The Egyptian Journal of Hospital Medicine (October 2024) Vol. 97, Page 3688-3693

PI3K / AKT / GSK-3β Pathway Mediates the Ameliorative Effect of Amentoflavone on Hepatorenal Injury Induced by Doxorubicin

Anwaar M. Shaban[1], Suzan A. Khodir[1*], Marwa M. Khalil[2], Fatma Eldesoky Ahmed[3], Nesma Mahmoud Afifi[4], Zainab Ibrahim Elakabawy[5], Alzahraa A.M. Sheref[1]

Departments of [1]Medical Physiology, [2]Medical Biochemistry and Molecular Biology, [3]Clinical Pharmacology, [4]Anatomy and Embryology and [5]Pathology, Faculty of Medicine, Menoufia University, Menoufia, Egypt.

***Corresponding author:** Suzan A. Khodir, **Mobile:** (+20) 01288266531,

E-mail: suzan.abdalhameed.12@med.menofia.edu.eg, **ORCID ID:** 0000-0002-2535-9445

ABSTRACT

Background: Doxorubicin (DOX) induced hepatorenal injury is a major health concern. Amentoflavone (AMF) is a biflavonoid with conspicuous pharmacological activities.

Objective: To clarify the possible protective AMF effect on DOX associated hepatorenal injury & the possible underlying mechanisms.

Material and methods: 30 rats were equally divided to three groups: control group. DOX group and DOX+AMF treated. After that blood samples were withdrawn for assessment of serum urea, creatinine, ALT, AST, MDA, TNF-α, caspase-3, SOD and IL10. Liver and kidney were preserved for AKT, PI3K and GSK-3β genes expression level detection. Finally, Histopathological assessment of hepatic and renal section was done.

Results: DOX group revealed a significant increase in urea, creatinine, ALT, AST, MDA, TNF-α, and caspase-3, along with a concurrent decrease in SOD and IL10 when compared to control rats. Serum levels of urea, creatinine, ALT, AST, MDA, TNF-α, caspase3 were significantly reduced, and SOD and IL10 were significantly increased in the DOX+AMF group compared to DOX group, while there was a significant reduction in the expression of the AKT and PI3K genes and a concurrent increase in the expression of the GSK-3β gene with DOX treatment. AMF significantly mitigated the expression of the AKT and PI3K genes with reduction of GSK-3β gene after 8 weeks.

Conclusion: AMF protects against doxorubicin induced hepatorenal injury by antioxidant, anti-inflammatory, anti-apoptotic mechanisms in addition to modulation in PI3K/AKT/GSK-3β signaling pathway.

Keywords: Amentoflavone, AKT, Doxorubicin, GSK-3β, PI3K, Hepatorenal injury.

INTRODUCTION

Commonly used as a strong chemotherapy, doxorubicin (DOX) has major adverse effects on various organs, particularly the kidney [(1)], and liver [(2)], hence its clinical use is limited.

Because DOX poisoning accumulates reactive oxygen species (ROS) in both organs, it causes oxidative stress, inflammation, impaired mitochondrial function, and activation of apoptotic pathways [(3)]. Thus, it is essential to find trustworthy chemo-protective medications to counteract the negative effects of DOX.

The distinctive function of phosphoinositide 3-kinases (PI3K) is to mediate a range of biological functions. Protein kinase B, often known as AKT, functions as a serine / threonine kinase. Phosphatidylinositol3, 4,5-trisphosphate (PIP3) is formed when PI3K is phosphorylated at its 30 hydroxyl groups in response to different activating stimuli. This procedure is essential for PI3K to activate Akt [(4)].

The PI3K/ AKT pathway plays a significant role in both metabolic and cellular processes. After translocating to the nucleus and cytoplasm, Akt controls several signaling pathways, one of which is Glycogen synthase kinase-3 beta (GSK-3β). Essential physiological functions, such as cell development and proliferation, programed cell death (apoptosis), and metabolism, are tightly regulated by these downstream effectors [(5)].

The apoptosis caused by DOX may be inhibited by activating the PI3K/Akt/GSK3-β signaling cascade can also open the mitochondrial permeability transition pore and consequent apoptosis [(6)]. Moreover, hepatocyte oxidative stress may be modulated by GSK3-β inhibition [(7)]. Amentoflavone (AMN) is mainly derived from different plant species. Its noteworthy pharmacological benefits such as anti-aging, anti-cancer, anti-diabetic, and anti-inflammation properties, as well as antioxidant and tissue protective effects as kidney, liver and heart [(8, 9)].

