AMELIORATIVE EFFECTS OF HESPERIDIN AND MELATONIN AGAINST ACETAMINOPHEN-INDUCED NEPHROTOXICITY IN ADULT ALBINO RATS

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ABSTRACT

   Background: Acetaminophen (APAP) is the most widely used analgesic antipyretic drug, either by prescription or over the counter. APAP overdose can damage multiple organs, especially the liver and kidney, due to its toxic metabolites' formation and clearance. **Aim:** To investigate and compare the ameliorative effects of hesperidin (HSP) and melatonin (MEL) in rats with APAP-induced nephrotoxicity. **Methodology**: This comparative experimental study was carried out on 72 adult male Wistar albino rats were divided into nine groups (eight/each): groups (1-5): controls "negative, solvent, N-acetyl cysteine (NAC), MEL and HSP respectively; group (6): APAP-nephrotoxicity "rats were given a single dose (900 mg/kg) of APAP intraperitoneally (i.p.); group (7): APAP+NAC (150mg/Kg); group (8): APAP+MEL (10mg/Kg) and group (9): APAP+HSP (200mg/Kg). All treated drugs (NAC, MEL, and HSP) were given i.p. 30 minutes before APAP administration. Renal blood flow (RBF), blood urea, serum creatinine, tissue malondialdehyde (MDA), reduced glutathione (GSH), tumor necrotic factor alfa (TNF-α), and interleukin-6 (IL-6) were estimated by colorimetric methods. Immunohistochemical expression of inducible nitric oxide synthase (iNOS) was detected, and histopathological examination of renal tissue specimens was performed. **Results**: HSP, MEL, and NAC partially improved APAP-induced nephrotoxicity, evidenced by biochemical, histopathological, and immunohistochemical findings. **Conclusion**: Both HSP and MEL alleviated APAP-induced nephrotoxicity; however, HSP was superior to MEL, and it was as equal as NAC (which was also superior to MEL) as regards the tested parameters. The ameliorative effects of HSP and MEL may be at least in part due to their antioxidant and anti-inflammatory properties.
Keywords: Hesperidin, Melatonin, Acetaminophen, Nephrotoxicity, N-acetylcysteine.

INTRODUCTION

   Acute renal injury is an alarming global public health issue affecting more than 10% of the whole population and constituting a major cause of morbidity and mortality worldwide **(Hayek et al., 2020; Liu et al., 2020).**

   Drug-induced acute kidney injury (nephrotoxicity) can result from a predictable dose-dependent mechanism or an idiosyncratic reaction **(Sales and Foresto, 2020).** As the kidney is the major organ of drug biotransformation and excretion, it is at a high risk of damage due to the generation of reactive oxygen species

(ROS) and toxic metabolites **(Delavar and Soheilirad, 2020; Tjon and Teoh 2020).**

Acetaminophen (APAP) -the most common over-the-counter antipyretic, analgesic drug- is well known to cause either acute nephrotoxicity or renal failure in nearly 1-2% of APAP overdose cases, even in the absence of hepatic injury **(Ilbey et al., 2009; Shen et al., 2020**).

Toxic effects of APAP are mainly due to accumulation of ROS, such as nitric oxide (NO), peroxides, superoxide, etc., with resulting oxidative stress due to the formation of its toxic metabolite [N-acetyl-p-quinone imine (NAPQI)] **(Chen et al., 2020; Mansourian et al., 2020).**

Traditional treatment of APAP toxicity is done using N-acetylcysteine (NAC), either by intravenous or oral regimens **(Hendrickson, 2019).** NAC is a potent antioxidant that encourages cellular glutathione (GSH) formation, attenuating NAPQI-mediated oxidative stress injury **(Long et al., 2020).**

However, NAC can result in several undesirable side effects, e.g., anaphylactoid reactions, seizures, gastrointestinal upset, cerebral edema, plus its low stability and bioavailability **(Soliman et al., 2019; Firozian et al., 2020).**

Hesperidin (HSP) is a safe and active type of bioflavonoids (secondary plant metabolites abundant in citrus species such as lemon, blood orange, orange, and lime). HSP has numerous useful pharmacological actions, e.g., antioxidant, anti-inflammatory, anticarcinogenic, antiviral, hypolipidemic, and hypoglycemic effects, without any side effects **(Tabeshpour et al., 2020; Tao et al., 2020).**

Melatonin (MEL) "N-acetyl-5-methoxy tryptamine" is a polypeptide derived from tryptophan, synthesized, and released by the pineal gland as the main hormonal controller of the circadian rhythm. Exogenous melatonin intake has immunostimulatory, anti-inflammatory properties, and antioxidant (direct free radical scavenger) activities, with a safe adverse-effect profile **(Biancatelli et al., 2019; Pal et al., 2019).**

Unfortunately, treatment of APAP-induced nephrotoxicity has not been well-established yet, as well as the molecular mechanism of APAP-induced nephrotoxicity is still unclear, so this work aimed to investigate and compare the ameliorative effects of hesperidin (HSP) and melatonin (MEL) against APAP-induced nephrotoxicity in adult male albino rats, as well as to illustrate their possible mechanisms of action. This may help to find an additional treatment strategy against APAP-induced nephrotoxicity.

MATERIAL & METHODS
Animals:

Seventy-two adult male Wistar albino rats of a locally breaded strain weighing between 150-200 g at the beginning of the study were used. They were acclimatized for one week in groups (four/cage) in a fully ventilated room, under a 12h light/dark cycle, at ordinary room temperature at the Pharmacology Department, Faculty of Medicine, with unlimited access to water and a balanced diet. All experimental protocols were conducted according to the local committee of the research ethics of Benha Faculty of Medicine.

