Original Article

Effect of Aging on the Histological Structure of the Colon of Male Albino Rat: Light and Electron Microscopic Study

Dina Mohamed Rashad, Safaa Mohammed Shaker, Nevine Bahaa and Faika Hassan EL Ebiary

Department of Histology, Faculty of Medicine, Ain Shams University, Egypt

ABSTRACT

Introduction: Aging is the impelling cause behind age related diseases. The colon, as a part of the gastrointestinal tract, is impacted by aging. Elders frequently complain of constipation, fecal incontinence and fecal impaction, that affects their life and causes morbidity.

Aim: To examine aging impact on the histological structure of the colon wall in male albino rats.

Materials and Methods: A total of thirty male albino rats were equally split into three groups: young, adult and senile, rats aged six weeks, 16 weeks and two years respectively. Colon specimens, one cm over the ileocecal valve, of all rats were collected, processed then examined by light and transmission electron microscopes. Hematoxylin and eosin, toluidine blue, alcian blue-PAS technique and Masson's trichrome were used to stain the sections then morphometrical measures and statistical analysis were carried.

Results: The layers of the colon were affected by aging on the microscopical level. Localized mucosal and epithelial interruption, mononuclear cellular invasion and goblet cells' mucous load change were revealed in senile rats. Collagen expansion in all layers was also depicted. Significant decline of myenteric plexus neurons' Nissls' granules and goblet cells' number were recognized. Colonocytes of aged rats displayed deformed nuclei, localized microvilli loss, vacuoles within cytoplasm and mitochondrial swelling with deformed cristae.

Conclusions: It was concluded that aging caused profound structural and ultrastructural changes in senile rats' colon. The changes in turn are thought to alter colonic function, which might contribute to aging associated diseases.

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Key Words: Aging, colon, rat, transmission electron microscope.

Corresponding Author: Dina Mohamed Rashad, MD, Department of Histology, Faculty of Medicine, Ain Shams University, Egypt, **Tel.**: +20 12 0442 5279, **E-mail:** dina.mohammad@med.asu.edu.eg

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INTRODUCTION

Aging is believed to be a predisposing factor for the majority of human disease. As people age, their body's capacity to manage stress and achieve homeostatic equilibrium declines. According to some researchers, ageing is a disease[1]. The aging process induces the impairment of organ functions because of the built up of various harmful changes in cells and tissues[2,3]. These changes are echoed as increased frequency of age-associated disorders, as cancer, cardiovascular disease and neurodegeneration[4,5].

Similar to all organs, gastrointestinal tract (GIT) does not escape the adverse effects of aging. The consequences of aging on the GIT might negatively affect general wellbeing and even cause morbidity in older people[6,7]. Aging induces functional and morphological changes in the GIT[8]. Difficult swallowing, slow gastric emptying and slow intestinal and colonic transit time were formerly described in elderly[9].

Unfortunately, there are no sufficient data describing the aging impact on the histological makeup of the colon. Therefore, the aim of this study was to examine aging impact on the histological structure of the colon in male albino rats.

MATERIALS AND METHODS

Materials

Experimental Rats

A total number of 30 male albino Wistar rats of different age groups; six weeks, 16 weeks and two years[10] were included in the study. The rats were acquired and maintained at MASRI, Faculty of Medicine, Ain Shams University (FM-ASU) with food and water adlibitum. The rats were split into three groups.

Group I (Young): accommodated 10 male albino rats aged six weeks (weighing 100-130 grams).

Group II (Adult): accommodated 10 male albino rats aged 16 weeks (four months) (weighing 180-200 grams).

Group III (Senile): accommodated 10 male albino rats aged two years (weighing 250-300 grams).

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Methods

Sample Collection

All rats were given one week for acclimatization, and then for the last twelve hours before scarification, they were only allowed to drink water. The animals were knocked out by ether inhalation, then scarified. A one cm of proximal colon (one cm distal to the ileocecal valve) per rat was obtained and flushed with saline[11]. The specimens were then fixed in proper fixatives, neutral buffered formaldehyde and 2.5% formol glutaraldehyde, and processed for examination by the light microscope and the transmission electron microscope respectively. Morphometry and statistics were then applied to study light microscopic sections.

Bodies of dead rats were discarded using the incinerator at Ain Shams University Hospitals. All rat practices were done following the Laboratory Animals Guide. The ethical approval (FMASU M D 355/2018) was granted by the local ethical committee at Ain Shams university (FMSU REC).

Light Microscopic Studies

The Colon specimens were submerged in neutral buffered formaldehyde for 5 days. Specimens were then passed through ascending alcohol and xylene then set in paraffin. Sequential 5µm thick sections were cropped. Sections were stained by hematoxylin and eosin (H&E), toluidine blue for Nissl's granules detection, alcian blue-periodic acid Schiff (PAS) technique to identify different types of mucins, and Masson's trichrome to distinguish collagen fibers[11].

Electron Microscopic Study

The colon specimens were sliced into 1mm^3 sections and were submerged in 2.5% formol glutaraldehyde. Sections were treated to be researched and photographed by TEM (JEM 1200 EXII) at "Center of Mycology and Biotechnology", AL- Azhar University, Cairo, Egypt[11].

Morphometric studies of the Colon

Obtained slides from all groups were assessed by morphometry. Measurements were collected from 5 slides for each rat. In each slide 5 non-intersecting random fields were assessed.

Morphometry was carried by Leica image analyzer program and microscope at Histology Department, FM-ASU.

The following parameters were measured:

- Mean crypt length and mean thickness of the colonic wall (H&E, X40).
- Mean optical density of Nissl's granules in neurons of the myenteric plexus (Toluidine blue, X40)
- Mean number of the goblet cells (PAS-Alcian blue, X40).
- Mean area percentage of the collagen fibers content (Masson's trichrome, X40).

Statistical analysis

All the obtained morphometrical data were statistically analyzed. IBM SPSS statistics program version 21 was used to calculate mean value and standard deviation (SD) of measurements obtained; from 5 fields/slide for 5 slides/rat. Means were compared by ANOVA with post-hoc test. Values were displayed in this study as mean ± SD. The significance was established by probability of chance (*P- value*) as $p<0.05$ was count significant and $p>0.05$ was count non-significant.

RESULTS

Light microscopic and Statistical results

Microscopic assessment of H&E-stained colon section of rats of all groups showed its layers: mucosa, submucosa, muscularis externa. The overall wall thickness was significantly higher in adult and senile rats compared to young rats, with non-significant difference between adult and senile rats. Crypts of Lieberkühn were seen extending through the mucosal thickness and were significantly increased in length in senile rats as compared to that of young and adult rats (Figures 1 a,b,c,g,h).

