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Histomorphmetric studies on the protective role of Maca (*Lepidium meyenii*) on New Zealand White Rabbits prostate gland under oxidative stress

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Abstract

The prostate gland considers the most important gland among rabbits' accessory genital glands and secretes many factors essential for sperm function. This study aims to show the impact of Maca as an antioxidant on the prostate gland's histomorphology and its capacity to reduce the effects of oxidative stress. Twenty-four healthy New Zealand white male rabbits, 6-8 months divided into 4 groups.1st group (control group), 2nd group (Maca), 3rd group (H_2O_2), 4th group (Maca+ H_2O_2). To evaluate these effects, we used histomorphometrical studies. In control and Maca groups, acini were lined by simple columnar epithelium and showed normal interstitium. However, in H_2O_2 treated rabbits, we observed cuboidal epithelium and epithelium height decreased significantly compared to other groups, and interstitium showed fibrosis. Also, in H_2O_2 treated rabbits, we observed mild degenerative changes in the epithelial lining the acini, with karyolitic nucleus and vacuolated cytoplasm. In concern to acini length, we observed that the secretory units in Maca groups was overcrowd due to increasing acini length. However, in H_2O_2 exposed rabbits the diameter of the glandular portion and the length of the acini decreased significantly compared to other groups. Moreover, in H_2O_2 treated rabbits we demonstrated other changes as congested and hyperemic blood vessels and muscular layer appeared disorganized and degenerated. In Maca+ H_2O_2 (H+M) treated rabbits the epithelium reverted to simple columnar and the secretory activity increased compared to H_2O_2 group. In conclusion, we can ensure the effect of Maca as an antioxidant and its ameliorative effect on oxidative stressed rabbits.

Keywords: Acini, Fibrosis, H_2O_2, Maca, Prostate.

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Competing interest: The authors have declared that no competing interest exists.

Introduction

The rabbit urogenital system is a good model that enables testing of new drugs or implants that have potential to be used in human or veterinary medicine (Skonieczna et al., 2019). The accessory sex glands, which include the prostate, seminal vesicles, ampullae of vas deferens and bulbourethral glands, play an important role in the reproductive process (Chughtai et al., 2005). The complex accessory sex glands secrete numerous substances found in the semen, including fructose, citric acid, glycerylphos-phorylcholine, and minerals **(**Holtz and Foote, 1978). Secretion of catalase is uniquely high in rabbit semen (Foote and Hare, 2000). The prostate gland is an exocrine gland found in almost all mammals. It secretes enzymes, amines, lipids and metal ions, essential for the normal function of the spermatozoa (Kindblom et al., 2003). The prostate in rabbits has a more intricate structure, as demonstrated by (Holtz and Foote, 1978). The gland is divided into four portions in this species of animal: proprostate, prostate, and 2 paraprostates. According to (Hafez, 1995) prostate contribute to the greater part of the volume of ejaculate. Each part of the gland plays a specific role in reproduction (Dimitrov, 2010). Oxidative stress has a special focus of interest in the last years as they consider one of causes of male infertility. Oxidative stress occurs by the excessive production of reactive oxygen species (ROS) in the cells and when the competence of the antioxidant defense is exceeded by ROS generation (Aguiar et al., 2008). Among a great variety of ROS, hydrogen peroxide (H_2O_2) plays a crucial role in male infertility (Barbouti et al., 2002). Under normal conditions, an elaborate antioxidant defense system made up of enzymes including superoxide dismutase (SOD), catalase, and glutathione neutralizes the free radicals and reactive oxygen species (ROS) production in the cells (Ji, 1995; Akimoto et al., 2010). Therefore, balance is a perquisite between the ROS production and antioxidant scavenging activity in the male reproductive organs (Mehrotra et al., 2013). Recent studies have investigated that a variety of antioxidant chemicals, which are derived naturally from plant sources, can reduce the damage exerted by oxidative stress. Lepidium meyenii (family Brassicaceae), known as Maca, has been cultivated and traditionally consumed in the Central Andes of Peru to improve male and female reproductive efficiency in both humans and animals (Flores et al., 2003; Valerio and Gonzales, 2005). Maca is observed in several varieties and characterized by different colors of the hypocotyls; Red Maca, Yellow Maca and Black Maca (Gonzales et al., 2005). The histo-morphometrical investigation of the prostate gland in animals administrated Maca still unpopular. As a result, the objective of current study to determine how Maca affected the prostate gland employing several histological and histochemical stains as well as morphometric analyses. Additionally, we wanted to demonstrate the protective effects of Maca on animals under oxidative stress.

