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Role of Melatonin in Arterial Blood Pressure Modulations and Renal Damage Induced By Ethanol Consumption in Adult Male Rats

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Keywords

- Ethanol
- Melatonin
- ABP
- TNF-α
- VEGF
- Renal caspase-3activity.

Abstract

Background and objectives: Ethanol exposure produces hypertension and many changes in the kidney. Melatonin has potent antioxidant and anti-inflammatory properties. The aim of this study is to detect the role of melatonin on arterial blood pressure (ABP) and its probable protective effects against renal dysfunction induced by ethanol in adult male rats. In addition, the possible involved mechanisms of melatonin using biochemical and histopathological methods were evaluated. ***Methods:*** Forty eight rats divided into three groups; control, ethanol treated and melatonin + ethanol treated groups. Half of the animals were sacrificed after six weeks (6Ws) and the other after twelve weeks (12Ws) for each group. Ethanol treated group: the animals of this group received 10 ml/kg of BW 30% ethanol by intra-gastric tube at alternate days. Melatonin+ ethanol treated group: the animals of this group received the pervious ethanol dose and 5 mg/kg melatonin injected subcutaneously at alternate days. ***Results:*** In ethanol treated group after the 6^{th} and 12^{th} week, there were significant decreased body weight (BW), significant increased (ABP) and tumor necrosis factor alpha (TNF-α). However, after the 6^{th}W serum urea, creatinine, vascular endothelial growth factor (VEGF) and renal caspase-3 activity were not significantly changed, but these parameters were significantly increased except VEGF which significantly decreased at (12Ws). In ethanol treated group for 6Ws and 12Ws dilatation, congestion of the peritubular vessels and dilated bowman's spaces were obvious. Dilated tubules lined by thinner epithelium with degenerated cells at some areas were also noticed. Degeneration and necrosis of renal tubular epithelia were more obvious following twelve weeks ethanol treatment. These damaging changes reflected toxic effects of ethanol with the duration of exposure. These parameters and histopathological effects were ameliorated by the melatonin. ***Conclusion*:** Melatonin has a protective role in elevated (ABP) and renal damage induced by ethanol.

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INTRODUCTION

In chronic alcoholic condition not only kidney but liver was also affected (1). While acute alcohol consumption has a nephrotoxic effect on the kidney, alternatively, chronic use in humans may result in alcohol-induced hypertension, indirectly increasing the risk of chronic kidney disease (2, 3). TNF-α is a primary cytokine that initiates the inflammatory cascades by inducing secondary cytokines and key enzymes. It has been shown to play an important role in apoptosis (4). Ethanol administration stimulates an increased Kupffer cells (KCs) mediated production and release of TNF-α and IL-6, cytokines that have been associated with inflammatory responses and hepatocellular damage in chronic disease states(5). Deterioration of renal function may be one of the most important factors associated with a significant increase in TNF-α activity (6). Ethanol intoxication in obese mice increases TNF-α which activate hepatic caspase-3(4).

Ethanol consumption can lead to cell apoptosis in various tissues such as liver (7), heart (8), stomach (9) and brain (10), which has been confirmed in rats, mouse and human experiments. Apoptosis is a complex process involving a variety of different signaling pathways and results in a multiple of changes in the dying cell (11). Most of the members of the caspase family are expressed in the kidney. Caspases-3 and -6 are localized predominantly in renal tubular epithelium (12).

Growth factors are signaling molecules playing crucial roles in the response to ethanol intoxication influencing the survival, differentiation, maintenance, and connectivity of cells of the brain, kidney, and liver (13). VEGF is one of the major growth factor regulating the pathophysiology of kidney (14). VEGF receptors can also be expressed in tubular epithelial cells, and the enhanced expression might provide survival benefit in situations such as renal ischemia and toxic injury (15). In addition, recent evidence indicates that VEGF plays an important role in endothelial cell proliferation and capillary repair in the glomeruli (16).

Melatonin activates various physiological functions causing sleep/wake rhythm, circadian rhythm, blood pressure control, immune system activity, detoxification of free radicals (17). It had been shown to improve apoptosis and cell injury in the liver of male rats (18). Melatonin acts upon the kidney through multiple pathways: as an antioxidant, as an apoptosis modulator, and as a circadian modulator of vascular function (19).