To the best of our knowledge, it's the 1st time to study the effect of AMF on DOX induced hepatorenal injury with its impact on PI3K/AKT/GSK-3β as a suggsted mechanism of action.

MATERIALS AND METHOD

All experiments were carried out in accordance with the guidelines for animal care and use given in the National Institutes of Health publication "No 85-23, which was amended in 1996".

Ethical approval:

The Menoufia University Ethics Committee for the Faculty of Medicine authorized the use and care of animals with IRB No:10/2024Bio18.

Animals

The study involved thirty male Wistar rats, sourced locally, with matching weights and ages ranging from 200 to 250 g. These rats underwent a 10-

Received: 21/05/2024
Accepted: 21/07/2024

day acclimatization period just before the commencement of the experiments. Rats got unrestricted access to regular dietary chow and water in a room with controlled temperature, twelve-hour cycles of light and darkness. Upon completion of the experiment, rats were killed via cervical dislocation.

Experimental design:

After acclimatization, the rats were divided into the designated groups, with ten rats in each group: Two milliliters of 0.9% normal saline were intraperitoneally injected into each of the rats in the control group, followed by an 8-week period of receiving 2 mL of distilled water through oral gavage.

Group II with DOX-induced hepatorenal toxicity: The DOX was obtained from Biozenta, Hetero Health Care LTD, India, in the form of red powder that was dissolved in 2 milliliters of 0.9% saline solution, and then administered through a single injection intraperitoneally at a dose of 20 milligram/kilogram [(10)].

AMF treated DOX-induced hepatorenal toxicity (DOX+AMF) group: From the first day of DOX administration, rats were delivered daily intragastric AMF (Sigma Aldrish, St Luis, Missouri, USA) in a dose of 40 milligram/kilogram for 8 weeks [(11)], with Dox used by at the same dose, duration, of group II [(10,11)].

Collection of Blood Samples:

5 milliliters of blood from the retroorbital venous plexus withdrawn under anesthesia for biochemical assessment and the rats then were euthanized by cervical dislocation. Each rat's blood sample was put into a basic, sterile tube and allowed to clot for nearly 30mints. at room temperature. The samples underwent centrifugation (3000rpm for 15mints.) to isolate the serum. They were then preserved at -80°C for additional biochemical analysis. The abdomens of all rats were opened, and both kidneys and liver were removed.

Colorimetric kits (Biodiagnostic Company; Dokki; Giza; Egypt) were used to determine S. liver enzymes (ALT, AST), serum urea, serum creatinine, serum MDA, and GPX.

ELISA kits from (BioSource, Sandiego., CA, USA), were used for measurement of Rat IL-10 and TNF-α. In addition to measurement of Caspse-3 was analyzed by utilizing ELISA kits from Cusabio Technology Llc, Houston; TX; USA (CSB-E08857r).

Real time PCR (rt-PCR) for detection of Akt1, PI3k and Gsk3β pathway genes expression:

The 7500 real-time PCR system (Applied Biosystems, CA, USA) was used to identify Akt1, PI3k, and GSK3-β. RNA was extracted from fresh blood using the A direct-zol RNA Miniprep kit (Cat. No.R2051; Zymo Research, USA), followed by the first PCR step. The QuantiTect Reverse Transcription Kit (205311, Qiagen, Applied Biosystems, USA) was used to synthesize complementary deoxyribonucleic acid (DNA), followed by the second step of PCR (rt-PCR step), which was performed using the QuantiTect SYBR Green PCR Kit with ready-made quantities Primer Assay (204143; Qiagen, USA) to measure gene levels.

The following primers were used: forward and reverse primers for *Akt1, PI3k and Gsk3-β* forward, 5′-TCATTGAGCGCACCTTCCAT-3′ and reverse 5′-TTCTGCAGGACACGGTTCTC-3′, forward 5′-CTGATTTTACGGCGGCATGG -3′ and reverse 5′-GCAGCACTTGGTCAACACTG -3 and forward 5′-AGCTGATCTTTGGAGCCACC -3′ and reverse 5′-AACGTGACCAGTGTTGCTGA -3 respectively. β-actin forward, 5′-CCCATCTATGAGGGTTACGC-3′ and reverse, 5′-TTTAATGTCACGCACGATTTC -3′ as endogenous control.