In the adult rats, the structure of all layers was comparable to the young rats, except for apparently increased mononuclear cells in lamina propria (Fig.1e). The luminal surface of colonic mucosa of young and adult rats appeared regular, lined mainly by columnar absorptive cells (Colonocytes) displaying oval basal vesicular nuclei. Goblet cells displaying apical bleached cytoplasm with basal nuclei were seen mainly lining the crypts of Lieberkühn (Figures 1 d,e).

As for the senile rats, luminal surface of colonic mucosa appeared corrugated in some areas and disrupted in others. Focal discontinuity of the surface covering epithelium was observed (Figure 1f). Broadening of the lamina propria and dispersed infiltration by mononuclear cells, mostly eosinophils, plasma cells and lymphocytes were observed. Moreover, multiple solitary lymphatic nodules were noticed beneath the crypts. Engorged blood vessels and cellular invasions were observed in the submucosa (Figures 1 c,f).

Myenteric plexus was depicted between the two layers of muscularis externa in all groups (Figures 2 a,b,c). In the young and adult rats, the myenteric plexus showed neurons, with light basophilic cytoplasm and rounded vesicular nuclei, associated with glial cells having fusiform nuclei (Figures 2 a,b). In senile rats, the neurons were apparently fewer and shrunken with densely stained nuclei (Figure 2c).

Toluidine blue-stained sections showed significantly decreased optical density of Nissl's granules in the cytoplasm of the neurons of the senile rats, when correlated to both young and adult rats (Figures 2 d,e,f,g,h).

Colonic sections stained with combined Alcian blue-PAS in young rats showed a mucous blanket depicted as thin blue layer of acidic mucin covering the mucosal luminal surface. Positively stained goblet cells containing variable mucin types were seen lining crypts of Lieberkühn. The upper part of the crypts showed goblet cells with acidic mucin staining deep blue, together with few mixed mucin-containing goblet cells stained purple. The lower part of the crypts showed light blue-stained, acidic mucin-containing goblet cells (Figure 3a).

In adult rats, positively stained acidic goblet cells were seen closely packed and were significantly elevated in number when correlated to that of young rats in the upper part of the crypts as well as in the base of the crypts. Few purple-stained goblet cells with mixed mucin were observed in upper part of crypts and base of crypts. In both sites, mixed mucin containing goblet cells were significantly declined when correlated to young rats (Figures 3 b,d,e).

As for senile rats, the mucosal luminal surface was lined by focally disrupted mucin blanket. Apparent decrease in acidic mucin enclosing goblet cells' population was observed. They were displaying significantly decreased number in the upper part of crypt as compared to both young and adult rats. However, in the base of the crypt, this decrease was non-significant as compared to the young rats. The purple-stained, mixed mucin-containing goblet cells were displaying significantly increased number correlated to that of young and adult rats, occupying most of the apical part of the crypts (Figures 3 c,d,e).

Masson's trichrome colonic sections exhibited few collagen fibers underneath the crypts, the submucosa and the muscularis externa in young rats. Moderate content of collagen fibers was detected in adult rats, with non-significant expansion in collagen fibers' area percentage when correlated to young rats. In senile rats, numerous collagen fibers content was observed, that were significantly increased as compared to that of young and adult rats (Figure 4).

Transmission electron microscopic results

Transmission electron microscopic assessment of colonic sections of young and adult rats exhibited colonocytes displaying regularly arranged, short, blunt, finger like microvilli on their apical surface. The cytoplasm contained basal oval euchromatic nuclei and apical and basal numerous mitochondria. Endocytic vesicles were noticed in the apical part of the cytoplasm. Junctional complexes were observed connecting the lateral border of apical parts of adjacent cells together (Figures 5 a,b).

On the other hand, colonocytes of senile rats showed nuclei with irregular nuclear envelopes and irregular chromatin distribution. Apparently swollen degenerated mitochondria with lost or irregular cristae were seen in some colonocytes. Other colonocytes showed disrupted or lost microvilli, and multiple cytoplasmic vacuoles (Figure 5c).

Goblet cells of all groups were studded with apical rounded mucin granules of variable electron densities (Figures 5 d,e,f). Mucin granules of adult rats were mostly electron lucent (Figure 5e). In senile rats, some goblet cells showed apical electro lucent mucin granules. Others showed apical electron dense or mixed mucin granules (Figure 5f).

Neurons within the myenteric plexus of young and adult rats showed euchromatic nuclei whereas, scattered widely separated neurons with heterochromatic shrunken nuclei plus vacuolated cytoplasm were observed in senile rats' myenteric plexus (Figure 6).