Material and methods

Ethical approval

All precautions for using and/or dealing with laboratory animals were performed in consideration with the Ethics Committee of Assiut University.

The current study was conducted at Experimental Farm of Poultry Production, Faculty of Agriculture, Assiut University, Assiut, Egypt.

Experimental animals:

Twenty-four healthy New Zealand rabbits were maintained with a commercial feed and at an appropriate temperature in accordance with the established guidelines for the care of laboratory animals. Four groups of rabbits were randomly selected, and each

group received one of the following treatments:

First group: bucks were fed on the commercial basal ration and received tap water (control group).

Second group: bucks received 1% hydrogen peroxide (H_2O_2) in tap water. The solution was freshly prepared daily to eliminate loss of efficacy due to degradation

Third group: bucks were orally administered with a daily dose of 75 mg **Maca**/Kg body weight (BW) in capsulated form.

Fourth group: bucks were administered orally with 75 mg **Maca** /Kg BW in capsulated form and were received 1% hydrogen peroxide (H_2O_2) in tap water.

Sample Collection:

Two months were spent on the experiment. The animals were sedated with a mixture of 35 mg/kg ketamine and 5 mg/kg xylazine. Then they were killed by heart puncture (Mokhtar et al., 2019).

Histological analysis and histochemical staining:

The prostate (anterior lobe or prostate part) was carefully dissected from other accessory glands. After being collected, the samples were quickly fixed in Bouin's solution for 18 to 22 hours. The fixed materials were dehydrated in ascending grades of ethyl alcohol, cleared in methyl benzoate, and embedded in paraffin wax. Transverse sections were obtained at 5–7 µm. After being deparaffinized in xylene, the sections were hydrated using ethanol in descending concentrations. The staining procedures were carried out in accordance with Bancroft and Steven's descriptions of the histology techniques (1996). On slices stained with Hematoxylin and Eosin (H&E), the general histological structure of the tissue could be seen. For the demonstration of the neutral and acidic mucopolysaccharides, respectively, periodic Acid Schiff (PAS) reaction with Alcian blue (AB) was used. Sections were also stained with Crossmon's trichrome to demonstrate the presence of muscle and collagenous fibers.

Morphometric and statistical analysis:

The morphometric studies were performed on the paraffin sections on the prostate gland. The measurements were applied in the different groups; control un-treated groups (C), groups administrated Maca alone (M), groups administrated 1% H_2O_2 (H), and groups administrated both Maca and H_2O_2 (H+M). The following measurement for each animal (3 animals, 10 sections representative for each group) were applied:

- The acini length.
- The diameter of glandular portion.
- The height of prostate epithelium.

Statistical analyses

Data obtained for the morphometric data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey post-hoc test. Values are presented as mean ± standard deviation (SD). The data were summarized in Graph 7 using "GraphPad Software" (Version 6.05, International Scientific Community) to compare between different variables. Differences were considered significant as $P < 0.05$ (*) and $P < 0.01$(**) and highly significant as $P < 0.001$ (***).

Results

Histomorphological findings

The prostate gland is a compound tubulo-alveolar gland. It consisted of stroma and parenchyma. The stroma consisted of connective tissue capsule (c.t) and interstitium. The connective tissue capsule made up mainly of collagen fibers. The parenchyma was divided into lobules