Drugs:

Hesperidin (powder of purity $\geq 97.0\%$, CAS No: 520-26-3), acetaminophen (powder, CAS No: 103-90-2), Melatonin (powder, CAS No: 73-31-4), N-Acetylcysteine (powder, of purity $\geq 99.0\%$, CAS No: 616-91-1) and polyethylene glycol (PEG)-400 (colorless, viscous, liquid of purity >99%, CAS No: 25322-68-3) were obtained from Sigma-Aldrich, Saint Louis, USA. Sterile Distilled water, diethyl ether, and formalin (neutral 10% formalin) were obtained from Al-Gomhoria Pharmaceutical Chemical Co., Egypt. Chemicals and drugs were of a high analytical category and were freshly prepared before each experiment.

Experimental design:

In this comparative experimental study, and following acclimatization, animals were arranged into nine experimental groups (eight/each):

Group I (negative control): animals in this group were kept in the same environment as the treated groups without any treatment to assess the tested parameters' normal values in this study.

Group II (solvent control): received drug vehicle [distilled water (four rats) or polyethylene glycol (PEG)-400] (four rats) via intraperitoneal (i.p.) injection in volumes comparable to that of tested drugs.

Group III (NAC control): treated with a single dose [150 mg/kg/body weight (b. wt.)] of N-acetylcysteine (NAC) via i.p. injection, according to **Sener et al. (2003).**

Group IV (MEL control): treated with a single dose (10 mg/kg/b. wt.) of melatonin (MEL) via i.p. injection, according to **Sener et al. (2003).**

Group V (HSP control): treated with a single dose (200 mg/kg/b. wt.) of hesperidin (HSP) via i.p. injection **(Ahmad et al., 2012).**

Group VI (APAP): treated with a single dose (900 mg/kg/b. wt.) of acetaminophen (APAP) via i.p. injection to induce nephrotoxicity, as described by **Sener et al. (2003).**

Group VII (APAP+NAC): received APAP and NAC in a dose-regimen, as illustrated in groups III and VI.

Group VIII (APAP+MEL): received APAP and MEL in a dose-regimen, as illustrated in groups IV and VI.

Group IX (APAP+HSP): received APAP and HSP in a dose-regimen, as illustrated in groups V and VI.

APAP, NAC, and MEL were dissolved in hot distilled water (70°C, subsequently cooled to 37°C at treatment) **(Sener et al., 2003)**; HSP was dissolved in PEG-400 **(Anwer et al., 2014).** All treated drugs (NAC, MEL, and HSP) were given 30 minutes before APAP administration **(Sener et al., 2003).**

After the end of the experiment (24 hours after APAP administration "the time needed to induce nephrotoxicity"), a renal incision was done under anesthesia (inhalation of ether) to expose the left kidney, and a bi-directional blood flowmeter (Hadeco ES 1000 SPM, Japan) was fit in its artery to measure the renal blood flow, by setting the mode of pulsed doppler blood flowmeter, using ultrasonic gel on the probe top and turn the volume control to the maximum, then the probe pressed softly to the measurement area at an angle of 45- 60°, and after hearing the optimal sounds for at least five seconds without moving the probe then press the freeze key to freeze the waveform **(Haywood et al., 1981).** After that, rats were sacrificed by decapitation under anesthesia. Blood samples were taken from abdominal aorta after dissection, and sera were obtained by centrifugation at 5000 round/minute for 5 minutes and kept at -4 C°. Kidneys were rapidly collected, washed with ice-cold saline, and longitudinally cut into two parts; one part was put in 10% formalin for immunohistochemical and histopathological examination, the other part was directly frozen at -80 C°, which latterly was minced and homogenized with tissue homogenizer, then it was centrifuged, and the resultant supernatant was used for assay of tissue oxidative stress markers.

Studied Parameters:

• Measurement of renal blood flow (RBF), using a doppler flowmeter (Hadeco ES 1000 SPM, Japan), according to **Haywood et al. (1981).**

• Biochemical measurements, using a spectrophotometer (model IL 143, Fischer Scientific, USA):

1- Blood urea, according to **Kaplan (1984).**

2- Serum creatinine, according to **Murray (1984).**

• Markers for oxidative stress:

1. Tissue reduced glutathione (GSH) was measured spectrophotometrically using commercial kits (Bio-diagnostic Co., Giza, Egypt), according to manufacturer's instructions **(Asghar et al., 1975).**

2. Tissue malondialdehyde (MDA) was measured spectrophotometrically using commercial kits (Bio-diagnostic Co., Giza, Egypt) according to the manufacturer's instructions **(Yagi, 1984).**

3. Serum tumor necrotic factor alfa (TNF-α) was measured by ELISA using commercial kits (USA & Canada R&D System, Inc) according to the manufacturer's instructions (**Corti et al., 1992**).

4. Serum interleukin-6 (IL-6) was measured by ELISA using commercial kits obtained from e-Bioscience (Vienna, Austria) according to the manufacturer's instructions (**Klinik et al., 1998**).

• Immunohistochemical expression of inducible nitric oxide synthase (iNOS):

Renal tissue sections (3mm thickness) were moisturized in xylene and subsequently, in graded ethanol. After that, they were soaked in 5% bovine serum albumin (BAS) in Tris buffer saline (TBS) for 2 hours, then they were immune-stained with a primary antibody polyclonal immunoglobulin-G (IgG) of rat iNOS (1mg/ml) with 5% BAS in TBS and incubated at 4°C. Subsequently, they were incubated at room temperature with a secondary antibody, diluted 1:2000 with 5% BAS in TBS for 2 hours. Lastly, sections were washed with TBS and incubated in a solution of 0.02% diaminobenzidine containing 0.01% hydrogen peroxide for 5-10 min. Staining was done by hematoxylin, and slides were examined using a light microscope [Olympus BX51, Hamburg, Germany] utilizing 10x and 40x magnifications (**Ahmad et al., 2012**).

