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A Brief Overview to Ageing-Related Organ Damage: A Light and Electron Microscopic Approach to Severeal Systems

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ABSTRACT

Ageing is thought to be a degenerative process caused by accumulated damage leading to cellular dysfunction, tissue, and organ failure, and eventually death. Although aging-related skin changes are considered the most important indicator of ageing, morphological and functional changes occur in all of the internal organs. Most of those changes are directly or indirectly associated with age-related decline of life quality. Histopathological features of the organs of old people are examined in postmortem tissues which are probably affected by diseases alongside ageing itself. Recent studies principally report the cellular changes obtained from rodents. The most commonly reported ageing-related ultrastructural changes are mitochondrial damage, lysosome and lipofuscin accumulation and dilatation or proliferation of endoplasmic reticulum. Here I summarize ageing-related changes in various organs such as skin, brain, heart, kidney, intestines etc. that have been revealed by light and electron microscopic examinations so far. Understanding ageing-related cell and tissue-based changes and related molecular mechanisms will contribute to the development of new strategies to prevent or eliminate age-related organ damage.

KEYWORDS: Ageing, cell, histology, microscopy, tissue damage

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INTRODUCTION

Ageing is thought to be a degenerative process caused by accumulated damage leading to cellular dysfunction, tissue, and organ failure, and eventually death. Some theories associate various factors with ageing and ageing rate, as changes of metabolic control (Lynch et al., 2015) and gene expression patterns (Shadyab and LaCroix, 2015) and production of high levels of reactive oxygen species (ROS) (Sergiev et al., 2015). Senescent cells proliferate in ageing, as a stress response primed by some changes such as telomeres shortening, DNA damage accumulation, abnormal oncogenes activities, metabolic alterations, and excessive reactive oxygen species (ROS) generation (Kuilman et al, 2010). Today it is known that there is a positive correlation between mitochondrial dysfunction and ageing. According to the mitochondrial free radical theory of ageing, ROS that are primarily derived from the mitochondria are undesirable toxic by-products of aerobic metabolism. ROS induce oxidative damage to various cellular macromolecules, including lipids, proteins, and nucleic acids, due to their high chemical reactivity, thus tissue damage occurs (Bratic and Larsson, 2013). One of the major prediction of the mitochondrial free radical theory of ageing is that oxidative stress shortens organisms' lifespan due to increased level of oxidants and their reactive metabolites, which are potentially damaging to macromolecules, thereby resulting in loss of tissue/organ functionality. Oxidative stress is suggested to be associated with age-related conditions and diseases such as sarcopenia, frailty, cardiovascular diseases, pulmonary diseases, kidney diseases, neurodegenerative disorders, diabetes, and cancer (Liguori, 2018).

Ageing-related organ damage is characterized by morphological and functional alterations caused by the accumulation of senescent cells. Although it is seen that the most affected organ by ageing is the skin, in fact, morphological and functional changes occur in all of the internal organs. Ageing-related histopathological changes in humans have been examined in postmortem tissues which are probably affected by various diseases. Here I have reviewed the ageing-related changes in various organs of mostly obtained from rodents that have been revealed by light and electron microscopic examinations so far.

1. Ageing-related skin changes

Age-related skin changes are considered the most important indicator of ageing. The skin of the aged individuals is wrinkled, and saggy, and pigmented age spots appear on the skin. Histologically, the epidermis and dermis are thinner with flattening of the dermal-epidermal junction (Montagna and Carlisle, 1979; Lovell et al., 1987; Esrefoglu et al., 2005). The number of fibroblasts and number and diameter of collagen fiber bundles decreases, and the ratio of type III collagen to type I collagen increases (Lovell et al., 1987; Langton et al, 2019; Smith, 1989). The number of elastic fibers, hair follicle, sweat and sebaceous glands (Langton, 2019; Smith, 1989), the papillae (Esrefoglu et al., 2005), and capillary loops are also reduced (Smith, 1989) **(Figure 1A-D).** Ultrastructure of age-related epidermal changes have not been extensively investigated. Nevertheless, loss of excepted organization and configuration of the epidermis because of the cytological atypia, nuclear irregularity, and heterochromatin condensation, tonofilament accumulation, mitochondrial edema and degeneration, and intraepithelial lymphocyte accumulation were reported. Swollen mitochondria containing dense granules or myelin figures were detected. Partial or total destruction of crests, increased or decreased translucence of the matrix were also reported (Esrefoglu et al., 2006). Not surprisingly, the mitochondria are the primary organelle affected during chronological and UV-induced skin ageing, the phenotypic manifestations of which are the direct consequence of mitochondrial dysfunction (Sreedhar et al., 2020). Esrefoglu et al. (Esrefoglu et al., 2006) detected many intraepithelial lymphocytes by electron microscopic investigation. Interestingly lymphocytes showed degeneration signs including nuclear irregularity and possession of swollen and/or degenerated mitochondria **(Figure 1 E-H).**

2. Ageing-related nervous system changes

Nervous system is highly vulnerable to the deleterious effects of age-related oxidative stress. A large body of research has consistently confirmed the implication of free radicals both in normal cerebral ageing and ageing-related pathologies (Esrefoglu et al., 2020). The high sensitivity to oxidative stress is probably because of the extended neuron surface, due to processes, increasing the possibility of lipoperoxidation, the oxygen metabolism is raised in cerebral areas and the glutathione content of the neurons is low (Christen, 2000). In nervous system, ageing-related volume change has been largely reported throughout years. Brain volume reductions increase from about 0.1–0.2 % per year at age 30–50 years to 0.3–0.5 % per year over the age of 70 years, in agreement with brain weight studies (Esiri, 2007). The volume and weight of the brain decline with age at a rate of about 5% per decade after the age of 40 years, the decline increasing with age over 70 years. The decrease in volume is relatively uniform in cerebral white matter but not grey matter. Changes in the gray matter of frontal and parietal cortex, and striatum are more evident. Conversely the occipital cortex is least affected (Mrak et al., 1997). The brain cortex and cerebellar cortex (especially granular layer) of pinealectomized aged animals was found to be thinner than that of young animals (Esrefoglu et al., 2010). The ventricular system expands to fill the space resultant of shrinkage of nerve tissue, partly the result of nerve cell loss (Esiri, 2007; Anderton, 2002). The leptomeninges thicken slightly and the subarachnoid space enlarges (Esiri, 2007).