by bands of connective tissue. The secretory end pieces of parenchyma consist of acini and ducts (Fig.1). In both the control and Maca groups, normal secretory cells and acini were observed (Fig. 1A, B). However, the secretory units and acini of the prostate gland degenerate to some extent in rabbits treated with H2O2 (Fig. 1C). Additionally, we showed that rabbits exposed to hydrogen peroxide and Maca (H+M) had normal architecture in the secretory unit and the glandular region of the prostate gland (Fig. 1D). The diameter of the glandular portion in the control and Maca groups was nearly similar (336±21, 327±20 respectively) (Fig.2A). The length of the acini was comparable between the control and Maca groups (Fig.2B). However, in H2O2 exposed rabbits the diameter of the glandular portion and the length of the acini decreased significantly compared to other groups (Fig.2 A, B). In both the control and Maca groups, the epithelial height was approximately comparable (Fig.2C). The glandular epithelium of the acini was lined with simple columnar cylindrical epithelium with basally located nucleus and acidophilic cytoplasm in control and Maca groups (Fig. 3A, B). However, in rabbits exposed to H2O2, the lining epithelium had modest degenerative alterations as the cytoplasm became vacuolated with karyolitic nuclei. Additionally, the height of the epithelium drastically decreased, and the lining epithelium changed into cuboidal shape (Fig. 2C; Fig.3C). In H+M treated rabbits the epithelium reverted to simple columnar (Fig, 3D). There was little connective tissue and a thin layer of smooth muscle fibers surrounding the glandular portion in the control and Maca groups (Fig. 4A, B; Fig. 5A, B). However, the muscular layer was disorganized and irregular in shape in the H2O2groups, and there was more fibrosis between the glandular portion (Fig, 4C; Fig. 5C). The muscle layer started to return to its original architecture and fibrosis levels decreased in the H+M group (Fig. 4D; Fig.5D). Apocrine secretions normally fill the prostate lumen in the control group when PAS & Hx staining is used to detect them (Fig, 6A). An increase in the amount of positive reactivity to the PAS & Hx stain with secretions is observed in rabbits treated with Maca (Fig, 6B). Although the secretion in H2O2 exposed rabbits was reduced compared to other groups and restricted to the apical border of the cells (Fig, 6C). However, there is a moderate reactivity to the secretion with PAS & Hx stain in rabbits that have been exposed to (H+M) (Fig, 6D).

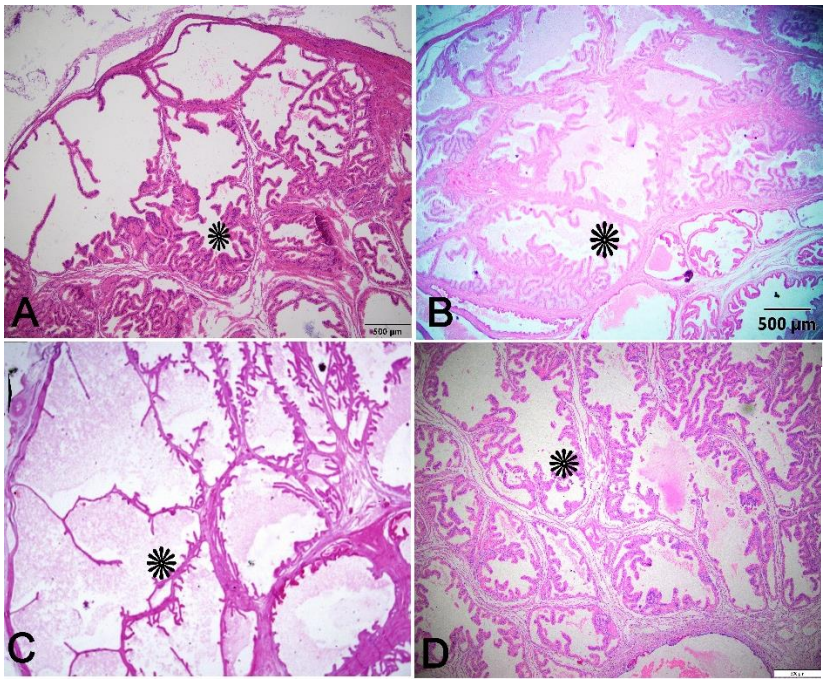

Fig 1. Photomicrographs of paraffin sections stained with Haematoxylin & Eosin showing the prostate gland. A) Control group B) Maca group C) H2O2 group D) H+M. Figures are showing the prostate acini (asterisks).