Most renal functions, including glomerular filtration rate, urine production, and solute excretion, exhibit circadian changes. There is an increase in the concentration of urine during the nocturnal period, which may be mediated by melatonin. Its receptors are predominantly localized in the proximal tubular segments (20). Melatonin has a protective effect on the structural alterations of proximal tubules of the kidney induced by gentamicin (21).

The aim of the present study is to evaluate the role of melatonin on blood pressure changes after exposure to ethanol, as well to detect its probable protective effects against cell death and tissue dysfunction in the renal injury induced by ethanol in adult male rats. Also, an attempt was undertaken to investigate the mechanisms responsible for these changes.

MATERIALS AND METHODS

Experimental animals

A total number of forty eight adult male albino rats about (175– 200 g) were purchased from animal house of Assiut University. The experimental protocol was approved by the Institutional Animal Research Committee of the Faculty of Medicine, Assiut University, Egypt. They were housed in an aerated room temperature (25°C) and a light/dark (12 h:12 h) cycle, food and water were provided ad-libitum. The body weight was carried out at the start of the experiment, at six weeks and twelve weeks before animal decapitation.

Chemicals

Melatonin was obtained from (Sigma Chemical Co., St Louis, MO, USA).

Experimental Design

After one week of acclimatization, rats were randomly divided into the following groups each of which was 16 rats. Control group: the animals of this group received saline orally by intra-gastric tube. Eight rats were sacrificed after six weeks, and the other after twelve weeks. Ethanol treated group: the animals of this group received 10 ml/kg of body weight 30% ethanol by intra-gastric tube (22) at alternate days. Eight of them were sacrificed after six weeks, the other after twelve weeks. Melatonin+ ethanol treated group: the animals of this group received the pervious ethanol dose and 5 mg/kg melatonin injected subcutaneously (23) at alternate days. Melatonin was dissolved in absolute ethanol and then the solution diluted with saline. Eight of them were sacrificed after six week, the other after twelve weeks.

Blood pressure measurement

Blood pressure, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in unanesthetized rats, at six week and at the end of experiments, with a pneumatic tail pulse transducer (Narco physiograph, model DMP4A, Biosystems, Inc., Houston, TX). The animals had been pre warmed in a metal chamber at approximately 30 °C and allowed to rest and allowed to acclimatize for 20 minutes before the recordings were made. Three consecutive measurements were made at the same time of day. Mean systolic and diastolic blood pressure values from three measurements were recorded as the pressure value for each animal.

Sample collection

After the end of the experimental period, blood was collected in glass tubes from orbital sinus and whole blood was centrifuged after clotting, and the serum was separated and the samples were maintained at -20 ^{o}C until used. Then the animals were sacrificed, kidneys were obtained. One of the kidneys was used for assessment of renal caspase-3 activity and the other was fixed in 4% gluteraldehyde solution.

a- Estimation of biochemical parameters in the serum

Serum urea and creatinine levels were measured with a spectrophotometric technique using commercial kits (Olympus, Hamburg, Germany) (24) and presented as milligram per deciliter. Serum TNF-α level was determined using ELISA kit (Invitrogen, Carlsbad, CA, USA) (25) according to manufactures instructions. Serum VEGF level was determined using ELISA mouse/rat VEGF assay kits (Quantikine, R&D

Systems, Minneapolis, MN, USA) (26) according to the manufacturer's instructions.

b-The renal activity of caspase-3

A specimen (50 mg) was taken from each frozen kidney and homogenized with 20 mM *N*-2-hydroxyethylpiperazine-*N*8-2- ethanesulfonic acid (HEPES, pH 7.5), containing 10% sucrose,0.1% 3-[(3- cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 2 mM dithiothreitol (DTT), 0.1% NP-40, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 μg/ml leupeptin, and 1 μg/ml pepstatin A. The supernatants obtained after centrifugation at 2,000 *g* were used to determine enzyme activity by caspase-3 colorimetric assay kit (Assay Design, Inc. Michigan USA) (27).The standard protocol was followed as detailed by the manufacturer. The caspase-3 activity was expressed as unit/mg tissue weight.

Histopathological examination

For light microscopic investigations, samples from the kidney were taken from all groups after scarification then fixed in 4% gluteraldehyde solution. Semithin sections were cut at 0.5-l μm and were stained with Toluidine blue (28) and examined with light microscope.