PCR was done as follow: Each reaction was carried out in a final volume of 20 µl, including 5 µl of RNase-free water, 10 µl SYBR Green 2× QuantiTect PCR Master Mixture, 3 µl cDNA plus one µl forward primer, and one µl reverse primer. Following three minutes of incubation at 94°C, the mixture was subjected to 55 cycles of denaturation for thirty seconds at 94°C, annealing for forty seconds at 55°C, and extension for thirty-one seconds at 72°C. Data analysis is done with Biosystems 7500 software, version 2.0.1. The relative quantification of gene expression assessment is done using the comparative ΔΔCt technique.

Histopathological analysis:

Fresh kidney and liver specimens were obtained, and they were promptly preserved in 10% neutral buffered formalin. Subsequently, 5 µm-thick paraffin slices were produced and histological features were verified by H&E staining.

Statistical analysis:

The data were tabulated and analyzed using the Statistical Package for the SPSS version 16.0. The mean ± standard deviation (SD) was used to express the statistical data. Following a one-way analysis of variance (ANOVA), a post-hoc Tukey testing was performed to determine the significance of the group differences. A statistical significance threshold of $P < 0.05$ was attained.

RESULTS

Comparing the DOX group's serum levels to those of the control group, our findings revealed a significant increase in urea, creatinine, ALT, AST, MDA, TNF-α, and caspase-3, along with a concurrent decrease in SOD and IL10. Serum levels of urea, creatinine, ALT, AST, MDA, TNF-α, caspase-3, and SOD and IL10 were significantly lower in the doxorubicin +AMF group than in the DOX group, however they were still significantly different from the control group (**Table 1).**

Table (1): The measured serum urea, creatinine, ALT, AST, MDA, SOD, TNF-α, IL10, and caspase-3 in the control, DOX, DOX+AMF groups.

	Control group	DOX group	DOX+ AMF group
Serum Urea (mg/dl)	38.2±5.6	120.7±7.2 *	**81.5±7.6** *#
Serum Creatinine (mg/dl)	0.4±0.07	1.8±0.07 *	**0.89±0.1** *#
ALT (U/L)	21.4±2.6	76.9±5.3 *	**40.2±3.9** *#
AST (U/L)	37.6±4.8	101.9±9.4 *	**74.9±5.8** *#
Serum MDA (nmol/ml)	21.7 ±2.9	59.9± 7.2*	**31.9± 2.04** *#
Serum SOD (U/ml)	15.53 ±2.6	4.78±0.91*	**9.88±0.7** *#
Serum TNF-α (pg/ml)	30.8±0.37	60.9±1.2*	**43.8±1.1** *#
Serum IL10 (pg/ml)	3.38±0.42	0.86±0.22*	**2.05±0.21** *#
Serum Caspase-3 (ng/ml)	37.76±2.08	93.38±2.13*	**64.08±1.22** *#

* Significant compared with control. # Significant compared with DOX.

When comparing the renal and hepatic tissues expression of the AKT and PI3K, GSK3-β genes of the doxorubicin group to those of the control group, there was a significant decrease in the expression of the AKT and PI3K genes and a concurrent increase in the expression of the GSK-3β gene. When comparing the renal and hepatic tissues expression of the AKT, PI3K, GSK-3β gene of the DOX+AMF group to those of the doxorubicin group, there was a significant increase in the expression of the AKT and PI3K genes and a concurrent decrease in the expression of the GSK-3 gene. However, the differences were still significant when compared to the controls. **(Fig 1a, b, c).**

