Effect of some antioxidants on the prostate of adult and aged albino rats: a histological and immunohistochemical study

Mohamed Sayed Gabry, Dina Helmy Abdel Kader*, Maiada Moustafa, Asmaa Abdel Hamed Elenany
Department of Zoology & Entomology, Faculty of Science, Helwan University, Cairo, Egypt.
*Department of Histology, Faculty of Medicine, Cairo University, Cairo, Egypt.

ARTICLE INFO

Article history:
Received on: 10/10/2013
Revised on: 27/12/2013
Accepted on: 22/11/2013
Available online: 27/02/2014

Key words:
Aging; prostate; zinc; vitamin E; vitamin C; prostatic hypertrophy; Rat.

ABSTRACT

Effect of some antioxidants on the prostate of adult and aged albino rats. Twenty-five adult and twenty-five aged male albino rats were divided into four groups: group I (control group) group II (zinc sulphate treated group), group III (vitamin E) & group IV (vitamin C) zinc administered in doses of 0.2673 mg for adult rat and 0.693 mg for aged rat, vitamin E administered in doses of 0.973 mg for adult rat and 2.52 mg for aged rat, vitamin C administered in doses of 1.215 mg for adult rat and 3.15 mg for aged rat. The prostate glands were processed and stained by H&E, Masson trichrome & immunoreaction of androgen receptor for light microscopic examinations. Morphometric analysis for collagen fibers and immunoreaction area percent was performed and statistically analyzed. Zinc showed improvements, in which decrease in number of mucosal fold and increase in immunoreactions of nuclear androgen receptor in ventral lobe also, Decrease fibrosis and increase in immunoreactions of nuclear androgen receptor in dorsolateral lobe, vitamin E showed improvements, in which decrease in number of mucosal fold, decrease size of acini, decrease of epithelial heights and increase in immunoreactions of nuclear androgen receptor in ventral lobe also, decrease of epithelial heights, decrease fibrosis and increase in immunoreactions of nuclear androgen receptor in dorsolateral lobe, vitamin C there were improvements, in which decrease in number of mucosal fold, dilatation of acini and slightly increase in immunoreactions of nuclear androgen receptor in ventral lobe also, rarified collagenous fibers and increase in immunoreactions of nuclear androgen receptor in dorsolateral lobe. It also ameliorated blood vessels congestion. Zinc, vitamin E and vitamin C exerted no harmful effects on adult prostate but ameliorated effects against aged prostate.

INTRODUCTION

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 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, 2003). The ageing process can be described as the gradual, lifelong accumulation of molecular damage to cells and tissues in response to exposure to stress associated with environment and lifestyle. Such damage results in loss of functions and an increased vulnerability to diseases, ultimately leading to death (Avelino-Silva et al., 2011). At the cellular level, aging can be described as gradual changes in the molecular physiology of the cell that causes a decline in the normal function of cells (Akbari et al., 2008). Exogenous factors such as nutrition and lifestyle are key environmental determinants able to modify the rate at which damage accumulates in cells (Avelino-Silva et al., 2011). The process of biological aging might have a free radical basis. Most free radical which damage the cells involve oxygen radicals or, more generally, activated oxygen species (AOS) which can damage genetic material, cause lipid peroxidation in cell membranes, and inactivate membrane-bound enzymes. The antioxidants, including trace elements, as Zn, Cu and Se, inhibit the oxidation of membrane fat polyunsaturated acids and DNA by oxygen radicals produced during aerobic metabolism (Savarino et al., 2001). Zinc is a constituent of the antioxidant enzyme superoxide dismutase and prevents the reactions between thiols and iron, which give rise to free radicals, and is also an essential
constituent of the nucleic acid-repairing enzymes and a stabilizing factor for biomembranes (Savarino et al., 2001). It may also indirectly act as an antioxidant by stabilizing membranes in some cell types (Platz and Helzlsouer, 2001). Vitamin E is a generic term for a group of compounds known as tocopherols (alpha, beta, gamma, and delta forms) and the corresponding four tocotrienols. The tocopherols are very efficient antioxidants localized in biological membranes and can reduce oxidative damage to a number of biomolecules including the DNA. Hence, numerous investigations have examined the role of vitamin E as a modulator of the risk of cancer and as a therapeutic adjuvant (Wilson et al., 2003). Vitamin E is thought to prevent the spread of peroxidation initiated by free radicals through the fatty acids of phospholipids as a chain-breaking antioxidant (Pekiner, 2003). Today vitamin E is known to possess many biological properties, including antioxidant activity and the ability to modulate protein function and gene expression (Farbstein et al., 2010). Vitamin C (Ascorbic Acid) is a water-soluble antioxidant. (Iqbal et al., 2004). It is an antioxidant found in both animals and plants. It cannot be synthesized in humans and must be obtained from the diet (Padayatty et al., 2003). Large doses of vitamin C have been found to reduce asthma symptoms significantly (Iqbal et al., 2004). The aim of the present work was to elicit the effect of ageing on the prostate gland of albino rat and to study the possible protective effect of three antioxidants: zinc, vitamin C and vitamin E.