Statistical analysis

Data were presented as means ± standard deviation (SD). Differences between groups were determined by non parametric Mann Whitney test. The level of significance was accepted with $P<0.05$. Prism computer program (graph pad version 3.0) was used for statistical analysis.

RESULTS

1- Body weight

Results clearly showed that the body weight of ethanol treated rats for six weeks and twelve weeks were significantly lowered ($P<0.001$ for each) when compared with the control. Melatonin administration in the melatonin+ ethanol treated group associated with significant improvement of the body weight at six and twelve weeks when compared to the ethanol treated group and the response was manifested more at twelve weeks ($P < 0.05$ and $P < 0.001$ respectively) but they were significantly lowered when compared to the corresponding control group ($P < 0.01$ for each). Table 1 shows results of 6 weeks and table 2 shows results of 12 weeks.

2- Biochemical markers

TNF-α level of the ethanol group at six and twelve weeks significantly increased ($P<0.001$ for each) when compared with the control. Treatment with melatonin reduced significantly the level of TNF-α ($P<0.001$ for each) in melatonin+ ethanol treated group as compared to the ethanol treated groups. However, TNF-α level of the melatonin+ ethanol treated group for six and twelve weeks were significantly increased as compared to control groups ($P < 0.05$and $P < 0.01$ respectively) (Table 1,2).

Estimation of serum level of VEGF in ethanol group at twelve weeks was significantly decreased as compared to the control group ($P< 0.001$). Treatment with melatonin for twelve weeks restored significantly its level ($P< 0.05$) when compared to the ethanol treated group, but it was still significantly lower than control group ($P< 0.01$). No significant difference in the serum level of VEGF between the ethanol treated, melatonin+

ethanol treated and control groups at six weeks were detected (table 1 and 2).

Compared with the control group Caspase-3 activity in renal homogenate was significantly increased in ethanol group at twelve weeks (P< 0.01). An administration of melatonin was associated with significant decrease in caspase–3 activity levels in the renal homogenate (P<0.05) as compared to the ethanol group. Comparing with control group, caspase-3 levels of the melatonin+ ethanol treated group at twelve weeks were significantly increased (P<0.01) (table 2). No significant difference in caspase-3 activity between the ethanol, melatonin+ ethanol treated group and control groups at six weeks (table 1).

Table (1): Mean ± SD of body weight, serum levels of TNF-α, VEGF, urea, creatinine, and caspase-3 in renal homogenate in different studied groups at six weeks.

	Control n = 8	Eth(6W) n = 8	Mel + Eth (6W) n = 8
BW **(g)**	178.50± 5.10	165.10± 5.20 ***	170.80± 5.20 **, #
TNF-α **(pg/mL)**	3.38± 0.90	14.50± 0.90 ***	4.90± 1.10 *, ###
VEGF **(ng/mL)**	56.25± 4.20	52.50± 3.20 NS	54.38± 2.20 NS, ns
caspase-3 **(U/mg tissue weight)**	4.00± 0.8	4.75± 0.70 NS	4.25± 0.70 NS, ns
Serum urea **(mg /dl)**	14.13± 1.60	15.38± 2.10 NS	14.75± 1.90 NS, ns
Serum creatinine **(mg /dl)**	0.47± 0.06	0.51± 0.03 NS	0.49± 0.02 NS, ns

* P <0.05, ** P<0.01, *** P<0.001, NS: non significant as compared to control group. # P< 0.05, ### P<0.001 and ns: non significant as compared to Eth. group at six weeks. BW= body weight, Et= ethanol, Mel+ Eth= melatonin+ ethanol, VEGF= vascular endothelial growth factor

As shown in the table (2), serum urea and creatinine increased significantly in ethanol treated group at twelve weeks compared to the control (P< 0.001 for each). The reduction were obvious and significant with the melatonin treatment (P< 0.001and P<0.01 respectively) at twelve weeks when compared to ethanol treated group, but their levels were still significantly higher than the control groups (P<0.05 for each).

Arterial blood pressure

According to figure (1a&b, 2a&b) the animals treated with ethanol for six and twelve weeks displayed significant increased in both SBP and DBP when compared to both the control (P<0.01for SBP at 6 w, P<0.05 for DBP at 6w and P<0.001for both SBP and DBP at 12 Ws). Melatonin supplementation ameliorated significantly both SBP and DBP when compared to both ethanol groups (at six weeks P<0.05 for each, at twelve weeks P<0.001for SBP and P<0.01 for DBP). In melatonin+ ethanol treated group for six and twelve weeks there were significant increase (P<0.05 for all) when compared with the control.