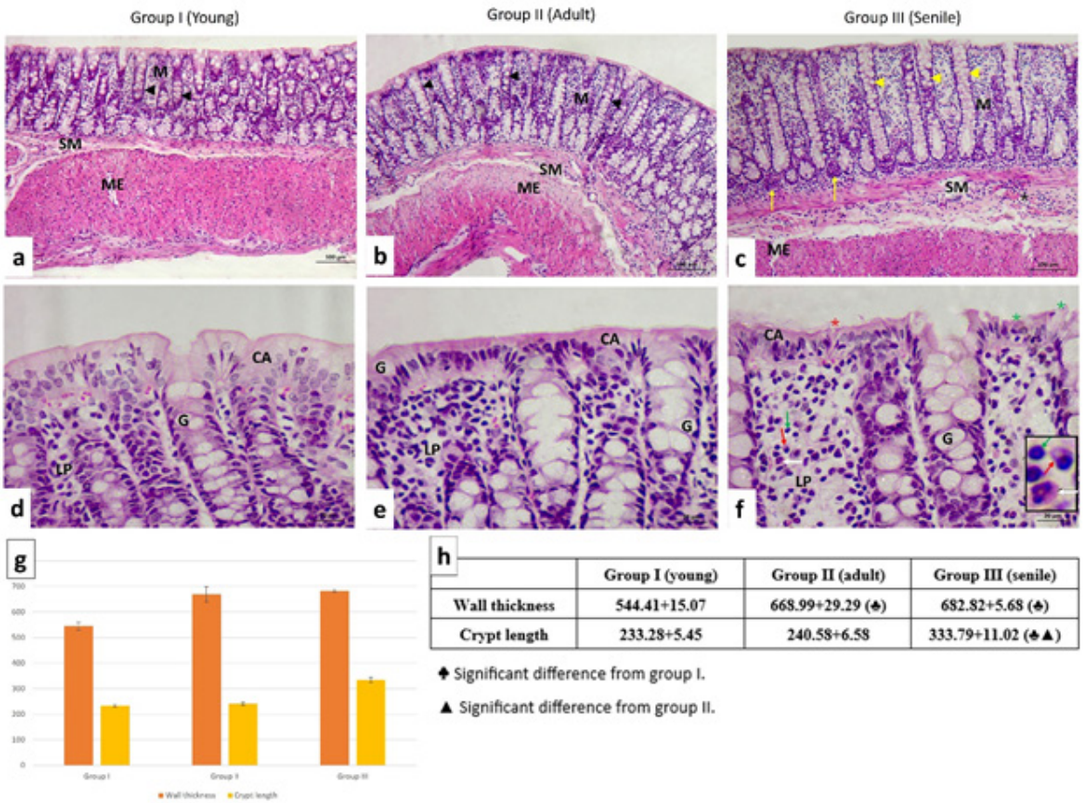


Fig. 1: (a, b, c) Photomicrographs of sections of the colon of rats of different groups showing colon layers: mucosa (M), submucosa (SM) and muscularis externa (ME) of young, adult and senile rats respectively. Crypts of Lieberkühn (▲) are seen extending through the mucosa. (c) Showing senile rat section with apparently elongated crypts of Lieberkühn (yellow▲), and multiple solitary lymphatic nodules beneath them (yellow ↑) with congeśted blood vessels and the cellular infiltration can be seen in the submucosa (black*). (d, e, f) Higher magnification sections: (d, e) young and adult rats of groups I and II respectively showing regular colonic mucosa lined by columnar absorptive cells (CA) and goblet cells (G). (f) showing senile rat section of group III, with corrugated surface epithelium (CA) in some areas (red *), and loss of the apical part of some colonocytes (green *). (e, f) Showing mononuclear cells in the lamina propria (LP) of adult and senile rats respectively. (f; inset) Showing eosinophils (white ↑), Plasma cells (red ↑) and lymphocytes (green ↑) in the lamina propria. (g, h) Hiśtogram and table showing the mean+ śtandard deviation of wall thickness and crypt length) of the colon in the different groups (in µm). (H&E; a, b, c x100- d, e, f x400; inset x1000)

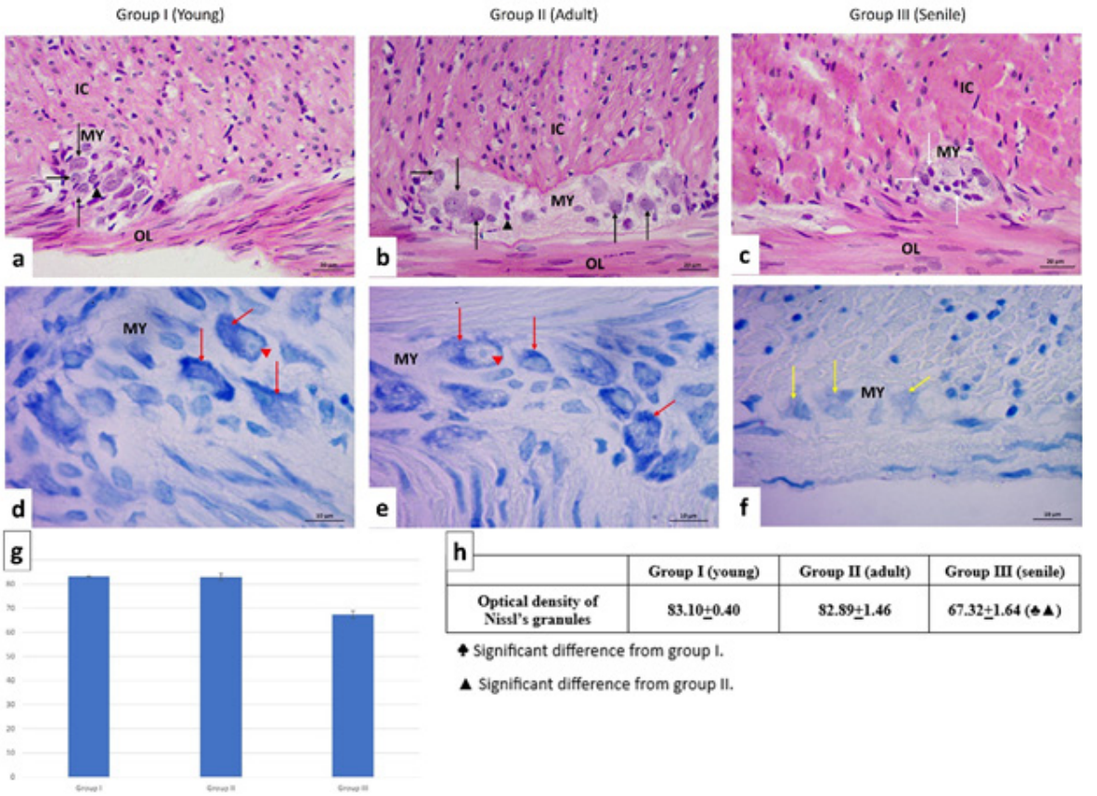
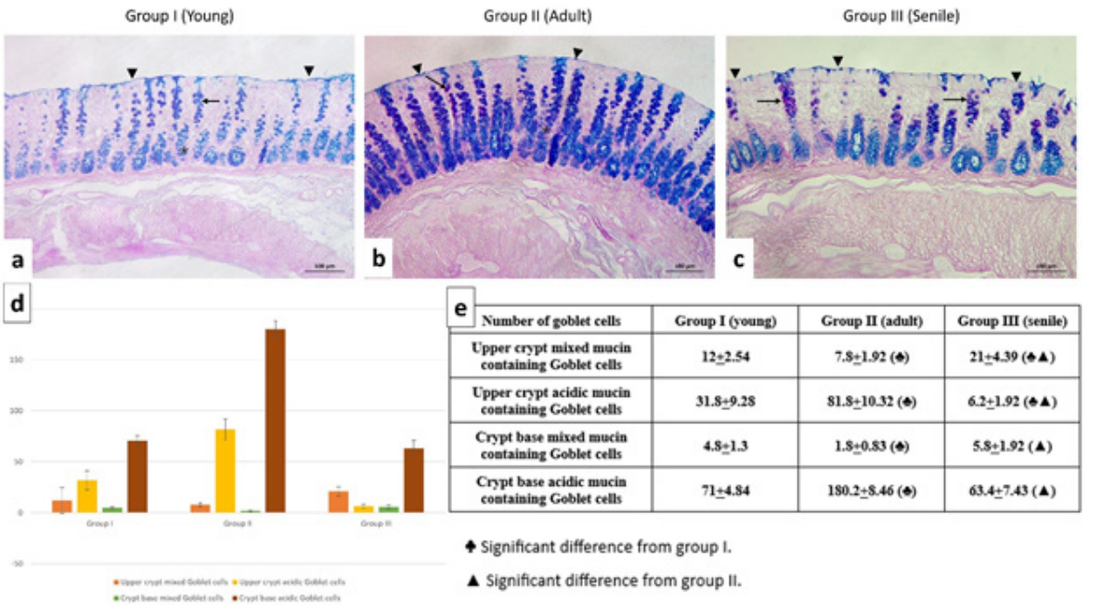
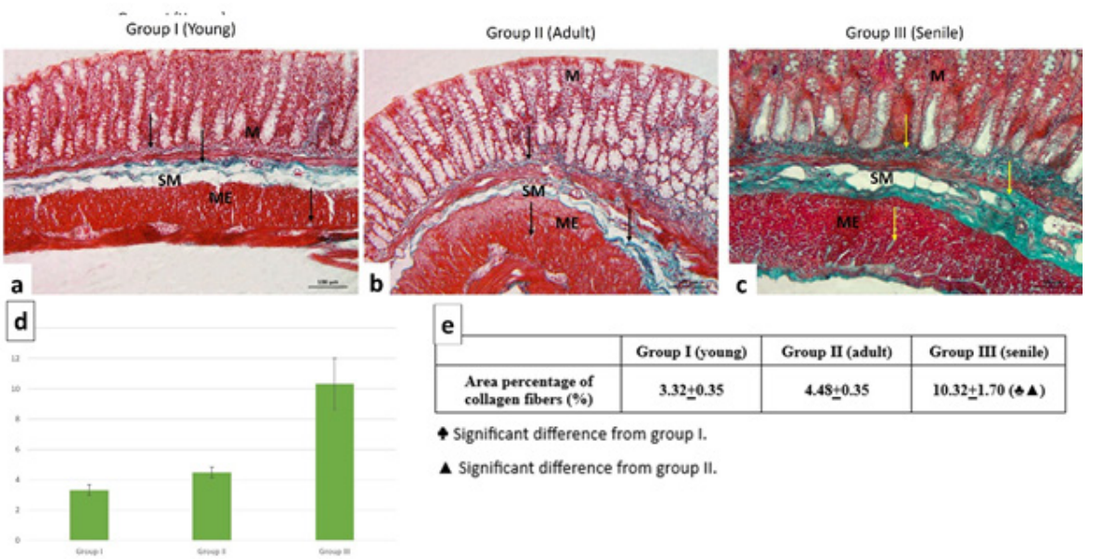