As expected, the results in terms of ageing related morphological and microscopic changes are obtained from primarily rodents. Morphological criteria do not allow close application of animal models to ageing problems in the human. For instance, the lesions including brain shrinkage, neuronal loss, alterations in the outline of neurons, lipofuscin accumulation and corpora amylacea are interpreted as non-specifically correlated with ageing; their correlation with functional loss is low in humans. On the other hand, the lesions including senile plaques and amyloid deposits, neurofibrillary and granulovacuolar degeneration and Hirano bodies are interpreted as characteristic, not of ageing itself, but of a widespread pathological condition and are strongly correlated with e.g., dementia (Foncin, 1981).

It is traditionally believed that humans loose numerous neurons per day deal with ageing. Since ageing-related changes in terms of nerve cell number in humans has been investigated via postmortem studies, neuron loss might be resulted or increased by undiagnosed neurodegenerative diseases. A substantial loss of neurons varying in the range 10–60% was reported depending on the study and on the neuronal population examined. Some regions e.g., cranial nerve nuclei showed no loss of neurons with age (Esiri, 2007). Pakkenberg et al. (2003) found the total number of neurons to be less than 10% over the age range from 20 to 90 years, while the glial cell number of 36 billion glial cells was not significantly different from the 39 billion glial cells in the neocortex of young individuals. The total myelinated fiber length varied from 150,000 to 180,000 km in young individuals and showed a large reduction with ageing. Nevertheless, the reduction of the area covered by neurons together with their extensions presumably contributes ageing-related volume and weight loss. The major form of ageing-induced neuron death is believed to be apoptosis. Ageing is under strict genetic control and therefore neuron death is truly programmed cell death. It is possible that a programmed mechanism becomes activated with ageing that triggers cell loss. However, senescent ageing may largely be due to “wear and tear” mechanisms (Pollack and Leeuwenburgh, 2001). Various studies have reported the coherence between increased DNA damage such as apoptotic DNA damage in nerve cells and ageing (Esrefoglu et al., 2010; Shimada et al. 2002; Isaev et al., 2018). Angleda et al. (1997) found morphological characteristics of apoptosis, such as cell shrinkage and chromatin condensation in approximately 2% neurons in human substantia nigra during normal ageing.

Esrefoglu et al. (2010) reported ageing-related increased number of TUNEL positive cells in both cerebral and cerebellar cortex. The results of a human study of Renovell et al. (1996) showed that in the human ageing a notable loss exists in quantitative (38%) as well as qualitative (cells are smaller) of the granule cells of cerebellar cortex.

It is well known that ageing is associated with widespread structural changes in both neurons and glia cells. Cytoplasmic edema, mitochondrial degeneration (edema, cristae loss, increased or decreased density of mitochondrial matrix, myelin figure formation, fusion, presence of megamitochondria etc.), dilatation of endoplasmic reticulum, fragmentation of Golgi apparatus, lysosome, and lipofuscin accumulation (Esrefoglu et al., 2020; Ridwan et al., 2010; García et al., 2013; Mann et al., 1978) have been reported **(Figure 2 A-D).** It has been reported that slowly developing apoptotic form of neuronal injury is associated with substantial increases in the number of mature lysosomes (Adamec et al., 2000). Some other age-related changes include nerve fiber loss from some fiber tracts, degeneration of myelin sheaths, and the loss of synapses and dendritic spines from upper layers of prefrontal cortex (Peters and Kemper, 2010; Burke and Barnes, 2006). The dendritic regression and spine loss may probably underlie the first signs of cognitive decline in learning and memory performance noted in normal ageing (Burke and Barnes, 2006). The size of astrocytes and microglia were found to be increased. Both cells were found in association with senile plaques. Additionally, thorn shaped, tau positive astrocytes were increased especially by the eight decades in subpial and subependymal zones (Schultz et al., 2004). Stereological cell counting study of Pelvig et al. (2008) applied to post-mortem neocortices of human brains showed that the different subpopulations of glial cells behave differently as a function of age; the number of oligodendrocytes showed a significant 27% decrease over adult life while the total astrocyte number is constant through life.

3. Ageing-related urinary system changes

Like the other organ systems, urinary system also suffers from ageing characterized by anatomical, microscopic, and functional changes. Ageing related changes include decreased glomerular filtration rate, changes in permeability of the glomerular capillary wall, changes in tubular reabsorption and secretory capacities, changes in urinary concentration, and production of kidney-derived hormones and molecules, and increased apoptosis (Wiggins et al., 2005; Esposito and Canton, 2005; Wiggins, 2012). Ageing is associated with glomerular and tubular damage. Glomerular damage is represented by sclerotic changes such as matrix expansion, narrowing or disappearance of the Bowman space, capillary collapse, and thickening of glomerular basement membrane (Parlakpinar et al., 2007; Esrefoglu et al., 2012). Glomerulosclerosis, associated with ageing which is identified in autopsy studies is present in more than 70% of people aged 40 years and older, with increasing prevalence and percentage of glomeruli involved by glomerulosclerosis with age (Kaplan et al., 1975). The density of non-sclerotic glomeruli decreases by 7% per decade, while the density of focal or global sclerotic glomeruli increases by 14% per decade (Kremers et al., 2015). These microscopic findings suggest that the progressive loss of nephrons results in decline in glomerular filtration rate due to ageing. A possible explanation for the progressive increase in sclerotic glomeruli with age is glomerular ischemia secondary to the changes in renal blood flow that occur with age (Dontas et al., 1972). Tubular damage is represented by tubular dilatation and tubular atrophy, epithelial degeneration, vacuolization, and tubular cast formation including thyroidization. Interstitial cell infiltration, interstitial fibrosis and congestion are also common (Parlakpinar et al., 2007; Esrefoglu et al., 2012) **(Figure 3 A-D).** Tubular atrophy, with thickening of the basement membrane, is a common feature of parenchymal change, and tubular "thyroidization," with dilatation of the lumen, flattening of tubular epithelium, and accumulation of eosinophilic hyaline cast material within the tubule is also a common feature of end-stage renal tubular damage (Lindeman and Goldman, 1986). By electron microscopy, the most prominent ageing-induced alterations are edema, massive vacuole formation, mitochondrial degenerative changes, such as edema, vacuole formation, cristae loss, or thickening of cristae membranes, lysosome and lipofuscin accumulation within the tubular cells, and thickening of the tubular basement membranes. Irregularity of tubular cells and microvillus loss and disorganization are also common. Rarely, tubular cell necrosis occurs (Esrefoglu et al., 2012) **(Figure 3 E-H).** Mitochondrial degeneration has been related to an increased generation of superoxide anion and hydrogen peroxide and a decline in the capacity for energy production (Boveris et al., 1999).