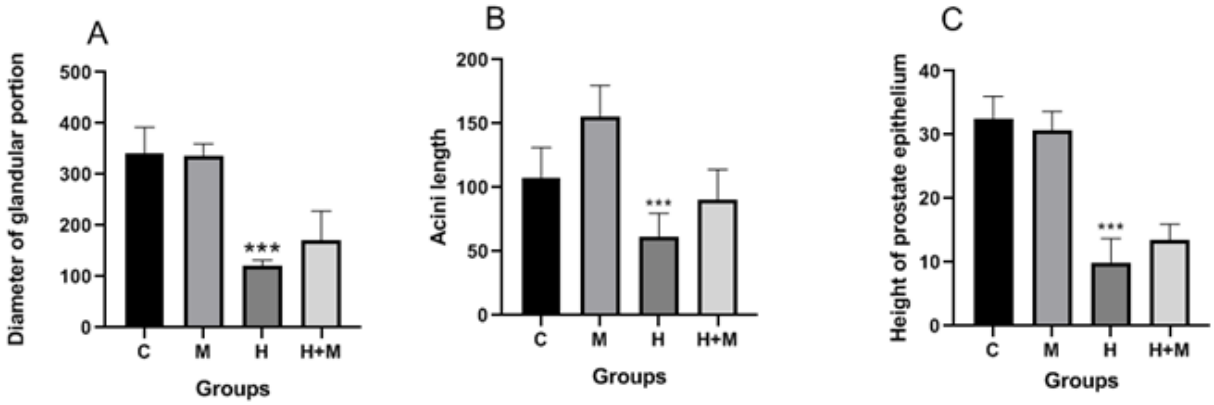

Fig 2. Effect of different treatments; Control group (C) Maca group (M) H_2O_2 group (H) and H+M. on the prostate epithelum. A) diameter of glandular portion, **B)** acini length, **C)** height of prostate epithelium. The measurements are expressed as the mean± SE. ***p<0.001**p<0.01, *p<0.05.

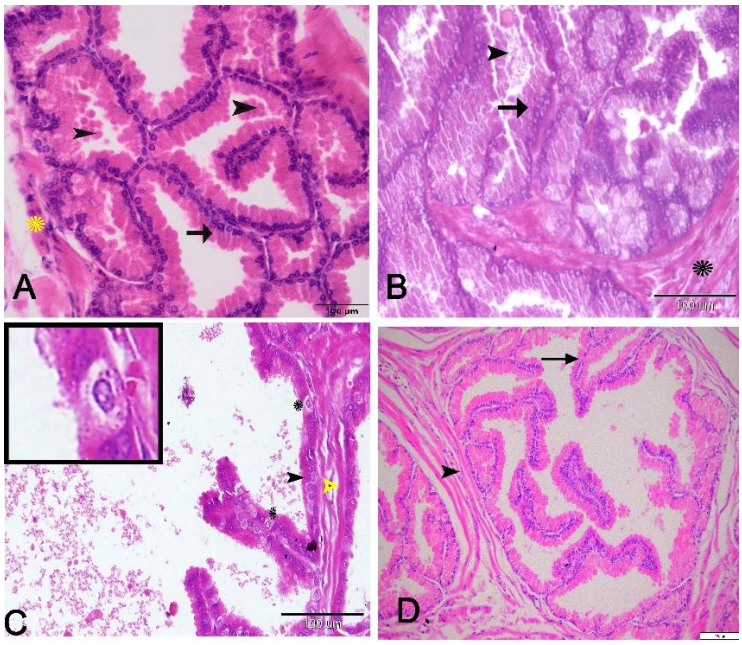

Fig 3. Photomicrographs of paraffin sections stained with Haematoxylin & Eosin showing the prostate gland. A) Control group showing the simple columnar epithelium (black arrow), apocrine secretion (black arrowheads), muscular layer (yellow astricks), B) Maca group showing the simple columnar epithelium (black arrow), apocrine secretion (black arrowheads), muscular layer (black Astricks), C) H_2O_2 group showing the cuboidal epithelium (black arrowheads), degenrated cells with karyolytic nuculeus (black astricks), muscular layer (yellow arrowhrads), D) H+M group showing the simple columnar epithelium (black arrow), muscular layer (black arrowheads).