Fig. 2: (a, b, c) Photomicrographs of sections of the colon of rats of different groups showing the myenteric plexus (MY) between the inner circular (IC) and the outer longitudinal (OL) layers of the muscularis externa. (a, b) The young and adult rat neurons (black↑) show light basophilic cytoplasm and rounded vesicular nuclei. Darkly stained fusiform nuclei of the glial cells (black▲) are noticed between neurons. (d, e) Photomicrographs of toluidine blue stained sections of the colon of young and adult rats showing numerous Nissl's granules (red↑) in the cytoplasm of the neurons of the myenteric plexus (MY). Vesicular rounded eccentric nucleus of a neuron is noticed (red▲). (c) Photomicrograph H&E stained section of the colon of senile rats showing most of the neurons (white↑) appear shrunken with darkly stained nuclei. (f) Photomicrograph of toluidine blue stained sections of the colon of senile rats showing decreased cytoplasmic Nissl's granules (yellow↑) in neurons of the myenteric plexus (MY). (g, h) Histogram and table showing changes in the optical density of Nissl's granules in the neurons of the myenteric plexus represented by mean + standard deviation in the different groups. (H&E: a, b, c x100- Toluidine blue: d, e, f x400)



Number of goblet cells	Group I (young)	Group II (adult)	Group III (senile)
Upper crypt mixed mucin containing Goblet cells	12±2.54	7.8±1.92 (♠)	21±4.39 (♠▲)
Upper crypt acidic mucin containing Goblet cells	31.8±9.28	81.8±10.32 (♠)	6.2±1.92 (♠▲)
Crypt base mixed mucin containing Goblet cells	4.8±1.3	1.8±0.83 (♠)	5.8±1.92 (▲)
Crypt base acidic mucin containing Goblet cells	71±4.84	180.2±8.46 (♠)	63.4±7.43 (▲)

Fig. 3: (a, b, c) Photomicrographs of PAS-Alcian blue sections of the colon of all groups showing positively stained goblet cells lining crypts of Lieberkühn. Goblet cells containing mixed mucin are stained purple, while those with acidic mucin content are stained blue. (a, b) Photomicrographs of sections of the colon of young and adult rats showing the mucosal surface lined by blue stained mucous blanket (▲). (a) Photomicrograph of sections of the colon of young rats showing goblet cells lining the upper part of the crypts (↑) darker than those at the base (*). (b) Photomicrograph of section of the colon of adult rats showing numerous acidic mucin-containing goblet cells lining crypts of Lieberkühn (*). Few mixed mucin-containing goblet cells (↑) are noticed in the upper part of the crypts. (c) Photomicrograph of section of the colon of senile rats showing the mucosal luminal surface lined by disrupted layer of blue mucin blanket (▲). Few goblet cells' population is observed. Most of goblet cells of the apical parts (↑) of the crypts appear purple. (d, e) Histogram and table showing changes in the mean number of goblet cells represented by mean + standard deviation in the different groups. (PAS-Alcian blue x100)



	Group I (young)	Group II (adult)	Group III (senile)
Area percentage of collagen fibers (%)	3.32±0.35	4.48±0.35	10.32±1.70 (♠▲)

Fig. 4: (a) Photomicrograph of a section of the colon of young rat showing minimal collagen fibers content (↑) in the mucosa (M), the submucosa (SM) and the muscularis externa (ME). (b) Photomicrograph of a section of the colon of adult rat showing apparent increase in the collagen fibers content (↑) in mucosa (M), submucosa (SM) and muscularis externa (ME). (c) Photomicrograph of a section of the colon of senile rat showing significant increase in collagen fibers content (yellow ↑) in mucosa (M), submucosa (SM) and muscularis externa (ME). (d, e) Histogram and table showing changes in the area percentage of the collagen fibers content represented by mean + standard deviation in different groups. (Masson trichrome, x100)