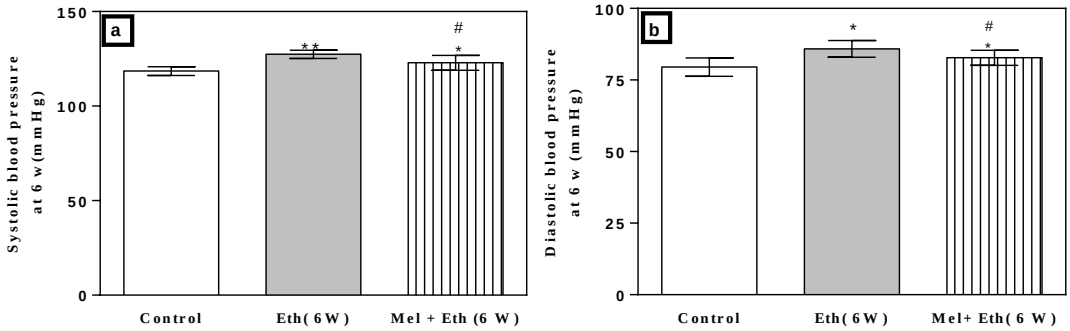

Figure (1), a: mean systolic blood pressure b: mean diastolic blood pressure in the studied groups at six weeks.*: P< 0.05 and **: P<0.01 as compared to control group.#: P< 0.05 as compared to Eth group.

Table (2): Mean ± SD of body weight, serum levels of TNF-α, VEGF, urea, creatinine, and caspase - 3 in renal homogenate in different studied groups at twelve weeks.

	Control n = 8	Eth(12W) n = 8	Mel+ Eth (12 W) n = 8
BW **(g)**	189.63 ± 7.46	166.75± 1.49 ***	178.38 ±3.42 **, ###
TNF-α **(pg/mL)**	3.31± 0.80	15.75 ± 1.00 ***	5.10± 0.99 **,###
VEGF **(ng/mL)**	55.88 ± 3.40	47.00± 4.40 ***	51.25± 2.50 **, #
caspase-3 **(U/mg tissue weight)**	3.90± 0.70	5.88± 0.80 **	4.88± 0.80 *, #
Serum urea **(mg /dl)**	14.25±1.40	22.13± 2.60 ***	16.75± 2.10 *,###
Serum creatinine **(mg /dl)**	0.47±0.05	0.57± 0.02 ***	0.52± 0.02 *, ##

* P <0.05, ** P<0.01, *** P<0.001, as compared to control group. # P< 0.05, ##P<0.01 and ### P<0.001 as compared to Eth group at twelve weeks. BW= body weight, Et= ethanol, Mel+ Eth= melatonin+ ethanol, VEGF= vascular endothelial growth factor

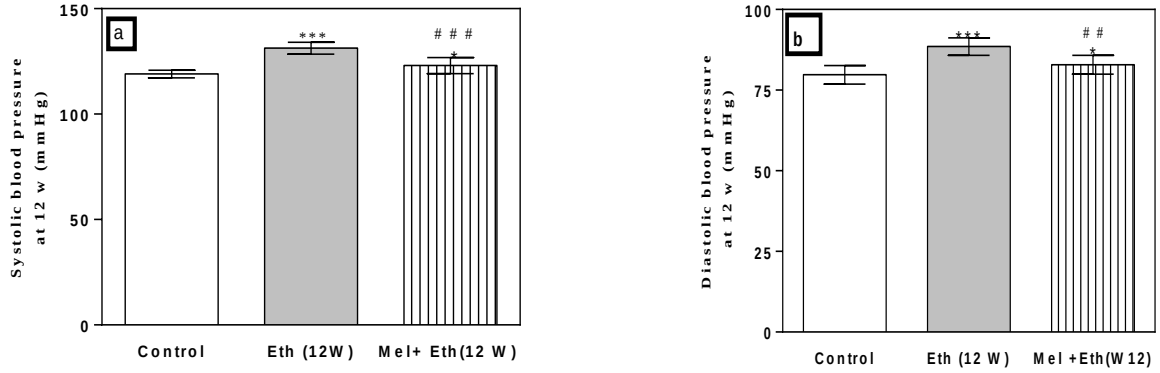

Figure (2), a: mean systolic blood pressure b: mean diastolic blood pressure in the studied groups at twelve weeks.*: P< 0.05 and ***: P<0.001 as compared to control group. ##: P< 0.01 and ###: P<0.001 as compared to Eth group.

