing with PBS for 5 min × 3 then incubation with (the terminal deoxynucleotidyl transferase) enzyme work solution at 37 °C \at 60 min in wet bow. After that incubate, slide washing with the PBS \ 5 min × 3. slides then incubation by anti-fluorescein antibody-HRP solution \37 °C \ 30 min in a wet bow, then washing by PBS\ 5 min × 3. Next, slide incubation with the DAB working solution \ RT \ 5 min. cell slide washing with PBS \5 min × 3 to stop color reaction. Cell counterstain by hematoxylin \ 30 s, then washing by PBS\ 5 min × 3. slides then dehydrate in the graded series 70% - 80%- 90% and 100% ethanol , II\ xylene I and xylene II\ for 2 min in each reagent. Then mounting, slide examine with the light microscope. Figures capturing on a microscope.

Results

Histological changes

Liver sections of the experimental rats' group shows (Figures 1 and 2):

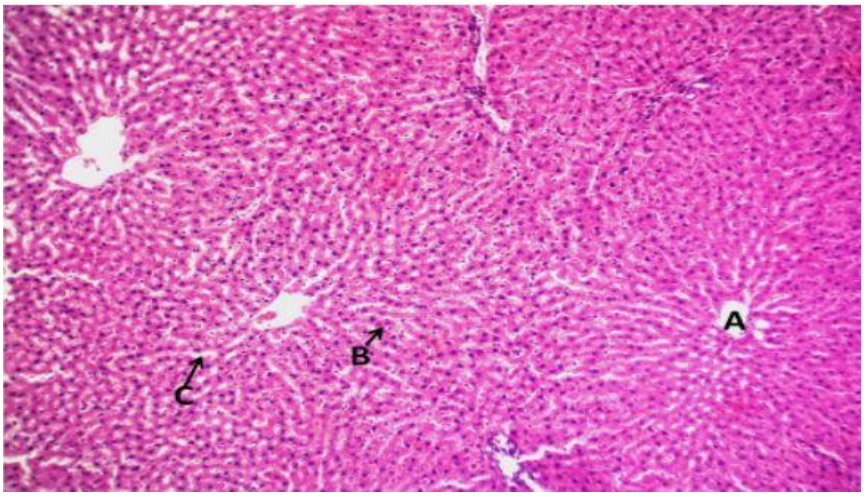

Figure 1. Photomicrograph of the rat liver of + control group shows normal structure of the liver tissues the central vein (A), hepatocytes (B), and sinusoids (C). 100X.

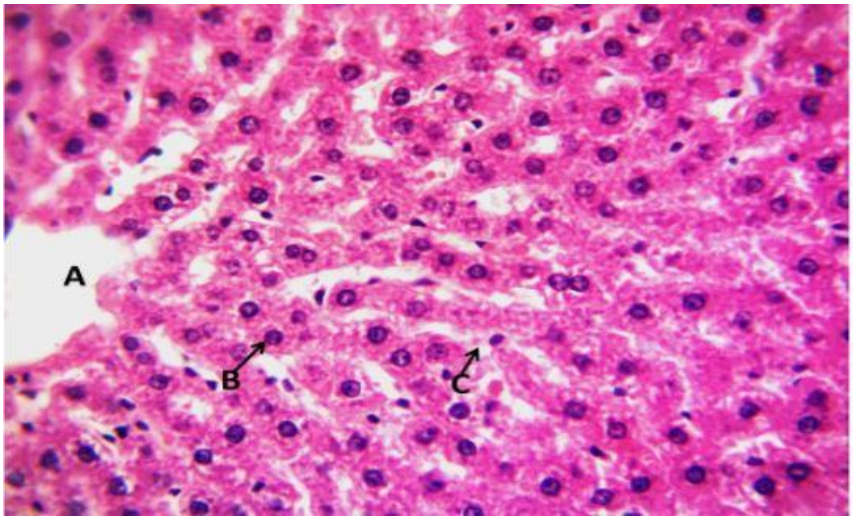

Figure 2. The liver of positive control shows the normal structure of the liver tissues: central vein (A), hepatocytes (B), and sinusoids (C). 400X.

In addition, results showed (Figures 3, 4, 5 and 6):

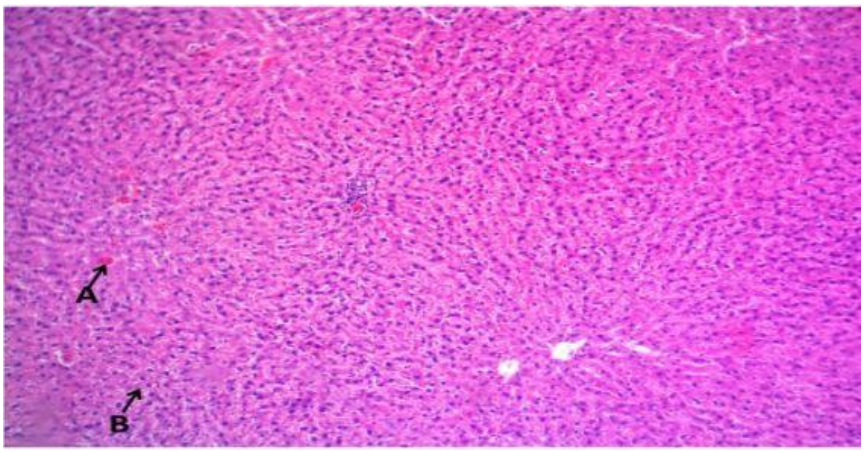

Figure 3. The rat liver of negative control treated with H_2O_2: the cell swelling (A), the coagulative necrosis of hepatocytes (B), infiltration of inflammatory cells (C), congested of central vein (D), 400X.

