Hesperidin mitigates Doxorubicin-induced hepatic toxicity in rats, targeting JAK-STAT signaling pathway

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ABSTRACT

Even though doxorubicin (DOX) is excellent cancer chemotherapy, its adverse impacts, including hepatotoxicity, restrict its clinical usefulness. The study's goal was to assess hesperidin's (HSP) hepatoprotective impact in rats exposed to DOX as well as any potential underlying mechanisms. Thirty male albino rats were split into three: DOX, DOX+HSP, and control (10/group). Serum liver enzymes, triglycerides (TG) and cholesterol, hepatic MDA, superoxide dismutase (SOD), serum TNF-α, IL-6, IL-10, liver-expressed JAK2, STAT3 genes, and organ index were assessed. There were additional evaluations of NF-kB and caspase-3 immunoreactions in the liver. The results revealed that hepatic SOD, IL-10, and liver index values all substantially declined in response to DOX-induced damage. But compared to the control group, there was a dramatic rise in the JAK2 and STAT3 genes, as well as hepatic MDA, TNF-α, and IL-6. There was also an increase in blood liver enzymes, serum cholesterol, and TG. In the liver, caspase-3 and NF-kB immunoreactions were also up-regulated. HSP dramatically improved DOX-induced changes in the liver. It can be concluded that, in addition to down-regulating the JAK2/STAT3 signaling cascade, HSP also employed antioxidant, anti-inflammatory, and anti-apoptotic mechanisms to mitigate the hepatotoxicity induced by DOX.

Introduction

Global data indicate that one of the primary causes of illness and mortality worldwide is neoplasms. The inaccessibility of diagnosis and obtaining medical attention in the latter stages of the illness are common problems. Systemic chemotherapy is still the cornerstone of cancer treatment, despite the ongoing development of other strategies (Markham et al., 2020).

Hepatotoxicity is a common side effect of the anthracycline antibiotic doxorubicin (DOX), commonly used as an anti-cancer medication (Tabeshpour et al., 2020). Since the liver is the primary tissue involved in detoxification, overusing anticancer medications, such as DOX, is the goal. Liver damage affects about 40% of individuals receiving DOX, which can lead to liver failure (Afsar et al., 2019).

The primary mechanisms of DOX-induced cytotoxicity are cellular apoptosis and reactive oxygen species (ROS) generation. Additionally, exposure to DOX stimulates nuclear factor kappa B cell (NF-kB) expression, which releases mediators that promote inflammation (Khodir et al., 2021).

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A multitude of genes and signaling pathways contribute to the pathophysiology of cytotoxicity generated by Dox. A proinflammatory cytokine's signaling aim is the janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway (Walker & Smith, 2005).

Furthermore, prior research has indicated that the overexpression of JAK2/STAT3 plays a vital part in the excessive activation of macrophages. This causes pro-inflammatory cytokines to be released more often (Li et al., 2014). The JAK/STAT pathway is a crucial regulator of cell division and apoptosis at the same time. (Lu et al., 2020). Furthermore, JAK2 inhibitors' suppression of STAT3 activation suppresses NF-κB activation (Liu et al., 2023).

Extensive research has demonstrated the importance of the JAK-STAT pathway in oxidative stress and apoptosis (Monroe, & Halvorsen, 2006). To mitigate the damage caused by Dox, several antioxidants or anti-apoptotic drugs have been used to control oxidative damage (Malekinejad et al., 2012).

One of the naturally occurring flavonoids promoted as a dietary supplement that is present in citrus fruits is hesperidin (HSP). Pharmacological activity of this substance includes anti-inflammatory, anti-oxidant, antihyperglycemic, and anti-carcinogenic effects (Roy et al., 2020).

The aim of the study was to assess the hepatoprotective impact of HSP in rats exposed to DOX as well as any potential underlying mechanisms involving the JAK2/STAT3 signaling pathway**.**

Materials and Methods

Animals

We conducted this study in accordance with the guidelines set out by the Faculty of Medicine at Menoufia University's Animal Experimentation Ethics Committee with registration number: 11/2024BIO13-1. We utilized thirty mature male Wister rats, weighing between 150 and 180 g. The rats were kept in housing that ranged from 20 to 24 °C, with a light and dark cycle of 12 hours each. They also had free access to tap water and regular rat food. Before the trials began, the rats were given ten days to acclimate.