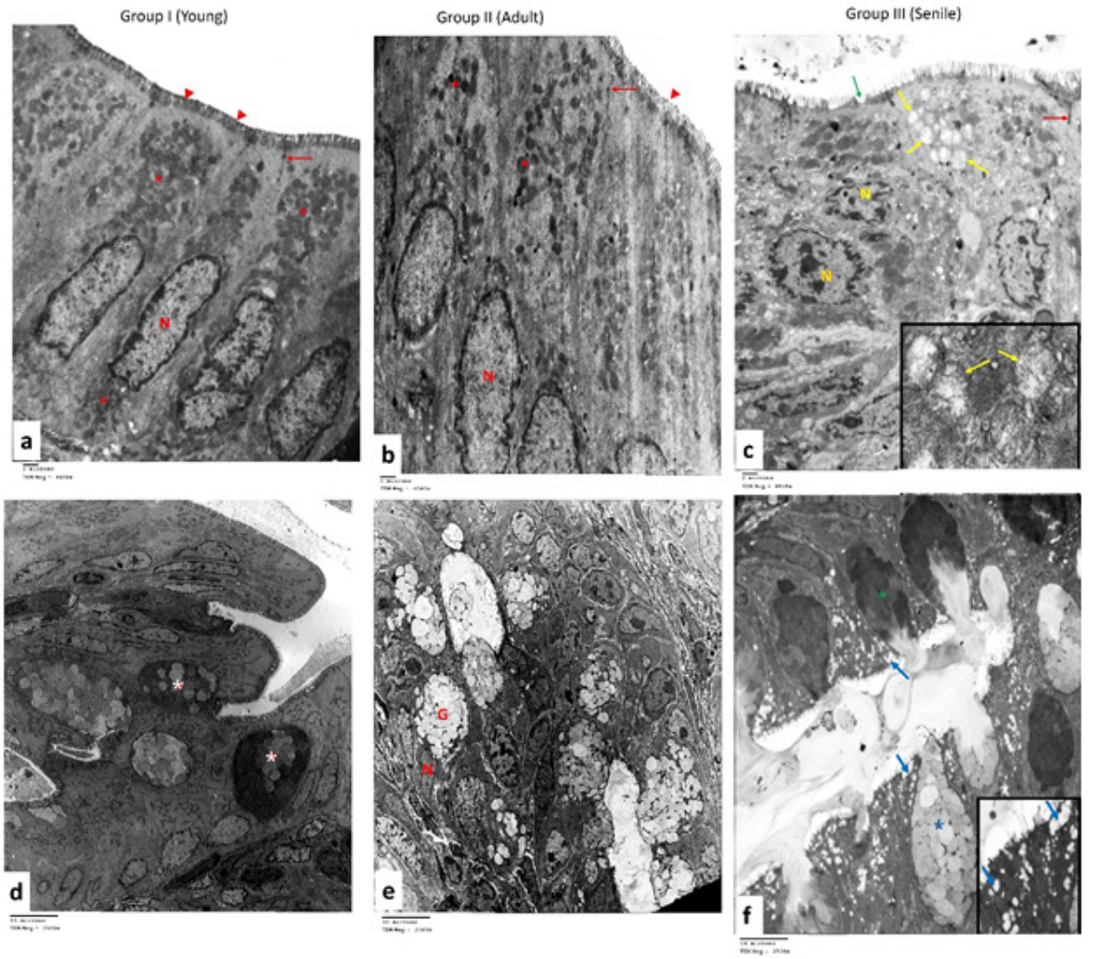


Fig. 5: (a, b) Electron micrograph of a section of the colon of young and adult rats showing colonocytes with basal oval euchromatic nuclei (red N). The cytoplasm showed numerous mitochondria (red *). The apical surface showed regularly arranged finger like microvilli (▲). (c) Electron micrograph of a section of the colon of senile rat showing colonocytes with nuclei displaying irregular nuclear envelopes and irregular chromatin distribution (yellow N). Notice the apparently swollen degenerated mitochondria with lost or irregular cristae (yellow↑). Areas of microvilli loss can be observed (green↑). (a, b, c) Notice the junctional complex (red↑). (d) Electron micrograph of a section of the colon of young rat showing some goblet cells with mucin granules of variable electron densities (white*). (e) Electron micrograph of a section of the colon of adult rats showing goblet cells (G) studded with apical rounded mucin granules of variable densities mostly electron lucent. (f) Electron micrograph of a section of the colon of senile rat showing goblet cells with apical electron dense (green*) or electron lucent granules (blue*). Notice colonocytes with disrupted microvilli and apical cytoplasmic vacuoles (blue↑). (TEM, a, b, c x4000 - d, e, f x2500 - Insests, c x15000 - f x4000)