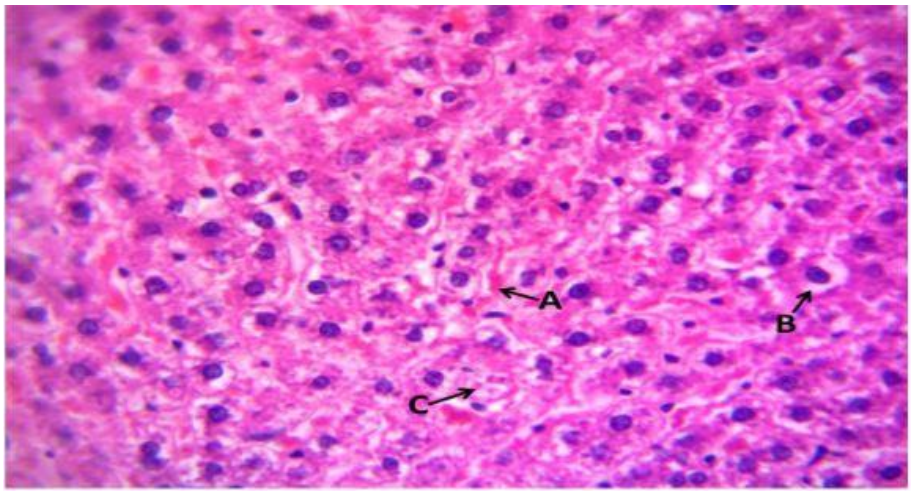

Figure 4. Rat liver of negative control with H_2O_2: the cell swelling (A), coagulative necrosis of hepatocytes (B) and infiltrate of inflammation cells (C). H&E stain, 400X.

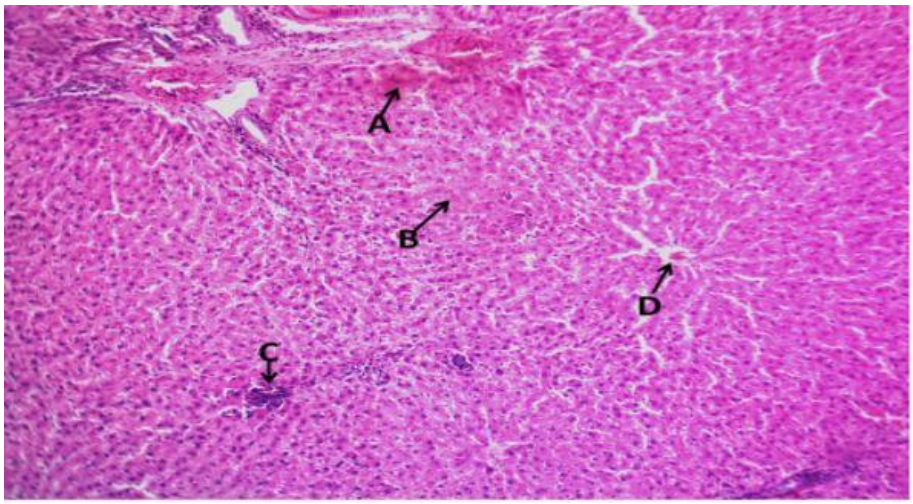

Figure 5. photomicrograph of liver treated with avocado + H2O2 shows congestion of sinusoids (A) and mild vacuolar degeneration of hepatocytes (B). 100X.

Kidney

Kidney sections of the experimental rats' group show (Figures 7, 8, 9 and 10):