MATERIALS AND METHODS

Drugs
Zinc sulphate capsule contained 110 mg zinc sulphate=25 mg zinc as white powder (October Pharma, 6 October City, Egypt), Vitamin C tablet contained 500 mg ascorbic acid (Kahira Pharma, Cairo, Egypt) and alpha tocopherol capsule contained 400 mg oily material (Pharcopharmaceutical, Alexandria, Egypt). The dose was calculated according to interspecies dosage conversion scheme of Goush (Paget and Barnes, 1964). Each dose was dissolved in 0.2 ml distilled water or sesame oil according to drug.

Experimental animals
Twenty adult (6 months) male albino rats (Rattus norvegicus) ranging in weights from 120-150gm and twenty aged (22 months) male albino rat ranging in weights from 300-400gm, were obtained from the farm of the Egyptian Organization of Biological products and Vaccines in Helwan, Cairo. All animals were kept under good hygienic conditions. They were fed and allowed free water supply.

Experimental design
The rats were divided into 4 groups: I, II, III and IV (5 adult and 5 aged rats each):

Group I (control group)
They did not receive any drug.

Group II (Zinc Sulphate treated group)
Adult rat received 0.2673 mg while aged rat received 0.693 mg therapeutic dose of Zn sulphate dissolved in sesame oil daily for 30 days.

Group III (Vitamin E treated group)
Adult rat received 0.973 mg while aged rat received 2.52 mg therapeutic dose of alpha tocopherol dissolved in sesame oil daily for 30 days.

Group IV (Vitamin C treated group)
Adult rat received 1.215mg while aged rat 3.15 mg therapeutic dose of ascorbic acid dissolved in sesame oil daily for 30 days.

Twenty four hours after the last dose all animals were sacrificed and then dissected for excision of prostate gland that was fixed in Bouin’s fluid for 24 hours. They were then subjected to the normal procedures for paraffin blocks formation, sectioned, and stained with haematoxylin & Eosin and Masson’s trichrome stains (Bancroft and Steven, 1982).

For immunohistochemistry, the ready to use rabbit polyclonal anti-androgen receptor primary antibody (Labvision Corporation, Fermont, USA) was applied. Then Power Stain™ 1.0 Poly HRP DAB Kit (Genemed Biotechnologies, CA-USA) was used to visualize the antigen-antibody reaction in the tissues. Then, DAB chromogen was prepared and applied for 2 min., rinsed and counterstained with Mayer Hematoxylin (Bancroft and Gamble, 2002).

Morphometrical studies
Morphometric measurements were performed using "Leica Qwin 500 C" image analyzer computer system (Cambridge, England) present in Histology Department, Faculty of Medicine, and Cairo University. The area percent for fibrosis in prostate section stained by Masson’s trichrome and positive AR immunoreaction was measured in 10 non overlapping fields for every specimen at magnification X 400 for all groups.

Statistical analysis
Results were expressed as the mean ± standard deviation (SD). Using the statistical program statistical package program (SPSS version 17.0), data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups “Tukey” was used as post hoc test. P value <0.05 was considered statistically significant (Petrie and Sabin, 2005).