Experimental design

Rats were randomly divided into three groups of ten each.

1. In the control group, rats received a single intraperitoneal (i.p.) injection of one milliliter of normal saline and distilled water, identical to the volume used to dissolve the medicines, via oral gavage daily.
2. DOX group: Rats were given a single intraperitoneal injection of 15 mg/kg DOX which cause hepatotoxicity (Khodir et al., 2021). HIKMA Specialized Pharmaceuticals in Badr City, Cairo, Egypt, offered DOX in vials under the brand name "Adricin." Each vial contains DOX HCL 50 mg/25 ml. Rats were given distilled water in a comparable volume to that used to dissolve the medicines via oral gavage once daily.
3. DOX/Hesperidin-treated (DOX+HSP) group: The rats received a single dose of DOX (15 mg/kg, i.p.) and HSP (CAS number: 520-26-3), obtained from Sigma-Aldrich (Germany) at a protective dose level of 50 mg/kg orally delivered by oral gavage for three weeks (Hozayen et al., 2014) starting the day after the DOX injection.

All of the rats were sacrificed at the conclusion of the research, and the liver was promptly dissected after weighing it.

The following formula was used to compute the organ indices: (Farsani et al., 2018)

$$\text{Organ index} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100$$

For the purpose of real-time PCR analysis of the JAK2 and STAT3 genes, the right lobe of the liver was kept at –80°C. While the remaining portions of the liver were prepared for histological and immuno-histochemical investigations, the remaining portions of the liver lobe were homogenized for biochemical studies.

Blood sampling and biochemical analysis

After an overnight fast, retro-orbital blood samples were collected, then centrifuged for 15 minutes at 2000 rpm and left to coagulate for 30 minutes at room temperature. Then rats were sacrificed and liver were dissected for pathological and biochemical analysis The serum liver enzymes (alanine transaminase [ALT], aspartate transaminase [AST], alkaline phosphatase [ALP], and gamma-glutamyl transferase [GGT]), serum cholesterol, and serum triglyceride (TG) were measured using colorimetric kits (Biodiagnostic Company, Dokki, Giza, Egypt) after the serum was collected and frozen at -80°C. The measures were assessed according to the manufacturer instructions.

Tissue homogenate preparation

Independent homogenization and weighing of liver tissues was accomplished using a tissue homogenizer (MPW120, MPW Medical Instruments, China). An ice-cold centrifuge was used to spin the crude tissue homogenate for 15 minutes at 10,000 rpm. The supernatant was then collected and kept at -80°C for further analysis. Hepatic MDA and SOD levels were measured using colorimetric kits (Biodiagnostic Company, Dokki, Giza, Egypt).

Hepatic IL-10, IL-6, and TNF levels were measured using rat enzyme-linked immunosorbent assay kits (ERI3010-1, Assaypro LLC, Saint Charles, Missouri, USA; ERT2010-1, Assaypro LLC, Saint Charles, Missouri, USA; and ab100772, Abcam, Cambridge, UK) according to the manufacturer instructions.

Quantitative assay of gene expression using reverse transcriptase polymerase chain reaction technique (RT-PCR)

The relative mRNA levels of JAK2 and STAT3 genes in the liver were measured using RT-PCR. TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from tissues, as directed by the manufacturer. RNA was isolated and stored at -80°C until needed. ThermoScriptTM RT reagent kits (Invitrogen) were utilized to synthesize complementary DNA (cDNA) (reverse transcription) during the initial PCR stage. Following that, cDNAs were amplified using SYBR Green Mix kits (Stratagene, USA) in PCR assays. Each amplification curve yielded a cycle threshold (Ct) value. The reference gene, glyceraldehyde-3-phosphate dehydrogenase, was used. Data analysis was performed using 7500 ABI PRISM (Applied Biosystems, USA) v.2.0.1. Dorak (2018) used the comparative ΔΔCt approach to measure STAT3 and JAK2 gene expression. The JAK2 and STAT3 gene primers were as follows:

JAK2,
Forward 5′-GTGGAGATGTGCCGCTATG-3′
Reverse 5′-CCTTGTACTTCACG ATGTTGTC-3
STAT3
Forward 5′-CACCCATAGTGAGCCCTTGGA-3′
Reverse 5′-TTTGAGTGCAGTGACCAGGACAG-3′;