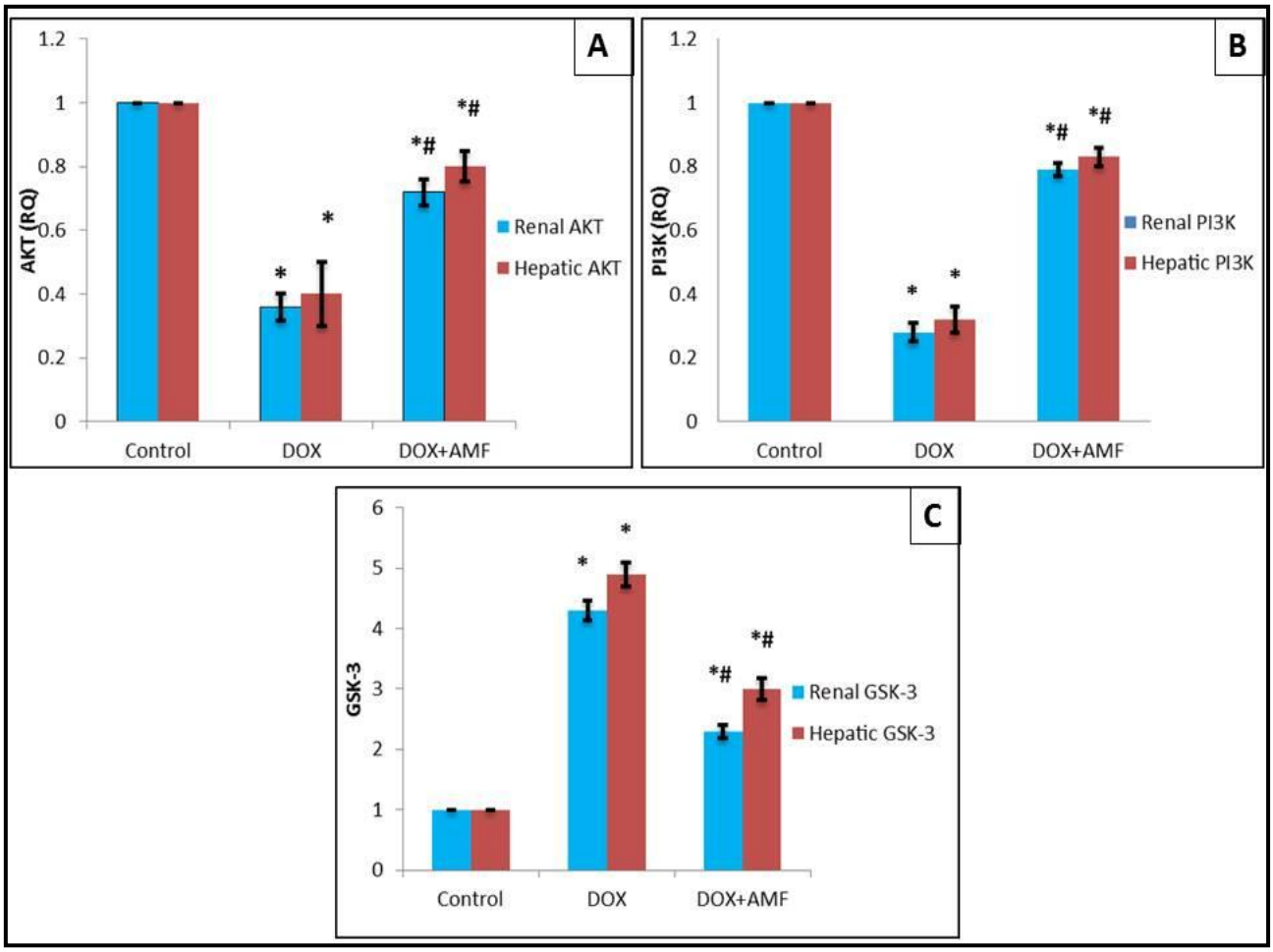

Figure (1): The impact of amentoflavone (AMF) on gene expression of AKT, PI3K, and GSK-3β in both renal and hepatic tissues in doxorubicin (DOX) induced hepatorenal injury. [* significant when compared with control group. # significant when compared with DOX group].

Histopathological results:

The control group's kidney section's histopathological examination revealed that the glomerulus, Bowmen's capsule, and convoluted tubules were all present in a normal, intact state (Fig. 2a). On the other hand, the kidney's tubular system underwent changes and Bowman's capsule suffered severe damage in the glomeruli of rats treated with DOX (Fig. 2b). On the other hand, the cotreated

group's histopathological analysis (DOX + AMN) showed ameliorated tubular renal epithelium lining and improved glomerular structure morphology (Fig. 2c).

Hepatocellular necrosis and hepatic cord atrophy with Kupffer cell hyperplasia (fig. 3b) were indicative of the DOX-induced alteration of the hepatic architecture, in accordance with the histopathological observation of the liver section of the control group, which showed the presence of a normal and intact hepatic histological architecture (fig. 3a). Nevertheless, concurrent exposure to DOX+AMF caused a very slight hepatic cord atrophy without necrosis with ameliorated hepatic architecture (3 c).