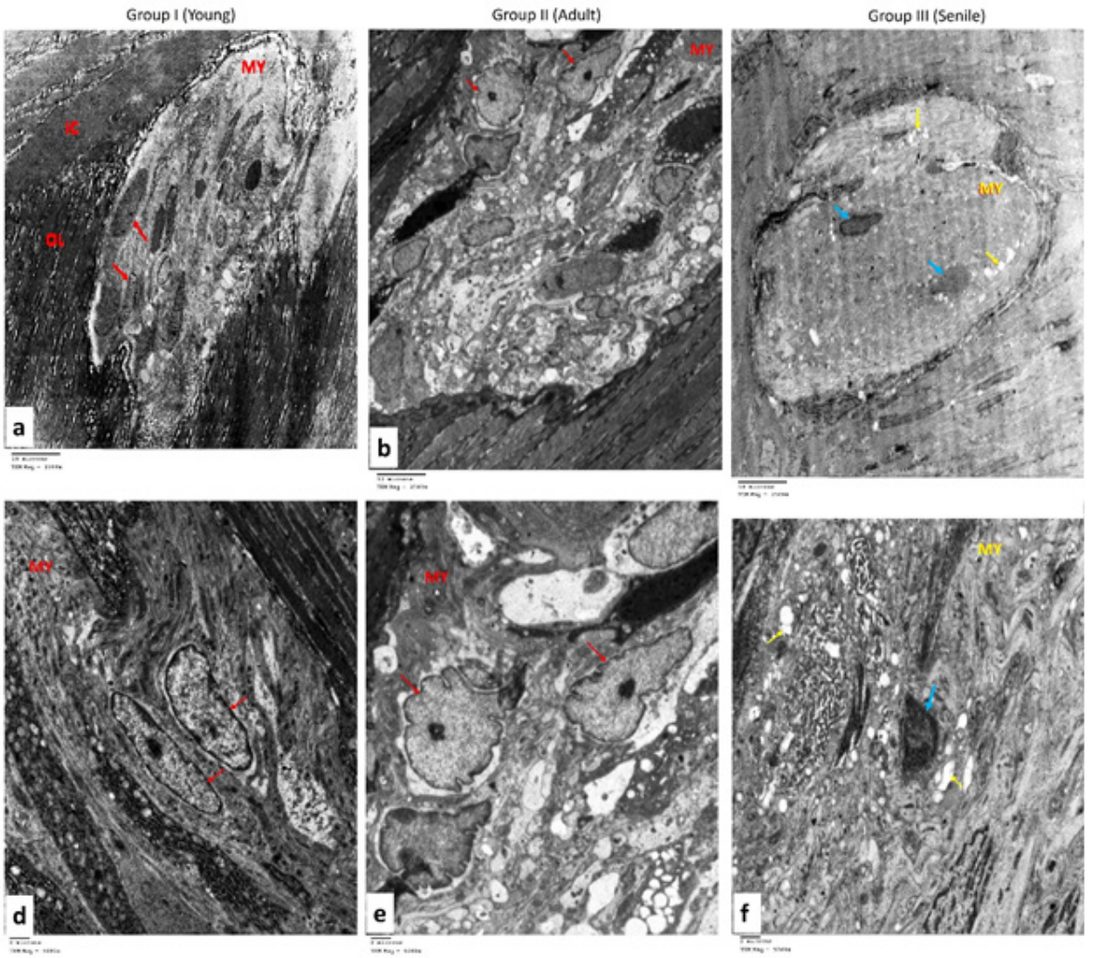


Fig. 6: (a, b, d, e) Electron micrographs of sections of the colon of young and adult rats showing the myenteric plexus (MY) between the inner circular (IC) and the outer longitudinal (OL) smooth muscle layers of the muscularis externa. Neurons display euchromatic nuclei (↑). (c, f) Electron micrograph of a section of the colon of senile rat showing myenteric plexus (MY). The neurons appear with shrunken heterochromatic nuclei (blue↑) and vacuolated cytoplasm (yellow↑). (TEM, a, b, c x2500 - d, e, f x5000)

DISCUSSION

This study was carried out to evaluate the impact of aging on the histological structure of the colon. This might help in understanding some of the GIT disorders presented by the elder population.

Rats chosen in this study were in three age groups; young (6 weeks), adult (4 months) and senile (2 years). According to Ghasemi *et al*. (2021) rats aging 70-150 days is equivalent to 18-25 years old in humans which represent emerging adulthood. While rats aging 600-730 days and 730+ days is equivalent to 65-75 and 75+ years in humans which represent older adulthood and late adulthood respectively[12]. According to Picut *et al*. (2018) rats aging 35-55 days is equivalent to 11-14 years in humans which represent prepubertal stage[13].

On examination of H&E colonic sections of senile rats (Group III) of this study, the mucosal surface appeared corrugated in some areas and disrupted in other ones. Discontinuity in some areas of the surface covering epithelium was also observed. This was concurrent with what observed earlier by Mostafa and Shaker (2014) in their study discussing the protective role of coenzyme Q10 on age associated alterations in rat colon[14]. Coinciding with these findings, Hendriks *et al*. (2021) stated that with aging, gastrointestinal tract is prone to hypoperfusion which affects intestinal integrity[15]. Moreover, aging affects signaling within the intestinal stem cells impairing their regenerative, self-renewal capacity[16,17] and aged intestinal epithelial cells' function[18].

Regarding the crypts of Lieberkühn in the present study, their length showed non-significant difference between that of the young (Group I) and adult rats (Group II). However, in the senile rats (Group III) the crypt length was significantly increased relative to groups I and II. This was parallel with the finding of Jasper (2020) and Stenvall *et al*. (2022) who found that with aging, crypt length increased in mice[8,19]. Saleem *et al*. (2023) also found that colonic crypt length increased with age in his study on pigs[20]. liang *et al*. (2017) thought that crypts elongation was to space

intestinal stem cells found in the crypt base from physical and metabolic harms that stem from infection, thus, conserving intestinal enterocytes[21]. Baker *et al*. (2019) added that crypts are dynamic structures under continuous fissions and fusions[22].

Broadening of lamina propria, dispersed mononuclear cells invasions with frequent solitary lymphatic nodules were also observed in mucosa of senile rats of this work. Moreover, engorged blood vessels and cellular invasions were observed in the submucosa. This coincides with Ceylani *et al*. (2023) who also found an increase in cellular infiltration, inflammatory markers in the ileum and colon in aged rats[23].

In this view, Malaquin *et al*. (2016) stated that cells develop an aging related secretory phenotype leading to secretion of variety of factors[24]. The aberrant aggregations of aging cells resulted in plausibly harmful impacts, mainly a defective immune response and hyperactivation of inflammation. Being the largest immune niche, the immune function of the mucosa of the intestine is greatly affected by aging[7]. These leads to a constant chronic low-grade inflammation in aged people, commonly known as inflammatory aging-process[25]. This stimulates the development of disorders, as intestinal infections and tumors, malnutrition, persistent constipation and other age associated disorders[7]. Borgoni *et al*., (2021) stated that the immunological dysfunction accompanied with aging is frequently known as immunosenescence process[26].

In current study, some senile colonocytes showed loss of their apical part. This was depicted in TEM as disrupted or lost microvilli. This finding was concomitant with Engevik *et al*. (2022) observations of microvilli shortening and loss with aging in mice[27]. Egge *et al*. (2019) explained that there is age-linked demolition of heat-shock transcription factor leading to disruption of the stress-activated kinases and protein phosphatase. This consequently increases a low-abundant actin variant (ACT-5) phosphorylation in troponin site. The phosphorylated ACT-5 speeds waning of the terminal web, weakens its connections with cell junctions and causes microvilli interruption. This jeopardizes intestinal barrier and causes pathology[28].

Otherwise, Engevik *et al*. (2022) proposed that telomere shortening in aging causes intestinal stem cells failure and defective enterocyte differentiation. Immature enterocytes showed structural defects as barrier disruption and short microvilli. These defects predispose to colitis, modulation of the microbiome and boosted absorption and flow of nutrient to the colon[27].

In present study, examination of colonocytes by TEM of senile rats of group III showed nuclei with irregular nuclear envelopes and irregular chromatin distribution. Change in nuclear shape and chromatin distribution was reported as one of the characteristic features of aging[29].

With aging, there is loss of lamin B1 (L-B1), that affects the integrity of the nucleus and chromatin distribution[30]. Lamin B1, along with different nuclear lamins form a condensed filamentous mech necessary for preserving nuclear structure and function[31]. Additionally, L-B1 anchors condensed chromatin to the internal membrane of the nucleus through lamin binding receptor LBR[32]. Decreased L-B1 and LBR expression was reported at the onset of senescence in cell lines. The loss of L-B1 causes dissociation of condensed chromatin from the inner membrane of the nucleus forming large heterochromatin foci in nucleoplasm[33]. Recently, Matias *et al*. (2022) reported that L-B1 reduction was coupled with deformities in the nucleus[34]. Lin *et al*. (2022) explained that with aging, lamin-B protein solubility increased and that in turn reduced lamin-B stability and caused its degradation[35].

In this study, senile rats` colonocytes showed apparently swollen degenerated mitochondria with lost or irregular cristae in opposition to normal mitochondria observed in that of the young and the adult rats. These findings were consistent with Miao *et al*. (2019) in their study on aged kidneys of mice. They observed mitochondrial swelling with disorganized and severed cristae in year-old mice, which was more pronounced in 2 years old mice[36]. Age-associated mitochondrial swelling and malfunction is one of the characteristic features of aging proposed[29].

