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Melatonin Mechanism to Mitigate Aging-related Changes in the Liver, Kidney, and Brain in Rats

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Abstract

THE STUDY aimed to investigate the potential anti-ageing effects of melatonin on the liver, kidney, and brain in rats with accelerated ageing induced by aluminum chloride (AlCl3) and D-galactose. Fifteen male albino Wistar rats were divided into three groups: a control group receiving normal saline, an accelerated ageing group receiving AlCl3 and D-galactose, and a melatonin-treated group receiving AlCl3, D-galactose, and melatonin. biochemical markers such as serum transaminases, oxidant /antioxidant enzymes, TNF-α, Bax and Bcl2 proteins, IL6 and SASP expression, and histopathological changes were assessed. Melatonin treatment mitigated hepatorenal and neural impairment in the aged rat group by reducing ALT, AST, urea, creatinine, TNF-α, and MDA levels. It also enhanced SOD activity and increased Bcl-2 levels while decreasing Bax and IL6/SASP expression. These findings suggest that melatonin may have therapeutic potential against oxidative stress and histopathological changes associated with ageing, likely due to its antioxidant, anti-apoptotic, and anti-inflammatory properties. Therefore, melatonin could be considered a protective agent against AlCl3 and D-galactose-induced ageing in rats.

Keywords: Melatonin, Aging, Oxidative Stress, Apoptosis, Senescence, Anti inflammatory

Introduction

Ageing is a progressive functional reduction of all body systems that is the God's law in all creation, characterized by cellular senescence and metabolic perturbation. Molecular deciphering of this cellular senescence revealed its occurrence through DNA damage, epigenetic alteration [1], DNA methylation [2], free-radical accumulation-induced oxidative stresses [3], telomere shortening [4] and mitochondrial dysfunction [5]. This ageing process involves progressive, destructive changes in one or more organs such as immune system impairment, nervous system dysfunction and cellular apoptosis. One of the leading key players in ageing-associated changes, including neurological disorders, is the accumulation of reactive oxygen species [6]. Aging-associated increased probability of multiple chronic diseases was documented to be correlated to increased pro-inflammatory markers levels in older people [7,8] and thus the intensity of aging-associated chronic disease morbidity and the expected severity of future disease was suggested to be related to the levels of inflammation and central obesity [9]. Although it has an essential role in protecting the body from confronting microbial attacks, the inflammation's normal course levels, being transient, appear when necessary and fade when unessential in a pattern that is essential to keep normal healing and body homeostasis [10]. However, being chronically persistent whether it is needed or not, inflammation causes the building up of its associated damages that finally leads to the homeostasis disturbances that occur in aged animals and humans, thus called (inflame-ageing) and is usually made a causal link to the ageing associated high probability of chronic diseases development such as cardiovascular and neurodegenerative disease [9,11,12] This was augmented by the reported increased serum levels of inflammatory markers like IL-6, IL-18, TNFα and CRP that were considered as characteristics of the so-called (inflammaging) and represent a high risk for chronic disease development and progression [13]. Simulating the normal process of ageing, prolonged overexposure to D-galactose in experimental animals was shown to cause the morphological and

