Original Article

COMPARISON OF POTENTIAL PROTECTIVE EFFECTS OF APOCYNIN AND MELATONIN AGAINST GENTAMICIN-INDUCED NEPHROTOXICITY IN ADULT MALE ALBINO RATS

Allia Mohammed Mahmoud[1], Zeinab Abdou Mohammed[1] and Mona Mostafa Ahmed[2]

[1]Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine – Zagazig University- Egypt.

[2]Pathology department, Faculty of Medicine – Zagazig University- Egypt.

Corresponding author:
Allia Mohammed Mahmoud
Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Zagazig University

Email address:
aliaMahmoud@gmail.com.

ABSTRACT

Background: Gentamicin (GNT) as an aminoglycoside antibiotic is commonly used against life threatening bacterial infections, however, the risk of nephrotoxicity is the main limitation of GNT therapeutic indication. **Aim of the Work**: This study was designed to evaluate the potential protective effects of apocynin (APO) and melatonin (MEL) against GNT induced nephrotoxicity in rats. **Material and Methods**: This study was carried out on thirty-two adult male albino rats randomly divided into 4 equal groups, GroupI (negative control), GroupII (GNT group) each rat was given intraperitoneal (ip) injection of (100mg/kg) of GNT daily for 7 days, Group III (APO/GNT) each rat was given (ip) injection of (10mg/kg) APO for 7 days started before administration of APO (10mg/kg) (ip) plus GNT (100mg/kg) (ip) for another 7 days. Group IV (MEL/GNT) each rat was given (ip) injection of (15mg/kg) MEL for 7 days started before administration of MEL (15mg/kg) (ip) plus GNT (100mg/kg) (ip) for another 7 days. **Results**: Gentamicin induced nephrotoxicity, as rats received GNT significantly presented an increase in the 24 -h urine volume, renal somatic index (RSI), urine protein, creatinine (Cr), blood urea nitrogen (BUN), serum lactate dehydrogenase (LDH), and malondialdehyde (MDA) Furthermore, GNT induced a significant decrease in the body weight gain percentage, creatinine clearance (CCr), superoxide dismutase (SOD) and glutathione peroxidase (GPX) in comparison to control rats. Gentamicin also induced hypercellularity in mesangial cells and renal tubular epithelium degeneration. Either APO or MEL significantly decreased 24- h urine volume, RSI, urine protein, Cr,BUN, , LDH, and MDA additionally, either APO or MEL caused a significant increase in body weight gain percentage, CCr, SOD, and GPX when compared to GNT group, moreover, either APO or MEL showed ren-oprotective effects histopathologically.

Conclusion: Apocynin and melatonin can attenuate nephrotoxic effects in rats treated with GNT possibly through anti-oxidant effect and keeping normal renal tissue morphology.

KeyWords: Apocynin,antioxidant,Gentamicin,melatonin,nephrotoxicity.

I. INTRODUCTION

Gentamicin (GNT) is a broad spectrum antibiotic described for treatment of serious infections. The rapid effect and affordable cost, make GNT a first line therapy for various infections (Ghaznavi et al., 2016), however, the nephrotoxicity is the most therapeutic limitations of GNT use. Gentamicin has been implicated in 30% of acute renal failure cases world- wide, specifically it is accumulated up to (5 to 50) times in renal convoluted tubules more

than in serum"(Abdel-Raheem et al., 2009 and Moreira et al., 2016).Therefore, a therapeutic approach to renal protection upon and before GNT therapy would have critical important consequences clinically. Several mechanisms could be related to GNT nephrotoxicity as, ischemia in renal tissue, inflammation, lipid peroxidation, and depletion of kidney antioxidant enzymes (El-Kashef et al., 2016).

Apocynin is extracted from (Himalayan herb Picrorhiza kurrooa). Apocynin is an inhibitor of the NADPH-oxidase complex (NOX)present in the cell membrane (Altintas et al., 2013). "Reactive oxygen species (ROS) are produced in the mitochondrial respiratory chain during cellular respiration and oxidative reactions, and catalyzed by (NOX),which is considered as the major enzyme used by NADPH to generate(ROS) and one of its efficient inhibitors is APO" (wang et al., 2021). Apocynin can, attenuate fibrosis in the models of heart failure that accompany myocardial infarction (Sánchez et al., 2021), APO has been described as one of the most encouraging therapies in experimental studies for neurodegenerative diseases and recentely has been supplied commercially as a novel antiaging agent, but its potential reno-protective effect against GNT has not been demonstrated (Abdelrahman,2018).

Melatonin is a hormone produced by the pineal gland, bone marrow, retina, and the immune system with a major role in modulating the circadian rhythm. It also has an anti-inflammatory, immune-modulatory effect, and might have a protective effect against oxidative stress (Ahmed et al., 2021).Recently, the melatonin supplements are widely used not only to prevent insomnia, but also to attenuate the aging however, the mechanism of melatonin antioxidant activity is still unknown (Chrustek and Olszewska-Słonina , 2021).

The present study was carried out to investigate the potential protective effects of Apocynin and Melatonin against Gentamicin induced nephrotoxicity in adult male albino rats.

II. MATERIAL AND METHODS

II.1 MATERIALS

II.1.1. Drugs and chemicals

Apocynin and Melatonin (powders) were purchased from Sigma-Aldrich Co. (St. Louis, Missouri,USA), Gentamicin:(40 mg) ampoules were obtained from Alexandria Pharmaceutical and Chemical Industries Company, Egypt.

II.1.2. Experimental animals

The study was carried out on thirty -two adult male albino rats with average weight of (200-230gm). They were obtained from the animal house of Zagazig Scientific &Medical Research Center of the Faculty of Medicine, Zagazig University. The study had been designed in the Faculty of Medicine, Zagazig University. Ethical consideration of experimental animals were according to recommendations of "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985). The Rats were randomly divided into four equal groups (n=8) as:

-Group I: (negative control group) to determine the basic values of tested parameters and did not receive any solvent or drug.

-GroupII: (GNT) group ,each rat injected by GNT (100mg/kg), (ip) for 7 days.

-Group III: (APO/GNT)group each rat injected by APO(10mg/kg) (ip) was dissolved in drinking water at a concentration of (1mg/ml) for 7 days then APO(10mg/kg)(ip) dissolved in drinking water at a concentration of (1mg/ml) plus GNT (100mg/kg))(ip) for another 7 days.

-Group IV: (MEL/GNT) group, each rat injected by MEL(15mg/kg)(ip) for 7 days dissolved in drinking water at a concentration of (1mg/ml) then MEL(15mg/kg)(ip) dissolved in drinking

water at a concentration of (1mg/ml) plus GNT (100mg/kg)(ip)for another 7 days. The doses of gentamicin, melatonin, and apocynin were chosen according to the previous dose–response studies of Karahan, et al**.** (2005), Ghaznavi et al. (2016) and Abdelrahman (2018) respectively.