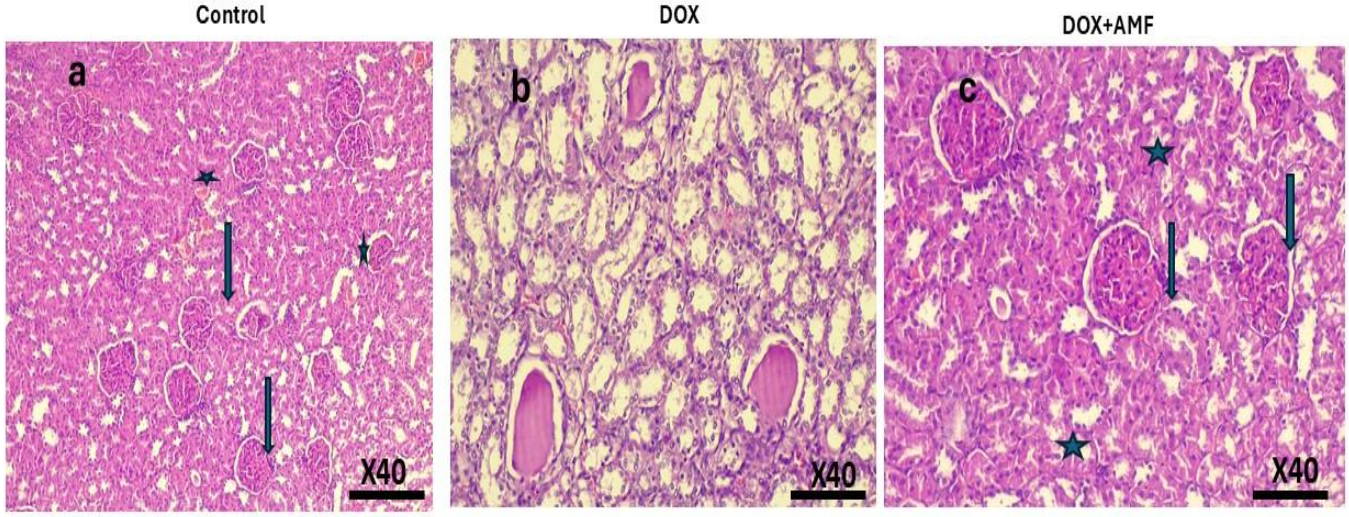

Figure (2) Showed renal-protective effects of amentoflavone on doxorubicin induced alterations in kidney histology. a) control group whose renal tissue architecture is normal b) DOX group: tubular epithelial degeneration (star), necrosis, nephrosis with casts, and congestion of glomerular capillaries were observed in renal sections treated with DOX (arrow). C) In DOX + AMF group; AMF treatment revealed ameliorated renal histology (H&E, X: 400).

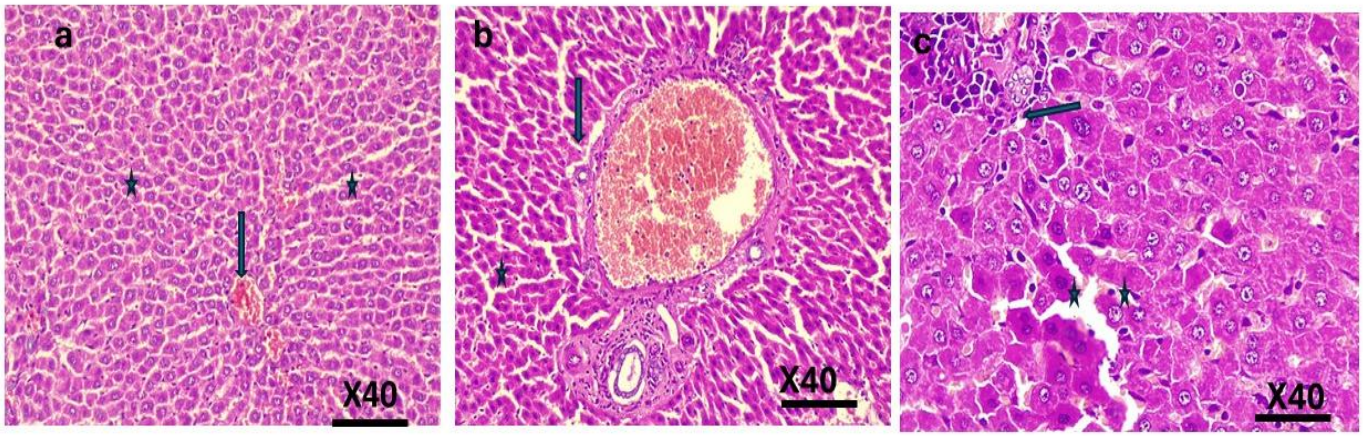

Figure (3): Demonstrated how AMF treatment affected the hepatic sections' histological examination. (a) section of the normal control group's liver displays central veins encircled by hepatocyte cords (star), some of which are binucleated. (b): section of DOX group demonstrated that hepatocytes displayed hydropic degeneration with spotty necrosis with confluent necrosis in certain areas, portal inflammation, periportal interface hepatitis (focal necrosis) (arrow). (c): Liver cords of ameliorated hepatocytes surround a section of the DOX + AMF rats with an average central vein, mild portal inflammation, and very mild atrophy without necrosis (H&E, X: 400).