4. Ageing-related cardiovascular system changes

Cardiovascular system is one of the most affected systems while ageing. Ageing is a well-recognized risk factor in the development of cardiovascular diseases, which are the primary cause of death and disability in the elderly population (Sung and Dyck, 2012). Increased arterial stiffness, elevated systolic pressure, lower heart rate and cardiac output, left ventricular hypertrophy, increased calcification of aortic and mitral valves, and massive myocyte loss converge toward deterioration of heart function (Venkataraman et al., 2013). Progressive cardiomyocyte hypertrophy, inflammation, and the gradual development of cardiac fibrosis are hallmarks of cardiac ageing. In the absence of a secondary insult such as hypertension, these changes are subtle and result in slight to moderate impaired myocardial function, particularly diastolic function (Meschiari et al., 2017). The increased senescence of cardiomyocytes centrally contributes to cardiac ageing, dysfunction, and failure. In aged or injured hearts, the senescent cardiomyocytes exhibit the hallmarks of DNA

damage, endoplasmic reticulum stress, mitochondria dysfunction, contractile dysfunction, hypertrophic growth, and senescence-associated secreting phenotype (Tang et al., 2020). As usual increased oxidative stress is essential in ageing-related myocardial changes. Hydroxyl radicals have damaging effect on the protein structure in cardiac mitochondria, myofibrils (Babusikova et al., 2008; Kaplan et al., 2003), and sarcoplasmic reticulum (Cebe et al., 2014) in adult rats. Various studies have shown that increased oxidative stress results in various alterations in both cardiac tissue and vessel wall (Babusikova et al., 2008; Cebe et al., 2014; Esrefoglu et al., 2011). Damage in proteins and lipids of cardiac sarcoplasmic reticulum increases during in vitro-generated oxidative stress in senescent rats. It has been suggested that the accumulation of oxidant-induced damage in interfibrillar mitochondria may be a major contributing factor to the age-related alterations in myocardial function (Judge et al., 2005). In senescent cardiomyocytes, the fission–fusion progress of mitochondria is imbalanced, and the function is declined (Nishimura et al., 2018). P53 inhibits Parkin-mediated mitophagy and promotes mitochondrial dysfunction to facilitate cellular senescence (Hoshino et al., 2013).

By light microscopic examination Cebe et al. (2014) detected some histopathological alterations including vacuolization, edema, myofilament loss, necrosis, and fibrosis in the cardiac samples obtained from chronologically and mimetically aged rats. By electron microscopic examination, Esrefoglu et al. (2011) detected prominent cellular alterations in cardiac myocytes such as nuclear irregularity, mitochondrial degeneration, myofilament disorganization and disruption, and lipofuscin accumulation in the sections obtained from aged rats. Mitochondrial swelling, cristae loss, myelin figure and vacuole formation, and a mottled matrix were particular in terms of mitochondrial alterations **(Figure 4 A-D).** Other cellular ultrastructural changes were interstitial edema and the appearance of intracellular vacuoles. Unverferth et al. (1986) reported ageing-related increase in cardiomyocyte size and some degenerative changes including lipid and lipofuscin deposition and tubular dilatation in the human myocardium upon endomyocardial biopsies. The most prominent ageing-related vascular changes at the aorta are elastic fiber loss (reduction and fragmentation) (Unverferth et al., 1986; Esrefoglu et al., 2011), irregularity in endothelial cells and their nuclei, divergence of endothelial cells from basement membrane area, basement membrane loss, peripheral heterochromatin condensation and mitochondrial degeneration **(Figure 4 E-H).** Increased expression of matrix metalloproteases, (MMP-2, MMP-1, MMP-9), as well as the decreased expression of tissue inhibitors of matrix metalloproteases contribute to the fragmentation of elastic fibers in ageing aorta (Tamarina et al., 1997).

5. Ageing-related digestive system changes

Ageing-related damage in digestive system involves the enteric nervous system, gastrointestinal motility, small intestinal permeability, mucosal defense system, and the gut-associated lymphoid tissue. Thus, various changes occur during aging in terms of absorption, digestion, appetite and postprandial blood pressure regulation and protection against ingested pathogens (Stijn et al., 2016). Some of the ageing-related changes are responsible for a variety of symptoms including alterations in taste and smell, in gastric motility which in turn cause anorexia, in swallowing which in turn cause aspiration, in gastric emptying which in turn cause postprandial hypotension, constipation, diarrhea, malabsorption, and delayed drug metabolism etc. (Bhutto and Morley, 2008). Altered gastric microbiota, reduced mucosal protective mechanisms, decreased gastric blood flow, and consequently compromised repair mechanisms are the hallmarks of age-related gastric changes (Newton, 2005; Pearson et al., 2017). These changes make older people more susceptible to the development of several diseases, such as gastric ulcer, atrophic gastritis, and peptic ulcer disease (Pearson et al., 2017). Previous studies showed that ageing gastric mucosa has impaired mucosal defense including reduced mucus and bicarbonate secretion, decreased prostaglandin generation, reduced nitric oxide synthase (NOS) activity; and impaired sensory nerve responses to luminal acid (Lee and Feldman, 1994; Grønbech and Lacy, 1995; Newton et al., 2000; Vogiagis et al., 2000). Tarnawaski et al. (2007) demonstrated partial atrophy of gastric glands and their replacement with increased connective tissue in the basal one third of the mucosa. The connective tissue in the lower one third of the gastric mucosa was significantly widened replacing glandular cells in ageing rats. Significantly increased apoptosis rate prominently involving epithelial cells at the basal mucosa explaining atrophy of the basal gastric glands was also detected. Farinati et al. (1993) demonstrate that the number of parietal cells tends to increase with age, and on the other hand, the number of mucous cells is reduced in elderly subjects undergoing endoscopy.