II.2. Methods:

Each rat was weighted at the start and at the end of experiment, weight difference was calculated then weight gain percentage was calculated by dividing the weight difference of each rat on its initial weight. After the last dose, rats were immediately kept in metabolic cages individually, for a collection of 24-h urine samples, Then urine samples were centrifuged for 15 min and kept at -20 C until analyzed, then, by using ether, the rats were anesthetized. Blood samples were collected and allowed to clot, Then serum samples were produced through centrifugation and stored frozen for biochemical measures, then rats were sacrificed by cervical dislocation. The kidneys were rapidly and carefully removed, rinsed with saline, then weighed for calculation of renal somatic index (RSI). RSI = (the weight of kidney (gm))÷ (the final weight of the body (gm)× 100). The right kidneys were homogenized in phosphate buffer centrifuged, and then the supernatants were used for the biochemical measures.

II.2.1. Biochemical measurements in the serum:

The Serum creatinine (Cr) and (BUN) levels were assessed according to Bartels et al.(1972) and Fawcett and Scott,(1960) respectively. The Serum creatinine and BUN were expressed as milligrams per deciliter (mg/dl). In addition,the lactate dehydrogenase (LDH) was assessed according to method of Henry et al., (1960) and expressed as a unit per liter (U/L).

II.2.2-Biochemical measurements in urine:

The urine (Cr) and protein levels were measured according to method of Bartels et al.(1972) and Daughaday et al.(1952) respectively, and expressed as milligrams per deciliter and milligrams per day, respectively. "the Creatinine clearance (CCr) was used to assess the glomerular filtration rate, calculated using this formula ,CCr= Cr in urine (mg/dl) ×urine flow (ml/min)/Cr in serum (mg/dl)." Urine flow was calculated by dividing urine volume of 24 h by 1440 (the number of minutes in day) and expressed in milliliters per minute.

II.2.3.Biochemical measurements in kidney tissue homogenate

The end product of lipid peroxidation (MDA) levels were assayed through monitoring of thiobarbituric acid reactive substance, and the biomarkers of oxidative stress (SOD and GPX) activities were determined in renal homogenate according to Ohkawa et al. (1979) Sun et al. (1988). and Paglia and Valentine (1967). respectively and expressed as nanomoles per gram tissue (nmol/g. tissue), units per gram tissue (U/g.tissue) and unit per mg protein (U/mg prot)

II.2.4. Histological examinations:

Samples from right kidneys were routinely processed, embedded in paraffin blocks and sectioned at 5µ thick sections. Sections were stained with haematoxylin and eosin and examined by light microscope **(**Bancroft and Stevens, 1996).

II.2.5.Statistical analysis

The results are reported as mean ± SEM. The statistical analyses were performed using one-way analysis of variance (ANOVA) by SPSS software (version 20). Group differences were calculated by post hoc analysis using Tukey-kramer test

III. RESULTS:

III.1. Weight gain percentage

No deaths were observed in all groups..A decrease in weight gain percentage caused by GNT was significantaly ($p < 0.05$,) observed on comparison to percentage of weight gain in control group, while Pretreatment with either APO or MEL significantly ($p < 0.05$,) attenuated the GNT-induced decrease in weight gain percentage , (Table 1).

III.2. Renal somatic index

(Table 1) shows a significant ($p < 0.05$,) increase in RSI in GNT group on comparison to the control group . while pre and concomitant treatment with either APO or MEL significantly lowered the RSI when compared to GNT group.

III.3- 24 h urine volume

Gentamicin caused a significant ($p < 0.05$) increase the 24-h urine volume on comparison to the control group while The 24-h urine volume was significantly ($p < 0.05$) decreased in APO/GNT or MEL/GNT group when compared to GNT-treated rats (Table 1).

III.4. Biochemical results:

(Table 2) shows a significant ($p < 0.05$,) increase in urine protein, serum, BUN and CR in GNT group on comparison to control group . however Pre and concomitant administration of either APO or MEL significantly ($p < 0.05$,) attenuated the GNT-induced increase in these parameters. GNT caused a significant ($p < 0.05$) decrease in (CCr) when compared to the control group while The CCr was significantly ($p < 0.05$) increased in either APO/GNT or MEL/GNT group on comparison to GNT-related rats(table2).

(Figure 1,2) show that GNT significantly ($p < 0.05$,) increased LDH and MDA on comparison to control rats while, Pre and concomitant injection with either APO or MEL in rats produced a significant ($p < 0.05$,) decrease in LDH and MDA, when compared with (GNT) group. (Figure 3,4) show that GNT significantly ($p < 0.05$) decreased SOD and GPX when compared to control rats, while Pre and concomitant administration of either APO or MEL significantly ($p < 0.05$,) increased SOD and GPX on comparison to GNT-treated rats. Furthermore, MEL/GNT group showed decrease in MDA and increase in SOD and GPX , however that decrease not reached statistically difference when compared to APO/GNT group yet.

III.4. Histopathological results:

Light Microscopic examination of (H&E) stained renal sections of control groups showed normal glomeruli and normal renal tubules with normal lining epithelium (Figure 5). Light Microscopic examination of (H&E) stained renal sections of rats received (100mg/kg GNT) ip for 7 days **(**group II), revealed prominent hypercellularity in mesangial cells with degeneration of renal tubular epithelium (Figure 6), while administration of either Apo or Mel 7days before combined administration of either APO or MEL with GNT showed normal glomeruli and normal renal tubules with normal lining epithelium (Figure 7,8) respectively

Table (1) : Statistical comparison between tested groups regarding (weight gain percentage,renal somatic index and 24 h urine volume) by one-way ANOVA followed by Tukey -Kramer multiple comparisons post hoc test.

Groups	Percentage of weight gain%	renal_somatic index (gm)	24 hours urine_vol (ml/100 g body weight)
negative control	6.99 ± 0.79	0.49 ± 0.01	2.85 ± 0.17
GNT	1.95 ± 1.68 (a)	0.62 ± 0.03 (a)	5 ± 0.33(a)
APO /GNT	5.65 ± 0.87(b)	0.54 ± 0.03(b)	3.58 ± 0.35(b)
MEL/GNT	4.51 ± 1.19(b)	0.57 ± 0.03(b)	3.61 ± 0.26(b)

GNT: gentamicin (100 mg/kg, i.p.); APO: apocynin (10 mg/kg, i.p.); MEL:melatonin(15mg/kg i.p.); SEM: standard error of the mean; ANOVA: analysis of variance. Data are expressed as mean ± SEM, n =8. (a): $p < 0.05$, significantly different from control group ; (b): $p < 0.05$, significantly different from GNT group .

Table (2): Statistical comparison between tested groups regarding (kidney functions tests) by one-way ANOVA followed by Tukey -Kramer multiple comparisons post hoc test.

