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| <b>Original Article</b> | <b>Effects of Estrogen in Comparison with Combined Estrogen and Vitamin E on the Structure of the Ascending Aorta of Senile Female Albino Rats: A Light and Scanning Electron Microscopic Study</b><br><i>Ghada A. Wassif, Seham H. Refaat, Azza K. Abou Hussien and Ashraf Ramzy</i><br><i>Anatomy Department, Faculty of Medicine, Ain Shams University</i> |
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### ABSTRACT

**Aim of the Work:** The present study aimed at illustrating the structural changes in senile ascending aorta and comparing the effects of combined estrogen and vitamin E and estrogen therapy alone in improving these changes by both light and scanning electron microscopy.

**Material and Methods:** Thirty female albino rats were used in the present work; adult rats (6 - 7) month old and senile rats (22 - 23)- month old. The senile rats were divided into three subgroups, control group and a group that received 0.05 ml of folone by intramuscular injection (one injection/ month). The last group received combined estrogen and vitamin-E 40 IU daily by gastric intubation. Animals were anaesthetized after 10 weeks and specimens were processed for microscopic studies.

**Results:** The ascending aorta of senile rats exhibited discontinuity of internal elastic lamina, large vacuolated foam cells in the tunica media and interrupted widely separated elastic lamellae of the tunica media. Collagen fiber deposition was noticed in the three layers. Scanning electron microscopy revealed loss of regular pattern of surface endothelium, desquamation, ulceration or focal shedding of endothelial lining. Upon administration of estrogen, internal elastic lamina appeared continuous. The elastic lamellae in the tunica media were less affected but the tunica adventitia still manifested cellular infiltration. Collagen was noticed in both the tunica intima and media. Scanning electron microscopy revealed that the surface endothelium was still rough with few blebs. Following combined estrogen and vitamin E administration, the characteristic finding was the appearance of elastic lamellae of the tunica media with no signs of separation between its layers. Collagen deposition was still prominent in some areas in the tunica media. Scanning electron microscopy revealed smooth endothelial surface with no signs of ulceration or bleb formation.

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**Key Words:** Ascending aorta, structure, senile, albino rats, female, estrogen therapy, estrogen-vitamin E combination, light microscopy, scanning electron microscopy.

### INTRODUCTION

Aging and estrogen deprivation induce deleterious effects on the cardiovascular system in females (Castillo, *et al.* 2005). Aging is associated with long term changes in arterial structure and function which is the dominant risk factor for cardiovascular diseases. Age related changes include intimal and medial thickening, arterial calcification and increased deposition of matrix substance thus leading to reduced compliance and increased wall stiffness (Nicita- Mauro, *et al.* 2007). These changes were mainly attributed to alterations in the content of collagen and elastin (Izzo and Mitchell, 2007).

Several preventive measures were proposed including postmenopausal hormone replacement

therapy, life style changes and the use of antioxidants. Hormonal replacement therapy in prevention of cardiovascular disease has evolved since estrogen was first proposed to be vasoprotective. It is believed that hormone replacement therapy exerts its potentially beneficial cardiovascular effects through multiple mechanisms as it can alter vasodilatation, coagulation, inflammation and vascular injury response (Ho and Mosca, 2002).

Many clinical studies revealed that estrogen did not decrease the occurrence of myocardial infarction or coronary heart disease (Hanke, *et al.* 1999), yet other studies proved that postmenopausal estrogen therapy has favorable effects on serum lipoprotein and slows the development of

atherosclerosis (Punnonen, et al. 1995; Chae, et al. 1997; Grodstein, et al. 1997; Mendelsohn and Karas, 1999). A possible explanation for these contradictory results is the possible genotypic effects on heart disease. The presence of apo E gene may influence the serum cholesterol response to hormone replacement therapy and consequently predispose to the development of atherosclerosis in postmenopausal women (Lehtimaki, et al. 2002).

On the other hand, antioxidant is defined as any substance which when present at low concentration compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell, 1995). The antioxidants including ascorbic acid, carotenoids, flavonoids, hydrolysable tannins, play an important role in the prevention of cardio- and cerebro-vascular diseases and certain types of cancers (Huxley and Neil, 2003; Johnsen, et al. 2003; Temple and Gladwin, 2003). Vitamin E is the major liposoluble antioxidant active in biological systems and inhibits the development of atherosclerotic lesions (Schwenke and Behr, 1998; Sharma, et al. 1999; Thomas, et al. 2001). Vitamin E has an anticarcinogenic effect by inducing apoptosis in tumor cells and modulates oncogenes (Dutta and Dutta, 2003).

So, it became the aim of the present work to illustrate the structural changes associated with aging in the ascending aorta by light and scanning electron microscopy. Furthermore to understand better the role of estrogen in the treatment and prevention of cardiovascular disease, the possible ameliorative effects of this hormone on the senile aorta will be investigated. In addition, the effect of combining estrogen with an antioxidant (vitamin E) will be compared to the effect of estrogen alone.

## MATERIAL AND METHODS

**Animals:** Thirty female albino rats were used in the present work. Five rats were adults (6-7)-month old and of average weight 150-170 gm. The remaining rats were senile (22-23)-month old and of average weight 240-250 gm.

The animals were bred in The Medical Research Centre and Bilharzial Research Unit at Faculty of Medicine, Ain Shams University and were allowed regular rat diet ad libitum and free water access.

**Drugs:** Folone (Misr Co for Pharm. Ind. SAE) was available as ampule 1ml. containing 5 mg. estradiol benzoate in oily solution. The effect of folone usually begins few days after the injection and is sustained for three weeks, and then the effect decreases gradually till the sixth week (Spicer, et al. 1994).

Vitamin E is a fat soluble vitamin that exists in eight naturally occurring compounds. The alpha-tocopherol is the principal biological active form of vitamin E. It is a highly effective antioxidant carried in the plasma by lipoproteins including low density-lipoprotein cholesterol (LDLc), where it works as a protector of polyunsaturated fatty acids, present mainly in phospholipids and cholesterol esters that protect against free radical damage (Janero, 1991; Wiseman, et al. 1995). Vitamin E was available in capsule each one containing 400 IU from the Arab Co for Pharm. & Medicinal plants (MEPA Co) Egypt.

**Experimental Groups:** The animals were divided into two groups:

- **Group I:** Included five adult female albino rats (6-7 month old) to examine the normal structural pattern of the ascending aorta.
- **Group II:** Included twenty five senile female albino rats (22-23 month old) which were further subdivided into three subgroups.
- **Group IIA:** Included five rats that were used as senile control
- **Group IIB:** Included seven rats that received 0.05 ml of folone by intramuscular injection (one injection/ month) (Kurokawa, 2007). The dose was equivalent to human therapeutic dose and was calculated according to the formula adapted by Paget and Barnes (1964). Three rats received 0.05 ml of corn oil once by intramuscular injection every month and were used as control for this subgroup. The experiment lasted for 10 weeks.
- **Group IIC:** Included seven rats that received 0.05 ml of folone by intramuscular injection (once/ month) followed by daily oral administration of vitamin E (40 IU/ rat) using gastric intubation for ten weeks (Wang, et al. 2007). Three rats received 0.05 ml of corn oil once by intramuscular injection every month and were used as control for this subgroup. The experiment lasted for 10 weeks.