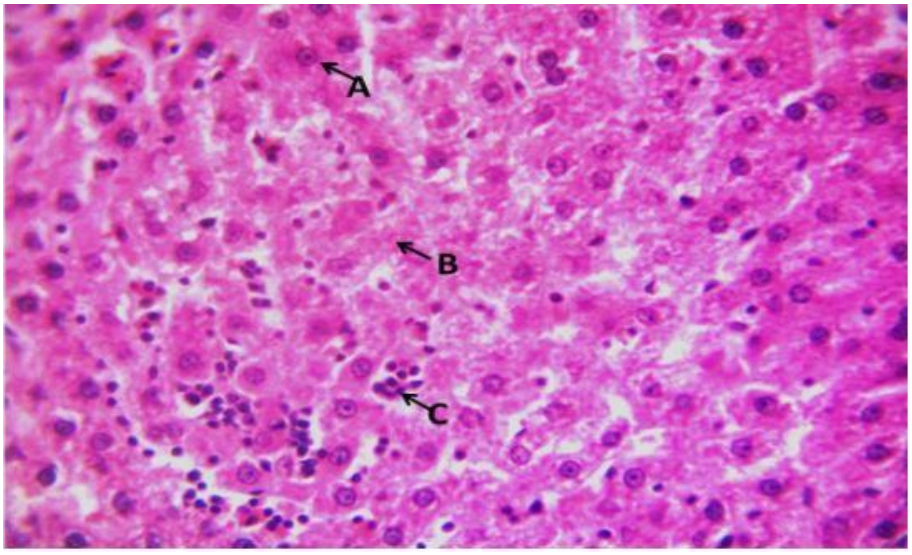

Figure 6. As well as, congestion of sinusoids (A) and mild vacuolar degeneration (B) and necrosis (C) of hepatocytes. H&E stain, 400X.

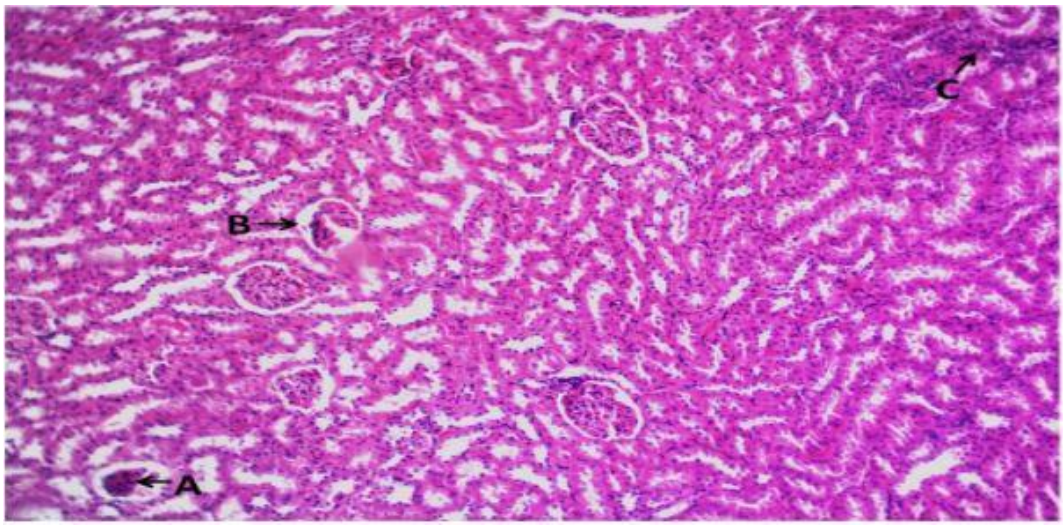

Figure 7. Rats kidney of negative control treating by H2O2: the atrophy of the glomeruli (A), dilation for Bowman's space (B), infiltration of the inflammatory cells (C). 100X.

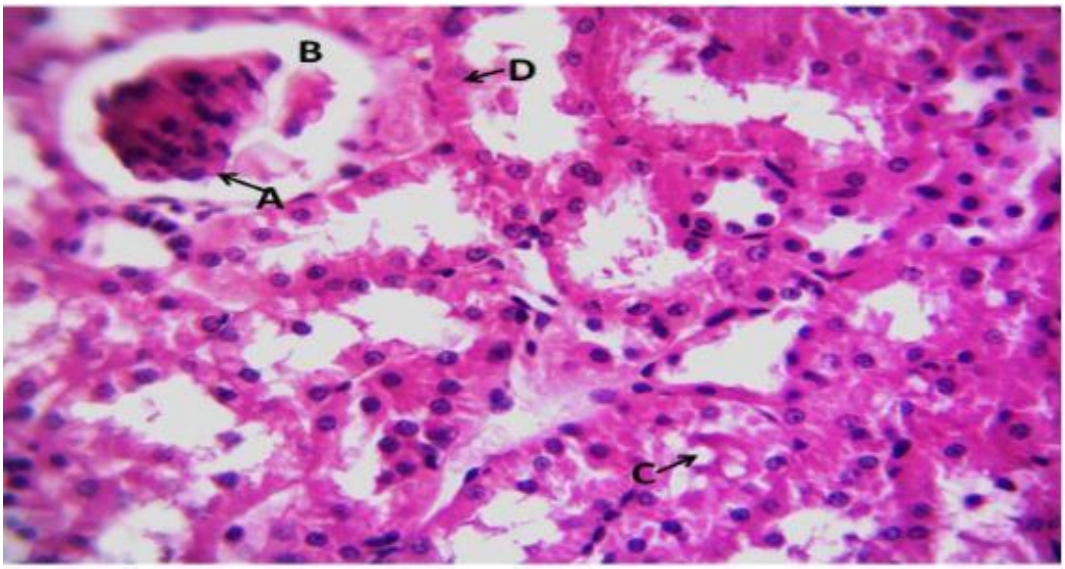

Figure 8. Atrophy of glomeruli (A), dilation of Bowman's space (B), and cell swelling of epithelial cells lining renal tubules (C) with coagulative necrosis of others (D). 400X.