DISCUSSION

One of the effects of DOX is hepatorenal injury [(2)]. Serum ALT, AST enzymes, blood urea, and creatinine were all significantly elevated in this study with liver and kidney damage when compared to control rats this agreed with previous studies [(11,12)]. This study revealed notable alterations in the liver and kidney's histopathology. Rats exposed to DOX had liver trabecular structure that was blurred, their hepatic plates had disappeared, and their pyknotic nuclei had condensed chromatin, indicating focal (single cell) necrosis. In addition, the kidney displayed necrotic atrophy, hydropic degeneration, and edema and vacuolation in the proximal and distal tubule epithelial cells. This was consistent with **Guo *et al.* study** [(12)] who reported that DOX induced large capsular space and atrophic glomerulus.

DOX causes lipid peroxidation that is triggered by ROS, which favors renal and hepatocyte destruction that cause ALT and AST to leak into the blood with

abnormal kidney function, this was agreed with previous studies [3,13].

According to **Neilan *et al.*** [14] AMF significantly reduced the damage to liver and kidney tissue caused by DOX and mitigated the histological profile seen in the DOX group. This was consistent with a prior investigation by **Afsar *et al.*** [15] who demonstrated that DOX induced redox homeostasis imbalance, disturbed antioxidant defenses that oxidized DNA and lipids causing tissue damage could be ameliorated by flavonoids [11].

AMF dramatically ameliorated oxidative stress caused by DOX, the antioxidant activity of AMF was previously shown by **Ijaz *et al.*** [3]**,** this could be attributed to its radicle scavenging ability.

Inflammation is also founded to be one of the processes involved in hepatorenal injury with chemotherapy [15]**.** In this study, there was significant increment of pro-inflammatory cytokines like TNF-α and decrease of anti-inflammatory cytokines as IL-10 with DOX medication compared to control rats, this was in line with a previous studies by [16, 17]. Free radicals generated by DOX stimulated TNF-α, which in turn activated the NF-kB inflammatory pathway in various tissues, including the kidney, liver and heart[18]. In this study, AMF significantly reduced tissue inflammation confirmed by its effect on pro-inflammatory cytokines TNF-α and significantly increased IL-10.

TNFα reduction and IL-10 elevation demonstrated the potent anti-inflammatory action of AMF this was agreed with **Menezes and Diederich**[19]**.** The master regulator of pro-inflammatory cytokine release, NF-κB, can be suppressed by AMF activation of SIRT1, which also controls P53 signaling and plays a major role in the regulation of inflammation, oxidative stress, and apoptosis [20]**.**

The induction of hepatorenal damage and apoptosis by DOX involved multifaceted mechanisms. One of the most prevalent causes of DOX-induced tissue damage might be attributed to decreased expression of HO-1 and Nrf2 proteins from the liver, as well as elevated MDA due to lipid peroxidation triggering increased ROS that might cause hepatocyte and nephrons apoptosis confirmed by significantly elevated Caspase-3. These findings corroborated those of previous study [21]**.** AMF effect on p53 could be the main antiapoptotic mechanism of flavonoids that significantly reduced Caspase 3 level in this study [19].

Xing *et al.* [22] discovered that GSK-3β may phosphorylate the serine residues of Nrf2, increasing Keap1-independent degradation and decreasing the antioxidant impact of the signaling pathway.

According to **Lu *et al.*** [23] DOX associated oxidative stress can be lessened by activating the Keap1/Nrf2-antioxidant pathway through GSK/3β suppression. This is consistent with the recent study that highlighted AMF's antioxidant response. In this investigation, GSK/3β was considerably lower in the AMF treatment group than in the DOX treated group.