Histopathological analysis

Fresh liver specimens from the right lobe were obtained and rapidly preserved in 10% neutral buffered formalin. To validate

histological characteristics, paraffin slices (5 µm thick) were stained with Hematoxylin and Eosin (Bancroft & Layton, 2013). The primary monoclonal antibodies employed were the mouse monoclonal antibodies against NF-kB (Abcam) and caspase-3 (Ab-7, Mouse Mab. MS). The cells' cytoplasm appeared brownish, indicating a cellular reaction. The immunostaining method was carried out exactly as specified by the manufacturer (Luedde and Schwabe, 2010).

Statistical analysis

The data was tabulated and analyzed using version 16 of the Statistical Package for the Social Sciences (SPSS) software (SPSS, Inc., USA). Statistical data were presented as mean ± standard deviation (X±SD). To determine the significance of group differences, a one-way ANOVA was utilized, followed by a post-hoc Tukey test. A significance level of $P < 0.05$ was deemed statistically significant.

Results

There were dramatically elevated serum liver enzymes, lipids, hepatic MDA, TNF-α, IL-6, and hepatic JAK2 and STAT3 genes expression, with a substantial decline in liver index, hepatic SOD, and IL-10 in the DOX group compared to the control. DOX+HSP showed dramatically declined serum liver enzymes, lipid, hepatic MDA, TNF-α, IL-6 and hepatic JAK2 and STAT3 gene expression with substantial elevation in liver index, hepatic SOD, and IL-10 compared to the DOX group.

Table (1): The measured serum liver enzymes, lipid, liver index, hepatic MDA, SOD TNF-α, IL-6, IL-10 , hepatic JAK2 and STAT3 genes expression (mean ± SD) in all studied groups.

	Control group	DOX group	DOX + HSP group
Serum ALT (U/L)	32.8±3.1	159±4.2 *	90±4.2 *#
Serum AST (U/L)	55.8±4.1	199±5.2 *	141±3.6 *#
ALP (U/L) Serum	100.1±5.9	291.5±4.8 *	180±7.1 *#
GGT (U/L) Serum	5.8±.08	21.5±2.09 *	12.3±2.01 *#
Liver index	2.82±0.11	1.96±0.2 *	2.31±0.19 *#
Serum cholesterol (mg/dl)	100±2.3	207±4.5 *	151±3.02 *#
TG (mg/dl)	40±3.15	157±7.8 *	93±7.5 *#
Hepatic MDA (nmol/ gm. Tissue)	12.1 ±2.9	37± 3.3*	21± 3.04*#
Hepatic SOD (U/gm. Tissue)	13.9 ±1.03	5.98±0.71*	8.18±1.01*#
Hepatic TNF-α (ng/ml)	21.8±1.37	47.8±2.91*	32.9±2.1*#
Hepatic IL-6 (pg/mL)	80±3.42	165±2.89*	119±3.1*#
Hepatic IL-10 (ng/mL)	18.2±1.42	8.9±0.89*	12.1±0.1*#
Hepatic JAK2 gene expression	1	3.1±0.01*	2.01±0.09*#
Hepatic STAT3 gene expression	1	4.2±0.11*	2.77±0.08*#

* Significant compared with control, # Significant compared with DOX. Data represented as mean ± SD. ALT: alanine transaminase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, GGT: Gamma-Glutamyl Transferase, TG: triglycerides, MDA: Malondialdehyde, SOD: superoxide dimutase, TNF-α: tumour necrosis factor alpha, IL-6: interleukin-6, IL-10: interleukin-10.

Histological evaluation:

Hematoxylin and Eosin staining:

Histopathological examination of all studied groups are shown in figure (1). Control showed normal histological liver structure (Fig. 1a). The DOX group showed a markedly dilated, congested central vein, focal cellular aggregations, and dissolution of hepatic cords (Fig. 1b,c). DOX+HSP showed normal hepatocytes and a mildly congested central vein (Fig. 1d).