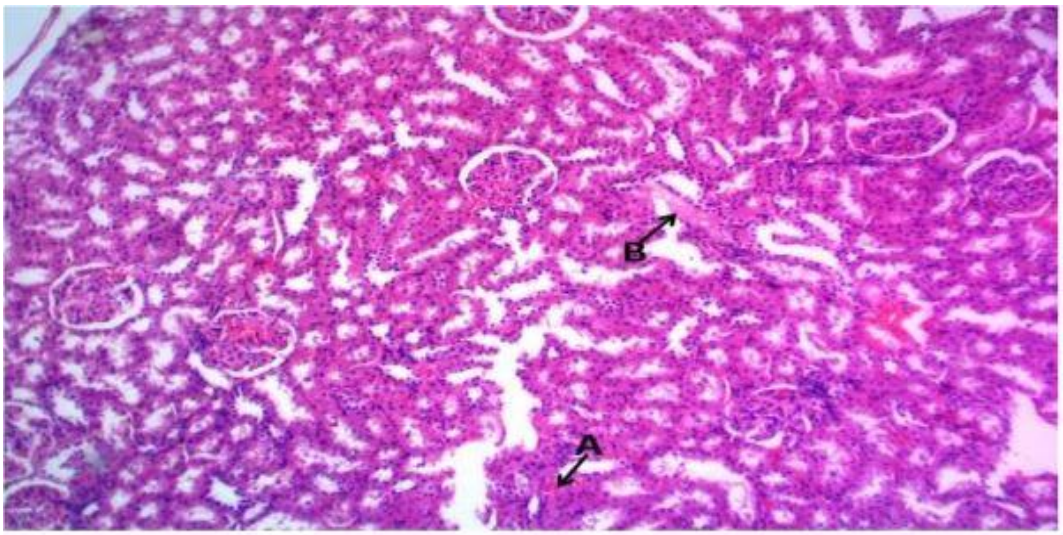

Figure 9. Photomicrograph of rat kidney treated with avocado + H2O2 group : the normal architecture of renal tissue except mild congestion of blood vessels (A) and oedema (B), 100X.

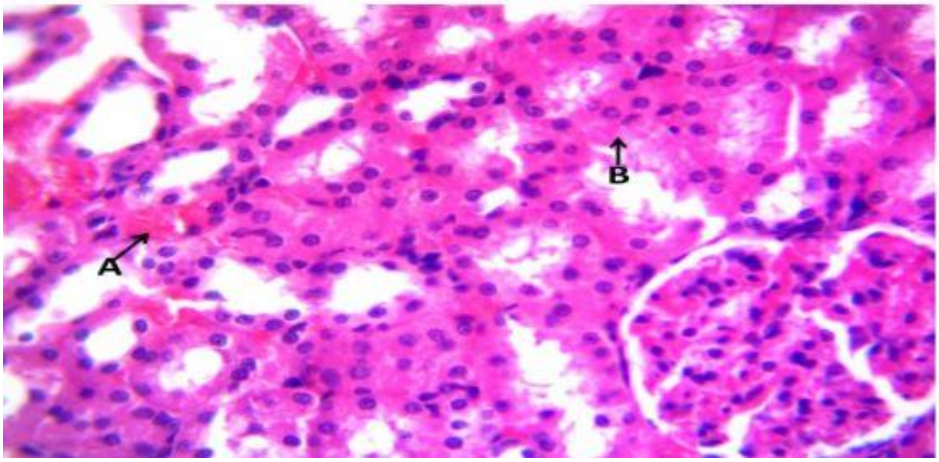

Figure 10. Photomicrograph of rat kidney treated with avocado + H_2O_2 group shows the normal architecture of renal tissue except mild congested of blood vessels (A) and mild cell swell the epithelial cells lining renal tubules (B). H&E stain, 400X.

Testis

Testis sections of the experimental rats' group show (Figures 11, 12, 13, 14 and 15):

Figure 11. Photomicrograph of rat testis control positive: normal structure for seminiferous tubule (A) spermatocytes (B) and spermatids (C).100X.