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molecular characteristics of ageing [14] through increasing oxidative stresses, disruption of normal immune response [15], and up-regulation of inflammatory cytokines production [16]. Of the numerous pathways through which prolonged overexpression of D-galactose induces ageing in animal, are the increased production of advanced glycation end products (AGEs) [17] and ROS [18] that are produced in its D-galactose metabolic pathways. To further worsen its neurodegenerative effects, D-galactose administration reduced serum glutathione (GSH) [19], Superoxide dismutase (SOD) [20] through their exhaustion by scavenging the increased free radical's production or through decreasing their production. Moreover, the D-galactose-induced neurodegenerative effects were shown to occur through stimulation of RAS and the subcellular signaling that activates NFκ-B, which finally leads to neurodegenerative with the result of memory deterioration [21]. The transcription activity of NFκ-B is the key player that induces senescent cells to attain a pro-inflammatory status named senescence-associated secretory phenotype (SASP) through P38, and therefore, NFκ-B has been proposed to have a pivotal role in ageing [22,23]. The D-galactose-induced NFκ-B was considered to be the base of the SASP-associated upregulation of the inflammatory cytokines such as IL-6 IL-8 [24]. Indeed, NFκ-B is a master player of various proinflammatory cytokines production as TNF- α, IL-8, IL-2, and IL-6 [25]. Moreover, persistent high TNF-α production was shown to be associated with enhancing the ageing process [26]. Although the normal ageing process is unavoidable, some environmental and epigenetic factors can hasten its development while others can slow its progress. Melatonin is the most critical internal body free radical scavenger and regulator of the ROS enzymes and thus has a significant aging-delaying effect [27]. Secreted mainly from the pineal gland, melatonin was also documented to be secreted from the retina, other brain areas, digestive system, bone marrow, white blood cells and skin [28,29,30,31]. Besides its free radical scavenging power [32,33], melatonin plays a significant part in adjusting multiple body performances as sleep cycle regulation [34] circadian rhythm [35] and body anti-carcinogenesis [36]. Melatonin was shown to downregulate inflammatory cytokines expression by decreasing the NFκ-B translocation to the nucleus and its DNA binding activity [37]. Additionally, melatonin was reported to inhibit the LPS-stimulated NFκ-B in macrophages [38]. By upregulating the free radical scavenging, melatonin protects the cell membrane from lipid peroxidation and thus reduces the deteriorating effect of oxidative stress on the cell membrane [39].

Material and Methods

Ethical Approval

The experiments received approval from the ethics committee at the Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt, and an ethical issue number was issued. KFS-IACUC/172/2023

Chemicals

D-(+)-Galactose (≥98% purity), anhydrous aluminium chloride (99.999% trace metal basis), and melatonin powder (≥98% purity) were sourced from Sigma-Aldrich and Thermofisher in Egypt. Melatonin was freshly prepared in normal saline before each treatment. Bio diagnostic in Cairo, Egypt, provided kits for superoxide dismutase (SOD), malondialdehyde (MDA), tumor necrosis factor-alpha (TNFα), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and urea tests, which were conducted following the manufacturer's instructions.

Animals grouping and Experimental design:

Fifteen healthy male Wistar rats, with an average weight of 175±2 grams, were bred and housed in well-ventilated plastic cages at the Department of Physiology, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt. Before treatment, the rats were left untreated for two weeks for acclimatization. Subsequently, the rats were randomly divided into three groups, each comprising five rats: Group I: Served as the control group and received oral saline daily. Group II (aging rat model): Received 100 mg/kg b.w/day of AlCl3 through intra-gastric administration according to [40] and 200 mg/kg of D-Galactose through intraperitoneal administration as stated by [41] over six weeks. Group III: Received the ageing rat model treatment and oral administration of 20mg/kg b.w/day of melatonin according to [42].

Sampling

After the sixth week of the experiment, the rats were put to sleep with isoflurane, and blood samples were taken from retro-orbital plexus of each rat. After that, the blood was allowed to clot and centrifuged for five minutes. Biochemical analysis was performed on the collected clear sera. Parts from each rat's liver, kidney, and brain were removed, washed in cold saline, and then either fixed in a 10% formalin solution for histopathological analysis or maintained frozen at -80◦C for molecular research and Western blotting.

Serum Biochemical, antioxidant, and oxidative stress biomarkers

Enzymatic activities of ALT and AST were measured using serum samples, following the method outlined by Reitman and Frankel [43]. The

commercial kits from Bio-diagnostic Co. were used to determine urea and creatinine, respectively, following the procedures described by Henry et al. [44] and Fabiny and Einghausen [45] (Giza, Egypt).

Serum samples were used for the determination of MDA content according to Ohkawa et al. [46], and SOD was evaluated according to McCord, and Fridovich [47]. Serum proinflammatory cytokine TNFα according to (ab46070) TNFα Rat Eliza kits.

Histopathological Examination

Liver, kidney, and brain samples were quickly fixed in a 10% neutral buffered formalin solution for at least 24 hours after collection. The fixed samples were subjected to the standard paraffin embedding procedure, which entails dehydration in increasing ethanol concentrations, clearing in three different xylene and melted paraffin solutions, and finally, embedding in paraffin wax at 60◦C. Sections of 3 µm in thickness were extracted from the produced paraffin blocks. Hematoxylin and eosin were used to stain these sections (H&E) [48].