Group	serum creatinine (mg/dl)	CCr (ml/min)	BUN (mg/dl)	Proteinuria (mg/day)
negative control	0.57 ± 0.03	0.28 ± 0.01	27.54 ±1.38	92.85 ± 2.82
GNT	1.14 ± 0.12(a)	0.18 ± 0.01(a)	47.34 ± 4.06(a)	141.21 ± 11.53(a)
APO/GNT	0.95 ± 0.14(b)	0.21 ± 0.02(b)	39.45 ± 4.48(b)	121.3 ± 8.42(b)
MEL/GNT	0.82 ± 0.09(b)	0.25 ± 0.02(b)	35.41 ±2.57(b)	119.79 ±7.04(b)

GNT: gentamicin (100 mg/kg, i.p.); APO: apocynin (10 mg/kg, i.p.). ; MEL:melatonin(15mg/kg i.p.). CCr: creatinine clearance; BUN: blood urea nitrogen; SEM: standard error of the mean; ANOVA: analysis of variance. Data are expressed as mean ±SEM, n = 8.

(a):$p < 0.05$, significantly different from control group ; (b):$p < 0.05$, significantly different from GNT group

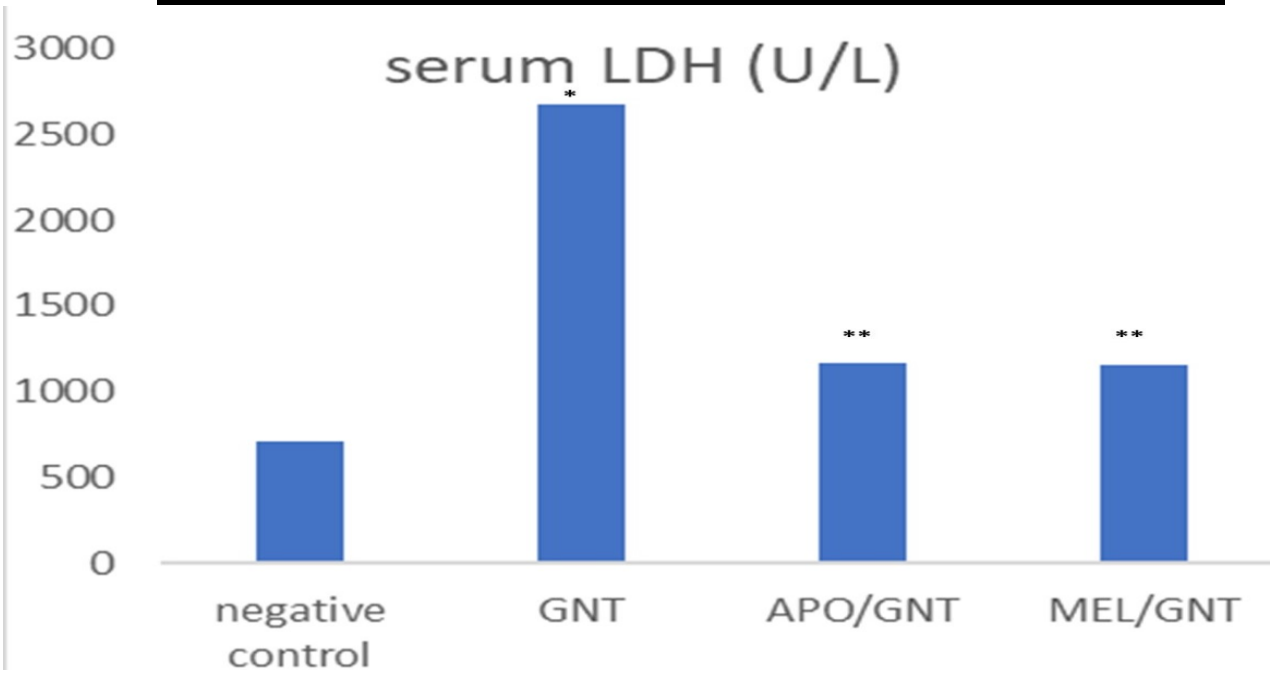

Figure (1). comparison of protective effects of APO and MEL against GNT-induced increase in serum LDH activity of rats. GNT:gentamicin, APO:apocynin ; MEL:melatonin ; SEM: standard error of the mean; ANOVA: analysis of varianc. Data are expressed as mean ±SEM, n = 8 *p < 0.05, significantly different from control group. **p < 0.05, significantly different from GNT group.

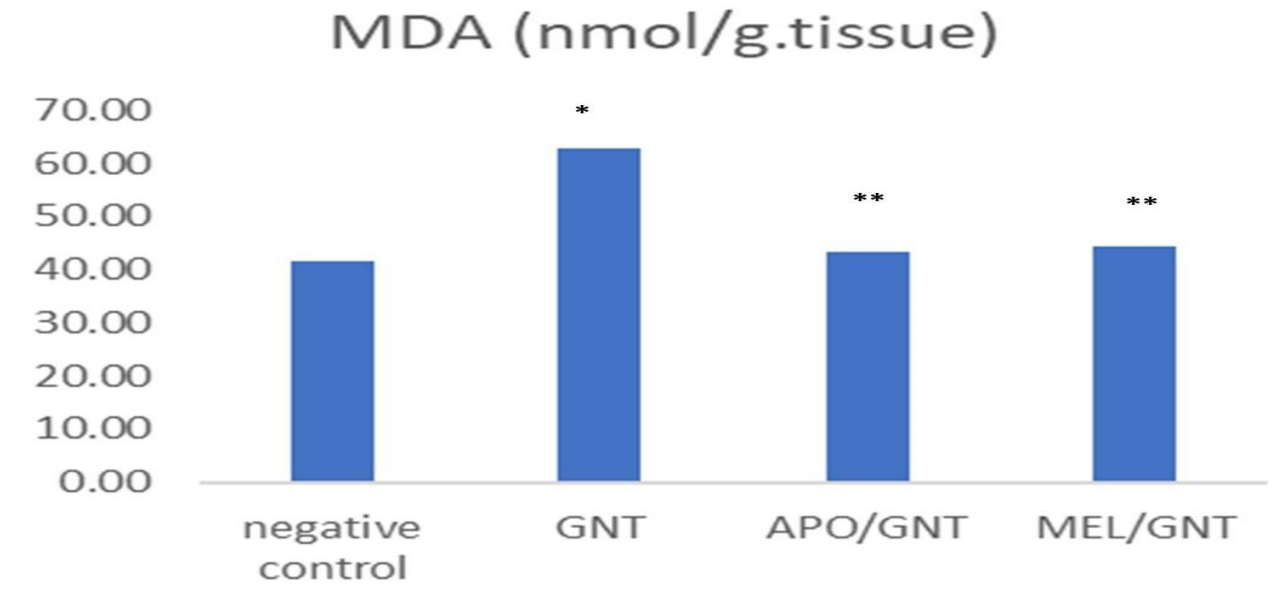

Figure (2): comparison of protective Effects of APO and MEL against GNT-induced increase in renal MDA activity of rats. GNT:gentamicin; APO:apocynin; MEL:melatonin. SEM: standard error of the mean; ANOVA: analysis of varianc. Data are expressed as mean±SEM, n = 8 ; *p < 0.05, significantly different from control group; **p < 0.05, significantly different from GNT group.