Sections were graded as regard both the diffuseness and the intensity of 3,3'-diaminobenzidine (DAB) staining as follows: zero (no staining), 1 [minimal (25% staining)], 2 [mild (25%-50% staining)], 3 [moderate (50%-75% staining) and 4 [marked (>75% staining)]. Slides were tested by two separate examiners with no previous knowledge of the study protocol.

• Histopathological examination:

The kidney specimens were fixed in formalin 10%, and histopathological examination was done using Hematoxylin and Eosin (H&E) stain (**Halliwell and Chirico, 1993**).

Statistical Analysis:

Obtained data were written as mean and standard deviation (±SD); the one-way analysis of variance was used for data evaluation. The differences between groups were compared using Student's t-test, with p <0.05 was chosen as a statistical significance level (**Khothari, 2004**).

RESULTS

In the current work, negative, solvent, NAC, MEL, HSP control groups showed a non-significant difference as regard all studied parameters (nearly normal values as the negative control group), as well as sections (histopathological and immunohistochemical) from the kidney tissue, showed nearly normal structure as the negative control group, without any significant changes. So, they were represented in tables and figures as one group (controls).

Administration of a single i.p. dose of APAP (900 mg/kg) caused marked impairment of renal function, as illustrated by significant (p<0.05) reduction in renal blood flow by 42.6%, significant (p<0.05) increase in blood urea and serum creatinine, renal MDA, serum TNF-α and IL-6 by 162.8%, 229%, 426%, 119%, 345% respectively, with a significant (p<0.05) reduction in renal GSH by 62.2% compared with the control group (tables 1-3). This was associated with marked immunohistochemical expression of iNOS and histopathological changes in the form of loss of renal architecture (severe degeneration with dense lymphocytic infiltrate and marked necrosis), as illustrated in fig. 1-3. The administration of NAC revealed a significant (p<0.05) elevation in renal blood flow by 53.1%, a significant (p<0.05) decrease in blood urea and serum creatinine by 45.3%, and 51.4% as compared with APAP-nephrotoxic rats. Renal MDA, serum TNF-α and serum IL-6 were significantly (p<0.05) reduced by 69.3%, 31.5%, and 49.6%, respectively, with a significant (p<0.05) elevation of renal GSH by 138.7% as compared with APAP-nephrotoxic rats (tables 1-3). This was

associated with a moderate immunohistochemical expression of iNOS, moderate histopathological improvements in

the form of mild tubular vacuolation, and inflammation (fig. 1-3).

Table (1): renal blood flow (RBF), blood urea, and serum creatinine levels in the different studied groups used to investigate and compare the ameliorative effects of hesperidin (HSP), and melatonin (MEL) in rats with APAP-induced nephrotoxicity (Mean ± SD):

Parameters / Groups	RBF (cm/s)	Blood urea (mg/dl)	Serum creatinine (mg/dl)
Controls	9.4 ±0.73	37.3±2.9	0.42±0.02
APAP group	5.4 ±0.31[a]	97.6±8.9 [a]	1.38±0.09[a]
(APAP+ NAC) group	8.3 ±0.54[a,b]	53.4±4.6 [a,b]	0.67±0.04 [a,b]
(APAP+ MEL) group	7.1 ± 0.42[a,b,c]	66.5±5.4 [a,b,c]	0.86±0.06 [a,b]
(APAP+HSP) group	8.8 ±0.56 [a,b,c,d]	49.7±3.8[a,b,c,d]	0.64±0.03[a,b,c,d]

a: significant difference versus controls (p<0.05); **b:** significant difference versus APAP group (p<0.05); **c:** significant difference versus APAP+ NAC treated group (p<0.05); **d:** significant difference versus APAP+ MEL treated group (<0.05); **±SD:** standard deviation; **APAP:** acetaminophen; **NAC:** N-acetylcysteine.

Table (2): renal tissue reduced glutathione (GSH) and malondialdehyde (MDA) levels in the different studied groups used to investigate and compare the ameliorative effects of hesperidin (HSP), and melatonin (MEL) in rats with APAP-induced nephrotoxicity (Mean ± SD):

Parameters / Groups	Renal GSH (nmol/g)	Renal MDA (nmol/g)
Controls	16.4 ± 1.5	0.73±0.06
APAP group	6.2 ±0.42[a]	3.84 ± 0.22[a]
(APAP+ NAC) group	14.8 ±0.91[a,b]	1.18 ± 0.11[a,b]
(APAP+ MEL) group	11,7 ± 0.78[a,b,c]	2.12 ± 0.16[a,b]
(APAP+HSP) group	15.2 ± 1.12[b,c,d]	1.15 ± 0.09[a,b,c,d]

a: significant difference versus controls (p<0.05); **b:** significant difference versus APAP group (p<0.05); **c:** significant difference versus APAP+ NAC treated group (p<0.05); **d:** significant difference versus APAP+ MEL treated group (<0.05); **±SD:** standard deviation; **APAP:** acetaminophen; **NAC:** N-acetylcysteine.