At the end of the experiment all animals were anaesthetized by intraperitoneal injection of sodium thiopental (25 mg/ kg body weight). The thoracic wall was incised and the ascending aorta was dissected and excised. Specimens for light microscopic examination were fixed in 10% formal saline embedded in paraffin blocks, 5  $\mu$ m transverse sections were cut and stained with haematoxylin and eosin, Masson's trichrome and orcein (*Bancroft and Gamble, 2002*).

For preparation of toluidine blue stained semithin sections, tissue samples (1mm) were washed in phosphate buffer, post fixed in 1% osmium tetroxide. After dehydration in ascending grades of alcohol, the specimens were cleared in propylene oxide and finally embedded in gelatin capsules filled with fresh Epon. Semithin transverse sections 0.5-1 $\mu$ m were cut and stained with toluidine blue to be examined by light microscopy.

For scanning electron microscopic study, the tissue samples were dehydrated in ascending grades of ethyl alcohol. Then in a mixture of 1:1 absolute alcohol and acetone 100% and then in 100% acetone. The specimens were then dried at critical point using liquid carbon dioxide in BAL-TEK CPD030. The specimens were fixed on aluminium stubs and then sputter coated with gold using BALTEK-SCD005 (*Robinson, et al. 1987*). Specimens were examined with Philips, XL 30 scanning electron microscope at the Electron Microscopic Unit, Ain Shams University.

**Statistical analysis:** Measurements were taken for the whole thickness of the aorta, media and adventitia of the different groups. All data were collected and analyzed using the Statistical Package for the Social Sciences (SPSS) software program (version 13; SPSS Inc., Chicago, IL, USA). All values were expressed as mean  $\pm$  SEM (standard error of mean). Anova test was used to detect any statistical significant values. Probability (P) value was considered statistically significant if  $< 0.05$  and highly significant if  $< 0.01$ .

## RESULTS

### I- Histological Results:

- **Group I (adult rats):** Light microscopic examination of sections of the ascending aorta revealed three identifiable layers (tunicae): intima, media and adventitia (Figs. 1, 2).

The tunica intima consisted of a continuous layer of flattened elongated endothelial cells with dark flat nuclei, with their long axis parallel to the direction of blood flow in the artery (Figs. 1-3). The subendothelial layer contained few wavy elastic fibers (Fig. 3). Also, some collagenous fibers could be detected in sections stained with Masson's trichrome (Fig. 4). The external border of the intima was delineated by an internal elastic lamina which is the innermost elastic layer of the aortic wall (Figs. 3, 4).

The tunica media was the thickest of the three layers of the ascending aorta and constituted the main bulk of its wall. The most conspicuous feature of tunica media was the presence of large number of concentric wavy elastic lamellae (Fig. 5). These lamellae were interspersed by pale oval nuclei of smooth muscle cells with few collagen fibers (Figs. 1, 3, 4).

The tunica adventitia was the outer layer of the ascending aorta and was about half the thickness of tunica media (Fig. 1). It consisted of irregularly arranged connective tissue with abundant collagen, few elastic fibers and smooth muscle cells (Figs. 4, 5). Small blood vessels (vasa-vasorum) were also detected (Fig. 2).

The scanning electron microscopy revealed that the luminal surface showed longitudinal folds and intact smooth endothelial lining. The endothelial cells were elongated, flat and arranged parallel to the long axis of the aorta with occasionally few cells that appeared branching (Figs. 6, 7).

- **Group II A (senile control rats):** Light microscopic examination of sections of the ascending aorta revealed apparent focal increase in the thickness of the intima as well as the media as compared to those of the adult group (Figs. 8, 9). Focal desquamation of surface endothelium and partial discontinuity of the internal elastic lamina were observed (Figs. 9, 10). The characteristic finding was the presence of large vacuolated foam cells mainly in the tunica media in addition to few cells noticed in the tunica intima (Figs. 10-12). Also, smooth muscle cells with longitudinally or obliquely arranged nuclei were seen along the luminal surface and in tunica media (Figs. 13, 14). In some areas muscle nuclei appeared rounded instead of being oval or spindle-shaped with perinu-

clear cytoplasmic vacuolations (Figs. 12, 14). In other areas muscle nuclei were aggregated together and sometimes existed in colonies (Figs. 15, 16). Fragmentation, thinning and variable separation of the concentric elastic lamellae of the tunica media were clearly observed. Frequently, the elastic fibers were disrupted and ill-defined (Figs. 13, 15, 17). The tunica adventitia showed apparent increase in thickness in comparison to the adult group. Moreover, cellular infiltration and excessive vascularization was encountered (Figs. 8, 11). Sections stained with Masson's trichrome revealed relative increase in collagen fiber deposition in the tunica intima, media and adventitia (Fig. 16).

Scanning electron microscopy of the ascending aorta of senile rats revealed that the luminal surface had irregular longitudinal folds and rough endothelial lining (Fig. 18). Most endothelial cells lost their regular pattern of arrangement and showed areas of ulcerations. The ulcers were small, rounded or oval in shape with some endothelial cells showing localized small rounded surface blebs (Fig. 19). Localized areas of desquamation of endothelial cells associated with aggregations of blood cells was noticed (Fig. 20). In some areas, the endothelial lining was lost with the exposure of the subendothelial layer (Fig. 21).

- **Group II B (senile rats that received estrogen):** Examination of sections of the ascending aorta of senile female rats which received estrogen showed that the increase in the intimal and medial thickness was less than that seen in Group II A (senile control rats) and in the control of this group. In some areas, discontinuity of the endothelial lining of tunica intima was seen (Fig. 22). Few vacuolated foam cells could still be observed in the tunica intima and tunica media (Figs. 22-24). Restoration of the internal elastic lamina with partial restoration of the elastic lamellae of tunica media was clearly observed (Figs. 23-25). However, some areas still exhibited ill-defined elastic fibers (Fig. 24). The tunica adventitia still manifested cellular infiltration (Fig. 24). Apparent increase in collagen could be noticed in both the tunica intima and media (Fig. 26).

Scanning electron microscopy of ascending aorta of this group revealed intact endothelial lining.

However, it appeared relatively rough (Fig. 27). The endothelial cells were elongated and flat and their parallel arrangement was almost restored. Few localized rounded surface blebs were still encountered (Fig. 28).

- **Group II C (senile rats that received combined estrogen and vitamin E):** Examination of sections of the ascending aorta of senile female rats which received combined estrogen and vitamin E revealed that the increase in the intimal and medial thickness was less than that seen in Group II A (senile control rats) and in the control of this group. The continuity of the endothelial lining of tunica intima was observed (Fig. 29). The internal elastic lamina was clearly seen (Fig. 30). The characteristic finding was the restoration of the elastic lamellae of tunica media with no signs of separation between its layers (Figs. 30, 31). However, some small oval vacuoles were obvious along the entire thickness of the tunica media (Fig. 30). Collagen fibers were also prominent in some areas in the tunica media (Fig. 32).

The scanning electron microscopy revealed that the luminal surface showed regular longitudinal folds. The endothelial lining was smooth and the cells were elongated and flat. No signs of ulceration or desquamation of endothelium could be observed, though few small rounded or oval umbilication could be noticed (Fig. 33).

**II- Morphometric Results:** Statistical analysis of the mean values of the whole thickness of the aorta in senile group (Group II A) revealed highly significant increase ( $P < 0.001$ ) in comparison to the adult group (Group I). The thickness of the media in senile group showed a highly significant increase ( $P < 0.001$ ) while that of the adventitia showed only significant increase ( $P < 0.05$ ) in comparison to the adult group (Table 1).