RESULTS
The prostate of control adult rats consisted of four lobes: ventral, dorsal, lateral and anterior according to their relation to urethra. The fibromuscular stroma consisted of fibroblasts, smooth muscle cells and extracellular matrix. The lumen of acini contained moderate amount of homogenous
acidophilic secretions (Fig.1.A.). The ventral prostatic acini were lined by cubical or squamous cells with central rounded nuclei (Fig.1.C). The dorsolateral acini were lined by folded columnar epithelium (Fig.1.B.). This epithelium consisted of three different cells (luminal cells which had apical brush borders with basal rounded nuclei, basal flattened cells and neuroendocrine cells with pale cytoplasm) (Fig.1.D.).

Aged prostate showed that the ventral prostate had markedly enlarged acini on the expense of thinned out fibromascular stroma and their lumina were filled with large amount of homogenous acidophilic secretions and lined with folded epithelium (fig.1.E.). These acini were lined by low cubical cells with rounded nuclei and congested blood vessels were also seen (fig.1.G.) Also, the ventral prostatic acini contained large amounts of rounded acidophilic secretions called prostatic concretions or corpora amylacea (fig.1.I.). The dorsolateral prostate showed increase in diameter of acini, the lumen was distended with secretions, highly folded mucosa, thick fibromuscular stroma between acini containing some inflammatory cells and congested blood vessels. The acini were lined by columnar cells with basal rounded nuclei (figs.1.F&H.).

In adult prostate treated with zinc, vitamin E and vitamin C showed approximately more or less similar results to those of control.

Zinc treated aged, the ventral prostatic acini showed decreased number of mucosal folds and small amount of fibromuscular stroma between acini which were lined by cubical cells with rounded nuclei (fig.2.A&B). The dorsolateral prostatic acini showed decreased number of mucosal folds and were lined by columnar cells with basal rounded nuclei (fig.2.C.).

Vitamin E treated aged, the ventral acini were decreased in size and secretions. Mild dilatation of prostatic acini with decrease number of epithelial folds and thin fibromuscular stroma separate them. The acini were lined by cubical cells with central rounded nuclei (figs.2.D&E). The dorsolateral acini showed slightly folded mucosa lined by columnar epithelial with basal rounded nuclei and separated with thin fibromuscular stroma (fig.2.F.).

Vitamin C treated aged, the ventral acini showed marked dilatation with small number of epithelial folds, large amount of secretions and thin fibromuscular stroma inbetween. The acini were lined by cubical cells with rounded nuclei (figs.2.G&H). The dorsolateral acini had small mucosal folds lined by columnar cells with basal rounded nuclei and rarified fibromuscular stroma (fig.2.I.).

In both the ventral and dorsolateral lobes of control adult, Masson's trichrome stain demonstrated moderate amount of collagen fibers in the septa between the acini (fig.3.A, B).

In the Aged group prostate, the ventral and dorsolateral lobe showed moderate and many thick collagen fibers respectively (figs.3.C, E) & (figs.3.D, F).

In adult prostate treated with zinc, vitamin E and vitamin C showed approximately more or less similar results to those of control. In the ventral lobe of Zinc, vitamin E&C treated aged prostate showed small amount of collagen fibers (figs.4.A, C&E). In the dorsolateral lobe showed moderate amount of collagen fibers (figs.4.B, D&F).

The morphometrical results are summarized as figures (7) & (8).

In a general view, AR immunoreactivity was verified predominantly in the epithelium and stromal cells of ventral and dorsal lobes of the prostate using anti-androgen receptor.

Control adult, the epithelial cells of ventral and dorsolateral prostatic lobe showed moderate immunoreactivity for AR in the nuclei and cytoplasm (fig.5&B). In the ventral and dorsolateral lobe of aged prostate, AR immunoreactivity was negative in the epithelial cells and the stromal (fig.5.C&D).

In adult prostate treated with zinc, vitamin E and vitamin C showed approximately more or less similar results to those of control.

In the ventral lobe of Zinc, vitamin E&C treated aged prostate showed moderate, intense &moderate immunoreactivity for AR in nuclear cells respectively and weak in stroma (fig.6.A, C&E).

In the dorsolateral lobe showed moderate, intense& moderate immunoreactivity for AR in nuclear cells respectively and weak in stroma while weak, moderate& intense respectively in the cytoplasm (fig 6.B, D&F).