Western blotting analysis for brain apoptotic and antiapoptotic markers

The tissue samples were blended in a cold lysis buffer and then centrifuged at 14,000×g for 20 minutes at 4°C. Subsequently, the protein content of the samples was assessed using the Bradford method developed by Bio-Rad Laboratories [49]. Samples with equal protein concentrations underwent electrophoresis using 10% SDS/PAGE and were then transferred to polyvinylidene difluoride membranes. At room temperature, these membranes were treated with 5% (w/v) skimmed milk powder in PBS/Tween-20 for 2 hours. The membranes were then exposed to anti-Bax, anti-Bcl2, and β actin and their respective secondary antibodies. Finally, the protein bands were quantified using ImageJ version 1.48 software (http://rsb.info.nih.gov/ij/) National Institutes of Health, Bethesda, MD, USA. The equivalent density of β-actin was used to normalize the band densities.

Real Time-PCR measurement of Inflammatory and Senescence-related gene expression

RNA extraction from brain tissue of control and treated rats was accomplished using the TRIzol reagent protocol and a DNase treatment kit from Invitrogen. This was done to assess Interleukin 6 (IL-6) gene expression levels and Senescence Associated Secretory Phenotype (SASP) genes. Subsequently, the first strand of cDNA was generated using the Superscript III First-Strand Synthesis System for RT-PCR from Invitrogen. Quantitative real-time reverse transcription polymerase chain reaction (RT-PCR) analysis was conducted using SYBR Premix Ex Taq™ from Takara Bio Inc. The primer sequences are available in [Table 1]. Each sample's value was normalized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The relative gene expression concentrations were assessed using the 2^(-ΔΔct) method [50].

Data Analysis

The data are displayed as mean ± SEM. Statistical analyses were performed using GraphPad Prism 5 and 9 (GraphPad Software, San Diego, CA, USA). Results underwent Tukey's multiple comparisons post-hoc, one-way ANOVA. Additionally, serum parameters, antioxidant status, and TNFα data were statistically evaluated using one-way ANOVA followed by Duncan's multiple range tests with the SPSS programming tool (IBM SPSS 20®, IBM Corp., Armonk, NY, USA). All significance statements were based on $p < 0.05$.

Results

Melatonin ameliorates body weight change in ageing rat model: Treatment of the rats with an ageing combination (D-galactose and AlCl3) led to a reduction of the body weight. While treatment of the ageing rat model with melatonin

There is a significant decrease in body weight in aged rat group compared with the control group. However, melatonin supplementation succeeded to raise body weight as compared with the aged rat group prevented this reduction in the body weight (Fig.1)

Melatonin ameliorates the ageing related deterioration of liver and kidney functions

The ageing rat model treatment showed elevated levels of the liver serum enzymes (ALT and AST) and kidney enzymes (urea and creatinine) compared to the control value (Table 2). Meanwhile, melatonin treatment in the ageing rat model returned these tests toward nearly the normal levels, indicating the potential protective impacts of melatonin on ageing-associated functional disturbances.

Melatonin antioxidant and anti-inflammatory effect on the ageing rat model

In this study, treatment of rats with an ageing rat model combination caused induction of the MDA and suppression of SOD enzymes in addition to elevation of the pro-inflammatory cytokine TNFα (Fig. 2). While melatonin treatment was able to revert these findings as linked with the aged rat group.

Discussion

The purpose of this study was to examine the impact of melatonin on the structural and functional decline of the brain, liver, and kidneys associated