Electron microscopy demonstrated degenerative changes in parietal and chief cells, hyperplasia of surface and foveolar mucous cells, and prominent accumulation of disorganized collagen fibrils in perivascular connective tissue (Hollander et al; 1989). We detected epithelial degeneration, squamous metaplasia, mucus reduction and dilatation of gastric glands in the stomach of aged Sprague-Dawley rats (unpublished data) **(Figure 5 A, B).** Most prominent electron microscopic changes were edema, endoplasmic reticulum dilatation, mitochondrial degeneration (edema, cristae loss and myelin figure formation etc.), and lysosome accumulation in the cytoplasm of the chief cells and mitochondrial degeneration and lysosome accumulation in the cytoplasm of the parietal cells (unpublished data) **(Figure 5 C, D).** The study of Zhang et al. (2013) illustrated that healthy aged individuals did have an intact parietal cell

ultrastructure and the molecular biological basis for the maintenance of the function of gastric acid secretion.

Some animal studies demonstrate age-related changes in small intestinal morphology such as increase in villous width and height and reduction in mucosal surface area whereas human studies did not find such changes (Lipski et al., 1992). Holt et al. (1984) detected increases in villous width throughout the small intestine, while increases in villous height were limited to the ileum of Fischer 344 rats. Martin et al. (1998) described histological changes such as large villi, reduced number of crypts, and decreased number of villi and crypts per mm along the murine small intestine. These changes were most pronounced in the distal, as opposed to the proximal small intestine. McGee et al. (2011) detected irregularity of intestinal lumen, degradation and later loss of microvilli, and significant loss of intestinal nuclei probably a clue of increased apoptosis rate in ageing *C. elegans*. We have not detected any cellular alterations in the intestines of aged Sprague-Dawley rats by light microscopic examination (unpublished data). However, by electron microscopic examination, edema, vacuolization, peripheral heterochromatin and cytoplasmic matrix condensation, microvillus disorganization and loss, mitochondrial degeneration (edema, cristae loss and myelin figure formation etc.), (**Figure 5 E, F**), lysosome and autophagosome accumulation were detected Additionally, proliferation of Golgi apparatus was obvious (unpublished data). Clinical manifestations of intestinal disorders, such as mucosal barrier dysfunction, intestinal dysmotility, and chronic obstipation, are highly prevalent in the elderly population and have been shown to be associated with an age-dependent decline of mitochondrial function (Schneider et al., 2020).

Ageing has an impact on liver morphology and function as well. Oxidative stress and inflammation are now widely accepted as the main mechanisms involved in the ageing process that may subsequently cause severe injury to mitochondrial DNA which leads to apoptosis (Azman et al., 2021). Ageing causes some morphological and physiological alterations, such as a decrease in liver volume and liver blood flow (Wynne, 1989). Ultrastructural analysis of the human liver has revealed that the integrity of mitochondria and enzymatic activity remain mostly unchanged with ageing (Anantharaju et al., 2002). The ageing liver can still maintain relatively normal metabolic functions under physiological conditions (. Pibiri, 2018). However, the regenerative capacity of the ageing liver is significantly reduced compared with the young liver. Increased numbers of damaged mitochondria, lysosomes, and lipid depositions were observed in the hepatocytes of elderly mice (Łysek-Gładysińska et al., 2021). Maeso-Diaz et al. (2018) detected differences between young and aged rat groups in all of the evaluated parameters including cytoplasmic vacuolation, nuclear pyknosis, cytoplasmic hyper-eosinophilia, loss of intercellular borders, necrosis, neutrophil infiltration, and fat accumulation. In addition, aged rats showed higher hepatic cell death as demonstrated by the TUNEL staining, with no significant changes in apoptotic proteins c-caspase-3. We recently detected minimal changes in aged Sprague-Dawley rats including condensation of mitochondrial matrix, rough endoplasmic reticulum proliferation, glycogen (**Figure 5 G, H**), lysosome, and lipid accumulation (unpublished data).

CONCLUSION

Ageing-related changes such as increased oxidative stress, telomer shortening, lipid peroxidation, protein damage, mitochondrial damage etc. might be effective for emerging morphological and eventually physiological changes. Aged tissues are characterized by morphological and functional alterations caused by the accumulation of senescent cells. Although it is seen that the most affected organ by ageing is the skin, in fact morphological and functional changes occur in all of the internal organs. Here I tried to review the histopathological changes represented by light and electron microscopic data in the skin, brain, cerebellum, kidney, heart, aorta, stomach, intestine, and liver of aged Sprague-Dawley rats. The most common changes are mitochondrial degeneration and lysosome and lipofuscin accumulation. Accumulating oxidative damage are expected to affect mitochondrial ultrastructure, which should be visible by electron microscopy. The formation of lipofuscin is mainly due to the iron-catalyzed oxidative damage of macromolecules. It is generally accumulating in lysosomes of most cells with age. I summarize the results of recent studies in terms of histology and pathology. Understanding ageing-related cell and tissue-based changes and related molecular mechanisms will contribute to the development of new strategies to prevent or eliminate age-related system changes.