Histological changes:

Control group: (Fig.3a)

The renal cortex revealed the renal corpuscle includes the glomerulus, Bowman's capsule and the vascular pole. The glomerulus was formed of collection of convoluted capillary blood vessels. Bowman's capsule deeply invaginated by the glomerulus. It was lined by a simple squamous epithelium on its outer wall.

The proximal tubules were lined with cuboidal epithelium with a high brush border. Cell borders were barely visible. The cytoplasm deeply stained and frequently appeared granular, turbid and diffuse. The distal tubules diameters were considerably smaller than those of proximal tubules. There was no brush border.

Ethanol treated for six weeks (Fig.3b) and for twelve weeks (Fig.3c):

Following ethanol treatment for six weeks dilatation, congestion and thick wall peritubular vessels was seen. Renal corpuscles with dilated bowman's space were obvious. Dilated tubules lined by thinner epithelium with degenerated cells at some areas were also noticed.

However, widened capsular space, degeneration and necrosis of renal tubular epithelia were more obvious following twelve weeks ethanol treatment. These damaging changes reflected toxic effects of ethanol with duration of exposure. Atrophic renal corpuscles in some of fields also noticed.

Melatonin+ ethanol treated group for six weeks (Fig.3d) and for twelve weeks (Fig.3e):

The co-administration of melatonin with ethanol for six and twelve weeks ameliorated the toxic changes of ethanol. Dilatation, congestion and thick wall peritubular vessels were less marked. Renal corpuscles were more or less healthy. These findings reflected the protective effects of melatonin on ethanol toxicity.

DISCUSSION

It is known that alcohol dependence and alcohol abuse causes substantial morbidity and mortality (29). Alcohol consumption has deleterious effects on many vital organs including the kidney (30).

In this work, suppression effects of ethanol on the body weight coincide with (31) who demonstrated a reduction in the body weight of the ethanol treated rats which may be adduced to malnutrition resulting from reduced absorption of nutrients from the intestine. Chronic alcohol consumption provokes greater energy expenditure for that, it was associated with body weight loss(3,32). Melatonin+ ethanol treated group showed restoration in the body weight that is consistent with the result of (33) who demonstrated that, melatonin promotes food intake in rats by decreasing in the food transit time which lead to empty gastrointestinal tract (GIT) more rapidly and trigger an increase in food intake. Also **Sokolovic et al.** (34) showed that melatonin administration to irradiated irritable rats caused an increased body weight which was attributed to melatonin mediated anti-stress effect.