Table (3): serum tumor necrotic factor alfa (TNF-α) and serum interleukin-6 (IL-6) levels in the different studied groups used to investigate and compare the ameliorative effects of hesperidin (HSP), and melatonin (MEL) in rats with APAP-induced nephrotoxicity (Mean ± SD):

Parameters / Groups	TNF-α (pg/ml)	IL-6 (pg/ml)
Controls	67.8 ± 4.8	200.2±13.2
APAP group	148.4 ± 12.4[a]	890.8±78.6[a]
(APAP+ NAC) group	101.6 ± 8.2[a,b]	448.7 ± 32.4[a,b]
(APAP+ MEL) group	120.5 ± 10.3[a,b,c]	656.8 ± 51.3[a,b]
(APAP+HSP) group	103.2 ± 8.4 [c,d]	452.4 ± 31.8[a,b,c,d]

a: significant difference versus controls (p<0.05); **b:** significant difference versus APAP group (p<0.05); **c:** significant difference versus APAP+ NAC treated group (p<0.05); **d:** significant difference versus APAP+ MEL treated group (<0.05); **±SD:** standard deviation; **APAP:** acetaminophen, **NAC:** N-acetylcysteine.

The administration of melatonin (10 mg/kg) showed a significant (p<0.05) elevation in renal blood flow by 31.2%, a significant (p<0.05) decreased in values of blood urea and serum creatinine by 32% and 37.7%, respectively as compared with APAP-nephrotoxic rats. Renal MDA, serum TNF-α and serum IL-6 were significantly (p<0.05) reduced by 45%, 18.8%, and 26.3%, respectively, with a significant (p<0.05) elevation of renal GSH by 88.7% as compared with APAP-nephrotoxic rats (tables 1-3). This was also associated with a moderate immunohistochemical expression of iNOS and moderate histopathological improvements in the form of mild tubular vacuolation and inflammation (fig. 1-3).