In current work, TEM examination revealed numerous cytoplasmic vacuoles in colonocytes of senile rats. Correspondingly, aging-associated vacuolar degeneration was observed by many researchers in various tissues and cells; renal cells[37], nerve cells[38], hepatocytes[39] and brain cells[40]. On the other hand, Engevik *et al*. (2022) added that the increased colonic absorption, due to aging-associated structural defects, led to apparently vacuolated colonocytes[27].

In the present study, examination of toluidine blue-stained sections revealed an apparent reduction of Nissl granules content in neurons of myenteric plexus of senile rats (Group III). This finding was confirmed by morphometric and statistical results. There was a highly significant reduction in Nissl granules' mean optical density in senile rats relative to young and adult rats. Similarly, Guo *et al*. (2021) observed age-related reduction of Nissl granules in neurons of the myenteric plexus[41]. Amer and Karam (2018). attributed this to dispersion of Nissl bodies and disassociation of ribosomes from rough endoplasmic reticulum with loss of their affinity to stain. The TEM examination revealed aging-associated vacuolar degeneration of neurons of the myenteric plexus[42].

In the current study, two types of goblet cells were noticed by combined Alcian blue & PAS technique; mixed mucin containing purple goblet cells and acidic blue goblet cells. Moreover, by TEM examination of the sections of the colon some goblet cells showed electrolucent granules, while others showed both electrolucent and electrodense mucin granules.

Nieto *et al*. (2002) stated that acidic mucin containing goblet cells are mature goblet cells, while mixed mucin

containing goblet cells are less mature[43]. Moreover, Soliman *et al*. (2010) found that acidic mucins are an exponent of sufficient colonic epithelial secretory function. They added that histochemical analysis of mucins may be used to evaluate colonic epithelial damage[44,45].

According to Gomes *et al*. (2017) in his study regarding goblet cells development in rat intestine, the expression of the distinct types of mucins depends on the food intake phases. Goblet cells differentiation starts intrauterine and continues postnatally. At birth, goblet cells are acidic, neutral and mixed. With weaning and introduction of solid food which is contaminated with pathogens, goblet cells tend to increase in number and acidic goblet cells predominates[46]. On the other hand, Sovran *et al*. (2019) in their study on mice proposed that aging alter post-translational modification, affecting mucin composition and decreasing its synthesis[47]. This reciprocal relationship was supported by Stefan *et al*. (2022) who explained that alterations in mucin profile is a reflection of the bacterial population[48].

Predominance of acidic goblet cells in colon was observed and statistically confirmed in all groups of this study. This was consistent with what reported earlier by Deplancke and Gaskins, (2001); Wani and Sahu, (2020) and Ma *et al*. (2022) in rats, humans and alpaca respectively[49,50,51]. The acidic mucins act as innate defense barrier against bacterial infection. Acidic mucin was reported to be less degradable by bacterial glycosidases and proteases protecting against transluminal bacterial translocation[52]. The type of mucin and its shifting to other types might help in diagnosis and prognosis of different colonic diseases[50].

Mixed mucin containing goblet cells were noticed mainly occupying the upper part of the crypts of the colon in the present work. This observation was in line with what observed by Liu *et al*. (2014) and Truter *et al*. (2017) in piglets and rats respectively[53,54].

The current study showed that adult rats of group II exhibited the most goblet cells load in colonic crypts mainly with acidic mucin content. This was also observed by Kini *et al*. (2020) in his study regarding mice colonic mucus barrier[55]. Meanwhile, significant reduced number of acidic mucin containing goblet cells in aged rats colonic sections was detected. Although there was significant increase in mixed mucin containing goblet cells, total count of goblet cells in senile rats was significantly decreased. This was consistent with Sovran *et al*. (2019) and Powell *et al*. (2020) who declared that aging alters colonic cellular composition and causes decrease in goblet cells. This in turn leads to disrupted intestinal barrier and increased baseline colonic inflammation[47,56]. Sovran *et al*. (2019) interpreted that the decrease is due to increased goblet cells' apoptosis with no redeeming increased epithelial proliferation[47].

In this context, Powell *et al*. (2020) clarified that goblet cells perform a crucial role in preserving colonic barrier stability. Goblet cells synthesize and release glycosylated mucin into the lumen forming a layer of mucus covering the epithelium of the colon. This mucus layer is resistant to bacterial penetration. The mucus also lubricates fecal matter thus limiting abrasions. When the mucus barrier is jeopardized, bacteria come indirect contact with the epithelium. This results in antigen and bacterial displacement to lamina propria, which provokes an increased inflammatory reaction[56].

In the current study, Masson's trichrome colonic sections displayed noticeable increased collagen fibers content in mucosa underneath crypts, submucosa and muscularis externa in senile rats compared to young and adult rats. This observation was proven by morphometry and statistics which showed significant increase in collagen area percentage in senile rats compared to young and adult rats. This was in accordance with Baidoo *et al*. (2022) who found increase in collagen fibers content within the layers of ageing human colon. They reported that these age-related alterations in turn may impact colonic motility[57]. Moreover, increase in collagen deposition in all colonic layers is thought to be the reason to the significant increase in colon wall thickness found in this study in senile rats.

Gautieri *et al*. (2017) reported that with aging covalently linked sugar molecules increasingly accumulate on different collagen proteins and diverse macromolecules[58]. Moreover, Birch (2018) added that collagen is affected by major age-related modifications including accumulation of glycation products, carboxylation and severing. All these modifications lead to an increased cross linkage resulting in less flexible, stiff or rigid collagen. Collagen also becomes more resistant to enzymatic degradation and loses its solubility property. This mechanism sequentially affects colonic biomechanics[59]. Other researchers work supported that the state of chronic inflammation provokes fibroblast conversion into active myofibroblasts causing increased collagen deposition and fibrosis[60].

CONCLUSION

It was concluded that aging caused profound structural and ultrastructural changes in senile rats' colon. The changes in turn are thought to alter colonic function, which might contribute to aging associated diseases.