Statistical analysis of the mean values of the whole thickness of the aorta as well as, the thickness of the media in estrogen group (Group II B) and estrogen & vitamin E group (Group II C) revealed highly significant decrease in comparison to the senile group (Group II A). The thickness of the adventitia in estrogen group (Group II B) showed a significant decrease while that in estrogen & vitamin E (Group II C) were highly significant in comparison to the senile group (Table 2).

**Note:** senile measurements of thickness of the aorta and its tunics were similar in senile group

II A and that of the control rats of group II B and II C.

**Table 1:** Mean thickness of the media & Adventitia and the whole thickness in group I and group II A.

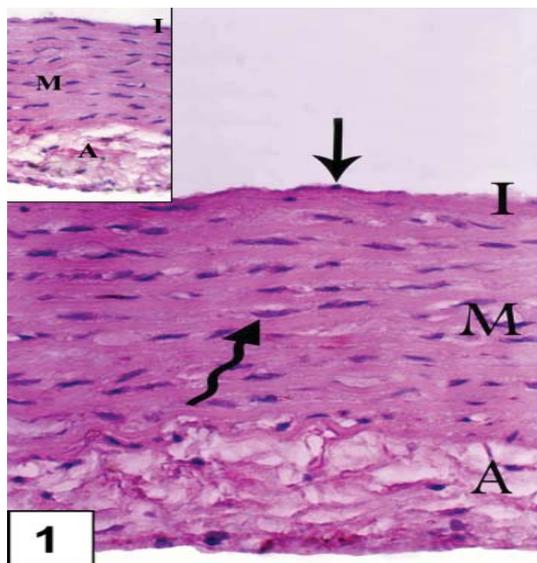
|                        | Groups        | Mean ( $\mu\text{m}$ ) | SDM       | P value | Significance |
|------------------------|---------------|------------------------|-----------|---------|--------------|
| <b>Whole thickness</b> | Adult (I)     | 122.2                  | $\pm 1.2$ | -       | -            |
|                        | Senile (II A) | 251.7                  | $\pm 7.5$ | < 0.00  | HS           |
| <b>Media</b>           | Adult (I)     | 93.1                   | $\pm 0.9$ | -       | -            |
|                        | Senile (II A) | 183.4                  | $\pm 3.3$ | < 0.001 | HS           |
| <b>Adventitia</b>      | Adult (I)     | 30.3                   | $\pm 1.7$ | -       | -            |
|                        | Senile (II A) | 51.8                   | $\pm 2.9$ | < 0.05  | S            |

Probability (P) value was considered statistically significant if <0.05 and highly significant if < 0.01.

**Table 2:** Mean thickness of the media & Adventitia and the whole thickness in group II A, group II B and group II C.

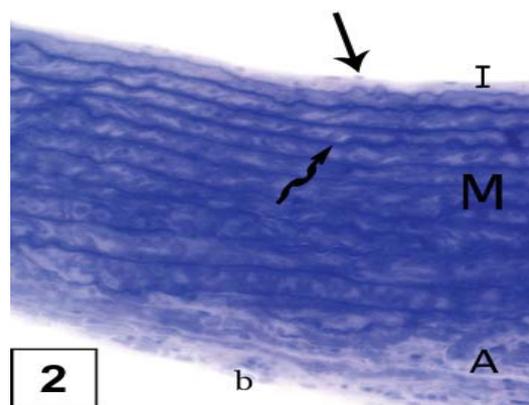
|                        | Groups                   | Mean ( $\mu\text{m}$ ) | SDM       | P value | Significance |
|------------------------|--------------------------|------------------------|-----------|---------|--------------|
| <b>Whole thickness</b> | Senile (II A)            | 251.7                  | $\pm 7.5$ | -       | -            |
|                        | Estrogen (II B)          | 147.9                  | $\pm 1.5$ | < 0.001 | HS           |
|                        | Estrogen & Vit. E (II C) | 142.0                  | $\pm 2.3$ | < 0.001 | HS           |
| <b>Media</b>           | Senile (II A)            | 183.4                  | $\pm 3.3$ | -       | -            |
|                        | Estrogen (II B)          | 127.8                  | $\pm 0.5$ | < 0.001 | HS           |
|                        | Estrogen & Vit. E (II C) | 117.7                  | $\pm 1.9$ | < 0.001 | HS           |
| <b>Adventitia</b>      | Senile (II A)            | 51.8                   | $\pm 2.9$ | -       | -            |
|                        | Estrogen (II B)          | 34.2                   | $\pm 1.7$ | < 0.05  | S            |
|                        | Estrogen & Vit. E (II C) | 20.5                   | $\pm 1.5$ | < 0.01  | HS           |

P value in each group was in comparison with the senile group. Probability (P) value was considered statistically significant if <0.05 and highly significant if < 0.01.



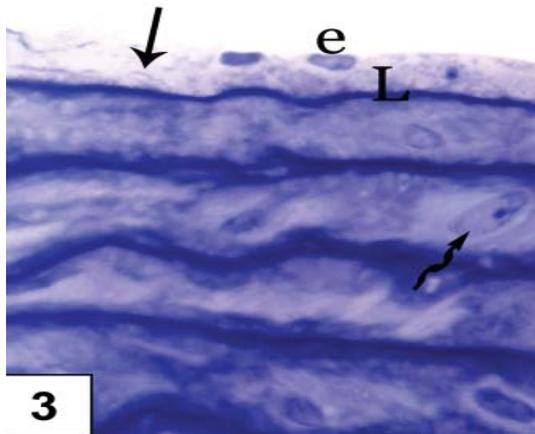
**Fig. 1:** A photomicrograph of a transverse section of aorta of adult female rat (group I) showing the three layers of ascending aorta, tunica intima (I), tunica media (M) and tunica adventitia (A). Note the endothelial cell nuclei (arrow) of tunica intima and the smooth muscle nuclei (spiral arrow) of tunica media. Inset shows the three layers of ascending aorta.

Hx& E; X400 - Inset; X200

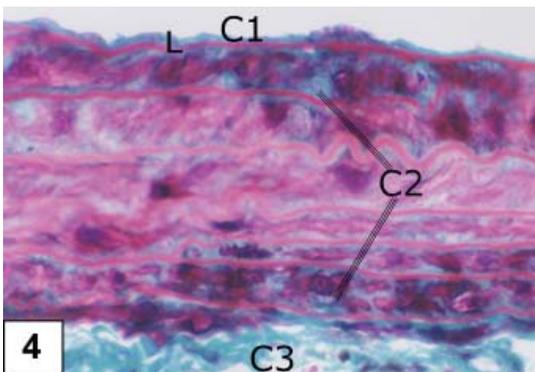


**Fig. 2:** A photomicrograph of a semithin transverse section of aorta of adult female rat (group I) showing intact endothelial cells (arrow) in tunica intima (I). Notice the parallel layers of elastic fibers (spiral arrow) in tunica media (M) and the presence of blood vessels (b) in tunica adventitia (A).