On the other hand, administration of hesperidin (200 mg/kg) revealed a significant (p<0.05) elevation in renal blood flow by 63.2%, a significant (p<0.05) reduction in blood urea and serum creatinine by 49.1% and 53.6% respectively as compared with APAP-nephrotoxic rats. Renal MDA, serum TNF-α and serum IL-6 were significantly (p<0.05) reduced by 70%, 30.5, and 49.2%, respectively, with a significant (p<0.05) elevation of renal GSH by 145.2% as compared with APAP-nephrotoxic rats (tables 1- 3). This was associated with a mild immunohistochemical expression of iNOS and marked histopathological improvements correlated with biochemical and immunohistochemical results (fig. 1-3) .
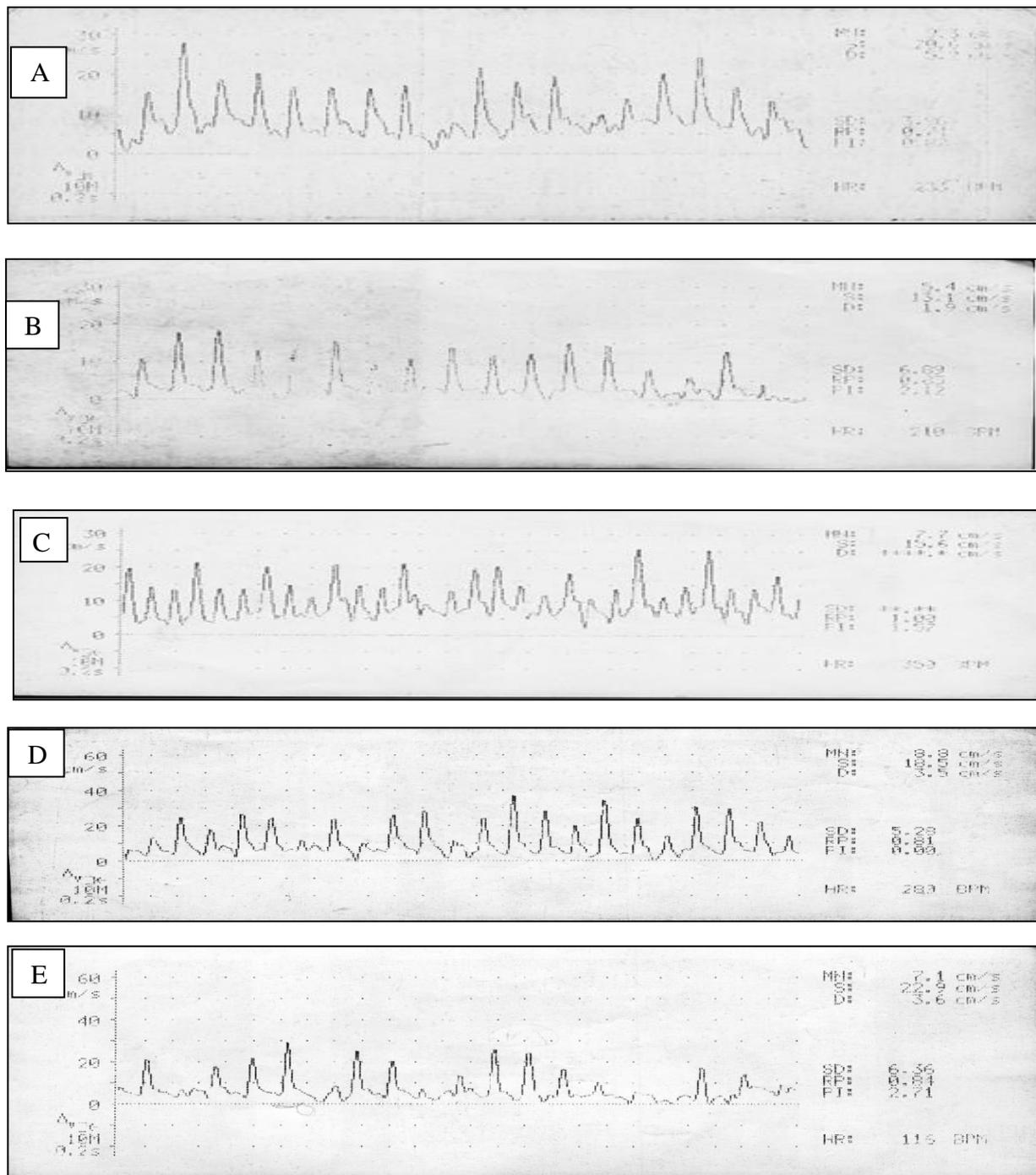
Figure (1): A trace (bi-directional blood flowmeter) showing renal blood flaw (RBF) in the different studied groups: **(A):** control rats **(controls)**; **(B):** APAP-nephrotoxic rats **(group 6)**; **(C):** APAP+NAC treated rats **(group 7)**; **(D):** APAP+MEL treated rats **(group 8)**; **(E):** APAP+HSP treated rats **(group 9)**; [**APAP:** acetaminophen**; NAC:** N-acetylcysteine; **MEL:** melatonin; **HSP:** hesperidin].
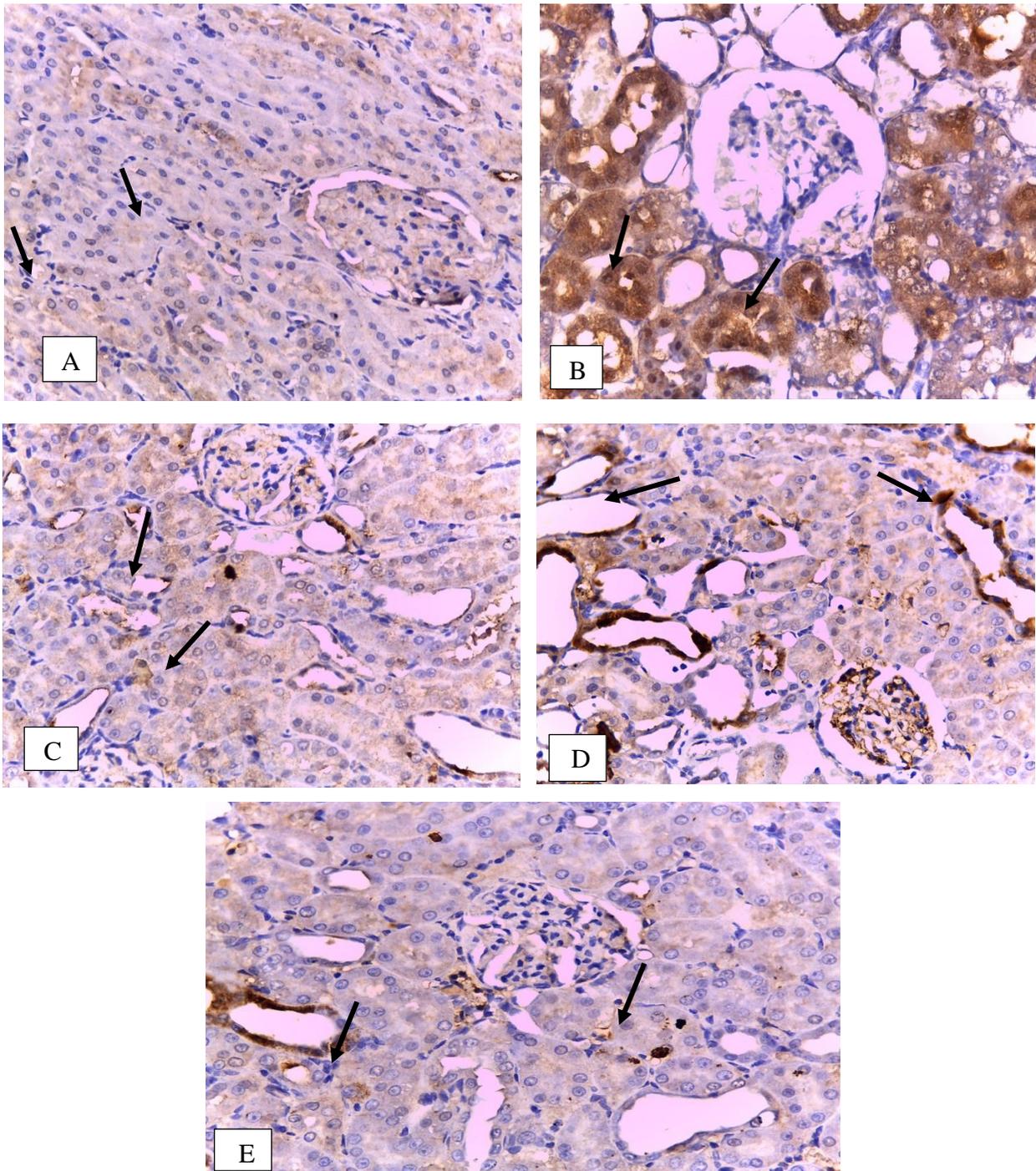