with ageing in rats using a model produced by D-galactose and AlCl3. Compared to the control group, the decrease in body weight of the ageing rat model is an indication of body entropy. It is consistent with the proposed increased entropy and ageing process [53]. Meanwhile, the aptitude of melatonin to alleviate the body weight decline in the ageing model indicates its ability to stop or at least delay ageing-related entropy. The hepatic index, ALT and AST concentration in the serum are the major parameters for liver health status [54,55]. Present research results showing the increased ALT and AST serum levels in the ageing rat model direct the reduced aptitude of the liver cells to withstand oxidative stress and is in agreement with the previously reported ageing allied hepatic decline of the metabolic ability and the high vulnerability of hepatic tissue to oxidative stress [56]. In the present investigation, the elevated levels of the hepatic tissue markers due to D-galactose supplementation are also in harmony with previous descriptions stating the induction of liver enzymes in a similar rat model [57-59]. The down-regulating effect of the co-treatment of the ageing rat model with melatonin against the D-galactose and ALC3-induced up-regulation of the hepatic markers ALT and AST, indicates the melatonin ameliorating effect on the ageing progression, especially in the liver tissue. This is consistent with the recent report of the ability of melatonin to counteract D-galactose-provoked ageing and its associated increased liver markers in the mouse model [15]. In the current research, the improved oxidative stress on the liver due to the D-galactose and ALC3-induced ageing process was manifested on the cellular levels by hepatocyte degeneration associated with cytoplasmic vacuolation, sinusoidal dilation and inflammatory cell infiltration. These D-galactose-induced histopathological changes confirm the toxic effect in the current ageing rat model and concur with the prior report [15]. Moreover, the nearly normalized histological nature of the hepatic tissue due to melatonin supplement in the ageing rat model indicates its ameliorating effect to counteract the ageing-associated hepatic disorders. These results also agree with a previous report stating the same aging-counteracting effect of melatonin on liver tissue [15]. This hepatoprotective effect of melatonin on the ageing-associated hepatic changes can be accredited to its anti-inflammatory and free radical scavenging effect following the previously assumed mechanisms of the hepatoprotective effect of melatonin [60]. Aging progression is inversely correlated with kidney performance due to the aging-associated reduction of the redox homeostasis ability [61]. The increased serum creatinine and urea concentration in the ageing rat mode mimics the ageing-related decline of the kidney's ability to wash out the body's waste product. This ageing-related decrease of kidney performance can be attributed to the accumulating stress due to increased AGE, MDA and ROS with the progressive reduction of the free radical scavenging power of the kidney manifested by downregulation of SOD and GSH [17] .The induced oxidative stress in the current ageing rat model was coherent with histopathological changes in the kidney manifested by glomerular atrophy, proximal and distal tubular necrosis, cells' pyknosis, and interstitial tissue hemorrhage. In the current study, this D-galactose-induced pathological changes in the kidney tissue is a reasonable consequence of the ageing process[62] and can be attributed to the ageing-associated excessive free radical accumulation and downregulation of the body's antioxidant power in the ageing process due to the upregulation of the serum oxidative injury markers as MDA and reduction of the serum antioxidant markers as SOD and GSH which represent a disturbance of the body redox system that participate in the kidney tissue senescence[63]. Moreover, the D-galactose persuaded pathological changes in the liver and kidney tissue is attributed to the ageing–associated inflammatory process based on the D-galactose-upregulation of the serum inflammatory marker TNF-α levels that were shown to be associated with enhancing ageing process [26] .Indeed, D-galactose was shown to induce NFκ-B [24] ;meanwhile, inducing NFκ-B was considered to be the master player of various pro-inflammatory cytokines production as TNF-α, IL-8 and IL-6 [25] . In the context of its anti-inflammatory, melatonin's ameliorating impact on the aging-associated orientation of TNF-α in this investigation concurs with the previous report of the ability of melatonin to suppress LPS-stimulated NFκ-B in macrophages[38] and its efficiency to downregulate inflammatory cytokines expression through decreasing the NFκ-B translocation to the nucleus and its DNA binding activity [37] and therefore its ability to downregulate the ageing-associated increase of TNF-α [15]. The brain is the most sensitive of all the body organs to ageing-associated oxidative injury due to its high fat and active metabolic activity [64]. Brain homeostasis is accomplished by astrocytes that guarantee nutrient and ion supplements, alleviate inflammation and maintain the blood-brain barrier (BBB) [65]. Nerveless, brain ageing decreases astrocyte efficiency to fulfil their duties with the enhancement of free radical release that accumulates and finally leads to neurodegeneration [66], which causes atrophy of the structure of the brain-sensitive region and thus affects the grey matter integrity as a hallmark of the normal ageing process [67]. In the current study, the induction of the mRNA expression of IL-6 and SASP indicates the severe inflammatory and oxidative effect of the ageing rat model