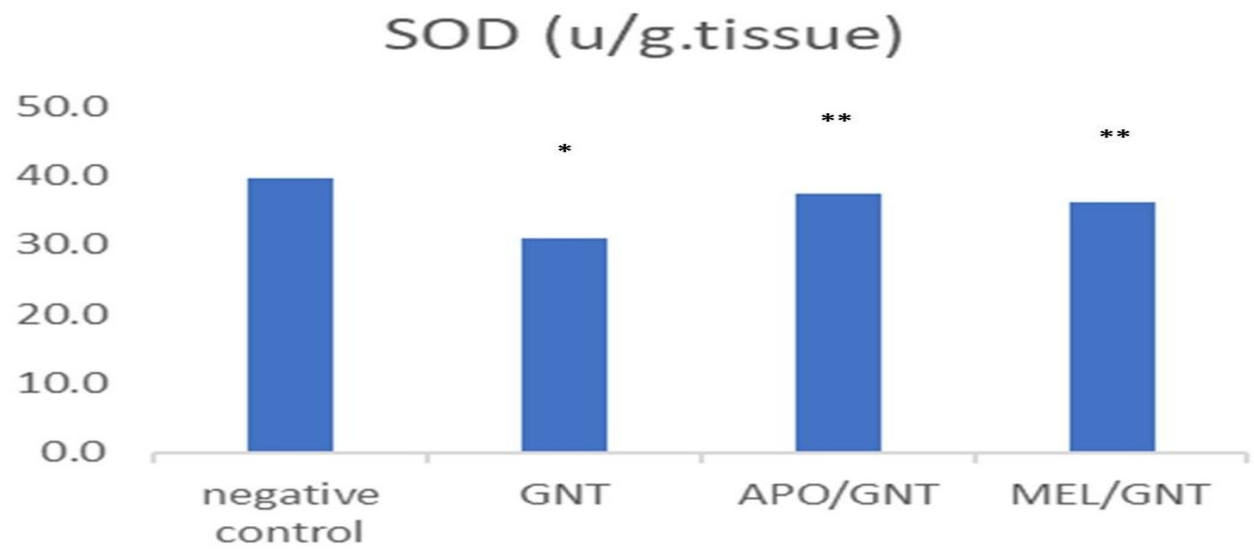

Figure (3): comparison of protective Effects of APO and MEL against GNT-induced decrease in renal SOD activity of rats. GNT: gentamicin. APO: (apocynin). MEL: (melatonin). SEM: standard error of the mean; ANOVA: analysis of varianc. Data are expressed as mean ±SEM, n = 8 ; *p < 0.05, significantly different from control group; **p < 0.05, significantly different from GNT group.

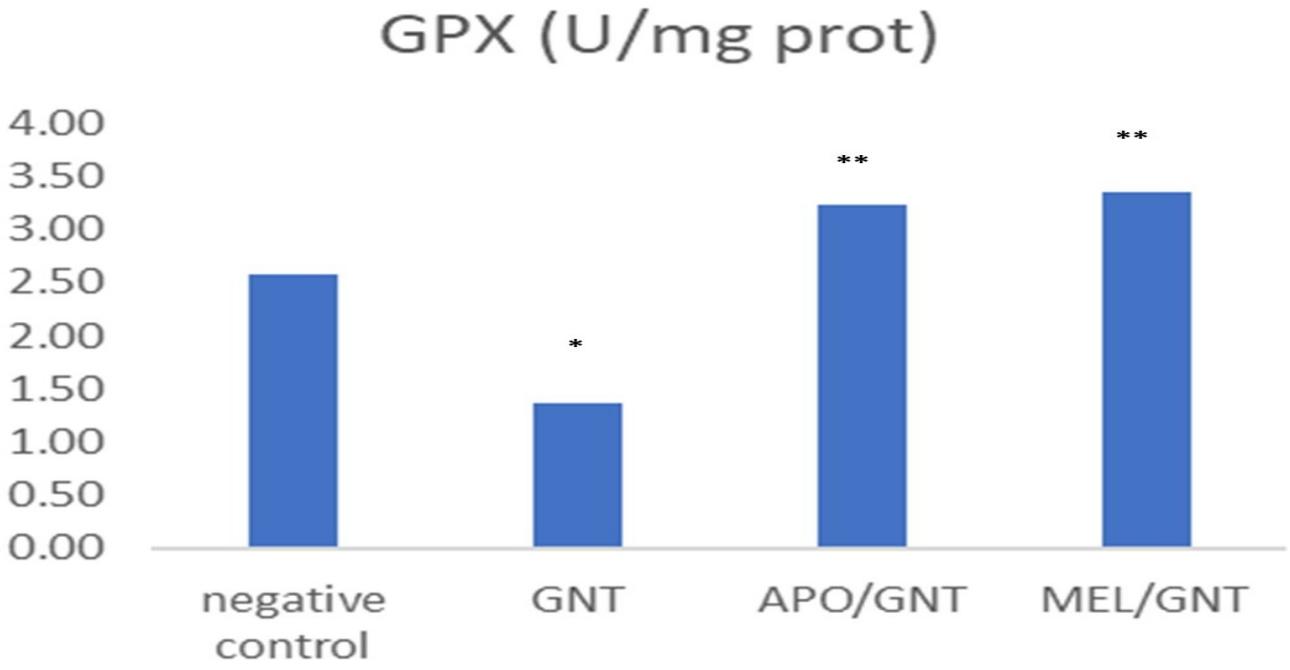

Figure (4): comparison of protective Effects of APO and MEL against GNT-induced decrease in renal GPX activity of rats. GNT: gentamicin. APO: apocynin. MEL: melatonin. SEM: standard error of the mean; ANOVA: analysis of varianc. Data are expressed as mean+SEM, n = 8 ; *p < 0.05, significantly different from control group ; **p < 0.05, significantly different from GNT group.

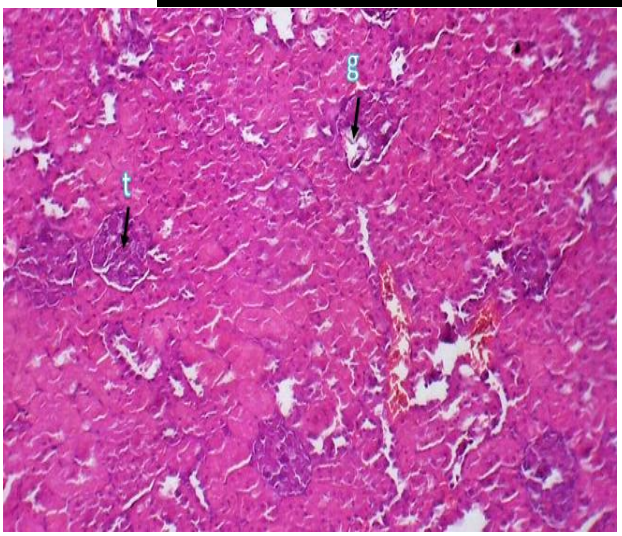

Figure (5): A photomicrograph of a section in the kidney from a rat of control group showed normal glomeruli (g) and normal renal tubules (t) with normal lining renal tubular epithelium (H&E x200).