Figure (2): A photomicrograph illustrating the immunohistochemical staining of inducible nitric oxide synthase (iNOS) among the studied groups; **(A):** control rats **(controls)** showing minimal expression of iNOS; **(B):** APAP-nephrotoxic rats **(group 6)** showing marked expression of iNOS; **(C):** APAP+NAC treated rats **(group 7)** showing mild expression of iNOS; **(D):** APAP+MEL treated rats **(group 8)** showing moderate expression of iNOS; **(E):** APAP+HSP treated rats **(group 9)** showing mild expression of iNOS. [**APAP:** acetaminophen**; NAC:** N-acetylcysteine; **MEL:** melatonin; **HSP:** hesperidin] [Black arrows] (x400).
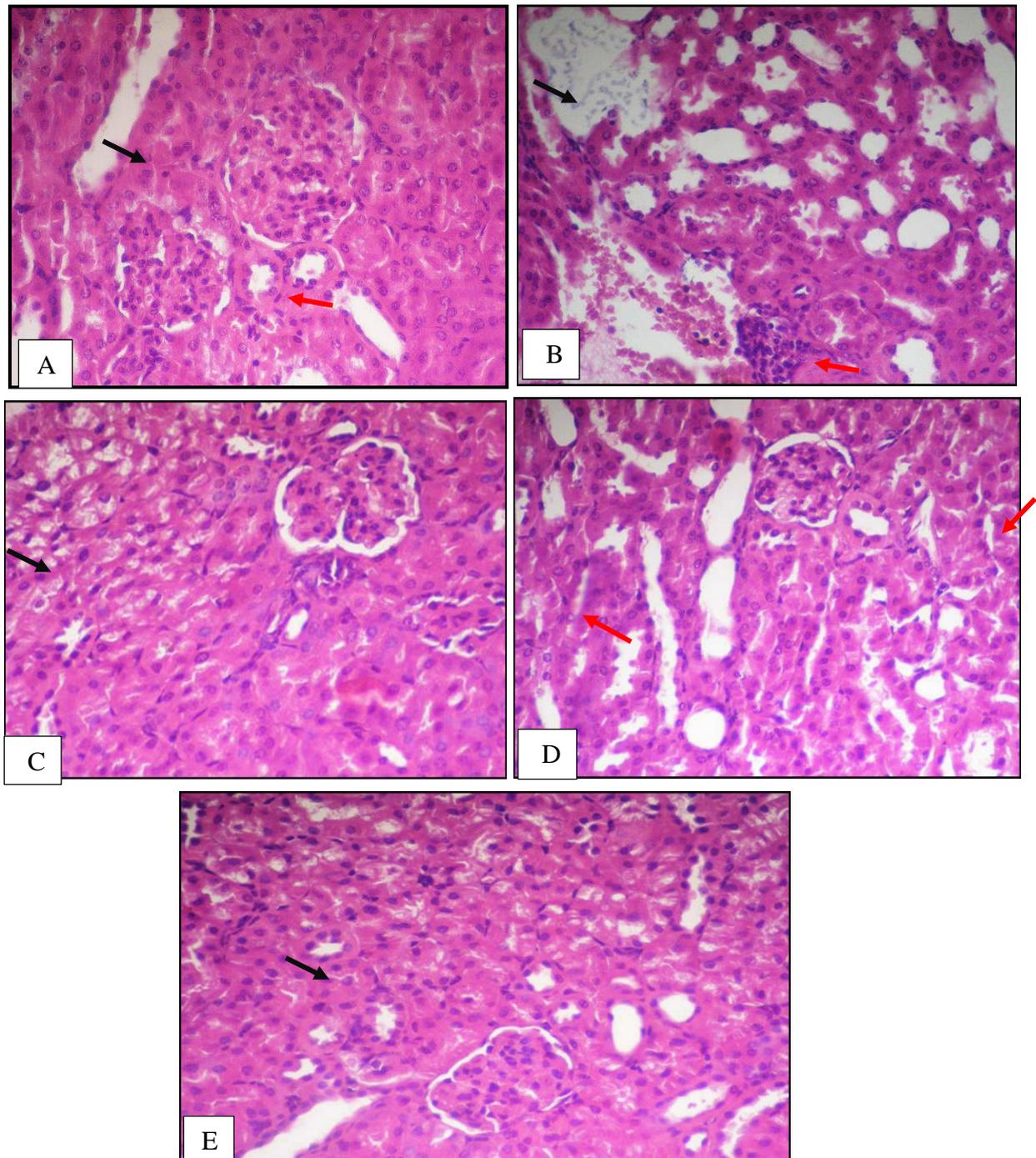
Figure (3): A photomicrograph of a transverse section of the kidney of **(A):** control rats **(controls)** showing central glomeruli surrounded by Bowman's space [black arrow], normal renal tubules lined by one layer of cuboidal epithelial cells [red arrow]; **(B):** APAP-nephrotoxic rats **(group 6)** showing marked tubular necrosis [black arrow], interstitial congestion & inflammation [red arrow]; **(C):** APAP+NAC treated rats **(group 7)** showing mild tubular vacuolation and inflammation [black arrow]; **(D):** APAP+ MEL treated rats **(group 8)** showing moderate tubular (vacuolation, congestion and inflammation) [red arrows]; **(E):** APAP+HSP treated rats **(group 9)** showing mild tubular vacuolation and inflammation with no congestion [black arrow]. [**APAP:** acetaminophen**; NAC:** N-acetylcysteine; **MEL:** melatonin; **HSP:** hesperidin] (H&E X40).