treatment on the brain tissue. This D-galactose-induced IL-6 and SASP is believed to be achieved through D-galactose-enhanced NFκ-B, which was considered the base of the SASP-associated upregulation of the inflammatory cytokines [24]. Therefore, the induction of IL-6 and SASP mRNA in the hippocampus tissue and serum TNF- α is assumed to be induced through NFκ-B induction based on its suggested role as the master player of various proinflammatory cytokines production as TNF- α, IL-8, IL-2, and IL-6 [25]. Moreover, the melatonin-mitigated effect on the D–galactose–induced IL-6 and SASP mRNA expression confirms the anti-ageing and anti-inflammatory effect and agrees with [15]. Moreover, using AlCl3 in the current ageing rat model added more burden and ageing stress, especially on the brain tissue. AlCl3 has been reported to activate inflammatory pathways and free radical production, causing various cellular signaling perturbations [68,69,70]. In addition to melatonin's ability to decrease the magnitude of free radical production, it also counteracts the oxidative non-free radicals as nascent oxygen, hydrogen superoxide and peroxynitrite [71,72]. Therefore, in the current study, the melatonin-ameliorating effect on the D-galactose and AlCl3 ageing rat model by suppressing the ageing rat model upregulated mRNA expression of IL-6 and SASP in the hippocampus tissue could be through its suppressive effect of the aluminum chloride-induced oxidation and its oxidative non-radical counteracting effects [27]. This effect was proposed to be through melatonin modulating effects on the mitochondrial membrane to ease its electron transfer and thus counteract the oxidative stress on the mitochondria [73]. The upregulation of the proapoptotic Bax and down regulation of the antiapoptotic Bcl2 mRNA levels in the hippocampus of the ageing rat model explain the hippocampus histological changes and indicate the mechanism of ageing-related brain affection as Alzheimer's disease (AD). Meanwhile, the suppressive effect on Bax and the enhancing effect on Bcl2 that have been revealed in the current study due to treatment of an ageing rat model with melatonin indicates the melatonin antiapoptotic through which it protects the brain oxidative-most vulnerable areas as hippocampus from the ageing deteriorating effects. This effect is consistent with melatonin's recently reported anti-apoptosis effect [74,75,76].

Conclusion

Here, we showed the melatonin protective effects against d-galactose and AlCl3 induced ageing in rat ageing models. This antiaging melatonin effect seems to be induced through the melatonin's anti-inflammatory, antioxidant, and anti-apoptosis effect. In particular, the induced oxidative markers MDA and inflammatory marker serum TNF-α, hippocampus IL-6 and SASP mRNA, proapoptotic marker (Bax) and the ageing-related histopathological changes in the liver, kidney and brain tissue indicated the ageing-related deterioration in these organs. Meanwhile, the current melatonin treatment for the ageing rat model revealed the protective effect against ageing-associated changes depending on the melatonin's antiapoptotic, anti-inflammatory and anti-oxidant effects. This suggests melatonin is a multi-organ protective factor that helps the body escape the severity of the harmful effects of ageing.

Conflict of interest statement

The authors have disclosed that they do not hold any conflicts of interest related to the publication of this article.

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TABLE 1. Primer sequences for gene under study.

Gene	Primer	References
IL-6	5′-CACAAGTCCGGAGAGGAGAC-3′ 5′-ACAGTGCATCATCGCTGTTC-3′	[51]
Senescence-Associated Secretory Phenotype (SASP)	5′-GAGCACACCAAGGAGTGATTT-3′ 5′-GAAGCTTCATGGTGCTCTCT-3′	[52]
β-actin	5′-CACCACACCTTCTACAATGAG-3′ 5′-TACGACCAGAGGCATACAG-3′	[51]

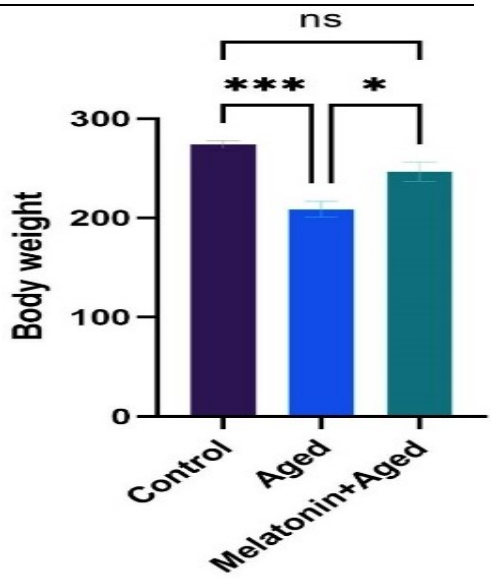

Fig.1. Impact of melatonin on body weight.