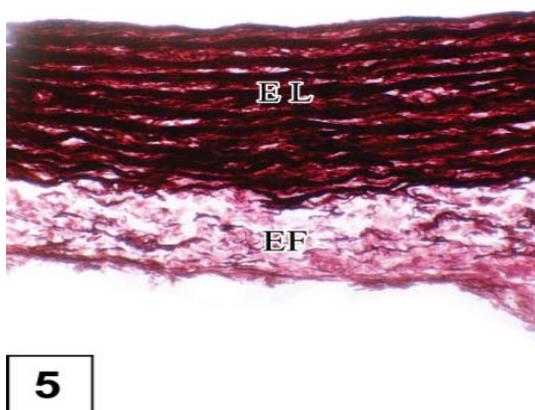
Toluidine blue; X200



**Fig. 3:** A photomicrograph of a semithin transverse section of aorta of adult female rat (group I) showing intact endothelial cell nuclei (e) with few wavy elastic fibers (arrow) in the subendothelial layer. Note the intact internal elastic lamina (L) and the smooth muscle nuclei (spiral arrow) of tunica media. Toluidine blue; X 1000



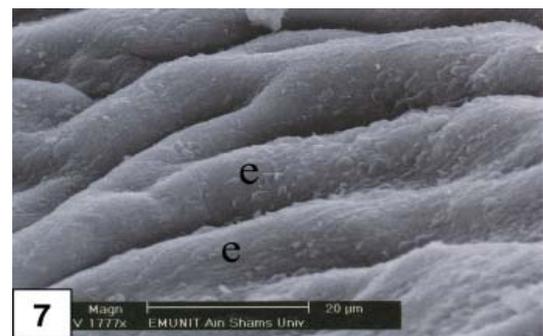
**Fig. 4:** A photomicrograph of a transverse section of aorta of adult female rat (group I) showing collagen fibers in tunica intima (C1) and tunica media (C2). Note the irregular arranged collagen bundles in tunica adventitia (C3). Note also the internal elastic lamina (L). Masson's trichrome; X 400



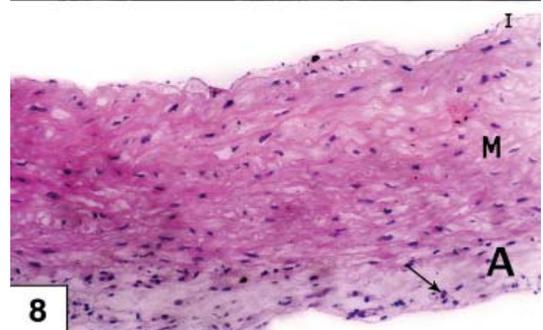
**Fig. 5:** A photomicrograph of a transverse section of aorta of adult female rat (group I) showing concentric wavy elastic lamellae (EL) in tunica media and the few elastic fibers (EF) in tunica adventitia. Orcein; X 200



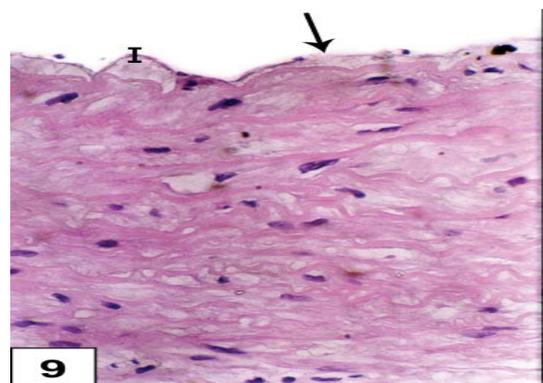
**Fig. 6:** A scanning electron micrograph of aorta of adult female rat (group I) showing intact smooth endothelial lining. X 231



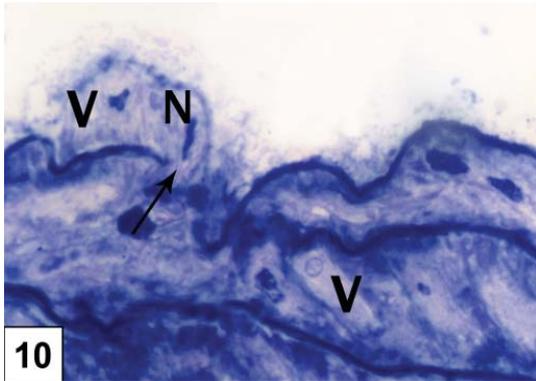
**Fig. 7:** A scanning electron micrograph of aorta of adult female rat (group I) showing elongated endothelial cells (e). X 1777



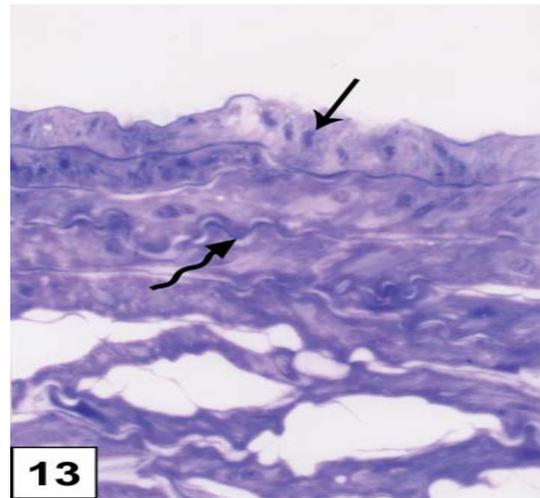
**Fig. 8:** A photomicrograph of a transverse section of aorta of senile female rat (group II A) showing apparent increases in the thickness of the intimal (I) and medial (M) layers of the aorta. Note cellular infiltration (arrow) in tunica adventitia (A). Hx& E; X 200



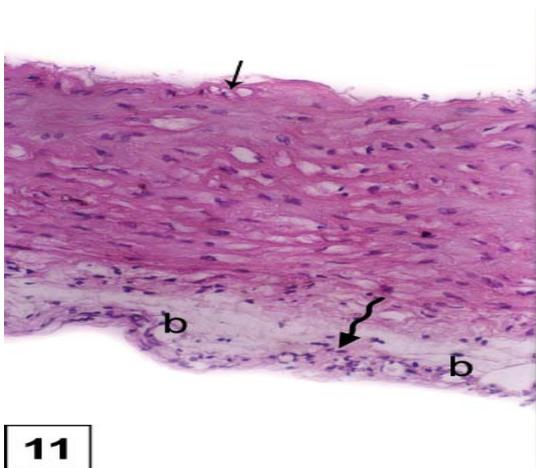
**Fig. 9:** Higher magnification of the previous figure showing thickened intima (I) with focal desquamation of the endothelial cells (arrow) of tunica intima. Hx& E; X 400



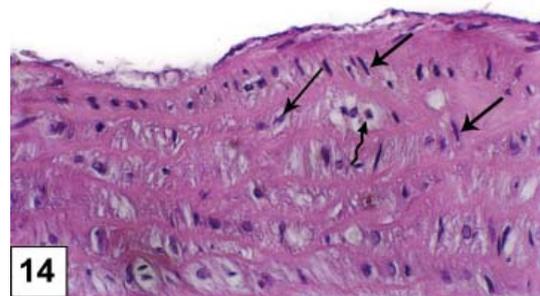
**10**  
**Fig. 10:** A photomicrograph of a semithin transverse section of aorta of senile rat (group II A) showing discontinuity of the internal elastic lamina (arrow). Note the large vacuolated foam cells (V) in tunica intima and media and the longitudinally arranged nuclei (N). Toluidine blue; X 1000