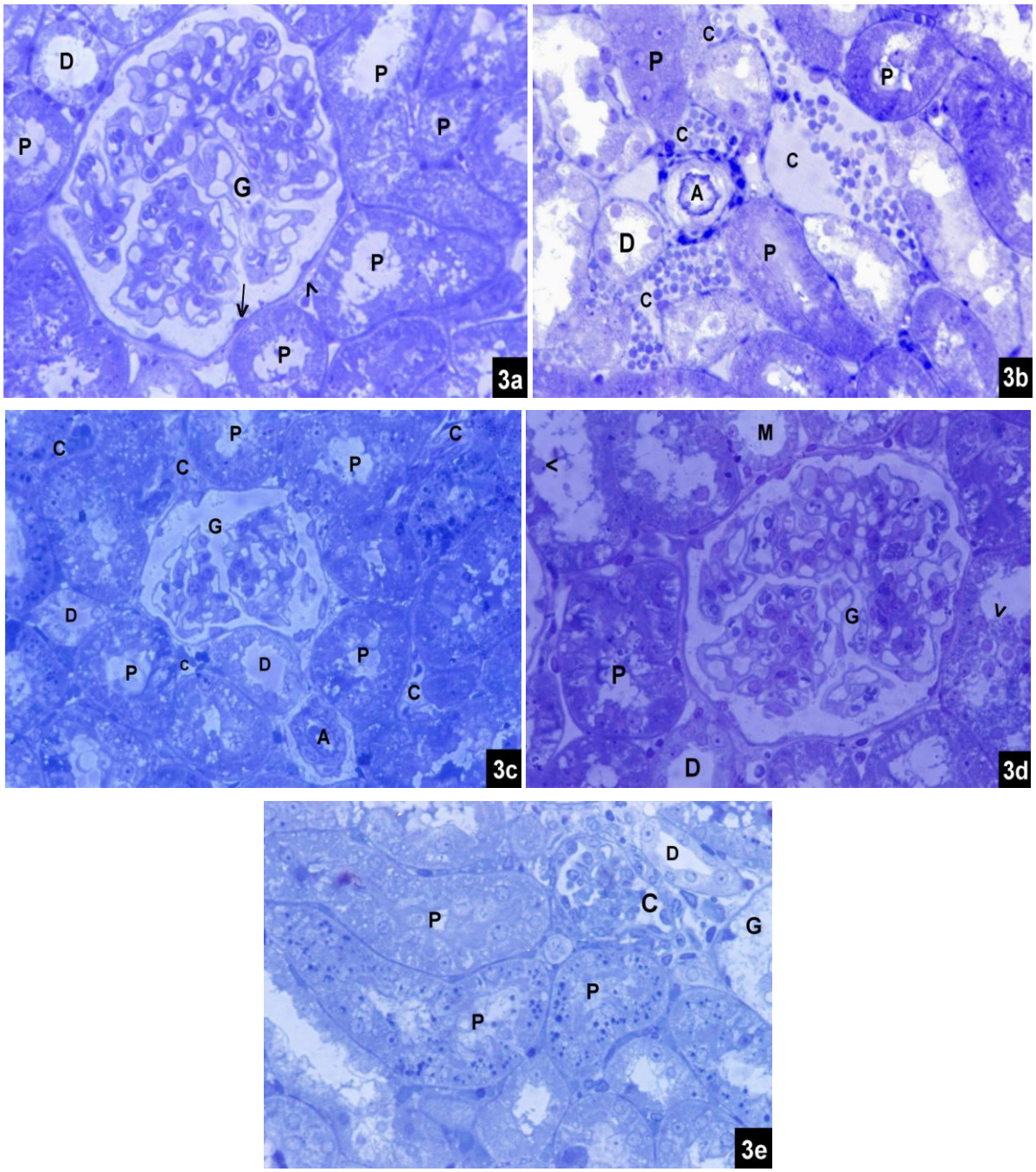

Fig. (3) Histopathological examination of kidney tissues, a) shows: glomerulus (G) lined by a simple squamous epithelium on its outer wall (arrow head) and specialized epithelial podocytes (arrow) on its glomerular wall, proximal convoluted tubules (P) and distal convoluted tubules (D) of control albino rat kidney, **(b)** shows: proximal convoluted tubules (P), distal convoluted tubules (D) with destruction of its epithelial lining at some areas, thick wall arteriole (A) and congested capillaries with blood (C) in ethanol treated rats for six weeks, **(c)** shows: atrophic glomerulus (G), proximal convoluted tubules (P), distal convoluted tubules (D) with destruction of its epithelial lining at some areas, thick wall arteriole (A) and congested capillaries with blood clots (C) in ethanol treated rats for twelve weeks, **(d)** shows: glomerulus (G) lined by a simple squamous epithelium on its outer wall and specialized epithelial podocytes on its its glomerular wall, proximal convoluted tubules (P), distal convoluted tubules (D) and macula densa (M) of melatonin+ ethanol treated group rats for six weeks and **(e)** shows: part of glomerulus (G), proximal convoluted tubules (P) with their epithelial lining rich in lysosomes, distal convoluted tubules (D) and congested blood capillaries (C) of melatonin+ ethanol treated group for twelve weeks. (Toludine blue X 400)

The increment of both SBP & DBP in ethanol treated rats at six and twelve weeks in this study are in agreement with (35) who found that long term ethanol treatment induced the development of pro-inflammatory and pro-atherosclerotic processes, with an increase in blood pressure, suggesting a potential direct implication of ethanol itself in induction of hypertension via inflammation processes.

Overall, the results of this study ethanol treatment for twelve weeks lead to significant increment of urea and creatinine concentration in serum are in lines with the observations of (36, 37) who found significant elevation in blood urea and creatinine levels after twelve weeks of ethanol exposure which indicate initiation of kidney damage, disruption in the structure and function of the kidneys.