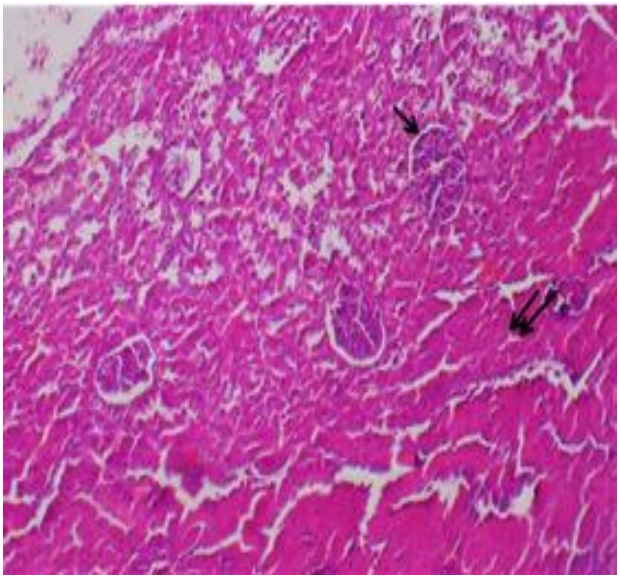

Figure (6): A photomicrograph of a section in the kidney from a rat of Gentamicin group: showed mesangial hypercellularity (arrow with degeneration of renal tubular epithelium (double arrows) (H&E (x200).

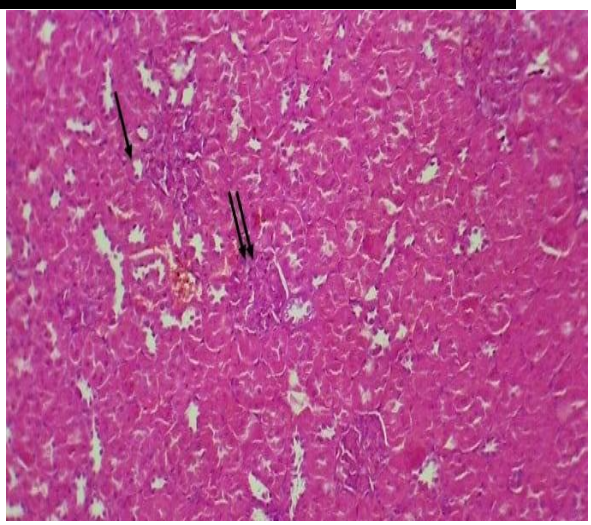

Figure (7): A photomicrograph of a section in the kidney from a rat of (Apo/GNT) group, GNT: gentamicin; APO: apocynin showed normal glomeruli (double arrows) and normal renal tubules with normal lining renal tubular epithelium (arrow) (H&E x200).

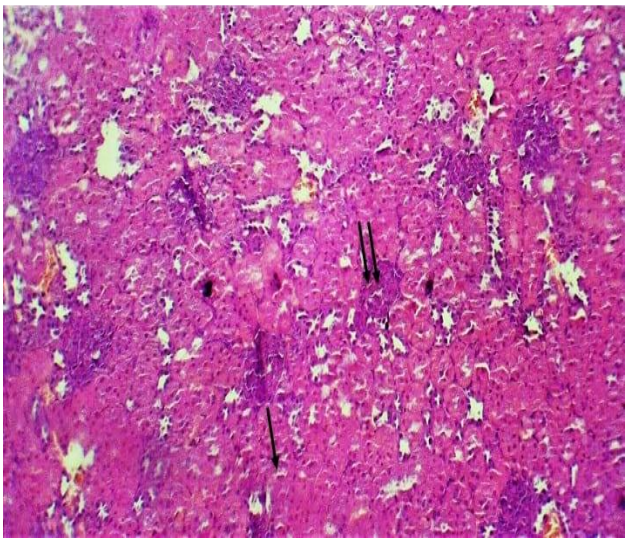

Figure (8): A photomicrograph of a section in the kidney from a rat of MEL/GNT group; MEL: melatonin; GNT: gentamicin showed normal glomeruli (double arrows) and normal renal tubules with normal lining renal tubular epithelium (arrow)(H&E x200).

IV. DISCUSSION

This study shows that either APO or MEL could protect and forming GEN-iron couples,which can be the first step in GEN-induced ROS formation (Priuska and Schacht, 1995).Lipid peroxidation is a proximal event in the cascade of GNT-induced nephrotoxicity. This is supported by an increase in MDA level, an index of lipid peroxidation, and decreases in antioxidant enzyme activities such as, SOD and GPX (Parlakpinar et al., 2005).

Either APO or MEL has a safe drug profile in the animal studies and utilized for long time without any signs of toxicity (Yu et al., 2008, Rodriguez et al., 2004 and Allegra et al., 2003).

In this work, either APO or MEL pretreatment caused a significant increase in the body weight gain percentages and a significant decrease in the RSI when compared to the GNTgroup, that is may attributed to either APO's or MEL's anti-inflammatory effects plus reversing histopathological changes of kidney upon GNT treatment (Kim et al., 2012 and Ghaznavi et al., 2016). These results are against GNT-induced nephrotoxicity, that is presented by APO or MEL ability to attenuate elevation in the RSI, serum Cr, BUN, urine protein, 24-h urine volume, LDH, and renal MDA induced by GNT, in addition to attenuation of GNT induced decrease in CCr, renal SOD and GPX.

Gentamicin is an effective aminoglycoside antibiotic however; toxic effects of this category limit its therapeutic indication (Mohamed and shenouda, 2021). In this work, GNT injection for 7days at dose of (100mg/kg ip) caused a significant decrease in the body weight gain percentages of rats and a significant increase in the RSI in comparison to control group. This restricted weight gain may be due to injury in renal tubules directly ,causing incapability of reabsorbing water by tubules, that led to dehydration and decrease in body weight (Ali et al., 2003).

The increase in weight of kidney in rats treated with GNT may be due to inflammation and edema (Erdem et al ., 2003). Gentamicin is absorbed through the renal tubular cells by anion transportation. Gentamicin accumulation in tubular cells finally leads to morphological changes, and increase in (ROS) in the kidney promoting inflammatory process (Ahmadvand et al.,2020). The body weight loss and increase in the kidney weight after administration of gentamicin to rats were also noted in other studies (Elkashef et al.,2016 and Nasri et al., 2013).