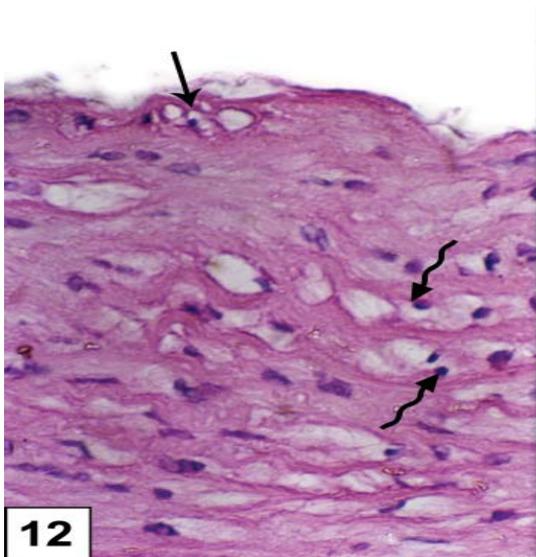
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**Fig. 13:** A photomicrograph of a semithin transverse section of aorta of senile female rat (group II A) showing smooth muscle cells with longitudinally or obliquely arranged nuclei (arrow) along the luminal surfaces. The elastic fibers in tunica media appear disrupted, fragmented and ill defined (spiral arrow). Note the variable degrees of separation of elastic fibers. Toluidine blue; X 400



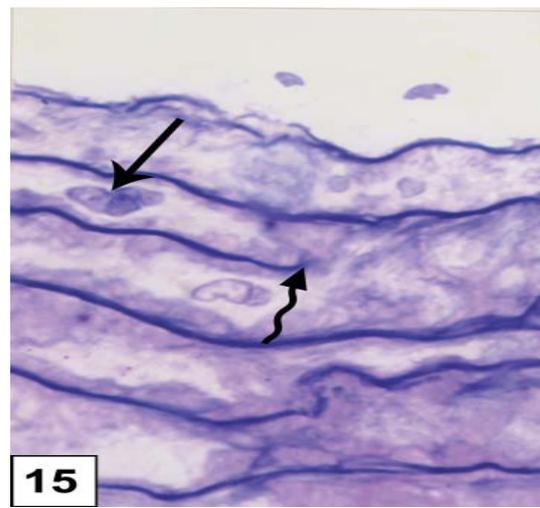
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**Fig. 11:** A photomicrograph of a transverse section of aorta of senile female rat (group II A) showing subintimal vacuolation (arrow). Note cellular infiltration (spiral arrow) in tunica adventitia with excessive vascularisation (b). Hx& E; X 200



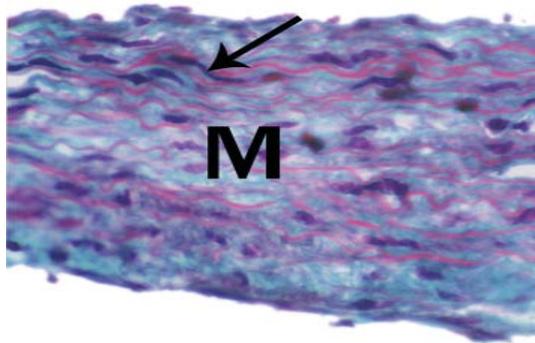
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**Fig. 14:** A photomicrograph of a transverse section of aorta of senile female rat (group II A) showing smooth muscle cells with longitudinally arranged nuclei (arrows) mainly in tunica media. Note the perinuclear cytoplasmic vacuolation (spiral arrow). Hx& E; X 400



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**Fig. 12:** Higher magnification of the previous figure showing subintimal vacuolation (arrow). Note some smooth muscle nuclei (spiral arrows) appear rounded with perinuclear cytoplasmic vacuolation. Hx& E; X 400

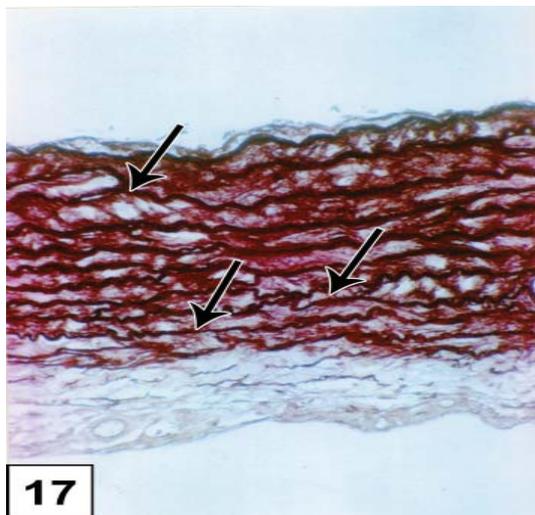


**15**  
**Fig. 15:** A photomicrograph of a semithin transverse section of aorta of senile female rat (group II A) showing aggregation of smooth muscle nuclei (arrow) with discontinuity of elastic lamellae (spiral arrow) in tunica media. Toluidine blue; X 1000



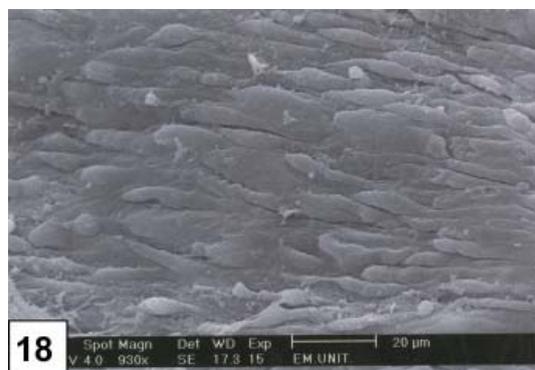
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**Fig. 16:** A photomicrograph of a transverse section of aorta of senile female rat (group II A) showing increase collagen deposition in tunica media (M). Some smooth muscles nuclei (arrow) existed in colonies. Inset shows the colonies of smooth muscle nuclei. Masson's Trichrome; X200 - Inset; X 400



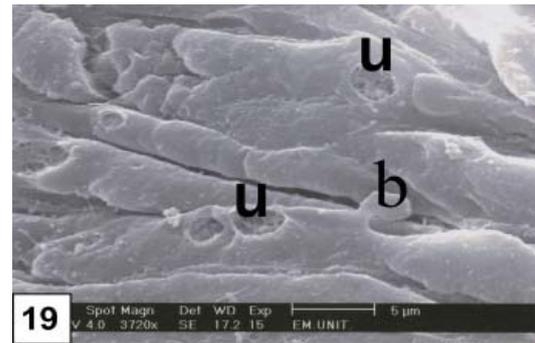
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**Fig. 17:** A photomicrograph of a transverse section of aorta of senile female rat (group II A) showing thinning, fragmented, disarrayed elastic laminae (arrows), which are more pronounced in the outer layer of tunica media. Orcein; X 200



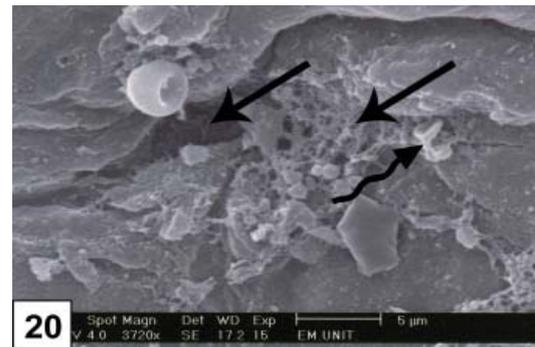
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**Fig. 18:** A scanning electron micrograph of aorta of senile female rat (group II A) showing rough endothelial lining with irregular longitudinal folds. X 930