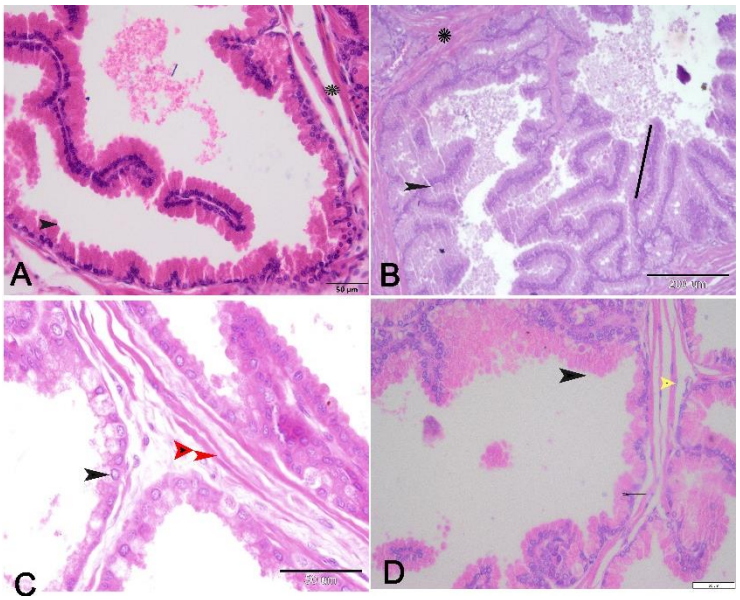

Fig 4. Photomicrographs of paraffin sections stained with Haematoxylin & Eosin showing the prostate gland. A, B) Control and Maca groups showing the simple columnar epithelium (black arrowheads), normal muscular layer (black astricks), acini length (Line tool). C) H_2O_2 group showing the degenrated cells with karyolytic nuculeus (black arrowheads), degenrated and disorganized muscular layer (red arrowheads), D) H+M group showing the simple columnar epithelium with apocrine secretion (black arrowheads), muscular layer begin to revert to normal (yellow arrowhrads).

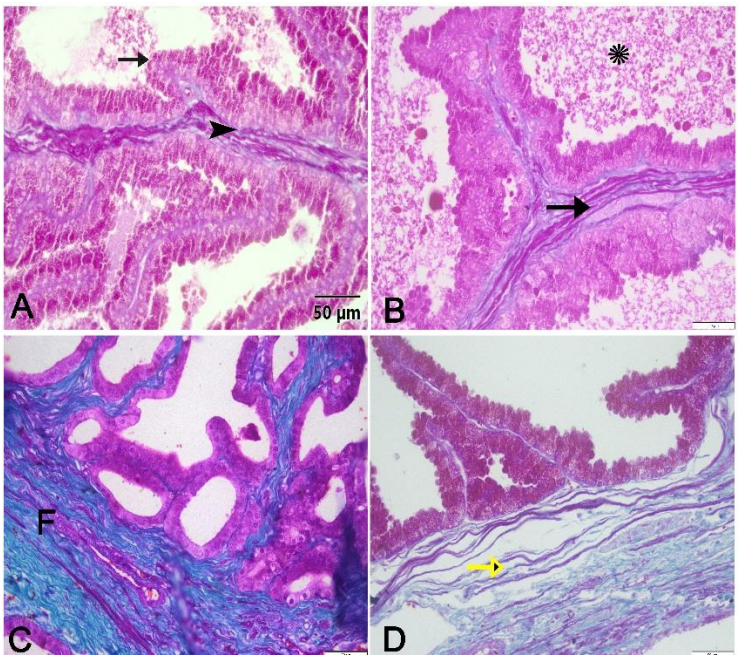

Fig 5. Photomicrographs of paraffin sections stained with Crossman's trichrome showing the prostate gland. A, B) Control and Maca groups showing the normal muscles and collagen fibers (black arrowheads), the secretory ganules in the epithelium lining the prostate (black arrow)and secrtion filling the lumen (astricks). C) H_2O_2 group showing the fibrosis (F), D) H+M group showing the intersitium have less degree of fibrosis (yellow arrow).