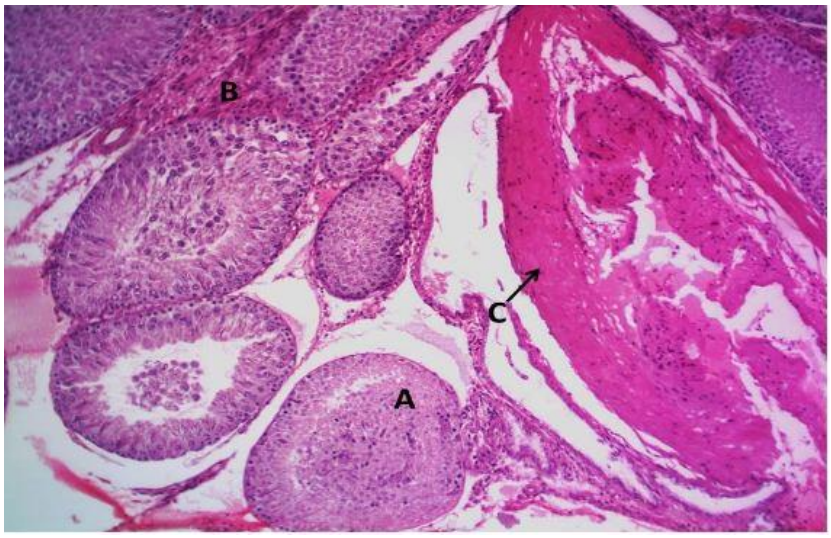

Figure 12. while negative control group treated with H_2O_2: :degeneration and the necrosis of cell of seminiferous tubules (A), increased fibrous tissue between seminiferous tubules (B), and thickening of blood vessels wall (C). 100X.

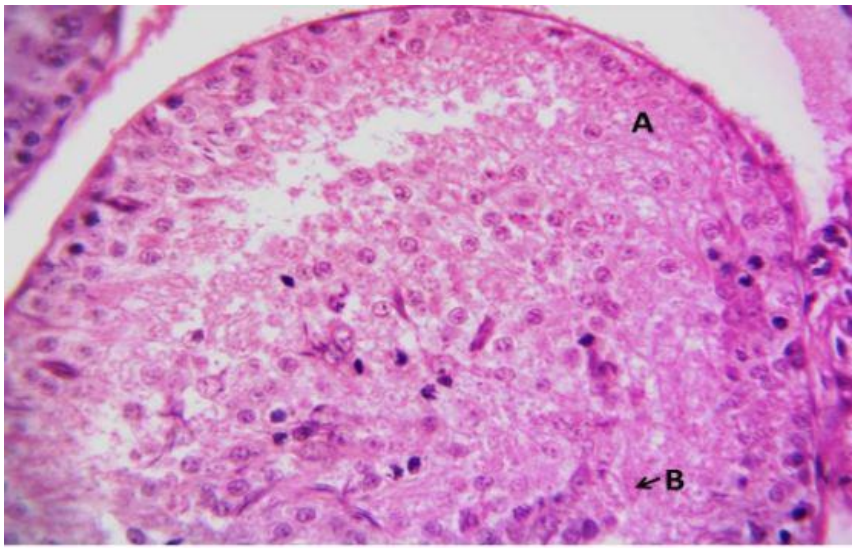

Figure 13. : In addition, treated group with H2O2 shows degeneration and necrosis of cells of spermatocytes (A) and spermatids in the seminiferous tubules (B).400X.

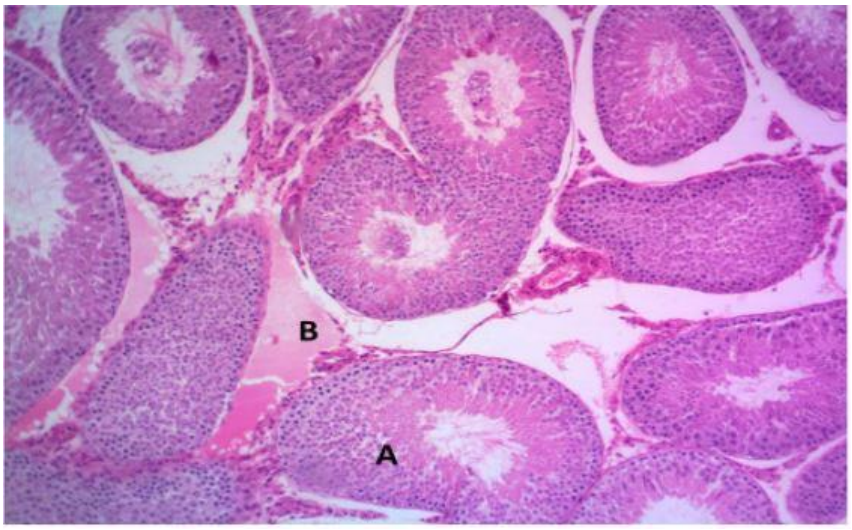

Figure 14. Photomicrograph of rat testis treated with avocado + H2O2 group shows normal architecture characterized by seminiferous tubules (A) except the presents of oedema among them (B).100X.