Morphometric and statistical results:

a- The intensity Percentage of Caspase-3 reaction:

Caspase-3 intensity in DOX was dramatically higher than control (38±0.54 vs. 9±0.74, respectively, P<0.05). DOX+HSP (15±0.54, P<0.05) was dramatically lower than that of the DOX; however, it was still substantially higher than control (P <0.05) (Figure 2d).

b- The intensity Percentage of NF-κβ reaction:

NF-κB intensity in DOX was dramatically higher than control (45±0.94 vs. 10±1.39, P <0.05). NF-κB intensity in the DOX+HSP group (19±2.7, P <0.05) was dramatically declined compared to DOX (Figure 2h).

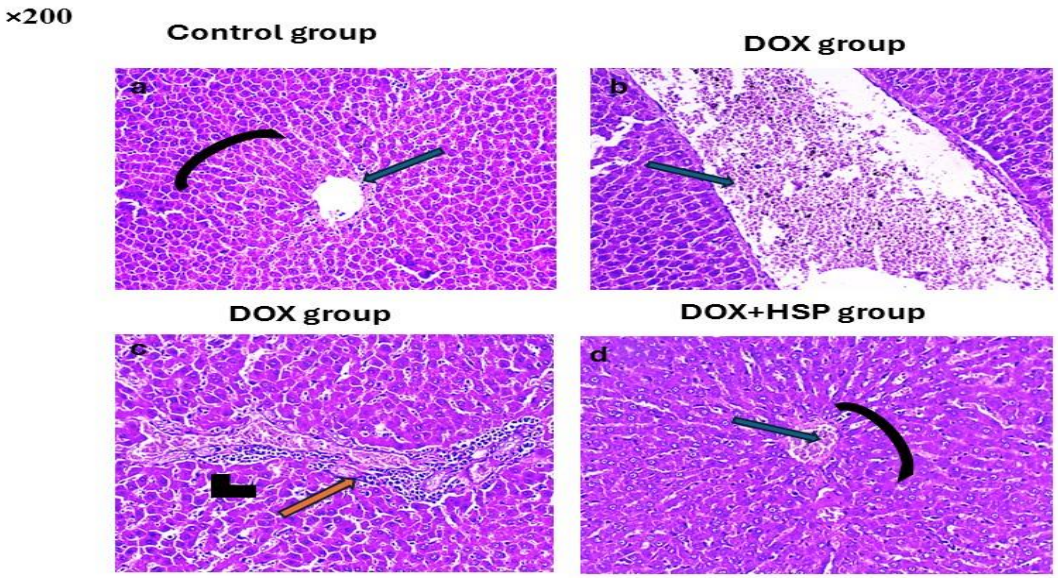

Fig. (1): H&E-stained liver sections in the studied groups (H&E ×200): Photomicrograph (a) shows the liver architecture of the control group, which includes a normal central vein (blue arrow) and hepatocyte with vesicular nuclei (curved arrow). The photomicrographs (b, c) of the DOX group demonstrated the disintegration of the hepatic cords (arrowhead), empty vacuoles, cells with shrunken, irregular nuclei, and dilated, congested central vein (blue arrow) with focal cellular aggregations (orange arrow). In (d), the hepatocytes (curved arrow) and mildly congested central vein (blue arrow) demonstrated an improvement in the liver tissue of the DOX+HSP group.