**19**

**Fig. 19:** Higher magnification of the previous figure showing small rounded or oval ulcers (u) and blebs (b). X 3720



**20**

**Fig. 20:** A scanning electron micrograph of aorta of senile female rat (group II A) showing localized desquamation of endothelium (arrows) with aggregation of blood cells (spiral arrow). X 3720



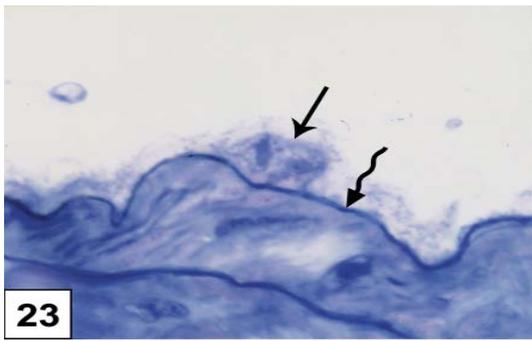
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**Fig. 21:** A scanning electron micrograph of aorta of senile female rat (group II A) showing area of shedding of endothelial lining with exposure of subendothelial layer. X 472

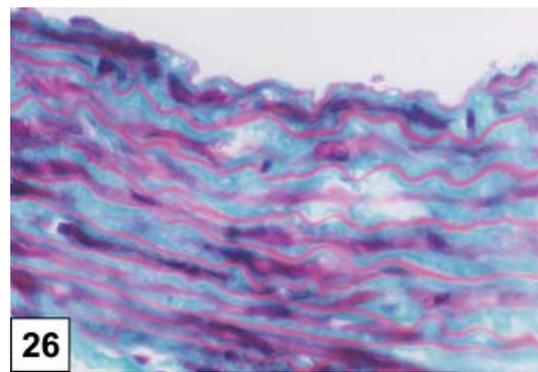


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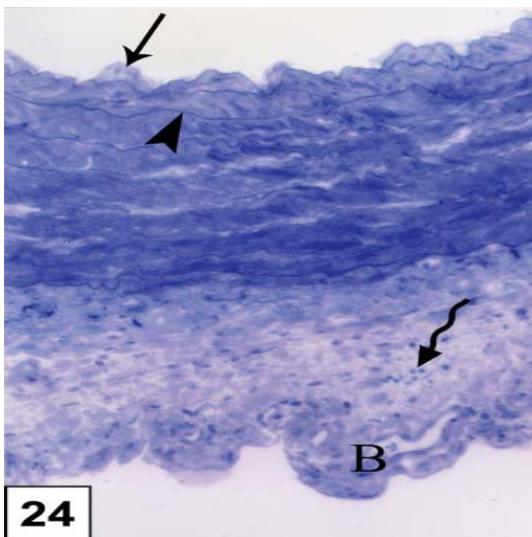
**Fig. 22:** A photomicrograph of a transverse section of aorta of senile female rat which received estrogen (group II B) showing some areas with desquamation of the endothelial cells (arrow). Note vacuolation (spiral arrows) in both tunica intima and tunica media. Hx&E; X 400



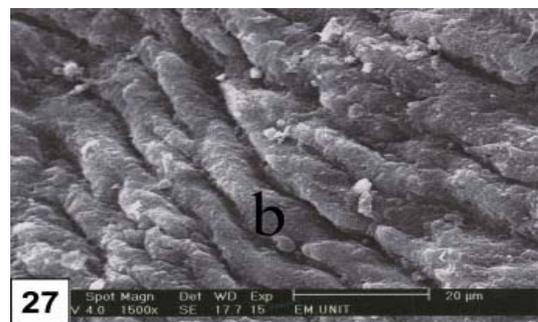
**Fig. 23:** A photomicrograph of a semithin transverse section of aorta of senile female rat which received estrogen (group II B) showing vacuolated foam cells (arrow) in tunica intima with continuous internal elastic lamina (spiral arrow). Toluidine blue; X 1000



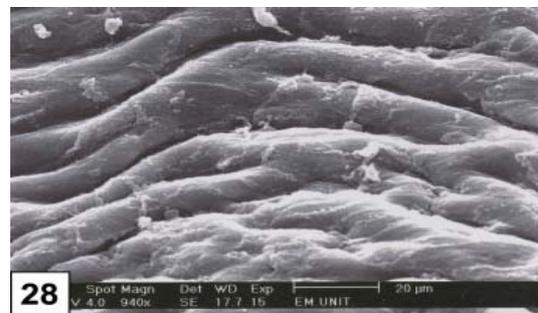
**Fig. 26:** A photomicrograph of a transverse section of aorta of senile female rat (group II B) which received estrogen showing apparent increase in collagen in tunica intima and media. Masson's trichrome; X 400



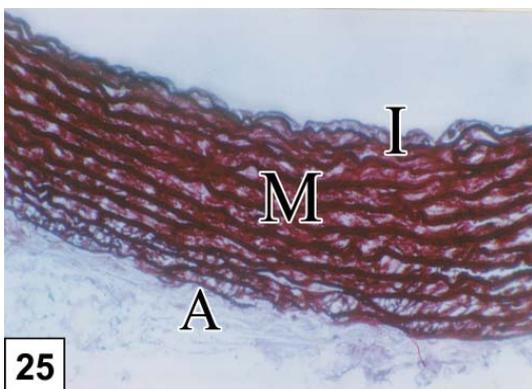
**Fig. 24:** A photomicrograph of a semithin transverse section of aorta of senile female rat which received estrogen (group II B) showing vacuolated foam cells (arrow) in tunica intima. Note the partial restoration of elastic lamellae in tunica media (arrowhead), the cellular infiltration (spiral arrow) in tunica adventitia and the blood vessels (B). Toluidine blue; X 200



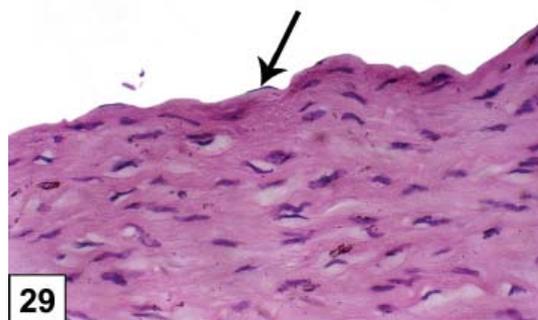
**Fig. 27:** A scanning electron micrograph of aorta of senile female rat which received estrogen (group II B) showing that the endothelial lining appears intact but rough with few surface blebs (b). X 1500



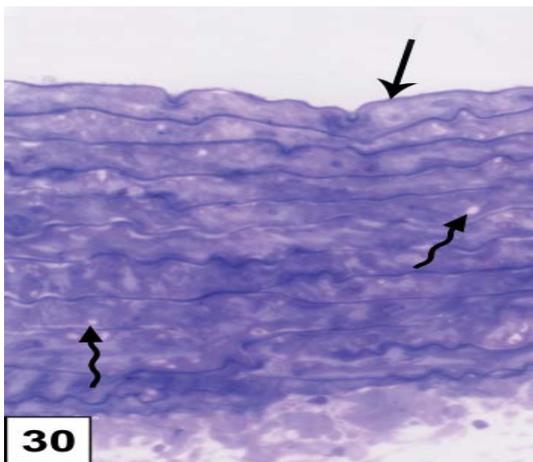
**Fig. 28:** A scanning electron micrograph of aorta of senile female rat which received estrogen (group II B) showing parallel arrangement of the elongated endothelial cells. X 940