DISCUSSION

Overdose of acetaminophen (APAP) can produce hepatic and renal failure; the latter could occur in about 1–2% of APAP overdose cases due to kidney damage because of oxidative stress (**Ansari et al., 2020**).

The results of this work revealed that i.p. administration of a single dose (900 mg/kg) of APAP resulted in nephrotoxicity, which was reflected by significant elevations in blood urea, serum creatinine, renal malondialdehyde (MDA), serum tumor necrotic factor alfa (TNF-α), and serum interleukin-6 (IL-6) levels as well as significant reductions in renal blood flow (RBF) and renal reduced glutathione (GSH) with marked immunohistochemical expression of iNOS and the histopathological findings. These results were in line with those shown by **Ilbey et al. (2009) and Molinas et al. (2010)**.

The mechanism of APAP-induced nephrotoxicity had gained a considerable argument. Conventionally, it is due to the production of NAPQI (a toxic metabolite) via cytochrome P450 enzyme in renal tissue. NAPQI depletes tissue GSH resulting in oxidative stress renal damage (**Bessems and Vermeulen, 2001; Hilal et al., 2019**).

Mazer and Perrone (2008) explained a different possible mechanism of APAP nephrotoxicity mediated by prostaglandin-endoperoxide synthase (PGES), which can also transform APAP into NAPQI. This mechanism is more common in the renal medulla, while the cytochrome P450 is more pronounced in the renal cortex.

Ilbey et al. (2009) stated that the N-deacetylase enzyme might also be responsible for APAP-induced nephrotoxicity, as it can convert either APAP itself or NAPQI to p-aminophenol, that is subsequently transformed to a free radical.

Despite the superfine variations, the endpoint of all these pathways is nearly typical (free radicals' formation), which resulted in oxidative stress tissue damage and necrosis. Such an assumption was confirmed by the significant increase in renal MDA (an end-product of free radical chain reaction) and a reduction in renal GSH (the main antioxidant). This was confirmed by the biochemical changes (elevated blood urea and serum creatinine) and the microscopic findings (hydropic degeneration and tubular necrosis).

Libby (2002) stated that inflammation and tubular necrosis might be explained by the pro-inflammatory effect of reactive free radicals, which induce the expression of leukocyte adhesive molecules, promoting leukocyte migration.

Free radical's formation promotes the expression of iNOS, cyclooxygenase-2 (COX-2), and the inflammatory cytokines such as TNF-α, interleukin-1 (IL-1), and IL-6 (**Reuter et al., 2010**).

Much more attention was made towards nitrosative stress or reactive nitrogen species (RNS), e.g., NO, and nitrogen dioxide in nephrotoxicity pathogenesis, a high level of NO production via iNOS is associated with an increased incidence of APAP-induced tissue inflammation and necrosis (**Ito et al., 2003; Abdel-Zaher et al., 2008; Xie et al., 2016**).

In this work and to prove this point, the results showed marked immunohistochemical expression of iNOS in immunohistochemical sections of the APAP-nephrotoxic group, mainly in renal tubules. This may be explained by the accumulation of the drug and its metabolites in the tubular cells, as previously confirmed by **Morris and Levey (1994)**, who stated that renal excretion of acetaminophen in humans and animals involves glomerular filtration and passive reabsorption.

Treatment with either melatonin (MEL), hesperidin (HSP), or N-acetylcysteine (NAC) resulted in partial improvement of APAP-induced nephrotoxicity represented by significant elevation of RBF, significant reduction of blood urea and serum creatinine, and a significant decrease in renal MDA levels, serum TNF-α and serum IL-6 and increase in renal GSH with a significant reduction in

iNOS expression, as compared with APAP-nephrotoxic group.

This was in line with **Sener et al. (2003)**, who reported that NAC, MEL, and vitamin E could ameliorate APAP-induced hepatotoxicity and nephrotoxicity, as they can scavenge free radicals.

Also, **Ahmad et al. (2012)** reported that HSP had hepato-protective and nephroprotective effects against APAP-induced toxicity and suggested that the antioxidant, anti-apoptotic and anti-inflammatory properties of HSP as mechanisms of action.

These results were also in harmony with **Subramanian et al. (2015),** who studied HSP's ameliorative effect against gentamicin-induced nephrotoxicity and concluded that hesperidin possesses anti-inflammatory properties and can significantly suppress TNF-α expression.

Renal tissue contents of MDA, GSH, and serum concentration of the inflammatory mediators (TNF-α and IL-6) were estimated as well as iNOS expression by immunohistochemical staining was recorded to explore the mechanism of the ameliorative effects of HSP and MEL against APAP-induced nephrotoxicity.

In the present study, both MEL and HSP showed a significant decrease in the renal tissue MDA, the serum concentration of TNF-α and IL-6 levels, and the immunohistochemical iNOS expression with a significant increase of the renal GSH levels as compared to the APAP-nephrotoxic group.

The ameliorative effect of MEL against free radicals and oxidative stress is multifactorial. It can act as a direct free radical scavenger for hydroxyl free radical, singlet oxygen, and peroxy-radicals, and it can also inhibit the pro-oxidant pathway of nitric oxide synthase (NOS). Hydroxyl free radicals combine with C_2 of the indole ring, forming 3-hydroxymelatonin, which is excreted in urine **(Reiter et al., 2001& 2016).**

Moreover, **Wang et al. (2004) and Rastogi et al. (2019)** stated that MEL acts indirectly by stimulation of antioxidant enzymes; the most important of which is glutathione peroxidase, which induces the recycling of glutathione from inactive oxidized form to active reduced form, MEL has shown a great activity as a free radical scavenger in mitochondria which is the major intracellular site for electron leak that produces free radicals.

Hardeland and Pandi-Perumal (2005) stated that MEL could increase intracellular GSH concentrations by activating γ-glutamylcysteine synthase.

Also, **Huang et al. (2007) and Koksal et al. (2012)** showed that NAC and MEL had beneficial effects against hepatic injury in a rat model due to cardiopulmonary bypass and methanol toxicity, respectively; as serum levels of liver enzymes, TNF-α, activities of iNOS, MDA, and myeloperoxidase in liver tissue were markedly reversed in both NAC and MEL groups. Furthermore, the glutathione content of liver and antioxidative enzyme activities markedly elevated after NAC or MEL treatment.