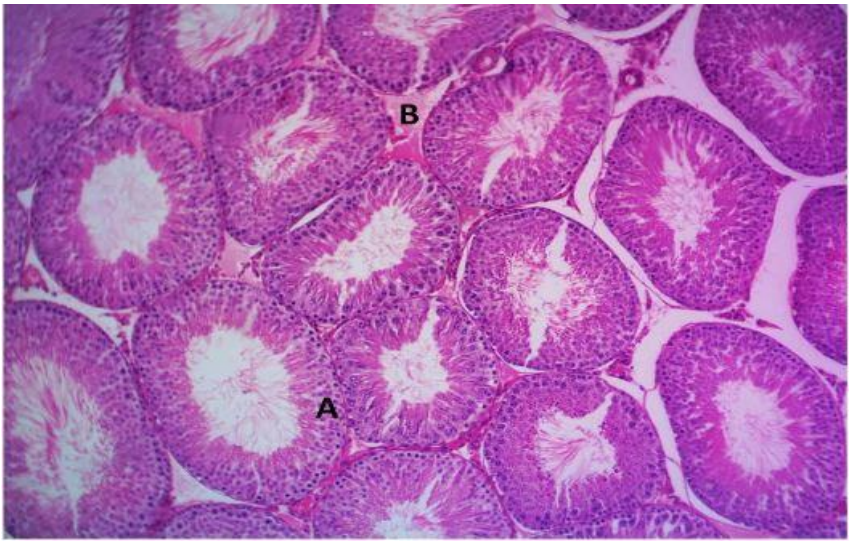

Figure 15. Additionally, rat testis treated with avocado + H2O2 group shows normal architecture characterized by seminiferous tubules (A) except presents of oedema between it (B). H&E stain, 100X.

Tunnel results

In TUNEL staining, to detect apoptotic cells in the liver sections of the experimental rats' group show (Figure 16):

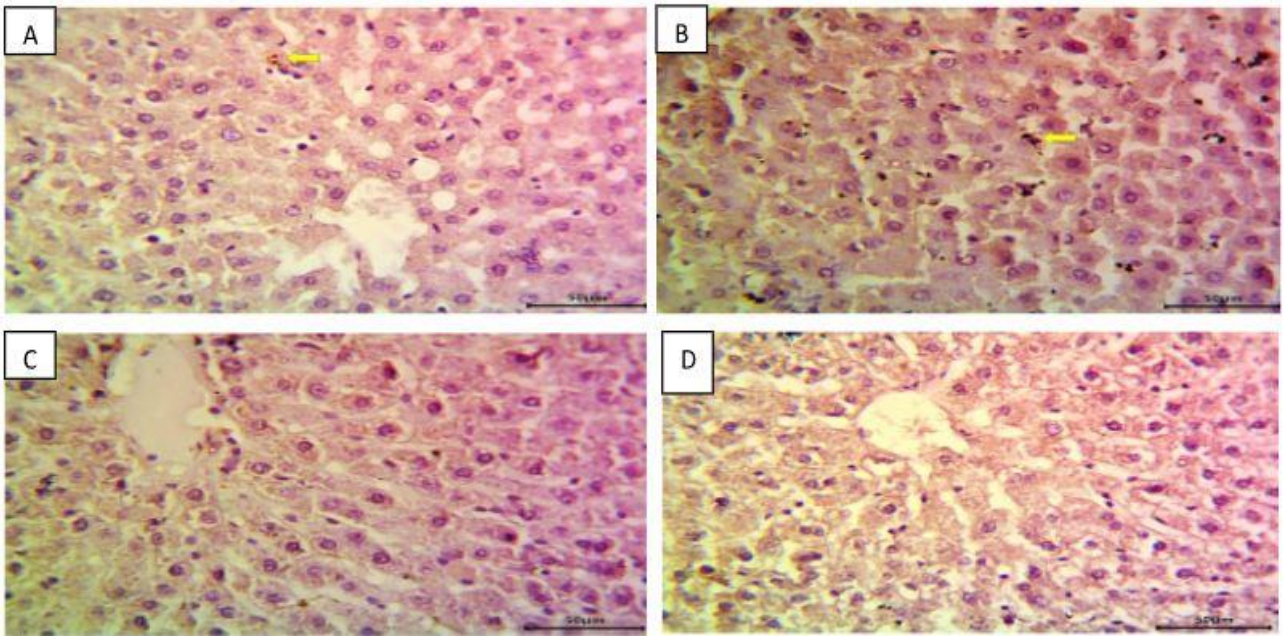

Figure 16. Photomicrographs of TUNEL staining for detecting apoptotic cells in the liver section of the control showing mild positive cells. 400X. A) while in liver section of the control group treated with H_2O_2 showing moderate positive cells. 400X; B) additionally, in the Avocado group showing negative cells. 400X; C) Finally, in the Avocado + H_2O_2 group showing negative cells. Hematoxylin stain, 400X;D).