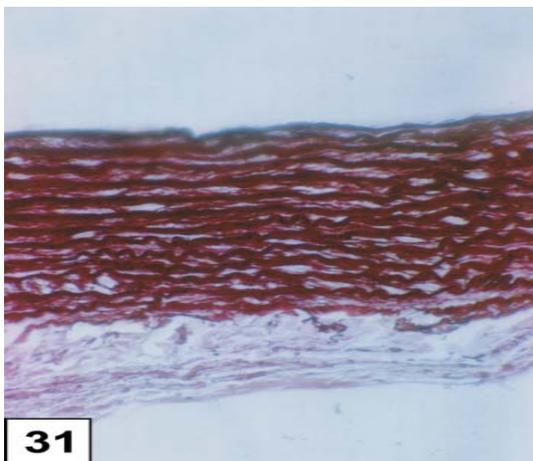
**Fig. 25:** A photomicrograph of a transverse section of aorta of senile female rat which received estrogen (group II B) showing the wavy appearance of elastic lamellae. Note tunica intima (I), tunica media (M) and adventitia (A). Orcein; X 200



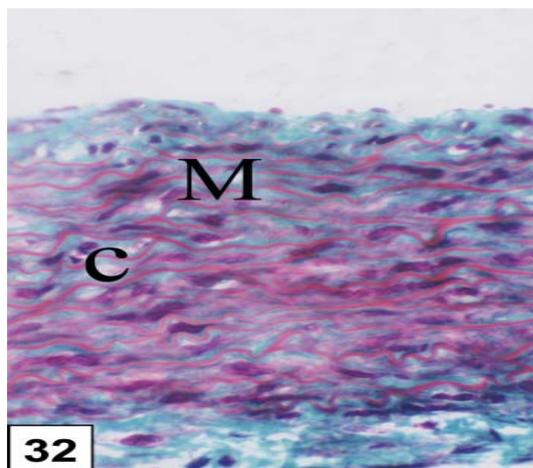
**Fig. 29:** A photomicrograph of a transverse section of aorta of senile female rat which received combined estrogen and vitamin E (group II C) showing intact endothelial lining (arrow) of the tunica intima. Hx&E; X 400



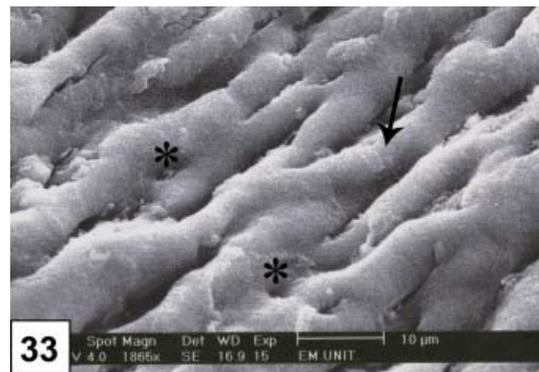
**Fig. 30:** A photomicrograph of a semithin transverse section of aorta of senile female rat which received combined estrogen and vitamin E (group II C) showing continuous internal elastic lamina (arrow) and the elastic lamellae in tunica media. Notice the few vacuoles (spiral arrows) in tunica media. Toluidine blue; X 400



**Fig. 31:** A photomicrograph of a transverse section of aorta of senile female rat which received combined estrogen and vitamin E (group II C) showing the concentric elastic lamellae in tunica media. Orcein; X 200



**Fig. 32:** A photomicrograph of a transverse section of aorta of senile female rat which received combined estrogen and vitamin E (group II C) showing collagen (C) deposition in some areas in tunica media (M). Masson's trichrome; X 200



**Fig. 33:** A scanning electron micrograph of aorta of senile female rat which received combined estrogen and vitamin E (group II C) showing smooth endothelial lining. Notice the elongated flat cells (arrow). Some areas show umbilication (\*) X 1865

## DISCUSSION

The present work clarified the changes that occur in the aorta by age. It manifested remarkable changes involving its three layers. The characteristic finding was the focal increase in the thickness of the intima and media of the ascending aorta. These results coincide with those reported by *Bilato and Crow (1996)*; *Nicita-Mauro et al. (2007)* in human. *Ross and Pawlina (2006)* explained the increase in intima thickness in atherosclerosis by migrating smooth muscle cells from the media to the intima producing large amounts of extracellular matrix (proteoglycans & collagen). Also, *Ueno et al. (2000)* added that in hypertensive animals with reduced blood flow, the smooth muscle cell proliferation has been considered a major mechanism responsible for the great arterial hypertrophy.

Focal desquamation of the surface endothelium was seen in the present work as a result of aging. *Kublickiene et al. (2005)* stated that nitric oxide was synthesized by the vascular endothelium. They added that nitric oxide plays a major physiological role in maintaining the endothelial cells and inhibition synthesis of the extracellular matrix.

In the present work, the discontinuity of the internal elastic lamina was seen. These alterations reflect the mode of migration of aortic smooth muscle cells in the development of atherosclerotic lesions in rat (*Nakatake and Yamamoto, 1987*).

A characteristic finding in the present work was the presence of vacuolated foam cells in the

tunica intima and media with the appearance of perinuclear vacuolations. These cells are probably the site of cholesterol accumulation. The excess cholesterol might be due to increased uptake of atherogenic lipoprotein and/or decreased cholesterol efflux from the cells. Other contributor to accumulation might include high plasma cholesterol and increased oxidative stress. The appearance of foam cells leads to the development of atherosclerotic lesions in mice (Kaplan, et al. 2001). Furthermore, Ross and Pawlina (2006) added that foam cells derived from both macrophages and smooth muscle cells accumulate low density- lipoproteins (LDLs) which cross the endothelial barrier and are oxidized by free radicals produced by endothelial cells. Thereby, inhibition of LDL oxidation is supposed to be one of the crucial steps in retarding the foam cell formation and development of aortic lesions (Chisolm and Steinberg, 2000).

In the present work, smooth muscle cells with longitudinally arranged nuclei were seen along the luminal surface and in tunica media. Similar findings were noted by Sims (2000) in human arteries. He mentioned that this took place through major defects of the internal elastic lamina and resulted in a change from transverse to longitudinal orientation of these cells and the accompanying elastin fibers of the intima. In addition, in this study, smooth muscles nuclei were noticed aggregated together in colonies. Tokunaga et al. (1989) described a similar aggregation in the nuclei of the endothelial cells of the human aorta. They explained this by adhesion of adjacent typical endothelial cells and added that this process was affected more by atherosclerosis in human aorta than by aging. Furthermore, Bilato and Crow (1996) observed increase in the amount of activated smooth muscle cells with advancing age. Ueno et al. (2000) added that in hypertensive animals smooth muscle cells proliferated.

In the present study, the elastic lamellae in tunica media were disrupted, thinned and ill- defined and the collagen deposition was relatively increased in the tunica intima, media and adventitia. Similarly, Groenink et al. (1999) noticed that in human the atherosclerotic degeneration in the thoracic descending aorta by age may result in fragmentation of the medial elastin network and repair with collagen. Collagen fibers are synthesized and secreted by smooth muscle cells (Ross and Pawlina, 2006). The breakdown of elastic fibers and

collagen replacement may lead to aortic dilatation and stiffness of arteries resulting in increased pulse pressure (Stevens and Lowe, 2004; Izzo and Mitchell, 2007). Moreover, the deposition of collagen substance may lead to reduced compliance that significantly contributes to the increase in systolic blood pressure in humans (Nicita-Mauro, et al. 2007).