Ganeshpurkar and Saluja (2019) stated that HSP is a potent antioxidant, and it has an alleviative effect against drug-induced nephrotoxicity, e.g., trichloroethylene and APAP nephrotoxicity rat models.

Ahmad et al. (2012) documented that HSP administration resulted in a significant reduction of iNOS expression in rats with APAP-induced nephrotoxicity.

Also, **Pari et al. (2015)** found that HSP significantly improved hepatic and renal functions, restored hepatic and renal antioxidants levels, and decreased the iron concentration in blood, suggesting antioxidant and heavy metal chelation mechanisms of HSP on iron-induced toxicity.

Moreover, **Khan and Parvez (2015); Hajialyani et al. (2019)** stated that HSP has a neuroprotective effect against the brain's toxic insults and neurodegenerative diseases, respectively, through amelioration of neuronal growth factors and antioxidant

activities, as well as decreasing inflammation and apoptosis.

CONCLUSION

- A single i.p. dose (900 mg/kg) of acetaminophen (APAP) induced nephrotoxicity in rats, evidenced by biochemical, histopathological, and immunohistochemical findings.
- Hesperidin (HSP) "200 mg/kg"; melatonin (MEL) "10 mg/kg" and N-acetylcysteine (NAC) "100 mg/kg", given i.p. 30 minutes before APAP administration partially improved the picture of APAP-induced nephrotoxicity in rats.
- HSP was superior to MEL, and it was as equal as NAC (which was also superior to MEL) regarding the tested biochemical parameters.
- HSP and MEL's ameliorative effects effect may be at least in part due to their antioxidant and anti-inflammatory properties.

RECOMMENDATIONS

Further studies are needed to detect any untoward effects of hesperidin on other vital body functions in animal models and human beings with renal impairment. Moreover, the effect of a possible combination of hesperidin with other nephrotoxicity-ameliorative drugs commonly used should be considered.

CONFLICT OF INTEREST (IF ANY)

'The Authors declare that there is no conflict of interest.'

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التأثيرات التحسينية للهسبيريدين والميلاتونين ضد السمية الكلوية الناشئة عن الأسيتامينوفين في الجرذان البيضاء البالغة

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عقار الأسيتامينوفين هو أكثر الأدوية المسكنة والخافضة للحرارة استخدامًا، سواء بوصفة طبية أو بدون وصفة طبية. يمكن أن يؤدي الاستخدام المفرط لعقار الأسيتامينوفين إلى إتلاف العديد من الأعضاء، وخاصة الكبد والكلى بسبب تكوين وإزالة المستقلبات السامة. هدف هذا العمل إلى دراسة ومقارنة التأثيرات المحسنة للهسبيريدين والميلاتونين ضد السمية الكلوية الناشئة عن الأسيتامينوفين في الجرذان البيضاء البالغة. أجريت هذه الدراسة على اثنين و سبعين جرذا" أبيض بالغ، تتراوح أوزانهم ما بين 150-200 جم, و تم تقسيمهم الى تسع مجموعات بطريقة عشوائية (خمس مجموعات تحكم "السالبة, والمذيب, والإن اسيتيل سيستايين و الميلاتونين و الهسبيريدين", و المجموعة السادسة: الأسيتامينوفين, والمجموعة السابعة: الأسيتامينوفين+ الإن اسيتيل سيستايين, والمجموعة الثامنة: الأسيتامينوفين+ الميلاتونين, والمجموعة التاسعة: الأسيتامينوفين+ الهسبيريدين). تم إحداث السمية الكلوية في الفئران عن طريق إعطاء جرعة واحدة (900 مجم/كجم) من عقار الاسيتامينوفين داخل الصفاق. وتم دراسة التأثيرات التحسينية للهسبيريدين (200 مجم/كجم) و الميلاتونين (10 مجم/كجم) والإن اسيتيل سيستايين (150 مجم/كجم) ، حيث أعطيت هذه المواد عن طريق الحقن بالصفاق قبل ثلاثين دقيقة من إعطاء الأسيتامينوفين, تم فحص تدفق الدم الكلوي ، واليوريا في الدم ، والكرياتينين في مصل الدم ، والمالوندا الديهايد (MDA) بأنسجة الكلية، والجلوتاثيون المنخفض (GSH) بأنسجة الكلية ، وعامل نخر الورم ألفا(TNF-α) بمصل الدم, والإنترلوكين -6 (IL-6) بمصل الدم, وتم الكشف عن التعبير النسيجي الكيميائي لـ iNOS , وكذلك تم إجراء الفحص الهستوباثولوجى لعينات من الأنسجة الكلوية بواسطة صبغة الهيماتوكسيلين والأيوزين. أظهرت النتائج أن كلا من الهسبيريدين والميلاتونين والإن اسيتيل سيستايين حسنت جزئيًا السمية الكلوية التي يسببها الأسيتامينوفين في الفئران، كما يتضح ذلك من النتائج البيوكيميائية والفحص الكيميائي المناعي. وقد خلصت الدراسة الى أن الهسبيريدين قد أعطى نتائج أفضل من الميلاتونين وكان متساويًا تقريبا" في الفعالية مع الإن اسيتيل سيستايين. وأنه قد يعزى التأثير المحسن للهسبيريدين والميلاتونين ضد السمية الكلوية الناشئة عن الأسيتامينوفين على الأقل جزئيًا بسبب خصائصهما المضادة للأكسدة والمضادة للالتهابات.