This study shows that administration of GNT to rats induced a significance increase in Cr, BUN, and urine protein in comparison to control group. Moreover, GNT induced a significant decrease in CCr in comparison to control group, these results indicate both glomular and tubular damage.

The decrease in glomerular filtration rate results in a reduction of the kidney's ability to filter Cr and the waste product of nonprotein is accumulated. Moreover, both urea and the urine protein also are increased which may pose to tubular dysfunction that impaired reabsorptive capacity of tubular protein(Abdelrahman, 2018) these results were also demonstrated in other studies(Christo et al., 2011 and El-kashef et al., 2016).

In the present study, GNT caused a significant increase in serum LDH on comparison to control groups that is may be

attributed to GNT induced renal inflammatory responses (Quiros et al., 2011) That increase in LDH upon GNT traetment also showed in El-Kashefe et al. (2015) study.

In this work, GNT induced a significant elevation in MDA and a significant decrease in SOD and GPX on comparison to control rats indicating GNT role in induction of oxidative stress. GNT is capable of generation of (ROS), and reactive nitrogen species (RNS). Several studies reported that GEN induces ROS formation by launching iron from mitochondria of renal cortex consistent with studies of Luchetti et al. (2010) and Connell et al. (2011).

In this study either APO or MEL pre and concomitant treatment with GNT caused a significant decrease in Cr. BUN, and urine protein and a significant increase in CCr when compared to GNT alone treatment .The ameliorative effect of APO on these kidney toxicity indicators may be attributed to the antioxidant effect and protection against both glomerular and tubular injury as "APO is a selective NOX inhibitor, which generates superoxide.The superoxide anion is a central ROS molecule and converts to more toxic reactive free radicals" (Altintas et al., 2013).

These findings come in line with Chirino et al.,2008 and Ghaznavi et al., 2016). The ameliorative effect of MEL may be also due to anti oxidant effects. several studies have demonstrated that the antioxidant effect of melatonin may be due to "its direct ability to detoxify free radicals and indirect antioxidant effect by increasing the activity and expression of antioxidative enzymes".(Allegra et al., 2003 and Rodriguezet al.,2004).

In the present study Treatment of rats with either APO or MEL attenuated the increase in serum LDH caused by GNT; this may be due to the anti-inflammatory effects of either APO or MEL (Dam et al., 2012 and Ghaznavi et al., 2016).

In this study, either APO or MEL could protect the kidney against GNT induce oxidative stress ,either APO or MEL induced a significant decrease in MDA and a significant increase in SOD and GPX on comparison to GNT rats . However in this study MEL is more efficient than APO in decreasing MDA and increasing both SOD and GPX yet, that effect difference not reached statistical difference, that may need longer duration and larger sample size to be fully investigated.

Apocynin attenuated cisplatin-induced nephrotoxicity through antioxidant effect (Chirino et al., 2008). Additionally, APO prevented the increase in oxidative stress in lung after bleomycin exposure (Gutterridge et al.,1995).

"Melatonin also is an efficient for scarvenging of ROS and RNS. Melatonin induces gene expression and activates multiple antioxidative enzymes as, GPX, and SOD (Reiter et al., 2003), SOD decreases free radical cellular damage that caused by producing H_2O_2, SOD converts H_2O_2 to water and oxygen; GPX also metabolize H_2O_2 to water (Lee et al., 2012).

Mitochondria has a major role in free radical generation. Superior ability of melatonin to accumulate into the mitochondria makes it an effective anti oxidant to treat oxidative stress related pathologies conversely to other

antioxidants with poor mitochondrial penetration (Raza and Naureen 2020).

The role of melatonin in scavenging (ROS), reducing (MDA) levels and promoting the production of antioxidant enzymes (SOD) and catalase (CAT) also documented by Tu et al.,(2021).

Many studies indicate that melatonin is able to attenuate the renal functional injury made by oxygen free radicals induced by cyclosporine in rats (Mun and suh, 2000) It has also decreased the renal oxidative insult caused by aluminum and lead (Mahieu et al., 2009) it has been shown that melatonin had protected against hamster kidney model of estradiol-induced DNA damage (Karbownik et al.,2001).

Histopathological investigation of the kidney of control rats showed normal glomeruli and normal renal tubules with normal lining renal tubular however ,GNT-induced hypercellularity in mesangial cells with degeneration of renal tubular epithelium in GNT group of rats, on other hand rats of either APO/GNT group or MEL/GNT group respectively showed normal glomeruli and normal renal tubules with normal lining renal tubular epithelium, these results point out the protective role of either APO or MEL against renal injury and reversing histopathological change induced by GNT through previous proven antioxidant and antiinflamatory effect . these findings also documented by Abdelrahman,(2018) and lee et al.,(2012).

V. CONCLUSION:

Either APO or MEL has a reno protective effect against GNT induced nephrotoxicity possibly through antioxiadant effect (persevering antioxiadant marker) and improving renal tissue morphology.

VI. RECOMMENDATION:

Further studies should be conducted on the human to confirm the efficacy of novel antioxidants (Apocynin and Melatonin) as curative and protective agents against nephrotoxicity of Gentamicin.

Acknowledgments:

The authors thank dr.Batoul Hegazy, lecturer of medical biochemistry, faculty of medicine, zagzig university for assistance in biochemical examination.

Conflicts of Interest:

The authors declared that they have no conflicts of interest.

VII. REFERENCES:

Abdel-Raheem, I.T.; Abdel-Ghany, A.A. and Mohamed, G.A. (2009): Protective effect of quercetin against gentamicin-induced nephrotoxicity in rats. Biol. Pharm Bull., 32:61-67.

Abdelrahman, R. S. (2018): Protective effect of apocynin against gentamicin-induced nephrotoxicity in rats. Human & experimental toxicology, 37(1): 27-37.

Ahmed, E.; Shaker, O. G.; Yussif, N.;et al., (2021): Effect of Locally Delivered Melatonin as an Adjunct to Nonsurgical Therapy on GCF Antioxidant Capacity and MMP-9 in Stage II Periodontitis Patients: A Randomized Controlled Clinical Trial. International Journal of Dentistry, 2021.

Ahmadvand, H.; Nouryazdan, N.; Nasri, M.; et al., (2020): Renoprotective Effects of Gallic

Acid Against Gentamicin Nephrotoxicity Through Amelioration of Oxidative Stress in Rats. Brazilian Archives of Biology and Technology, 63.

Ali, B.H.; Al-Qarawi, A.A.; Haroun, E.M.; et al., (2003):The effect of treatment with gum Arabic on gentamicin nephrotoxicity in rats: a preliminary study. Ren. Fai., 25: 15–20.

Allegra, M.; Reiter, R.; Tan, D.X; et al., (2003): The chemistry of melatonin's interaction with reactive species. J. Pineal. Res., 34:1-10.

Altintas, R.; Polat, A.; Vardi, N.; et al., (2013): The protective effects of apocynin on kidney damage caused by renal ischemia/reperfusion. Journal of endourology, 27(5): 617-624.