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FIGURE LEGENDS

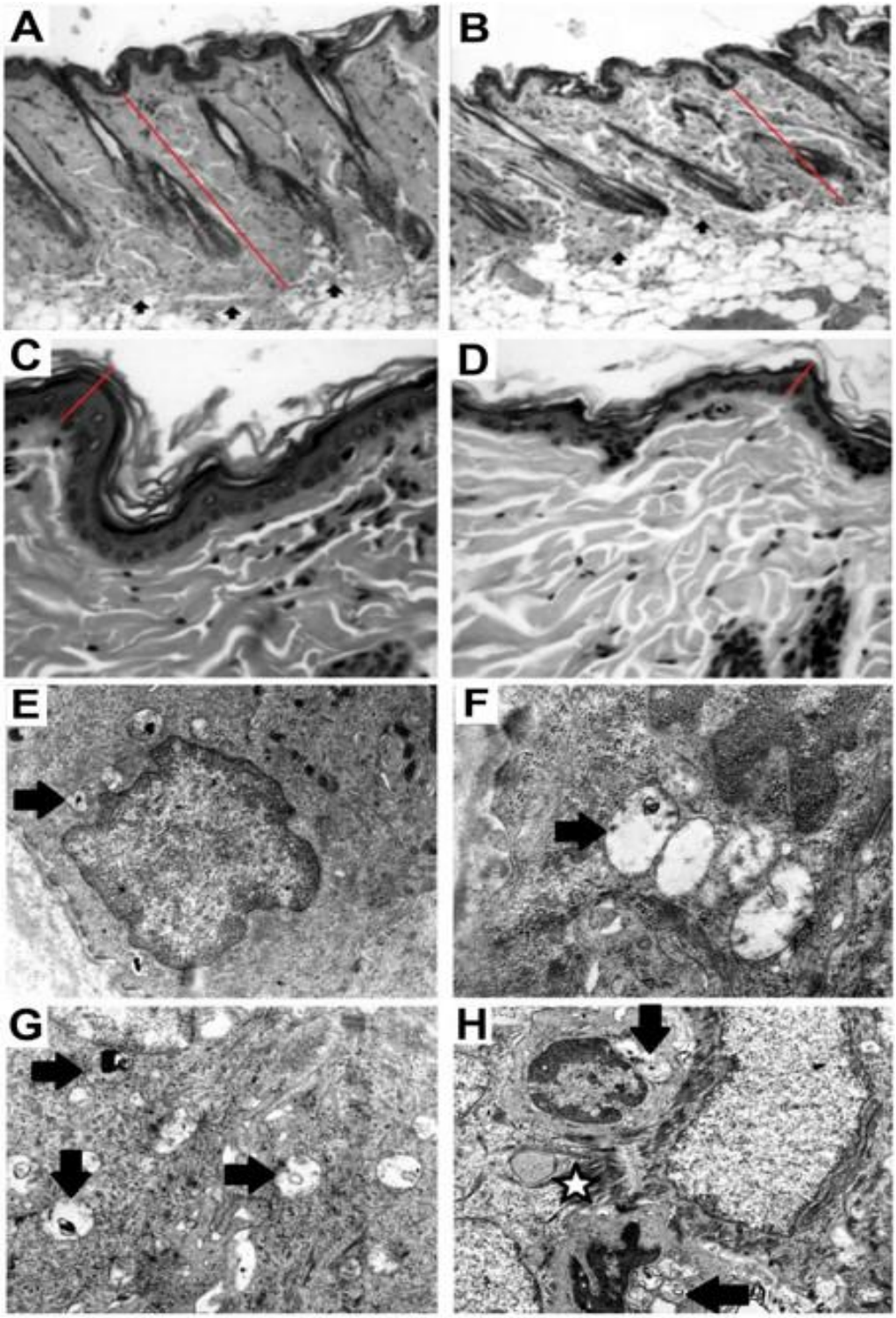

Figure 1. Light (A-D) and electron microscopic (F-H) changes in the skin of pinealectomy-induced aged rats. Decrease in the thickness of epidermis and dermis is obvious. Mitochondrial degeneration including edema, cristae loss and myelin figure formation are observed. **A, B:** Overall thickness of dermis is marked by red line in the skin samples of young and aged rats respectively. The bottom of dermis is marked by arrows. **C, D**: Overall thickness of epidermis is marked by red line in the skin samples of young and aged rats respectively. Hematoxylin and eosin; X 10 (upper pictures), X 40 (bottom pictures); respectively. **E:** Crista loss and myelin figure formation (arrow) are observed. X 6000; **F:** Mitochondrial edema and cristae loss are obvious. One of the edematous mitochondria containing myelin figure is marked by arrow. X 10.000; **G:** Degenerated mitochondria are marked by arrows (edema, cristae loss and formation of myelin figures). X 8.000; **H:** Intraepithelial lymphocytes containing degenerated mitochondria as well are marked by arrows. Tonofilament accumulation is marked by asterisk.