The morphometrical results are summarized as figures (9) & (10).

**DISCUSSION**

The present study, showed histological changes in the senescent rat prostate that were ameliorated after administration of the antioxidants. Senescence is a period of life which is associated with important changes in the hormonal environment in the prostatic gland from different species (Roy-Burman et al., 2004). As the age advances, the oxidative stress increases and with any pathological condition it may aggravates owing to damage in tissues causing additional complication (Meredino et al., 2003).

The present study showed some morphological changes in ventral lobe of prostate (VP) during senescence, including cystic dilatation of prostatic acini, the lumen was distended with secretions with the presence of corpora amylacea, in addition to highly folded epithelia with some pyknotic nuclei, thinned out fibromuscular stroma, and congestion of blood vessels as compared with adult. These results are inagreement with those of Acosta et al. (2004) who found similar changes in the ventral prostate during senescence, including epithelial atypia and atrophy, epithelial and stromal hyperplasia, the presence of amylaceous bodies and infiltration of inflammatory cells. This is also in accordance with Simanainen et al. (2008) who detected that the VPs showed focal atrophy of a small number of acini, lined with cuboidal cells, coexisting with morphologically normal acini and atypical hyperplasia, involving only a small section of the acinar
epithelium. The lumen of some acini was found to contain concretions (ie, corpora amylacea). These changes could be attributed to progressive reduction in testosterone level with senescence. This could be supported by the studies of Montico et al. (2011) who stated that the reduction of testosterone serum levels in the senescence which were associated with harmful changes in the prostatic stromal microenvironment.

Also, García-Flórez et al. (2005) stated that androgen deficiency leads to involution of the prostate, intense activation of apoptosis and remodeling of the extracellular matrix of this organ. Accordingly, it could be suggested that the prostatic hypertrophy could be attributed to accumulation of secretions as manifested by cystic dilatation and to hyperplasia from prostatic glandular proliferation in the form of highly folded epithelium.

The increased detection of corpora amylacea (CA) reconcile with Kate M Young et al. (2007) who reported that corpora amylacea (CA) are a feature of aging. It could be also supported by the study of Yanamandra et al. (2009) who reported that prostatic CAs could contain amyloid structures resulting from calcification of precipitated prostatic secretion. Recently, Gharibyan et al. (2012) found that S100A8/A9 proteins are involved in amyloidogenic process in the ageing prostate, contributing to the formation of calcified corpora amylacea (CA) inclusions, which commonly accompany age-dependent prostate tissue remodelling and cancer.

In the present study, the presence of vascular congestion and inflammatory cellular infiltration could be explained by the work of Adamson et al. (1990) and Krouwer et al. (2012) who referred to changes in the integrity of blood vessels with old age causing disruption of the endothelial barrier and increased capillary permeability evoking an inflammatory response through activation of oxidative stress-sensitive signaling pathways.

In this study, the dorsolateral lobe (DL) of prostate showed some changes during ageing including increased height of epithelial folds, thick fibromuscular stroma, inflammatory cellular infiltration and congestion. Also, Masson’s trichrome stain showed thick longitudinal collagen bundles interbetween acini. The observation of a significantly increased deposition of collagen fibres in the stroma of prostate is similar to the results of Monnier et al. (2005) who stated that ageing was associated with an abundant, highly disorganized and fragmented collagen matrix in the prostate. These collagen alterations may be a consequence of age-associated changes of collagen cross-linking as reported by Ruangpanit et al. (2001). They also added that impairment of collagen degradation would render an accumulation of partially degraded fibrils. Also, in accordance with the present study Salminen et al. (2008) found clustered foci of inflammatory cells in the interglandular regions infiltrating into the fibromuscular stroma as well as the luminal epithelium, and could be effectors of age-related pathologies. In addition, Sivakumar and Das (2008) suggested that inflammation can result in increased collagen deposition in the stroma. In fact, it was mentioned that aging is associated with increased inflammatory activity reflected by increased cytokines which leads to further cell recruitment, inflammation, and eventual matrix remodeling (Braunsgaard et al. 2001). On the other hand, the present study showed a decrease of intensity of the nuclear immunostaining for androgen receptors (AR) in both ventral and dorsolateral lobes of aged rats prostate. This is in partial agreement with Banerjee et al. (2001) who reported that AR immunoreaction decreased in the ventral lobe and increased in the dorsal and lateral lobes in elderly rats. Cândido et al. (2012) suggested that the intensified estrogen and testosterone ratio imbalance could sensitize the AR in this gland, which could be an important signaling pathway in the glandular microenvironment in senescence.