Our findings for the PI3K-AKT pathway showed that DOX treatment dramatically reduced the level of their gene expression, which in turn raised GSK-3β phosphorylation and reduced the amount of antioxidant enzymes with pronounced ROS-associated oxidative stress. This is closely related to **Long *et al.*** [24]**.**

According to **Zeraik *et al.*** [25] AMF showed promising outcomes in the treatment of DOX-induced oxidative associated hepatorenal injury by inhibiting the production of superoxide anion and total ROS in human neutrophils as well as oxidant hemolysis and lipid peroxidation.

In the current study, oral AMF treatment was associated with restoration of hepatic levels of PI3K and AKT and suppression of renal and hepatic GSK-3β levels when compared to the doxorubicin treated group. This was in line with **Saleh *et al.*** [2].

One characteristic that distinguishes DOX-induced hepatorenal damage is the dysregulation of the PI3K/AKT/GSK-3β signaling pathway, which is crucial for regulating cell survival, proliferation, and metabolism [7].

Ultimately, one potential factor to consider when combining DOX with an antioxidant treatment is whether the antioxidant would obstruct the intended tumor cell death. But according to research by **Hovorka *et al.*** [26] DOX toxicity in tumor cells is mainly caused by DNA damage and intercalation, whereas its toxicity to the heart, kidneys, and/or liver is primarily caused by the production of free radicals ROS, ROS scavengers, such as AMF, can block this.

CONCLUSION

By AMF modulatory effect on the AKT/PI3K/GSK-3β signaling pathway, AMF's anti-inflammatory, apoptotic amelioration, and oxidant stress protection qualities may be linked to its protective effect against DOX-induced hepatorenal injury.

Acknowledgments: We appreciate and thank the Central Research Laboratory of Medicine, Menoufia University, for its support and precise results.

Conflict of Interests: No conflict of interests is declared.

Fund: Non-fundable.

REFERENCES

1. **Qi W, Boliang W, Xiaoxi T *et al.* (2020):** Cardamonin protects against doxorubicin-induced cardiotoxicity in mice by restraining oxidative stress and inflammation associated with Nrf2 signaling. Biomedicine & Pharmacotherapy J., 122: 109547. doi: 10.1016/ j.biopha.2019.109547.
2. **Saleh D, Mahmoud S, Hassan A *et al.* (2022):** Doxorubicin-induced hepatic toxicity in rats:

Mechanistic protective role of Omega-3 fatty acids through Nrf2/HO-1 activation and PI3K/Akt/GSK- 3b axis modulation. Saudi Journal of Biological Sciences, 29: 103308. doi: 10.1016/j.sjbs.2022.103308.