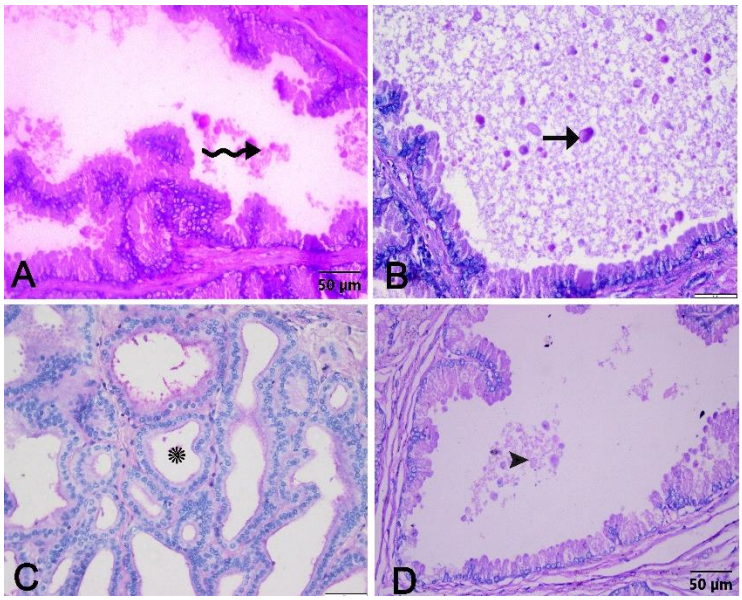

Fig 6. Photomicrographs of paraffin sections stained with PAS&HX showing the prostate gland. A) normal reaction of the luminal secretion to PAS stain in the control group (Zigzag arrow), B) increased reaction of the luminal secretion to PAS stain in Maca group (black arrow), C) No raction could be detected in the lumen with PAS stain in H_2O_2 group (Astricks). D) revert in positive reaction with PAS stain in (H+M) exposed rabbits (arrowhead).

Changes in H_2O_2 group resulted from the oxidative stress:

This study demonstrated macrobiotic changes in the lining epithelium of the prostate. Excessive accumulation of Corpora amylacea in the lumen of the

The present study showed the histological changes in the normal rabbit prostate after exposing to the oxidative stress which ameliorated after administration of Maca. Epidemiological studies have found that consumption of cruciferous vegetables is associated with a

gland was observed. The blood vessels become congested and hyperemic. The muscular layer become disorganized and degenerated (Fig. 7 A-D).

Discussion

reduced risk of prostate cancer (Kolonel et al., 2000). The essential role of the prostate gland is the secretion of the fluid, which comprises almost half the volume of semen. This liquid is rich in citric acid and proteolytic enzymes therefore; it is an energy source for sperm and stimulates

their motility (Young et al., 2006). Therefore, we aimed in the current study to examine for the first time the in-vivo effect of antioxidant on the prostate gland and its role to ameliorate the effect of the oxidative stress.