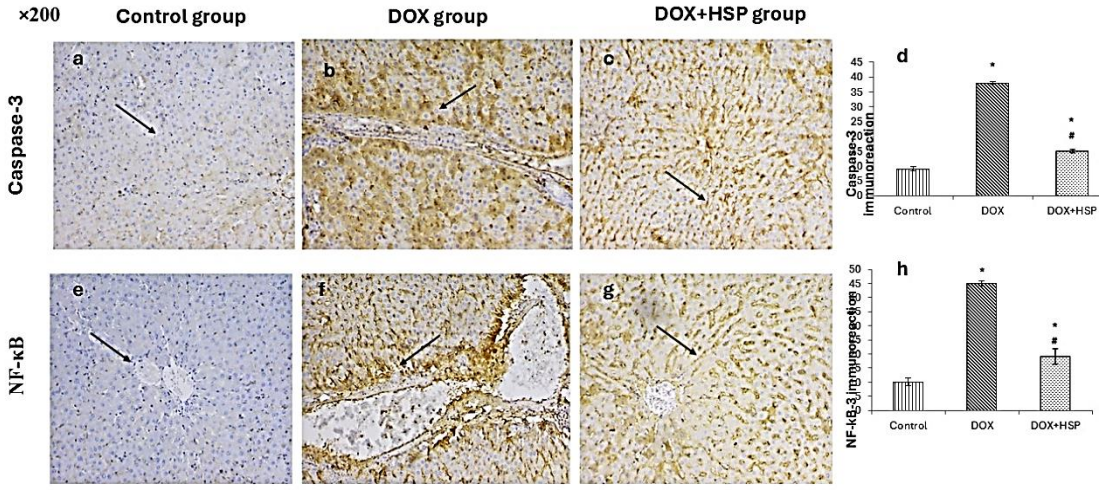

Fig. (2): Photomicrographs (a, b, and c) depict liver slices stained with Caspase-3; (a) The control group exhibits negative immunostaining for Caspase-3. (b) The liver tissue in the Dox had a deep, dark hue that was spread out. (c) The Dox+HSP group had weak Caspase-3 cytoplasmic immunostaining (Caspase-3 x 200) in liver tissue. The pictures (e, f, and g) show liver sections that have been stained with NF-κB. (e) The control group does not have any cytoplasmic immunostaining for NF-κB. (f) The liver tissue of the DOX group has a deep brown color, indicating a favorable response to NF-κB. (g) NF-κB cytoplasmic immunostaining is somewhat visible in the DOX+HSP group (NF-κB x 200). (d) represent the data for caspase-3, and (h) represent the data for NF-κB.

Discussion

Despite being effective cancer chemotherapy, adverse effects such as hepatotoxicity limit the practical utility of DOX-based treatment (Afsar et al. 2019).

The release of hepatocellular enzymes is a sign of hepatotoxicity. In keeping with our findings from previously published study, the DOX group's results demonstrated a significant increase in blood liver enzymes and a decrease in liver index value (Khodir et al., 2021).

According to Neilan et al. (2007), the free radical-induced oxidative stress pathway is responsible for the DOX-induced hepatotoxicity. This induces lipid peroxidation that is initiated by ROS, promoting hepatocyte destruction and causing ALT and AST to leak into the blood. Our liver histology results supported this and were consistent with earlier findings by Barakat et al. (2018).

Liver function improved considerably after HSP treatment. This was consistent with prior research by Hozayen et al. (2014) and Turk et al. (2014). Our histopathological results verified the improvement, which could be attributed to HSP's anti-inflammatory and antioxidant characteristics (Ciftci, et al. 2015). In a previous study by Aboraya et al. (2022), it was found to be an effective pretreatment for cisplatin-induced liver damage.

In Dox group's, it was found that there was a substantial spike in blood cholesterol and TG. These outcomes were consistent with earlier findings that were published by Khodir

et al. (2021). DOX changed HSP's lipid profile considerably. Donia et al. (2019) have previously demonstrated HSP's hypolipidemic influence.

DOX-induced damage is mostly dependent on oxidative stress (Arunachalam et al., 2021). The Dox group's data showed a considerable spike of hepatic MDA levels and a drop in hepatic SOD. These findings are consistent with earlier research that was reported by Barakat et al. (2018). The primary cause of DOX's negative effects is its inherent propensity to generate free radicals and inhibit antioxidant enzymes (Yeh et al., 2009).

In keeping with earlier research, HSP significantly reduced the oxidative stress brought on by DOX (Hozayen et al., 2014). Hesperidin's antioxidative actions include activating Nrf2, scavenging free radicals, suppressing enzymes that produce ROS, preventing DNA damage, and boosting endogenous antioxidant defenses (Mahmoud et al., 2017).

The Dox group's serum levels of the pro-inflammatory markers were dramatically elevated, while the anti-inflammatory marker IL-10 was significantly decreased. Furthermore, the liver of the DOX group exhibited an increased NF-kB immunoreaction. These results align with other published studies by Ma et al. (2019). According to Abd El-Aziz et al. (2012), exposure to DOX triggers the production of NF-kB, which in turn produces pro-inflammatory mediators.