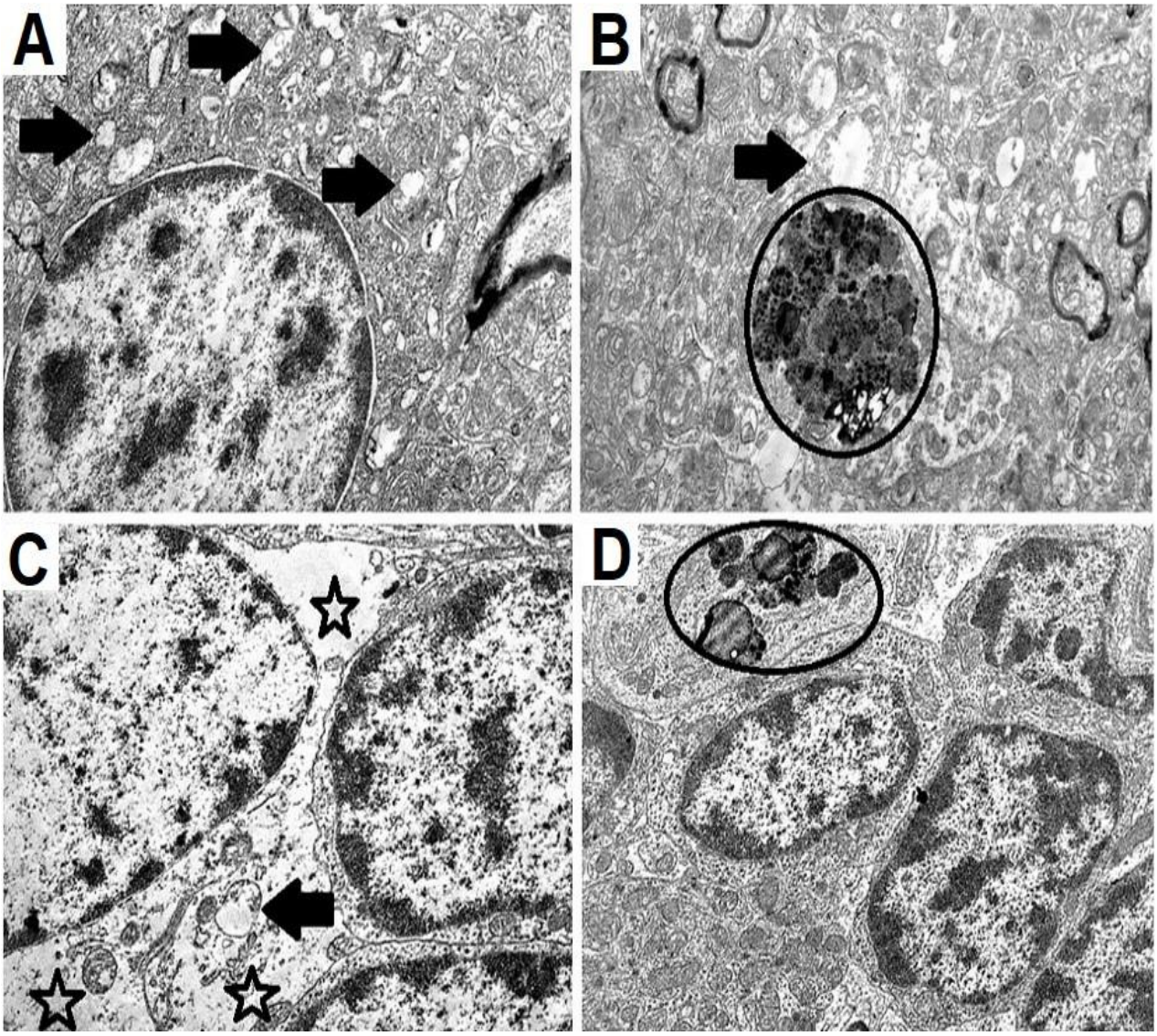

Figure 2. Ultrastructural changes in the brain (A, B) and cerebellum (C, D) of pinealectomy-induced aged rats. Mitochondrial changes including edema and cristae loss, lipofuscin accumulation and cytoplasmic edema are obvious. **A**: A few of the degenerated mitochondria are marked. X 12.500; **B**: A few of the degenerated mitochondria are marked by arrow; lipofuscin accumulation is encircled. X 10.000; **C**: Cytoplasmic edema is marked by asterisks, one of the degenerated (edema, cristae loss) mitochondria is marked by arrow. X 10.000; **D**: Lipofuscin accumulation is encircled. X 8.000.