As well as, TUNEL staining was used to detect apoptotic cells in the kidney sections of the experimental rats' group show (Figure 17):

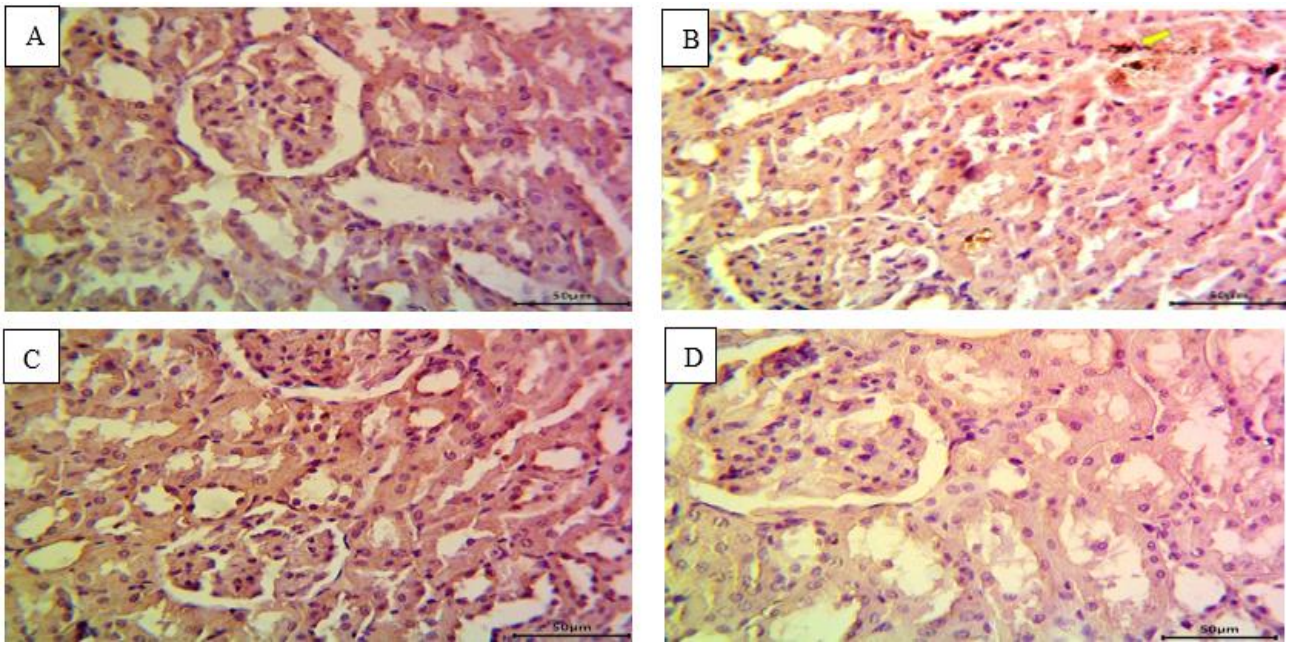

Figure 17. Photomicrographs of TUNEL stain for apoptotic cells in kidney section of control group: negative cells. 400X. A) While in the kidney section of the control group treated with H_2O_2 showing mild positive cells. 400X. B) As well as, in the Avocado group showing negative cells.400X. C) Finally, in the Avocado + H_2O_2 group showing negative cells. Hematoxylin stain, 400X.D).

In the image obtained from TUNEL staining to detect apoptotic cells in the testicle's sections of the experimental rats' group show (Figure 18):