RECOMMENDATION

Farther research is vital to assess aging effect on colonic structure to correlate ageing associated structural changes to functional defects.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير التقدم بالعمر على التركيب الهستولوجي لقولون ذكر الجرذ الأبيض : دراسة بالمياكروسكوب الضوئي و الالكتروني

دينا محمد رشاد، صفاء محمد شاكر، نيفين بهاء، فايقة حسن الابياري

قسم الهستولوجيا، كلية الطب، جامعة عين شمس

الهدف من الرسالة: هدفت هذه الدراسة إلى تقييم تأثير التقدم في العمر على تركيب القولون لدى ذكور الجرذان البيضاء.
مواد وطرق البحث: استخدم في هذه الدراسة ثلاثون من ذكور الجرذان البيضاء من فئات عمرية مختلفة و هي ستة أسابيع و ستة عشر أسبوعا و عامين. وقد تراوحت أوزانهم من ١٠٠ إلى ٣٠٠ جرام. و تم تقسيم هذه الجرذان إلى ثلاث مجموعات حسب أعمارهم:
المجموعة الأولى (مجموعة صغار السن): ضمت هذه المجموعة عشرة من ذكور الجرذان البيضاء و التي بلغت من العمر ستة أسابيع (وزن ١٠٠-١٣٠ جرام).
المجموعة الثانية (مجموعة البالغين): ضمت هذه المجموعة عشرة من ذكور الجرذان البيضاء و التي بلغت من العمر ستة عشر أسبوعا (وزن ١٨٠-٢٠٠ جرام).
المجموعة الثالثة (مجموعة المسنين): ضمت هذه المجموعة عشرة من ذكور الجرذان البيضاء و التي بلغت من العمر سنتان (وزن ٢٥٠-٣٠٠ جرام).
تم جمع عينات القولون- واحد سم من القولون الأدنى (بعد واحد سم من الصمام اللفائفي الأعوري)- من هذه الحيوانات ومعالجتها للفحص المجهري الضوئي والإلكتروني النافذ. ولقد تضمن الفحص المجهري الضوئي استخدام صبغة الهيماتوكسلين والايوسين و صبغة التولويدين الأزرق و صبغة الالشيان الازرق وحمض البير أيودك شيف و صبغة ماسون ثلاثية الألوان. كما تم إجراءالتحليل القياسي باستخدام محلل صور لايكا.
نتائج البحث: أظهر الفحص المجهري الضوئي للعينات المصبوغة بالهيماتوكسلين و الإيوسين الخاصة بالجرذان المسنة (المجموعة الثالثة) تموج أو تقطع في الغشاء المخاطي للقولون، كما لوحظ تقطع في النسيج الطلائي في بعض المناطق. و لقد زاد طول خبايا ليبركون زيادة ذات دلالة إحصائية مقارنة بالجرذان صغار السن (المجموعة الأولى) و البالغين (المجموعة الثانية). لوحظ أيضا اتساع الطبقة المخصوصة و انتشار الخلايا أحادية النواة فيها. هذا بالإضافة إلى وجود عقيدات ليمفاوية أسفل الخبايا. كما لوحظ احتقان للأوعية الدموية و انتشار للخلايا أحادية النوايا في الطبقة تحت المخاطية. و ظهرت الخلايا العصبية الخاصة بالضفيرة العضلية المعوية أقل عددا و أصغر حجما و ذات أنوية داكنة. كما وجد زيادة ذات دلالة إحصائية في سمك جدار القولون الخاص بالجرذان البالغة(المجموعة الثانية) و الجرذان المسنة(المجموعة الثالثة) مقارنة بالجرذان صغار السن(المجموعة الأولى).

ولقد أظهرت مقاطع القولون المصبوغة بالتولويدين الأزرق الخاصة بالجرذان المسنة (المجموعة الثالثة) انخفاضًا ذو دلالة إحصائية في كمية حبيبات نيسل في سايتوبلازم الخلايا العصبية الخاصة بالضفيرة العضلية المعوية مقارنة بالجرذان صغار السن (المجموعة الأولى) و البالغين (المجموعة الثانية).

أظهرت مقاطع القولون المصبوغة بالالشيان الأزرق وحمض البير أيودك شيف الخاصة بالجرذان المسنة (المجموعة الثالثة) تقطعا بؤريا في البطانة التي تغطي السطح المخاطي و انخفاضا ذا دلالة إحصائية في الخلايا الكأسية مقارنة بالجرذان صغار السن (المجموعة الأولى) و البالغين (المجموعة الثانية). بالنسبة الى الخلايا الكأسية التي تحتوي على مخاط مختلط فقد زادت زيادة ذات دلالة إحصائية في الجرذان المسنة (المجموعة الثالثة) مقارنة بالجرذان صغار السن (المجموعة الأولى) و البالغين (المجموعة الثانية). بينما وجد انخفاض ذو دلالة إحصائية في الخلايا الكأسية التي تحتوي على مخاط مختلط في مقاطع القولون الخاصة بالجرذان البالغين (المجموعة الثانية) مقارنة بالجرذان صغار

السن (المجموعة الأولى). أما بالنسبة الى الخلايا الكأسية التي تحتوي على مخاط حمضي، فقد كان هناك انخفاضا ذو دلالة إحصائية في الجرذان المسنة (المجموعة الثالثة) مقارنة بالجرذان صغار السن (المجموعة الأولى) و البالغين (المجموعة الثانية). بينما وجد زيادة ذات دلالة إحصائية في الجرذان البالغين (المجموعة الثانية) مقارنة بالجرذان صغار السن (المجموعة الأولى).

أظهرت مقاطع القولون المصبوغة بالماسون ثلاثية الألوان الخاصة بالجرذان المسنة (المجموعة الثالثة) زيادة ذات دلالة إحصائية في محتوى ألياف الكولاجين في الطبقة المخاطية أسفل الخبايا و الطبقة تحت المخاطية و الطبقة العضلية الخارجية مقارنة بالجرذان صغار السن (المجموعة الأولى) و البالغين (المجموعة الثانية).

ولقد أظهر الفحص باستخدام الميكروسكوب الإلكتروني النافذ لمقاطع القولون الخاصة بالجرذان المسنة (المجموعة الثالثة) أنوية بعض خلايا القولون بها غشاء نووي غير منتظم و توزيع غير منتظم للكروماتين. كما احتوت بعض الخلايا على ميتوكوندريا منتفخة متحللة بها ثنايا مفقودة أو غير منتظمة. بينما أظهرت بعض الخلايا الماصة زغيبات متقطعة أو مفقودة، و العديد من التجويفات في السيتوبلازم.

أما الخلايا الكأسية فقد احتوت على حبيبات مخاط شفافة في الجرذان البالغة (المجموعة الثانية). بينما احتوت الخلايا الكأسية الخاصة بالجرذان صغار السن (المجموعة الأولى) و المسنة (المجموعة الثالثة) على حبيبات مخاط شفافة و داكنة اللون.

ظهرت الخلايا العصبية الخاصة بالضفيرة العضلية المعوية في الجرذان المسنة (المجموعة الثالثة) بنوايا منكمشة تحتوي على كروماتين داكن و سيتوبلازم ذي فجوات.

الاستنتاج: يستنتج من ذلك أن التقدم في العمر يسبب تغير في التركيب النسيجي و التركيب الدقيق لقولون الجرذان المسنة.وبالتالي يعتقد أن هذه التغيرات قد تؤثر على وظائف القولون مما قد يساهم في ظهور الأمراض المصاحبة للشيخوخة.