The present work further revealed variable extent of separation of the elastic lamellae in the senile aorta. This separation may be attributed to the degeneration of elastic lamellae. In addition, Pereira et al. (2003) stated that in hypertension the media increased in thickness and the distance between each concentric elastic lamella increased due to enlargement of the intermingled smooth muscle cell layers. Sudoh et al. (2001) reported that smooth muscle proliferation followed by increase media thickness occurs as a result of impaired nitric oxide production from injured endothelial cells. Nitric oxide inhibits smooth muscle growth.

The present study demonstrated the presence of cellular infiltration in tunica adventitia of the senile aorta. The infiltration might follow the injury of medial elastic fibers (Groenink, et al. 1999).

Scanning electron microscopy of the aorta of senile rats clarified that the endothelial cells lost their regular pattern of arrangement and showed numerous ulcerations and surface blebs. Occasionally, the endothelial cells were desquamated. The structural changes in the rat endothelium and its disruption might reduce the contractile power of the arteries due to a defect in nitric oxide release from the damaged endothelial cells (Wong, et al. 2006).

In the present work, administration of estrogen to senile rats showed less affection in the elastic lamellae of tunica media than in the control senile group. However, collagen deposition and cellular infiltration could still be encountered. The protective effects of estrogen therapy could be due to the ability of estrogen to delay plaque formation (Seli, et al. 2007). Scanning electron microscopy revealed that endothelial cell lining was intact with parallel arrangement, yet it appeared relatively rough and showed few localized blebs. The intact endothelium is essential to enhance the ability of estrogen to prevent accumulation of cho-

lesterol in blood vessels in rabbits (Holm, et al. 1999). Li et al. (2008) stated that estrogen plays a dual role in the regulation of local dilator and constrictor mechanisms important in the control of normal cardiovascular homeostasis in female rats. Although, in the present study, estrogen proved to be beneficial in improvement the morphology of the aorta in senile rat, Koledova and Khalil (2007) claimed that estrogen replacement therapy could not exert a total anti- atherogenic protective effect due to changes in vascular specific estrogen receptors associated with aging. Instead, it might aggravate the pre- existing cardiovascular disease in postmenopausal women

Comparing estrogen therapy to the administration of combined estrogen and vitamin E in the present study clarified that this combination was more protective to the structure of the aorta in senile rats. The internal elastic lamina was almost continuous. Restoration of elastic lamellae in the tunica media was a characteristic finding. Scanning electron microscopy confirmed the less affection after combined estrogen and vitamin E administration. The luminal surface had regular longitudinal folds. The endothelial cells were elongated with no apparent signs of ulceration or desquamation. The improvement induced by this combination could be attributed to the decreased LDL susceptibility to aggregation and consequent reduction of the atherogenic changes. The potent antiatherogenic effect is attributed to its antioxidant properties (Carr, et al. 2000) as vitamin E protects against free radical peroxidation of the carrier protein (HDL and LDL) by trapping peroxy radicals (Dutta and Dutta, 2003). On the contrary, Suarna et al. (2006) stated that the protective effect of vitamin E supplements on experimental atherosclerosis occurs only in hyperlipidemic mice with severe vitamin E deficiency and independent of lipid oxidation in the vessel wall. Furthermore, alpha- tocopherol also reduces the release of inflammatory cytokines, and inhibits monocyte- endothelial cell adhesion (Jialal, et al. 2001). In addition, Islam et al. (1998) stated that alpha- tocopherol mediated inhibition of monocyte receptors and transcription factor which are essential for proper adhesion between monocytes and endothelial cells enhancing the process of atherogenesis. Also vitamin E reduces aortic macrophage infiltration (Gavrila, et al. 2005).

The present work further demonstrated that foam cells were markedly reduced following

combined estrogen and vitamin E administration. These findings are in accord with those of Devaraj et al. (1996) who added that alpha- tocopherol decreases the release of reactive oxygen species and inhibits the release of interleukin-1B from activated monocytes which promote cholesterol esterification in macrophages. So, vitamin E leads to the alteration of macrophage cholesterol accumulation and consequently reduces foam cell formation.

Upon comparing the ameliorating effects of combined estrogen and vitamin E to estrogen alone, the present work clarified that the combination caused almost complete restoration of elastic lamina in the tunica media unlike estrogen alone. Moreover, the endothelial cells were smooth with no signs of ulceration or surface blebs. Combined estrogen and vitamin E was also more effective in reducing collagen deposition.

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## تأثير هورمون الأستروجن مقارنة بتأثير هورمون الأستروجن مع فيتامين هـ علي تركيب الشريان الأورطي الصاعد في أنثي الفأر الأبيض المسن: دراسة باستخدام كل من المجهر الضوئي والمجهر الإلكتروني الماسح

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### ملخص البحث

كان هدف الدراسة توضيح التغيرات التركيبية في الشريان الأورطي الصاعد في الفئران المسنة ومقارنة تأثير إعطاء هورمون الأستروجن بالإضافة إلى فيتامين هـ بهورمون الأستروجن وحده علي تحسن هذه التغيرات وذلك باستخدام كلا من المجهر الضوئي والمجهر الإلكتروني الماسح.

استعملت في هذه الدراسة ثلاثون أنثي فأر أبيض تتراوح أعمارها بين 6-7 شهور وأخري مسنة يبلغ عمرها 22-23 شهرا حيث قسمت الفئران المسنة إلي ثلاث مجموعات فرعية : مجموعة ضابطة وأخري أعطيت 0.05 مليلتر فولون ( بنزوات استراديول) جرعة واحدة كل شهر وأعطيت المجموعة الثالثة هورمون الأستروجن مع فيتامين هـ بجرعة مقدارها 40 وحدة دولية يوميا لكل فأر عن طريق أنبوبة معدية. وقد تم تخدير الفئران بعد عشر أسابيع واستخراج العينات ثم حضرت لدراستها بكل من المجهر الضوئي والمجهر الإلكتروني الماسح.

وقد أظهرت النتائج أن الشريان الأورطي الصاعد في الفئران المسنة به تهتك في الطبقة الداخلية المطاطية مع وجود خلايا متعددة التجاوب في الغلاف الأوسط العضلي وتباعد في الطبقات المطاطية بها. كما لوحظ ترسب الألياف الكولاجينية في الأغلفة الثلاثة لجدار الشريان، بينما أظهر المجهر الإلكتروني الماسح فقدان الشكل المنتظم للبطانة مع وجود تقشر أو تساقط لهذه البطانة.

أما في حالة إعطاء هورمون الأستروجن، فقد ظهرت الطبقة الداخلية المطاطية متكاملة مع إستعادة شبه كاملة للألياف المطاطية في الغلاف الأوسط العضلي، بينما لا يزال هناك تغلغل خلوي في الغلاف الخارجي للشريان ، كما لوحظ وجود الكولاجين في كل من الغلاف الداخلي والأوسط. وأظهر الفحص بالمجهر الإلكتروني الماسح أن بطانة الشريان لاتزال خشنة مع وجود بعض البثرات.

أما بعد إعطاء هورمون الأستروجن مع فيتامين هـ فقد حدث تقارب في الطبقات المطاطية في الغلاف الأوسط العضلي للشريان فيما لا يزال ترسب الكولاجين مستمراً في بعض المواقع في هذا الغلاف. وأظهر المجهر الإلكتروني الماسح أن البطانة كانت ناعمة مع غياب التقشرات أو البثرات.