In prostate of aged rats treated with zinc there were observable improvements, in the form of decreased number of mucosal folds, and increased AR immunoreactivity both in ventral and dorsolateral lobes. The antioxidant role of zinc (Zn) may be one of the key mechanisms to elucidate its protective effect by inhibiting the production of reactive oxygen species like H2O2 by transition metals (Oteiza et al., 1995).

It may play a key role in the prevention of prostatic disease by ameliorating oxidative stress, which can subsequently result in DNA damage, increasing the risk of mutation and malignant transformation. In fact, there is a relationship among advanced age, decreased prostate Zn content, and increased oxidative stress (Bianchi-Frias et al., 2010). The detected increase in AR immunoreaction is similarly reported by Hatch et al. (1987) who observed upregulation of AR in zinc-treated aged rat. This could be explained by the work of Jara et al. (2004) who reported that zinc content in the prostate gland is known to be regulated by testosterone, and that testosterone concentration in serum generally decreases with age. This is further supported by the work of Rawy and Seif Al Nassr (2013) who reported that zinc supplementation increased testosterone level.

In vitamin E-treated aged rats, there was decrease in the number of mucosal folds, size of acini, epithelial heights and fibrosis and increase in AR immunoreactions in ventral and dorsolateral lobes. This is in accordance with Kolleck et al. (2002) who reported that vitamin E prevented inflammatory cellular infiltration and thickening of the prostatic interstitial spaces. Also, Zidan. (2011) observed that dietary vitamin E supplementation substantially reduced the extent of pulmonary collagen deposition and histological damage. Vitamin E (VE) is widely promoted for their antioxidant properties (Galan et al., 2005). It was reported to play a major protective role against oxidative stress and prevents the production of lipid peroxides by scavenging free radicals which are toxic byproducts of many metabolic processes in biological membranes (Akiyama, 1999). Moreover, it was also observed to increase testosterone level in aged animals (Chen et al., 2009). In addition, it is essential in maintaining the physiological integrity of testis, epididymis and accessory glands, which has vital role in spermatogenesis and sperm maturation (Cerolini et al., 2006). There are reports in the literature supporting the protective role of VE against damage related to aging in Sprague-Dawley rats through removal of 8-isoprostane and reduction of oxidative damage (Fartes et al., 2012). In aged
prostate treated with vitamin C (VC), there were improvements, in the form of a decrease in the number of mucosal folds, rarified collagenous fibers and a slight increase in AR immunopositivity in ventral and dorsolateral lobes. It also decreased blood vessels congestion, which reconcile with the results of Farshid et al. (2013) who reported that the blood congestion of bladder induced by cyclophosphamide was improved by VC supplementation which they attributed to increased total antioxidant capacity (TAC) levels of plasma. The detected decrease in fibrosis after VC administration is in accordance with Alkhamees (2013) who observed that the degree of hepatic fibrosis was lower in vitamin c-treated rats. They attributed this to induction of endogenous antioxidants. Meanwhile, the increased AR immunoreaction could be justified by the work of Murugesan et al. (2007) who reported that vitamin C administration led to restoration of testosterone to normal level. In the current study, the prostate of adult rat groups, treated with zinc, vitamin E and vitamin C showed approximately more or less similar results to those of control. This is in disagreement with Edger et al. (2005) who reported that dietary supplementation of antioxidant to normal tissue induced harmful effects due to storage of excess in adipose tissue of the body which might become toxic. This contradiction could be attributed to the little (therapeutic) doses of antioxidants used in the present work.