Bartels, H.; Bohmer, M. and Heierli, C.(1972):Serum creatinine determination without protein precipitation. Clin. Chim. Acta., 37: 193–197.

Bancroft, J. and Stevens, A. (1996): Theory and practice of histological technique (fourth ed.) .Churchill, Livingston, Edin burgh, London, Melbourne and New York pp. 50–56.

Chirino, Y.I.; Sanchez-Gonzalez, D.J.; Martinez-Martinez, C.M.; et al., (2008): Protective effects of apocynin against cisplatin-induced oxidative stress and nephrotoxicity. Toxicology, 245: 18–23.

Christo, J.S.; Rodrigues, A.M.; Mouro, M.G.; et al., (2011): Nitric oxide (NO) is associated with gentamicin(GENTA)nephrotoxicity and the renal function recovery after suspension of GENTA treatment in rats. Nitric Oxide; 24(2): 77–83.

Chrustek, A. and Olszewska-Słonina, D. (2021): Melatonin as a powerful antioxidant. Acta Pharmaceutica, 71(3), 335-354.

Connell, B.J.; Saleh, M.C.; Khan, B.V.; et al.,(2011): Apocynin may limit total cell death following cerebral ischemia and reperfusion by enhancing apoptosis. Food Chem. Toxicol., 49:3063–3069.

Dam, V.P.; Scott, J.L.; Ross, A.; et al., (2012): Inhibition of cystathionine gamma-lyase and the biosynthesis of endogenous hydrogen sulphide ameliorates gentamicin-induced nephrotoxicity. Eur. J. Pharmacol., 685: 165–173.

Daughaday, W. H.; Lowry, O. H.; Roskbrough, N. J.; et al., (1952). Determination of cerebrospinal fluid protein with the Folia phenol reagent. Journal of Laboratory and Clinical Medicine, 39: 663-665.

El-Kashefe, D.H.; El-Kenawi, A.E.; Rahim, M.A., et al. (2016):Agmatine improves renal function in gentamicin induced nephrotoxicity in rats. Can. J. Physiol. Pharmacol., 94: 278–286.

El-Kashefe, D.H., El-Kenawi, A.E., Suddek, G.M., et al.(2015): Flavocoxid attenuates gentamicin-induced nephrotoxicity in rats. Naunyn- Schmiedebergs Arch. Pharmacol., 388: 1305–1315.

Erdem, A.; Gundogan, N.U.; Usubutun, A.;et al., (2003): The protective effect of taurine against gentamicin-induced acute tubular necrosis in rats. Nephrol. Dial. Transplant,15(8):1175-1182.

Fawcett, J.K. and Scott, J.E. (1960):A rapid and precise method for the determination of urea. J. Clin. Pathol., 13:156–159.

Ghaznavi, H.; Mehrzadi, S.; Dormanesh, B.; et al.,(2016): Comparison of the protective effects of melatonin and silymarin against gentamicin-induced nephrotoxicity in rats. Journal of evidence-based complementary & alternative medicine, 21(4): NP49-NP55.

Gutterridge, J.M.(1995): Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin. Chem., 41: 1819–1828.

Henry, R. J.; Chiamori, N.; Golub, O. J.; et al., (1960): Revised spectrophotometric methods for the determination of glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, and lactic acid dehydrogenase. American journal of clinical pathology, 34(4_ts): 381-398.

Karahan, İ.; Ateşşahin, A; Yılmaz, S.; et al., (2005): Protective effect of lycopene on gentamicin-induced oxidative stress and nephrotoxicity in rats. Toxicology, 215(3): 198-204.

Karbownik, M.; Reiter, R.J.; Cabrera, J.; et al.,(2001): Comparison of the protective effect of melatonin with other antioxidants in the hamster kidney model of estradiol-induced DNA damage. Mutat. Res., 474:87-92.

Kim, S.Y.; Moon, K.A.; Jo, H.Y.; et al. (2012):Anti-inflammatory effects of apocynin, an inhibitor of NADPH oxidase, in airway inflammation. Immunol. Cell Biol., 90: 441–448.

Lee, I.C; Kim, S.H; Lee, S.M; et al.,(2012): Melatonin attenuates gentamicininduced nephrotoxicity and oxidative stress in rats. Arch Toxicol., 86:1527-1536.

Li, M.; Liu, Z.; Zhuan, L.; et al., (2013): Effects of apocynin on oxidative stress and expression of apoptosis-related genes in testes of diabetic rats. Mol. Med. Rep., 7: 47–52.

Li, Z. and Wang, Y. (2015): Effect of NADPH oxidase inhibitor-apocynin on the expression of Src homology-2 domain-containing phosphatase-1 (SHP-1) exposed renal ischemia/reperfusion injury in rats. Toxicol. Rep., 2: 1111–1116.

Luchetti, F.; Canonico, B.; Betti, M.; et al. (2010): Melatonin signaling and cell protection function. F.A.S.E.B. J., 24:3603-3624.Pediatr Infect Dis J.,30: 347–349.

Mahieu, S.; Contini, M.C.; Gonza´lez, M.; et al., 2009): Melatonin reduces oxidative damage induced by aluminium in rat kidney. Toxicol, Lett. 190:9-15.

Mohamed, H. Z. and Shenouda, M. B. (2021): Amelioration of renal cortex histological alterations by aqueous garlic extract in gentamicin induced renal toxicity in albino rats: a histological and immunohistochemical study. Alexandria Journal of Medicine, 57(1): 28-37.

Moreira, M. A.; Nascimento, M. A. and Bozzo, T. A. (2014): Ascorbic acid reduces gentamicin-induced nephrotoxicity in rats through the control of reactive oxygen species. Clinical nutrition, 33(2): 296-301.

Mun, K.C. and Suh, S.I.(2000): Effect of melatonin on renal function in cyclosporine nephrotoxicity. Transplant Proc.,32:1919-1920.

Nasri, H.; Nematbakhsh, M.; Ghobadi, S.; et al., (2013): Preventive and curative effects of ginger extract against histopathologic changes of

gentamicin-induced tubular toxicity in rats. Int. J. Prev. Med., 4(3):316-321.

Ohkawa, H.; Ohishi, N. and Yagi, K.(1979): Assay for lipid peroxides in stress in rats. Arch. Toxicol., 86(10):1527–1536.

Paglia, D.E. and Valentine, W.N.(1967): Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med.,70:158–170.

Parlakpinar, H.; Tasdemir, S. and Polat, A. (2005): Protective role of caffeic acid phenethyl ester (cape) on gentamicin-induced acute renal toxicity in rats. Toxicology, 207: 169–177.

Priuska, E.M. and Schacht, J. (1995): Formation of free radicals by gentamicin and iron and evidence for an iron/gentamicin complex. Biochem. Pharmacol., 50:1749-1752.