3. **Ijaz M, Fatima T, Batool M *et al.* (2023):** Therapeutic Efficacy of Chrysoeriol on Doxorubicin-induced Liver Damage by Improving Biochemical and Histological Profile in Rats. Journal of Food and Nutrition Research, 11(1): 57-62.
4. **Cao Y, Lv W, Ding W *et al.* (2020):** Sevoflurane inhibits the proliferation and invasion of hepatocellular carcinoma cells through regulating the PTEN/Akt/GSK-3β/ β-catenin signaling pathway by downregulating miR-25-3p. Int J Mol Med., 46 (1): 97–106.
5. **Zhoua L, Mob Y, Buc X *et al.* (2018):** Erinacine facilitates the opening of the mitochondrial permeability transition pore through the inhibition of the PI3K/akt/ GSK-3β signaling pathway in human hepatocellular carcinoma. Cell Physiol Biochem., 50: 851-867.
6. **Jakobs P, Serbulea V, Leitinger N *et al.* (2017):** Nuclear factor (erythroid-derived 2)-like 2 and thioredoxin-1 in atherosclerosis and ischemia/ reperfusion injury in the heart. Antioxid Redox Signal, 26(12): 630–644
7. **Guo J, Jiang X, Lian J *et al.* (2024):** Evaluation of the effect of GSK-3β on liver cancer based on the PI3K/AKT pathway. Front Cell Dev Biol., 12:1431423. doi: 10.3389/fcell.2024.1431423
8. **Yu S, Yan H, Zhang L *et al.* (2017):** A Review on the Phytochemistry, Pharmacology, and Pharmacokinetics of Amentoflavone, a Naturally-Occurring Biflavonoid. Molecules, 22: 299. doi: 10.3390/molecules22020299.
9. **Mancarz G, Prado M, de Santi Pazzim M (2023):** Flavonoids: an alternative therapy for oxidative stress-related diseases. Studies in Natural Products Chemistry, 77: 37-64.
10. **AlAsmari A, Alharbi M, Alqahtani F *et al.* (2021):** Diosmin Alleviates Doxorubicin-Induced Liver Injury via Modulation of Oxidative Stress-Mediated Hepatic Inflammation and Apoptosis via NfkB and MAPK Pathway: A Preclinical Study. Antioxidants, 10(12): 1998. doi: 10.3390/antiox10121998.
11. **Alher F, El-Masry T, Negm W *et al.* (2022):** Potential cardioprotective effects of Amentoflavone in doxorubicin-induced cardiotoxicity in mice. Biomed Pharmacother., 154: 113643. doi: 10.1016/j.biopha.2022.113643.
12. **Guo H, Liu Y, Wang G *et al.* (2016):** Alleviation of doxorubicin–induced hepatorenal toxicities with sesamin via the suppression of oxidative stress. Human and Experimental Toxicology, 35(11): 1183–1193.
13. **Ibrahim Fouad G, Ahmed K (2021):** The protective impact of berberine against doxorubicin-induced nephrotoxicity in rats. Tissue Cell, 73: 101612. https://doi.org/10.1016/jtice.2021.101612.
14. **Neilan T, Blake S, Ichinose F *et al.* (2007):** Disruption of nitric oxide synthase 3 protects against the cardiac injury, dysfunction, and mortality induced by doxorubicin. Circulation, 116: 506–514
15. **Afsar T, Razak S, Almajwal A *et al.* (2020):** Doxorubicininduced alterations in kidney functioning, oxidative stress, DNA damage, and renal tissue morphology; improvement by Acacia hydaspica tannin-rich ethyl acetate fraction. Saudi J Biol Sci., 27(9): 2251–2260.
16. **El-Moselhy M, El-Sheikh A (2014):** Protective mechanisms of atorvastatin against doxorubicin-induced hepato-renal toxicity. Biomed Pharmacother., 68 (1): 101–110.
17. **Prasanna P, Renu K, Gopalakrishnan A (2020):** New molecular and biochemical insights of doxorubicin-induced hepatotoxicity. Life Sci., 250:117599. doi: 10.1016/j.lfs.2020.117599.
18. **Han D, Ybanez M, Ahmadi S *et al.* (2009):** Redox regulation of tumor necrosis factor signaling. Antioxid Redox Signal., 11: 2245–63.
19. **Menezes J, Diederich M (2021):** Bioactivity of natural biflavonoids in metabolism-related disease and cancer therapies. Pharmacological Research, 167: 105525. doi: 10.1016/j.phrs.2021.105525.
20. **Sirwi A, Shaik R, Alamoudi A *et al.* (2021):** Mokko lactone attenuates doxorubicin-induced hepatotoxicity in rats: emphasis on Sirt-1/FOXO1/NF-κB axis. Nutrients, 13(11): 4142. https://doi.org/10.3390/ nu13114142
21. **Barakat B, Ahmed H, Bahr I *et al.* (2018):** Protective effect of boswellic acids against doxorubicin induced hepatotoxicity: impact on Nrf2/HO-1 defense pathway. Oxid Med Cell Longev., 18: 8296451. doi: 10.1155/2018/8296451.
22. **Xing H, Cai Y, Wang X *et al.* (2015):** The cytoprotective effect of hyperoside against oxidative stress is mediated by the Nrf2-ARE signaling pathway through GSK-3b inactivation. PLoS One, 10(12): e0145183. https://doi.org/10.1371/journal.pone.0145183.
23. **Lu M, Ji J, Jiang Y *et al.* (2016):** An inhibitor of the Keap1-Nrf2 protein-protein interaction protects NCM460 colonic cells and alleviates experimental colitis. Sci Rep., 6:26585. doi: 10.1038/srep26585.
24. **Long H, Cheng Y, Zhou Z *et al.* (2021):** PI3K/AKT Signal Pathway: A Target of Natural Products in the Prevention and Treatment of Alzheimer's Disease and Parkinson's Disease. Front Pharmacol., 12:648636. doi: 10.3389/fphar.2021.648636.
25. **Zeraik P, Ximenes M, da Fonseca V *et al.* (2015):** Redox-active bioflavonoids from Garcinia brasiliensis as inhibitors of neutrophil oxidative burst and human erythrocyte membrane damage. J Ethnopharmacol., 174: 410–418.
26. **Hovorka O, Subr V, Větvička D *et al.* (2010):** Spectral analysis of doxorubicin accumulation and the indirect quantification of its DNA intercalation. European Journal of Pharmaceutics and Biopharmaceutics, 76(3):514–524.