It is widely accepted that ethanol consumption causes markedly increase in the serum TNF-α level after six and twelve weeks that in accord with finding of (38, 39, 40) Who demonstrated that ethanol feeding resulted in increased TNF-α. It triggers release of mitochondrial cytochrome C, sequentially activating caspases-9 and -3, and resulting in DNA fragmentation and cell death (41).

In patients with hypertension there was an increased production of TNF-α and IL-6, which might play an important role in the initiation and progression of hypertension by a variety of mechanisms such as contraction of vessels, leading to thickening of vascular wall, and proliferation of endothelial cells and smooth muscle cells (42) TNF-α has a role in the pathogenesis of acute and chronic renal disease (43).

Furthermore, it is accepted that the significant increase in TNF-α from six weeks in ethanol treated group may be contributed to increase the blood pressure then this increment lead to risk of renal damage as evident by the marked increase in urea and creatinine concentration in serum of ethanol treated group for twelve weeks . This finding was in agreement with **Parekh and Klag** (2) who found that Chronic alcohol consumption has been linked with hypertension and therefore indirectly with chronic kidney disease.

It seems probable that the observed, significant increased in renal caspase-3 activity with ethanol treatment for twelve weeks in the present work has a role in the pathogenesis of the renal damage this agrees with study of (44) who revealed that acute and chronic ethanol treatment resulted in significant increases of the hepatocyte apoptosis. Increment of caspase-3 with ethanol consumption may be due to mitochondrial damage, which induces a signaling cascade that activates downstream apoptosis mediators, such as caspase-3, and promotes chromatin degradation, decreases oxygen consumption and ATP production, which can ultimately lead to the initiation of apoptosis (29). Hypertensive renal injury was associated with evidence of activation of the apoptotic pathway (increased activation of caspase-3) (45).Apoptotic death of renal endothelial cells could conceivably lead to a decline in GFR by causing changes in renal vascular tone or by exposing procoagulant subendothelial surfaces(46).

Taking into account that, the marked decrease in serum VEGF level in ethanol treated rats after twelve weeks is in consistent with (47) who

reported that a decrease in VEGF induced by ethanol in rats' vascular wall. This decrement of VEGF could be due to decreased expression of VEGF receptors and their phosphorylation with ethanol exposure (48). In rodent and human kidneys, VEGF mRNA and/or protein were detected predominantly in glomerular podocytes, distal tubules, and collecting ducts, and to a lesser extent in some proximal tubules (49).In addition, experimental nephron reduction resulted in early peritubular and glomerular endothelial cell proliferation followed by a progressive loss of peritubular and glomerular capillaries, which associated with a decreased VEGF staining in tubules and glomerular podocytes (50).

Moreover, the structural injury as atrophic renal corpuscles, dilatation and congestion of the peritubular vessels and a few foamy-appearing tubules recorded by the histopathological study in this work in the ethanol group that coincides with (30, 51, 52).

In the present study, supplementation with melatonin showed decrease in the arterial blood pressure in melatonin+ ethanol treated group for six and twelve weeks which can be attributed to, the anti-inflammatory, anti apoptotic and angiogenic properties of melatonin. This observation was confirmed by **Nava et al.**(53) who showed that in spontaneously hypertensive rats, blood pressure decreased after six weeks of melatonin treatment, which was associated with a reduction of interstitial renal tissue inflammation, decreased oxidative stress and attenuation of expression of NF-κB in the kidney.

Adewole et al.(23), found that the results of the control and melatonin groups were similar on kidney function parameters and morphology so we exclude the administration of melatonin as a separate control group.

It is important to highlight in this work that the rats in melatonin + ethanol group for twelve weeks which received melatonin decreased significantly serum urea and creatinine levels and preserved more or less the normal morphology of the kidney as compared to the control group. This study is in line with **Altintas et al.**(54) who found that melatonin significantly diminished serum levels of urea, creatinine and improve the histopathological changes induced by acetylsalicylic acid on kidney.