Fig.1: Photomicrograph of the ventral lobe (A-C), dorsolateral lobe (B-D) prostate of adult control group and ventral lobe (E-I), dorsolateral lobe (I-H) of aged control group. (A) The normal histological appearance of prostatic acini (arrow), fibromascular stroma (s) and moderate amount of homogenous acidophilic secretions inside their lumens (L) (X100). (C) Acini lined with cubical cells (curved arrow) with central rounded nuclei (X400; inset, X1000). (B) Short folded prostatic acini (green wavy arrow) with lumens (L) contained acidophilic secretion (SC) (X100). (D) the acini lined with three different cells luminal with apical brush border cells with basal rounded nuclei (green head arrow), basal flattened cells (yellow split arrow) and neuroendocrine cells with pale cytoplasm (blue arrow) and had homogenous secretion (X400; inset, X1000). (E) Folded epithelial lining (green wavy arrow) and markedly enlarged prostatic acini on the expense of thinned out fibromascular stroma (S) and their lumens (L) is distended with homogenous acidophilic secretions. (G) acini lined with simple cubical cells with rounded nuclei and separated by the fibromuscular stroma (S) with some congested blood vessels (BV) (X400; inset, X1000). (I) Large amounts of darkly acidophilic prostatic concretions (corpora amylacea) (CA) inside the acini lined with cuboidal epithelium with rounded nuclei (X1000). (F) Highly folded (green wavy arrow) prostatic acinar lining with thick fibromuscular stroma (S) inbetween acini and the lumen is distended with homogenous acidophilic secretion congested blood vessels (BV) and few inflammatory cells (star) could also be seen within the stroma (X100). (H) highly folded mucosa and thick fibromuscular stroma with inflammatory cells (IM) and congested blood vessels (BV) between acini lined by columnar cells with basal rounded nuclei (H&E X 400).
Fig. 2: Photomicrograph of the ventral lobe of aged rats treated with zinc, vitamin E&C (A-B), (D-E) & (G-H) respectively. Dorsolateral lobe of aged rats treated with zinc, vitamin E&C (C, F&I) respectively. (A), (D) & (G) Prostatic acini with decreased amount of fibromascular stroma (S) between acini and decrease in number of mucosal folds (green wavy arrow) (X100). (B), (E) & (H) acini with decreased amount of fibromascular stroma (S) between acini and decrease in number of mucosal folds (X400; inset, X1000). (C), (F) & (I) showing decrease number of mucosal folds and lined by columnar cells with basal rounded nuclei (H&E: X400; inset, X1000).

Fig. 3: Photomicrograph of the ventral lobe (A), dorsolateral lobe (B) prostate of control adult group and ventral lobe (C-E), dorsolateral lobe (D-F) of control aged group. (A, C & E) showing moderate amount of collagen fibers (CF). (B, D & F) showing moderate and thick longitudinal collagen bundles (LCB) fibrosis respectively (Masson’s trichrome: X 100, 400).
Fig. 4: Photomicrograph of the Ventral lobe of aged treated with zinc, vitamin E &C (A, C&E) respectively and dorsolateral lobe of aged treated with zinc, vitamin E &C (B, D&F). (A), (C) & (E) showing small amount of collagen fibers. (B), (D) & (F) showing moderate amount of collagen fibers (Masson's trichrome: X400).

Fig. 5: Photomicrograph of the Ventral lobe of control adult and aged (A-C) and dorsolateral lobe of control adult and aged (B-D). (A)&(C) showing moderate and negative immunoreactivity for AR respectively in the nuclei (arrowhead) of the lining epithelial cells and a mild one in the stroma (S) (AR immuno: X400; inset, X1000).
Fig. 6. Photomicrograph of the Ventral lobe of aged treated with zinc, vitamin E & C (A, C&E) respectively and dorsolateral lobe of aged treated with zinc, vitamin E & C (B, D&F). (A), (C) & (E) showing moderate, intense & moderate immunoreactivity for AR in nuclear cells and weak in stroma. (B), (D) & (F) showing moderate, intense & moderate immunoreactivity respectively for AR in nuclear cells and weak in stroma(S) while weak, moderate & intense respectively in the cytoplasm(arrow) (AR immuno: X400; inset, X1000).

Fig. 7: Effect of zinc, vitamin E & vitamin C on collagen content in the ventral lobe of prostate.

①Significant change at p < 0.05 with respect to adult control group; (*) Significant change at p < 0.05 with respect to aged control group.
REFERENCES