TABLE 2. Effect of Melatonin on liver and kidney functions in an ageing rat model

	ALT (U/L)	AST (U/L)	Creatinine (mg/dl)	Urea (mg/dl)
Control	65.24[c]	91.33[b]	0.49	45.6[c]
Aged	114.7[a]	115.2[a]	0.6	62.69[a]
Aged+ Melatonin	81.33[b]	95.6[b]	0.5	49.12[b]
SEM	0.548	1.65	0.0878	0.548
P- value	0.001	0.01	0. 021	0.024

(a) significance at (p 0.001), (b) non-significance compared to control.

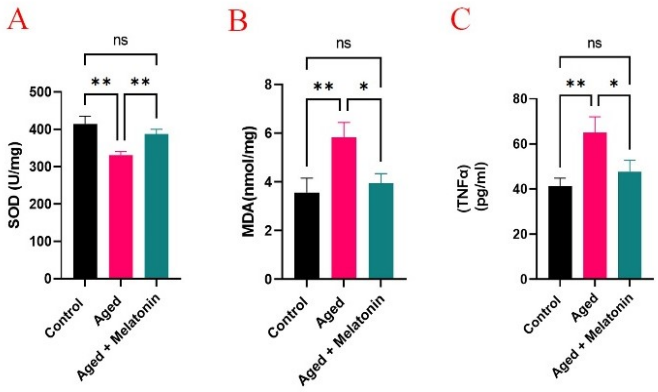

Fig. 2. The impact of melatonin on serum oxidative and antioxidant markers: Superoxide dismutase (SOD), malondialdehyde (MDA), and tumor necrosis factor-alpha (TNF-α) were measured in the serum of the control, ageing rat model and the ageing rat model plus melatonin treatment. *P < 0.05 and, **P < 0.01 in relation to the aged group

Melatonin alleviates the ageing related histopathological changes in the liver.

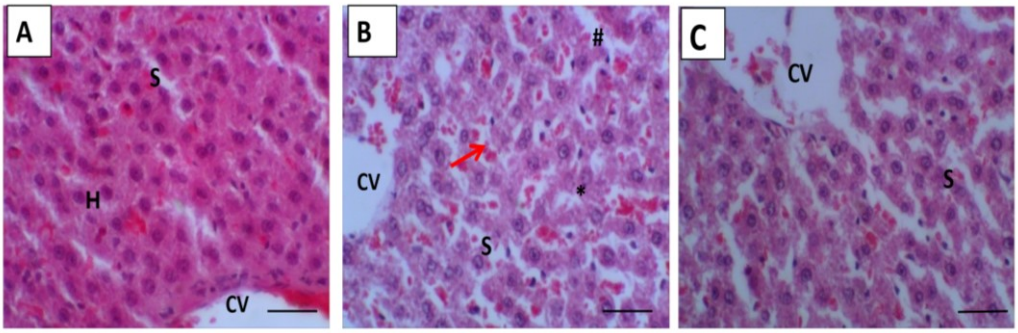

Fig. 3. Melatonin protection of liver histopathological changes induced in an ageing rat model: H &E-stained Liver. (**A**)The control group showed normal hepatic lobules where hepatocytes (H), central vein (CV), and sinusoids (S). (**B**) The ageing rat model shows the degeneration of hepatocytes (*) associated with cytoplasmic vacuolation and sinusoidal dilation (#), and infiltration of inflammatory cells (arrow). (**C**) The melatonin-treated ageing rat model shows the liver's nearly normal architecture with fewer hepatocyte degeneration (scale bar = 40X).

Melatonin alleviates the ageing related histopathological changes in the kidneys:

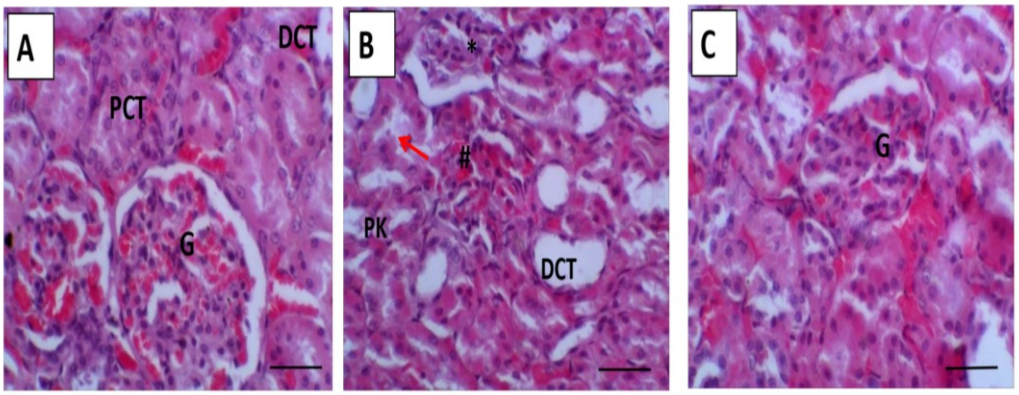

Fig. 4. Melatonin protection of kidney histopathological changes induced in an ageing rat model: H & E-stained kidney tissue. (**A**)The control group indicates normal histological structure with glomerulus (G) proximal convoluted tubules (PCT) and distal convoluted tubules (DCT). (**B**) ageing rat model showing glomerular atrophy (#), proximal and distal tubular necrosis (arrow), pyknotic cells (PK), and hemorrhage in interstitial tissue (*). (**C**) Melatonin-treated to ageing rat model showing marked reduction in pathological lesions in the glomerulus. (scale bar = 40X).

Melatonin protects brain from the ageing associated histopathological changes

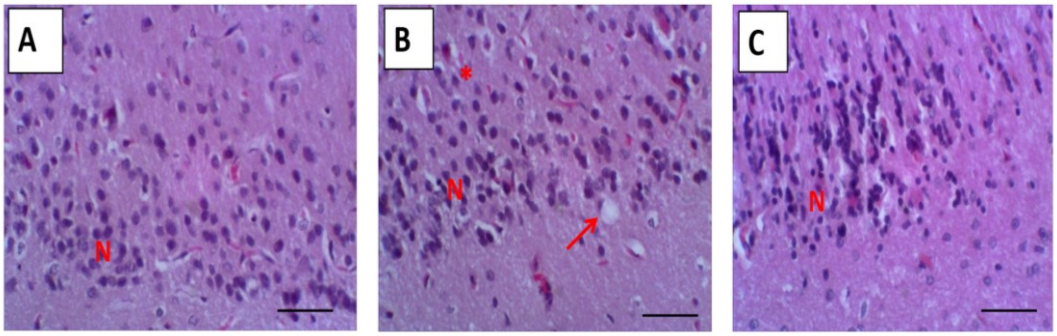

Fig. 5. Melatonin protection of cerebral cortex histopathological changes induced in an ageing rat model: H & E-stained cerebral cortex sections. (**A**)The control group showed an intact cortex with well-arranged neurons (N), and the nuclei were centered with apparent staining. (**B**) ageing rat model showing apoptotic cells (*) and cytoplasmic vacuolations (arrow) and distortion of neuron arrangement. (**C**) Melatonin treatment in the ageing rat model showed reduced damage levels seen in the ageing rat model and nearly normalization of the neurons (scale bar = 40X).

Melatonin down regulates ageing–associated enhancement of apoptosis in hippocampus: administration of the rat with ageing rat model treatment upregulated the apoptotic factor, Bax. It downregulated the anti-apoptotic factor Bcl-2 compared to control. Meanwhile, melatonin treatment in the ageing rat model ameliorated the ageing-induced deterioration of these markers, indicating the melatonin's ability to slow down the aging-associated apoptotic change (Fig.6)

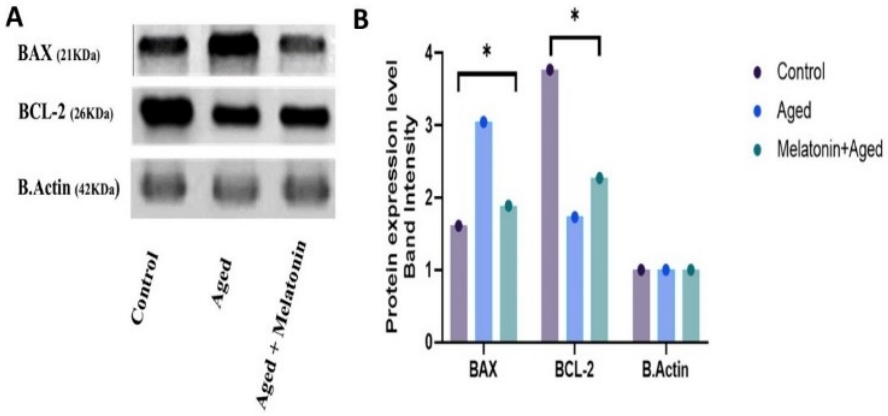

Fig. 6. Melatonin antiapoptotic effect on aging rat model. *P<0.05 in relation to Aged group.