Quiros, Y.; Vicente-Vicente, L.; Morales, A.I.; et al. (2011): An integrative overview on the mechanisms underlying the renal tubular cytotoxicity of gentamicin. Toxicol. Sci., 119: 245–256.

Raza, Z. and Naureen, Z. (2020): Melatonin ameliorates the drug induced nephrotoxicity: Molecular insights. Nefrología, 40(1): 12-25.

Reiter, R.J.; Tan, D.X.; Mayo, J.C.; et al., (2003): Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans. Acta. Biochim. Pol. 50:1129-1146.

Rodriguez, C.; Mayo, J.C.; Sainz, R.M.; et al., (2004): Regulation of antioxidant enzymes: a significant role for melatonin. J. Pineal. Res., 36:1-9.

Sánchez, E.; Peña-Montes, D.; Sánchez-Duarte, S.;.et al., (2021): Effects of Apocynin on Heart Muscle Oxidative Stress of Rats with Experimental Diabetes: Implications for Mitochondria. Antioxidants, 10(3): 335.

Sun, Y.; Oberley, L.; and Li, Y. (1988):A simple method for clinical assay of
superoxide dismutase. Clin. Chem., 34:497–500.

Tu, Y.; Song, E.; Wang, Z.; et al., (2021): Melatonin attenuates oxidative stress and inflammation of Müller cells in diabetic retinopathy via activating the Sirt1 pathway. Biomedicine & Pharmacotherapy, 137, 111274
Wang, Y.; Song, Y.; Zhong, Q.; et al., (2021): Suppressing ROS generation by apocynin inhibited cyclic stretch-induced inflammatory reaction in HPDLCs via a caspase-1 dependent pathway. International,Immunopharmacology, 90:107-129

Yaman, I. and Balikci, E.(2010): Protective effects of nigella sativa against gentamicin-induced nephrotoxicity inrats. Exp. Toxicol. Pathol., 62: 183–190.

Yu, J.; Weiwer, M.; Linhardt, R.J.; et al., (2008): The role of the methoxyphenol apocynin, a vascular NADPH oxidase inhibitor, as a chemopreventative agent in the potential treatment of cardiovascular diseases. Curr. Vasc. Pharmacol., 6: 204–217.

الملخص العربي

مقارنه الآثار الوقائيه المحتمله لعقارى الأبثيونين والميلاتونين ضد التسمم الكلوى المصاحب لعقار الجنتاميسين فى الجرذان البيضاءالبالغه

***علياء محمد محمود ,*زينب عبده محمد ،**منى مصطفى احمد**
قسم الطب الشرعى والسموم الإكلينيكية*/ **قسم الباثولوجى
كليه الطب البشرى- جامعة الزقازيق- مصر

مقدمة البحث:يعد عقار الجنتاميسين من اوائل العلاجات المستخدمه فى العدوى الخطيره ولكن يبقى التسمم الكلوى المصاحب لتناوله من اهم المحاذير التى تحد من استخدامه.
هدف البحث: الهدف من هذا البحث هو مقارنه الاثار الوقائيه المحتمله لعقارى الأبثيونين والميلاتونين ضد التسمم الكلوى المصاحب لعقار الجنتاميسين فى الجرذان البيضاء البالغه.
طريقة البحث:أجرى البحث على اثنين وثلاثين جرذ أبيض بالغ وقد تم تقسيمهم الى أربع مجموعات متساوية:
-المجموعة الأولى :(مجموعة الضابطة السالبة)-المجموعة الثانية:(الجنتاميسين) تم حقن كل جرذ بريتونيا ١٠٠ مجم من الجنتاميسين لمده سبع أيام-المجموعه الثالثه: (مجموعةالأبوثينين/جنتاميسين) تم إعطاء كل جرذ ١٠مجم / كجم من الأبوثينين بالحقن البريتونى لمده سبع ايام ثم حقن كل جرذ بريتونيا ١٠٠ مجم من الجنتاميسين مع ١٠مجم من الأبوثينين لمده سبع ايام آخرين. المجموعة الرابعه: (مجموعة الميلاتونين /جنتاميسين) تم إعطاء كل جرذ ١٥مجم / كجم من الميلاتونين بالحقن البريتونى لمده سبع ايام ثم حقن كل فاربريتونيا ١٠٠ مجم من الجنتاميسين مع١٥مجم من الميلاتونين لمده سبع ايام آخرين.**النتائج:.** لقد تسبب الجنتاميسين فى حوث التسمم الكلوى بالفئران التى تناولته حيث كان هناك زيادة ذو دلالة إحصائية فى كل من (مؤشر وزن الكلى بالنسبه للجسم، حجم البول المجمع فى ٢٤ ساعه،الزلال بالبول،الكيرياتينين،بولينا الدم،لاكتات الهيدروجين المنزوع و المالوندياالاهيد الدال على التأكسد) لمجموعه الجنتاميسين وذلك بالمقارنة بالمجموعة الضابطة بالاضافه الى انخفاض ذو دلاله إحصائية فى كل من [النسبه المئويه للزياده بوزن الجسم،معدل التخلص من الكيرتانين،دلائل مضادات الأكسده(فوق أكسيد الديسماتوز و الجلوتاثيون بيركسوديز)] كما وجد بالفحص المجهرى الضوئى لأنسجه الكلى ان الجنتاميثين تسبب فى تحلل خلوى للانابيب الكلويه.فى حين ان تناول اى من الأبوثينين أو الميلاتونين تسبب فى انخفاض ذو دلاله إحصائية فى كل من(مؤشروزن الكلى بالنسبه للجسم، حجم البول المجمع فى ٢٤ ساعه،الزلال بالبول،الكيرياتينين،بولينا الدم،لاكتات الهيدروجين المنزوع و المالونديالاهيد الدال على التأكسد) بالمقارنه بمجموعه الجنتاميسين كما أن تناول اى من الابوثينين أو الميلاتونين تسبب فى زياده ذو دلاله إحصائية فى كل من[النسبه المئويه للزياده بوزن الجسم،معدل التخلص من الكيرتانين،دلائل مضادات الأكسده(فوق أكسيد الديسماتوز و الجلوتاثيون بيركسوديز)] بالمقارنه بمجموعه الجنتاميسين . كما وجد بالفحص المجهرى الضوئى لأنسجه الكلى أن أى من الأبوثينين أو الميلاتونين أستطاع وقايه أنسجه الكلى من التأثير السام للجنتاميسين. وبمقارنه التأثير الوقائى للابوثينين بالنسبه للميلاتونين وجد أن الميلاتونين كان افضل فى تأثيره الوقائى بالنسبه لدلائل مضادات التأكسد ولكن هذا الفرق لم يصل بعد لدلاله احصائيه .
الخلاصة: وجد آن تناول اى من الأبوثينين أو الميلاتونين أستطاع الحد من التسمم الكلوى المصاحب لأستخدام عقار الجنتاميسين عن طريق التاثير المضاد للتأكسد وكذلك وقايه الانسجه الخلويه للكلى من التحلل.