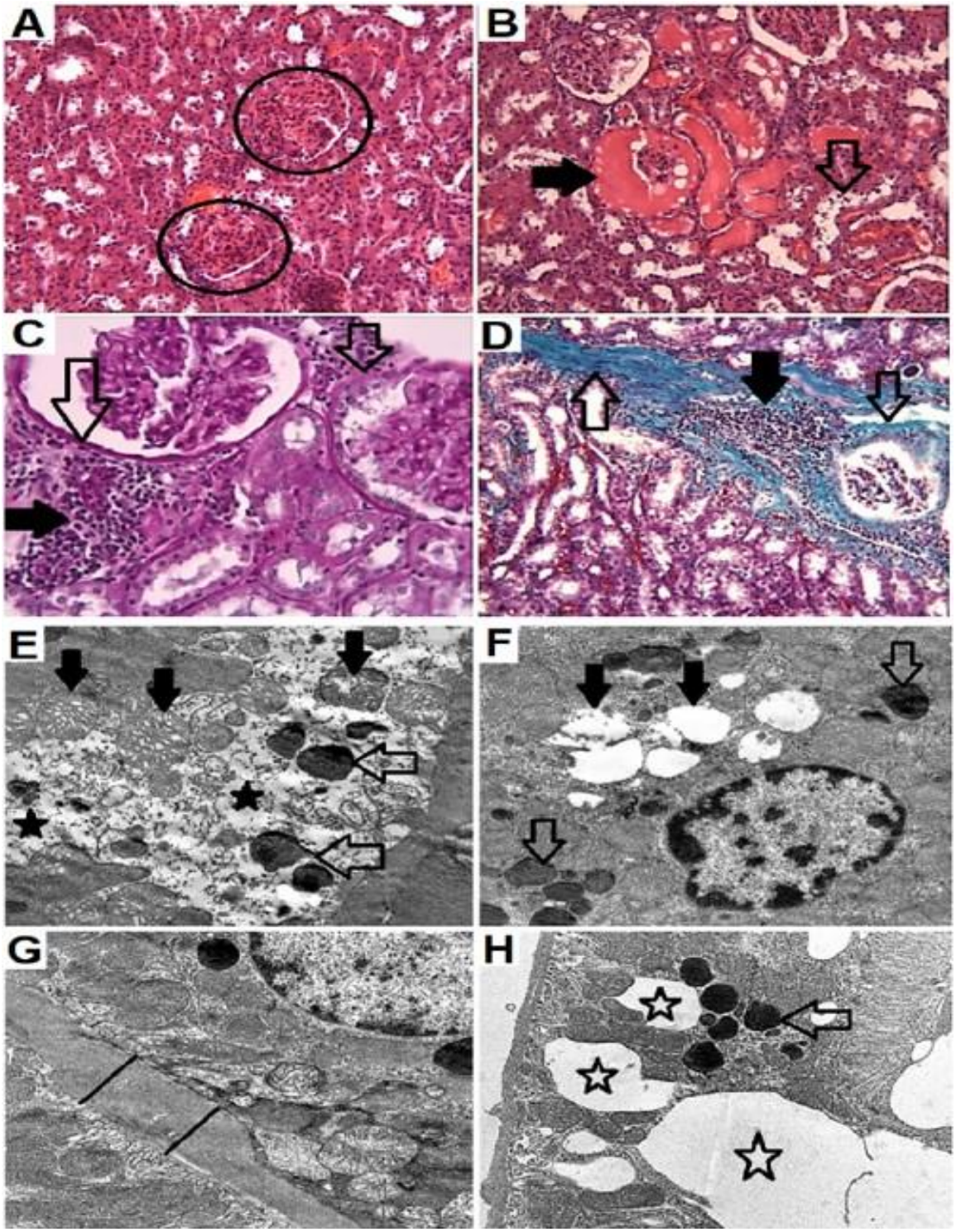

Figure 3. Light (A-D) and electron microscopic (F-G) changes in the kidneys of chronologically aged rats. Sclerosis, epithelial degeneration, mitochondrial changes (edema, crista loss, or thickening of the cristae etc.), intracellular edema (decreased cytoplasmic matrix density), lysosome and lipofuscin accumulation, and basement membrane thickening are obvious. **A:** Sclerotic glomeruli are encircled (narrowing, or disappearances of the Bowman's space, adhesion of capillary tuft to the Bowman's capsule are obvious). Hematoxylin and eosin; X 20; **B**: Tubular epithelial degeneration (clear arrow) and thyroidization (arrows) are obvious. Hematoxylin and eosin; X 20; **C:** Thickening of the basal membrane of Bowman's capsule (clear arrows) and interstitial cell infiltration (arrow) are observed. PAS; X 40. **D:** Periglomerular and glomerular collagen deposition (clear arrows) and cell infiltration (arrow) are obvious. Tubular epithelial degeneration is also observed. Masson's trichrome method; 20.
E. A few of the degenerated mitochondria (thickening of cristae) are marked by arrows; lysosome accumulation is marked by clear arrows. Cytoplasmic edema is also obvious (asterisks). X 10.000; **F:** A few of the degenerated mitochondria (edema and complete loss of cristae) are marked by arrows; lysosome and lipofuscin accumulation are marked by arrows. X 10.000; G**:** Thickening of tubular basement membrane is obvious. Note the lysosomes in the cytoplasm. X 10.000; H**:** Cytoplasmic edema (vacuole formation) (asterisks) and lysosome accumulation are marked (clear arrows). X 8.000.