In the current study, treatment with melatonin significantly decreased serum level of TNF-α in melatonin + ethanol groups that is in consistent with (55). They indicated that melatonin administration lowered the expression of TNF-α and IL-1β in hepatic ischemia/reperfusion injury. In addition, melatonin may be exert its anti-inflammatory effects through the inhibition of the activated NF-κB, limitation of excessive production of leukotrienes and other inflammatory mediators such as cytokines, chemokines, and adhesion molecules (56, 57). Melatonin protects rats from damage to proximal tubular mitochondria by attenuating oxidative stress and inflammation (58). It has been shown to ameliorate inflammation by blocking transcriptional factors and TNF-α(59).

Importantly, the major findings presented in this work demonstrate that melatonin reduced caspase-3 concentration with consequent reduction of apoptotic and necrotic cell death in the kidney. This results is in accordance with the other studies that confirmed melatonin decreased the release of

cytochrome c into the cytoplasm, thus decreasing the activation of caspase-3 in a rat model of obesity and hepatic steatosis(60). It inhibits caspase-3 activation and prevents apoptotic and necrotic cell death in the kidney (61). Melatonin induced angiogenesis via upregulation of VEGF (62).It brings about significant proangiogenic activity by increasing VEGF expression (63).The ability of melatonin to enhance angiogenesis has been elucidated in gastrointestinal mucosa (64) and during bone repair (65).

Conclusion: The present study clearly demonstrated the renoprotective effects of melatonin in a model of chronic ethanol consumption induced renal damage in adult male rats which could be due to its anti-inflammatory, anti-apoptotic and its angiogenic properties. Also, it may have therapeutic benefits in treatment of hypertension.

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دور الميلاتونين في تعديل ضغط الدم الشريانى والتليف الكلوى الناجم عن استهلاك الإيثانول فى ذكورالفئران البالغة

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مقدمة: تناول الإيثانول يؤدي إلي ارتفاع ضغط الدم والعديد من صور الخلل الكلوي الوظيفي . يعتبر الميلاتونين من مضادات الأكسدة وله تأثير مضاد للالتهابات.

الهدف من الدراسة: تهدف هذه الرسالة للكشف عن دور الميلاتونين على ضغط الدم الشرياني وآثاره الوقائية المحتملة ضد الخلل الكلوي الوظيفي الناجم عن تناول الايثانول في ذكور الفئران البالغة مع محاولة تفسير هذا التأثير الوقائي باستخدام أساليب بيوكيميائية وهستولوجية لتوضيح هذا التأثير .

الأساليب: أستخدمت في هذه الدراسة ثمانية وأربعون من الفئران البيضاء قسمت إلى ثلاث مجموعات. المجموعة الأولي: هي المجموعة الضابطة ، و المجموعة الثانية: هي مجموعة الإيثانول حيث أعطيت حيوانات هذه المجموعة 10 مل / كغم من وزن الفأر 30٪ من الإيثانول بواسطة أنبوب داخل المعدة في أيام متبادلة. و المجموعة الثالثة: مجموعة الميلاتونين + الإيثانول حيث أعطيت حيوانات هذه المجموعة الايثانول بنفس الحرعة السابقة مع جرعة 5 ملغ / كغ من الميلاتونين حقن تحت الجلد في أيام متبادلة. تم التضحية بنصف الحيوانات من كل مجموعة بعد ستة اسابيع والباقى بعد اثني عشر أسبوعا.

النتائج: الحيوانات التي أعطيت الايثانول لمدة 6 ،12 أسبوع كان هناك انخفاض ملحوظ في الوزن الجسم ، إرتفاع معنوي في ضغط الدم وعامل نخر الورم ألفا .(TNF-α) . و أوضحت المجموعة التي أعطيت الايثانول لمدة 6 أسابيع أن اليوريا في الدم والكرياتينين، وعامل النمو البطاني الوعائي (VEGF) ، والكاسباس الكلوى-3 لم يتغير تغيرا معنويا ، أما التي أعطيت الايثانول لمدة 12 فقد لوحظ إرتفاع هذه المعايير إلى إرتفاعا معنويا باستثناء VEGF التي انخفضت بشكل ملحوظ. كما لوحظ تغييرات هستولوجية مرضية في الحيوانات التي أعطيت الايثانول لمدة 6 ،12 أسبوع بينما تحسنت هذه التغيرات المرضية في المجموعة التي تناولت الميلاتونين مع الإثانول.

التوصيات: خلصت الدراسة إلى ان الميلاتونين له دور وقائي في ارتفاع ضغط الدم الشريانى و ضد التلف الناتج عن تناول الايثانول فى الكلى.