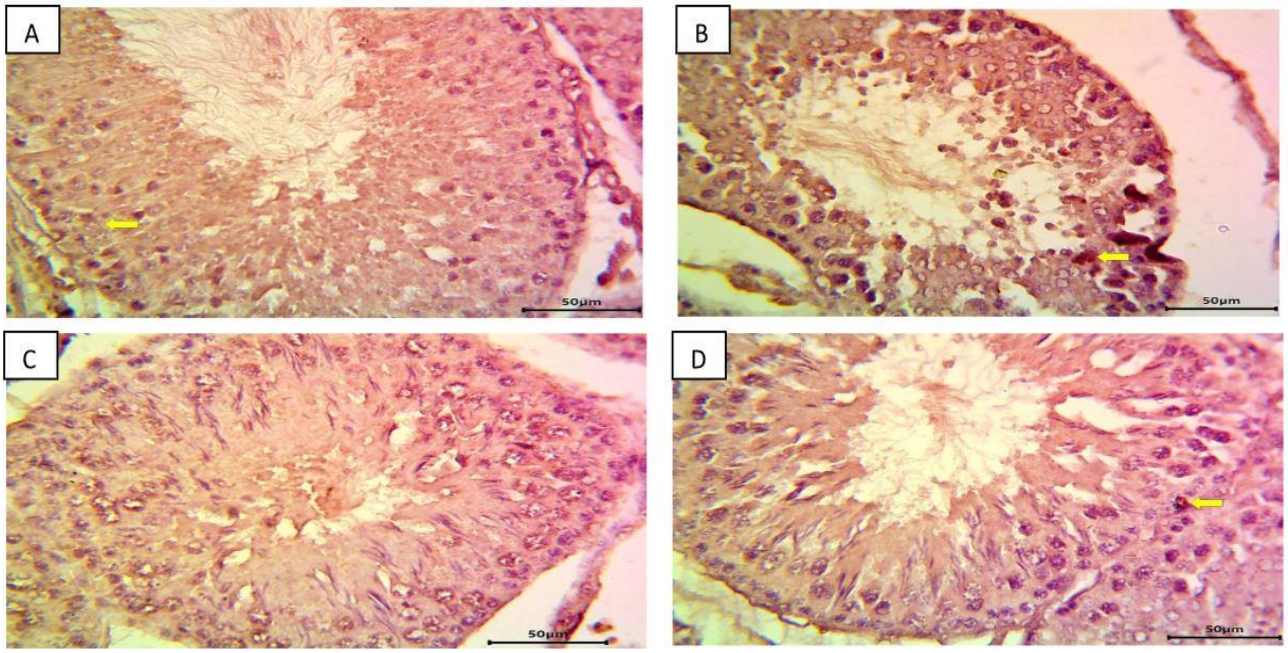

Figure 18. Photomicrographs of TUNEL staining for detecting apoptotic cells in the testes section of the control group (Without treating) showing negative cells. 400X. A) While in the testes section of the control group treated with H_2O_2 showing moderate positive cells. 400X. B) Additionally, in the testes section of the Avocado group showing negative cells. 400X. C) Finally, in the Avocado + H_2O_2 group showing mild positive cells. Hematoxylin stain, 400X.D).

Discussion

In this study, four groups of rats included the control group (healthy group) that had free feeding; The second group included the group of male rats that only consumed ethanolic avocado for four weeks; The third group of male rats that were treated with 0.5% of hydrogen peroxide H_2O_2; And the fourth group of male rats who were treated with both 0.5% H_2O_2 and avocado ethanolic extract (50 mg/kg BW) for four weeks participated. These four groups of rats were examined after examining the histological changes through the TUNEL assay to investigate changes in cell apoptosis. In this study, histology results in four groups of rats showed that were treated with avocado, there were minor tissue changes in their liver, kidney, and intestine, and the tissues of these organs were healthy. The results of variable analysis of TUNEL staining showed that there are no apoptotic cells in the liver, kidney, and testis cells in mice treated with avocado; Therefore, these results showed that the avocado plant prevents cell apoptosis in different organs of the body of rats and will prevent the creation of cancerous tissues and other diseases.

It has been shown in animals that avocado will prevent cell metastasis and cancerous tissue (Khoogar et al., 2016; Bhuyan et al., 2019), and the components of avocado, its leaves and seeds are cytotoxic for cancer cells (Bangar et al., 2022: Setyawan et al., 2021).

Ahmed et al. (2022), conducted the study to investigation preventive effect of the avocado plant on liver cancer caused by diethyl nitrosamine (DEN)/2-acetylaminofluorene (2AAF) in rats. In this study, the creation of cancer in mice was done through injection and feed. Mice under care were treated with avocado extract. The results showed the avocado reduces liver carcinogenesis in mice through the activation of antioxidant, anti-inflammatory, and apoptosis properties, which are in line with the results of our study; Because cell changes were not observed in mice treated with avocado.

To investigate the antioxidant and anticancer effects of the avocado plant in laboratory conditions, a study was conducted by Sahyon et al. (2023). In this study, the avocado was used through chitosan nanoparticles (Cs-NPs) to treat cancer in mice. The results of this research are agreement with results of the present study; Because the results showed that avocado has strong anti-cancer effects and can inhibiting proliferation of the cancer cells.

In this study, it was well shown that avocados can play the important and key role in the process of balance and maintenance of homeostasis in different tissues and cell life. These results are in line with the results done to investigate the effects of avocados.

The effects of avocados have been well demonstrated in animals and laboratory conditions. Due to the presence of polyphenols, which is a strong antioxidant, Avocado reduced the congenital malformations and

improved the growth disorder, the memory, and the cognitive abilities in the studied mice that were treated with Avocado (Kim et al., 2023). The effect of the ethanolic extract of the Avocado seeds as an adjunctive treatment to prevention tamoxifen- endometrial hyperplasia through breast cancer treatment in mice has been shown (Mvondo, Mbollo, & Njamen, 2021). Also, its protective effects on the heart of rats after taking long-term drugs that had toxic effects have also been well demonstrated (Shamlan, 2021).

The positive impact of avocados on humans have also been well demonstrated. Considering that avocado is rich in phytochemicals, it can prevent breast (Butt, et al.,2011), prostate (Lu et al., 2005), mouth (D'Ambrosio, Han, Pan, Kinghorn, & Ding, 2011), and esophagus (Vahedi Larijani et al., 2012) cancers. For this reason, it seems necessary to conduct studies and clinical trials in humans to find out more about its benefits.

Conclusion

Based on the findings of this research, ethanolic avocado is useful against oxidative stress damage and avocado can be used to protect tissue upon oxidative stress. Results of this study confirm the results of other studies in the direction of the beneficial effects of avocados.

Acknowledgement

We would like to express our sincere appreciation to all those who have contributed to the successful completion of our research project. We are grateful to the Department of Biology for providing us with the laboratory facilities and resources necessary to conduct this research. Our heartfelt thanks go to our supervisors for their invaluable guidance and support throughout the project. We also acknowledge the contributions of our collaborators and the technical staff of the laboratory for their assistance in conducting the experiments.

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