Melatonin down regulates ageing–associated inflammatory and senescence gene expression in cerebral cortex:

Administration of the rat with ageing rat model treatment upregulated the mRNA expression level of IL6 and SASP compared to the control group. Furthermore, melatonin supplementation initiated a substantially reduced mRNA expression level in IL6 and SASP compared to the aged rat group, as portrayed in (Fig.7).

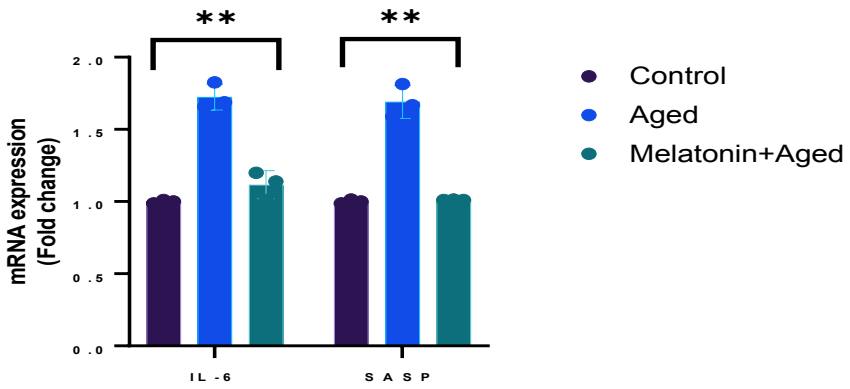

Fig. 7. Melatonin inhibition of the ageing-related inflammatory marker induction in the rat cerebral cortex. interleukin6 (IL6) and senescence-associated secretory phenotype (SASP). **P<0.001 in relation to Aged group

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ميكانيكية الميلاتونين لتقليل التغيرات المرتبطة بالشيخوخة فى الكبد والكلى والمخ فى الجرزان

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المستخلص

هدفت الدراسة إلى التحقيق في الآثار المضادة للشيخوخة المحتملة للميلاتونين على الكبد والكلى والدماغ في الفئران التي تعاني من تسريع الشيخوخة المستحثة بواسطة كلوريد الألومنيوم (AlCl3) و-Dجالاكتوز. تم تقسيم خمسة عشر فأراً من الفئران البيضاء الذكور من نوع ويستار إلى ثلاث مجموعات: مجموعة السيطرة التي تلقت محلول ملحي طبيعي، ومجموعة الشيخوخة المسرعة التي تلقت AlCl3 و-Dجالاكتوز، ومجموعة معالجة بالميلاتونين تلقتAlCl3 ، -D جالاكتوز، والميلاتونين. تم تقييم معايير مثل ترانسامينازات الدم، المؤشرات الكيميائية الحيوية، مستويات المضادات والمؤكسدات، TNF-α، بروتينات Bax وBcl2، تعبير IL6 وSASP، والفحص النسيجي المرضي. ساهمت معالجة الميلاتونين في التخفيف من الضرر الكبدي والكلوي والعصبي في مجموعة الفئران المسنة من خلال تقليل مستويات ALT، AST، اليوريا، الكرياتينين، TNF-α، و .MDAكما زادت من نشاط SOD ورفعت مستويات Bcl-2 بينما قللت من تعبير Bax و .IL6/SASPتشير هذه النتائج إلى أن الميلاتونين قد يمتلك إمكانية علاجية ضد الإجهاد التأكسدي والتغيرات النسيجية المرضية المرتبطة بالشيخوخة، وذلك على الأرجح بسبب خصائصه المضادة للأكسدة والمضادة للموت الخلوي المبرمج والمضادة للالتهابات. يمكن اعتبار الميلاتونين عامل وقائي ضد الشيخوخة المستحثة بواسطة AlCl3و-Dجالاكتوز في الفئران.

الكلمات الدالة: الشيخوخة – الميلاتونين – الفئران - مضادات الاكسدة - التغيرات النسيجية.