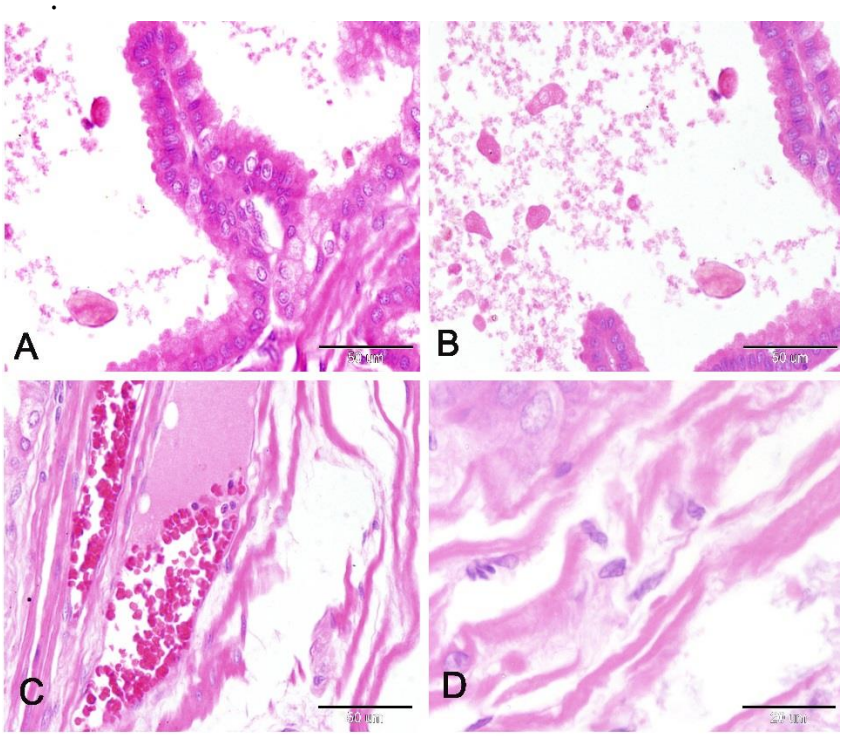

Fig 7. Photomicrographs of H_2O_2 treated rabbits stained with Haematoxylin & Eosin showing changes occurred in the prostate gland due to oxidative stress. A) degenrated cuboidal epithelium with karyoltic nuculeus, B) Excessive accumulation of Corpora amylacea in the lumen of the gland, C) blood vessels become congested and hyperemic, D) disorganized and degenerated muscular layer statical anaylsis in rabbit prostate gland.

Our data reveled that in control and Maca groups, prostate glands consist of bands of connective tissue divided the parenchyma into lobules and the glandular portion was lined with a single cylindrical epithelium with oval basally located nuclei. This observation was in line with (Gabry, 2014). Moreover, Skonieczna et al., (2019) mentioned that prostate in adult prostate treated with zinc, vitamin E and vitamin C showed approximately similar results to those of control. We observed that the columnar epithelium with its apical part of the epithelial cells contained granular material, which was excreted into the lumen of the gland in an apocrine manner. Apocrine secretion involves the formation of a bleb-like protrusion of cytoplasm at the apical pole of the cell referred to as an apical bleb. Apical blebs have been described in various species, namely the rabbit prostate gland (Nicander et al., 1974), turkey efferent ducts, epididymis, and vas deferens (Hess et al., 1976), rat prostate (Thompson et al., 1978).

Some common features are specific for the apocrine-synthesized proteins, transglutaminase and carbonic anhydrase (CAH) studied in the rat-coagulating gland (Wilhelm et al., 1997; Groos et al., 1998). The secreted proteins play a role in the regulation of human sperm function (Aumuller et al., 1997). However, androgen deprivation is rapidly followed by an almost complete loss of transglutaminase immunoreactivity in secretory cells and all blebs disappeared (Steinhoff et al., 1994). In Maca treated rabbits, we observed increase in secretory units and secretory activities appeared in increasing acini length and increased diameter of the glandular tissue, giving overcrowded appearance of gland, this might be due to increased testosterone secretion in this group (un-published data). In contrast, H_2O_2 treated rabbits, we demonstrated nearly empty gland from

secretory units which results in decreased secretory activity and acini length and this may be due to decreased testosterone secretion in oxidative stressed rabbits (Rovira-Llopis et al., 2017).

In H_2O_2 group, we observed a significant decrease in the height of epithelium and decrease in the secretory activities of the cells. Degenerative and disorganized smooth muscle fibers as well as an increase in collagen fibers, both of which point to fibrosis were observed. This result was supported with (Alvarez et al., 2004), who said that there is significant reduction in the height of epithelial cells was noted in the atrophic epithelium. In comparison to control prostates, Maca groups were found to have much more secretion in the gland's lumen. However, in H_2O_2 groups, the acini's epithelium shown degenerative alterations and the secretion reduced markedly. So, this implies that the gland isn't functioning, which results in a lower secretory capability (Nepomnyashchikh, 2014). Rats given BPA at a dose of 100 g/kg showed identical deterioration of the muscle and blood vessel congestion as those in the H_2O_2 groups, and this alteration may lead to tissue damage (Hasanluyi et al., 2016). Our findings indicated a clear rise in corpora amaylacea in the H_2O_2 group, and we hypothesized that this phenomenon was caused by epithelial degeneration or that H_2O_2 exposure may have made the glands appear older (Marx et al., 1965). According to Andrews (1951), 25% of males between the ages of 20 and 40 have Corpora amylacea in their benign acini of the prostate.

Conclusion

In conclusion, the findings of this research show the significance of Maca as an antioxidant for male fertility. Maca also demonstrated a protective effect against H_2O_2 in rabbit prostate glands.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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