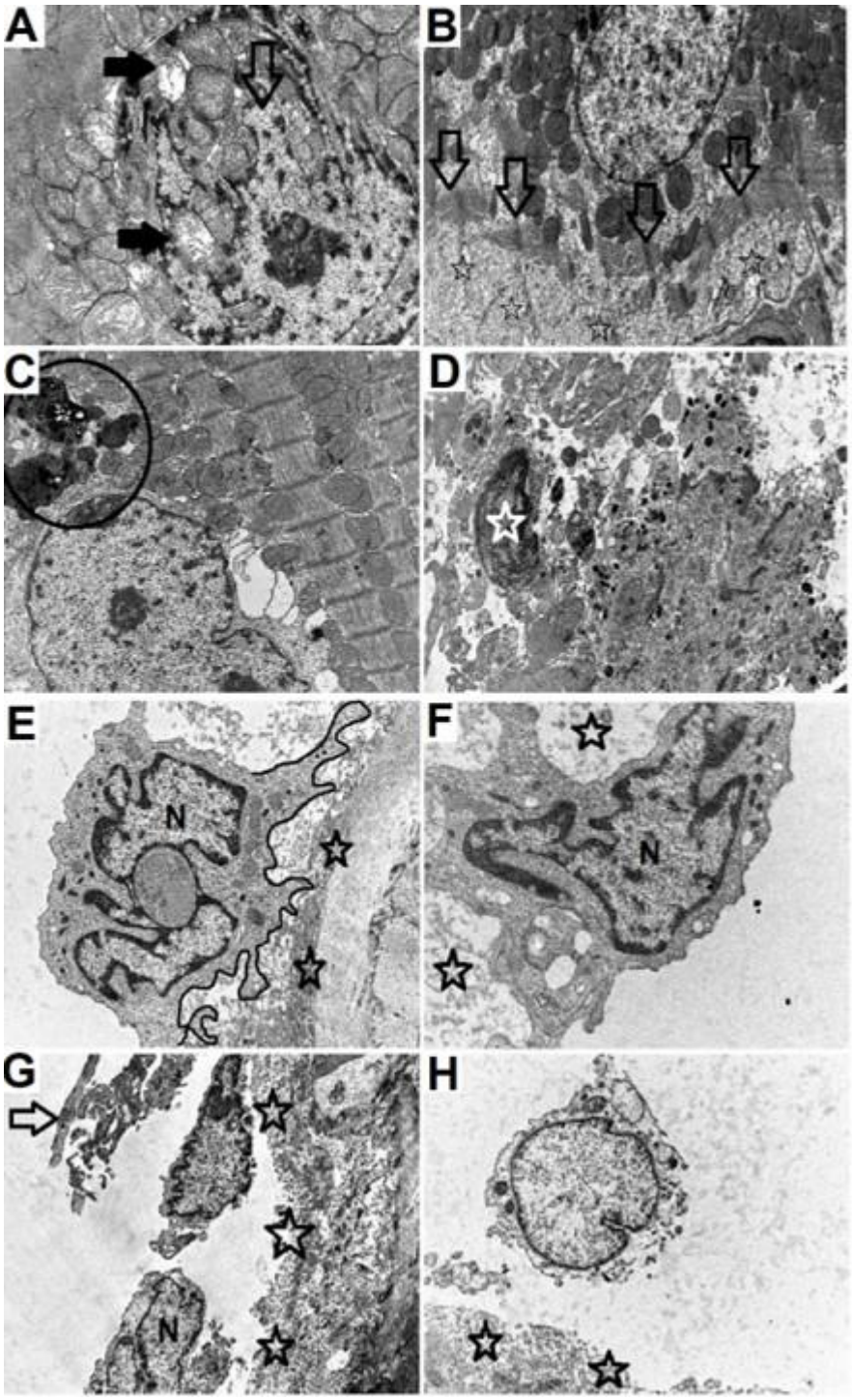

Figure 4. Ultrastructural changes in the heart (A-D) and aorta (E-H) of chronologically aged rats. Mitochondrial changes (edema and cristae loss etc.), nuclear irregularity, myofilament disorganization, lipofuscin accumulation, necrosis, endothelial changes such as cellular irregularity, pseudopod formation, nuclear irregularity and basement membrane fragmentation are obvious. **A:** Severe nuclear irregularity is marked by clear arrow; a few of degenerated mitochondria (edema and cristae loss) are marked by arrows. X 12.500; **B:** Myofilament disorganization is obvious (arrows). Cytoplasm that is devoid of myofilaments is marked by asterisks. X 8.000; **C:** Lipofuscin accumulation is encircled. Note the large vacuoles next to the nucleus. X 8.000; **D:** Necrosis is observed. Degenerated organelles are spilled into intercellular area. Pyknotic nucleus is marked by asterisk. X 6.300; **E:** Cellular and nuclear (N) irregularity is obvious at the endothelial cell of the aorta. Basal plasma membrane is lined in order to emphasize the formation of pseudopods. Basement membrane is degenerated (asterisks). X 12.500; **F:** Cellular and nuclear (N) irregularity is obvious at the endothelial cell of the aorta. Abnormal gap at the base of the endothelial cell is marked by asterisks. X 16.000; **G:** Endothelial and basement membrane degeneration (asterisks) are obvious. As a result of necrosis, cellular fragments are observed at the lumen (arrow). X 6.800; **H:** Basement membrane degeneration (asterisks) is obvious. A cell undergoing necrosis is observed at the lumen. X 8.000.

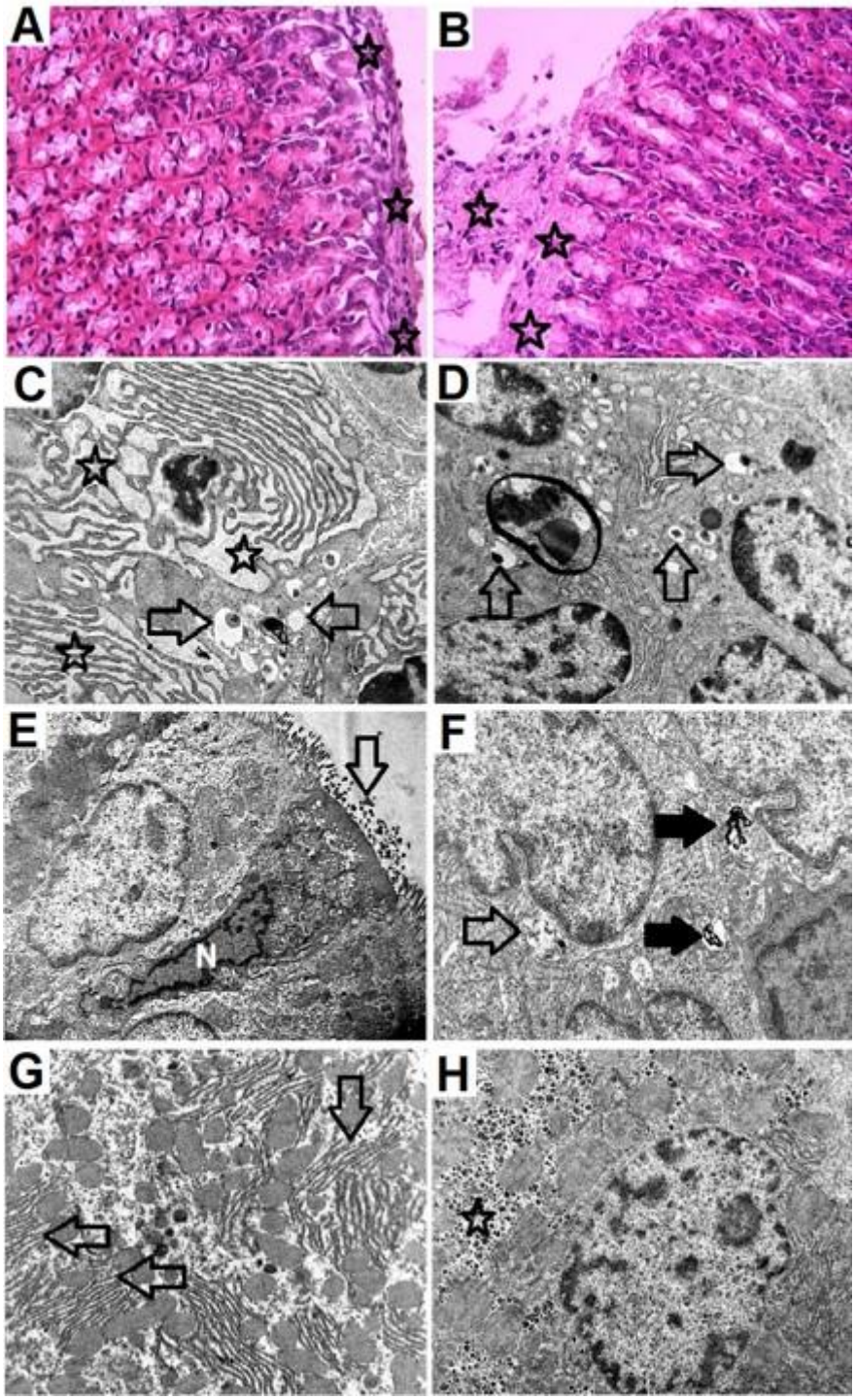

Figure 5. Pinealectomy-induced ageing-related changes in stomach (A-D), small intestines (E, F), and liver (G, H). **A:** Squamous metaplasia is obvious (asterisks). Hematoxylin-eosin; X 20; **B:** Degeneration (necrosis) at the apical part of the mucosa is obvious (asterisks). Hematoxylin-eosin; X 40; **C:** Nuclear pyknosis, dilatation of rough endoplasmic reticulum (asterisks), and mitochondrial degeneration (arrows) (cristae loss, formation of inclusions at the matrix) are observed in a gastric glandular chief cell. X 8.000; **D:** Mitochondrial degeneration (cristae loss, formation of inclusions at the matrix) is marked by arrows and lipofuscin accumulation is encircled in a gastric glandular parietal cell. X 8.000; **E:** Nuclear pyknosis and condensation, cytoplasmic matrix condensation, microvillus fragmentation and loss (arrow) at the enterocytes of the epithelium of the small intestines are prominent. X 5.000. **F:** Degenerated mitochondria representing edema, cristae loss (clear arrow) and myelin figure formation (arrows) are marked. X. 12.500; **G:** A few groups of proliferated rough endoplasmic reticulum sacs are marked in the cytoplasm of a hepatocyte. X 7.000; **H:** Glycogen accumulation (asterisk) is observed in the cytoplasm of the hepatocyte. X 6.730.