The HSP, however, significantly reduced the inflammatory processes brought on by DOX. HSP's anti-inflammatory properties have been documented before (Alherz et al., 2024 and Mahmoud et al., 2017). In the liver of rats given DEN/CCl4, HSP significantly reduced collagen deposition, NF-kB, and TGF-β1 (Mahmoud et al., 2017). Additionally, activated Nrf2 and HO-1 may mediate hesperidin-induced NF-kB suppression and inflammatory abrogation in the liver. According to reports, Nrf2 inhibits the NF-kB pathway (Cuadrado et al., 2014). Researchers discovered that hesperidin inhibited the synthesis of inflammatory mediators. Wei et al., (2012) demonstrated that hesperidin significantly reduces Th2 cytokines and the proportion of inflammatory cells that infiltrate.

Our findings linked the administration of DOX to a dramatically higher level of caspase-3 immunohistochemistry staining of the liver. This result aligns with previous published research (Khodir et al., 2021).

However, the combination of HSP with DOX resulted in a reduction in the caspase-3 immunoreaction in the liver, which was similar with previous research (Ali et al., 2023). They discovered suppression of both extrinsic and intrinsic apoptotic mechanisms. HSP has been reported to link endoplasmic reticulum stress pathways, hence inhibiting cell cycle progression and apoptosis (Wang et al., 2015).

Several studies have linked the JAK/STAT signaling pathway to a variety of diseases. JAK2 inhibitors have been shown to reduce STAT3 activity, which inhibits NF-κB activation (Liu et al., 2023).

Furthermore, previous study has shown that overexpression of JAK2/STAT3 is critical for excessive macrophage activation and inflammation (Li et al., 2014). Furthermore, the JAK/STAT system regulates both apoptosis and cell proliferation (Lu et al., 2020). As a result, blocking the JAK2/ STAT3 pathway may reduce DOX-induced hepatic inflammation and regulate both innate and acquired immune responses. In line with previous studies, the DOX group discovered that JAK2/STAT3 genes were elevated in the liver (Li et al., 2023).

In contrast to DOX, HSP effectively inhibits the JAK2/STAT3 signaling pathway, which is consistent with Yang et al. (2024). Another study by Cincin et al. (2018) discovered that when hesperidin was injected, the STAT genes were considerably reduced. HSP was expected to slow cancer cell proliferation by inhibiting STAT3 activity.

Conclusion

DOX exerts multiple deleterious and toxic effects on the liver. Hesperidin offers significant protective advantages against DOX-induced hepatotoxicity by down-regulating JAK2 and STAT3, inhibiting apoptosis, and exhibiting antioxidant, anti-inflammatory, and hypolipidemic properties.

Conflict of interest

Authors declare that there is no conflict of interest

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References

Abd El-Aziz, T.A., Mohamed, R.H., Pasha, H.F, et al. (2012). 'Catechin protects against oxidative stress and inflammatory mediated cardio-toxicity in adriamycin-treated rats'. *Clin. Exp. Med.*; 12(4), pp.233-240.

Aboraya, D.M., El Baz, A., Risha, E.F. et al. (2022). 'Hesperidin ameliorates cisplatin induced hepatotoxicity and attenuates oxidative damage, cell apoptosis, and in ammation in rats'. *Saudi Journal of Biological Sciences*, 29(5), pp.3157-3166.

Afsar, T., Razak, S., and Almajwal, A. (2019). 'Effect of Acacia hydaspica R. Parker extract on lipid peroxidation, antioxidant status, liver function test and histo-pathology in doxorubicin treated rats'. *Lipids Health Dis*;18(1):126.

Alherz, F.A., El-Masry, T.A., Oriquat, G.A. et al. (2024). 'Hesperidin nanoformulation: A potential strategy for reducing doxorubicin-induced renal damage via the Sirt-1/HIF1-α/VEGF/NF-κB signaling cascade'. *Pharmaceuticals*, 17(9), p.p. 1144.

Ali, Y.A., Soliman, H.A., Abdel-Gabbar, M. et al. (2023). 'Rutin and hesperidin revoke the hepatotoxicity induced by paclitaxel in male wistar rats via their antioxidant, anti-inflammatory, and anti-apoptotic activities'. *Evidence‐Based Complementary and Alternative Medicine*, (1) p.p. 2738351.

Arunachalam, S., Meeran, M.F., Azimullah, S. et al. (2021). 'Nerolidol attenuates oxidative stress, inflammation, and apoptosis by modulating Nrf2 /MAPK signaling pathways in doxorubicin-induced acute cardiotoxicity in rats', *Antioxidants*.; 10(6) p.p.984.

Bancroft, J.D. and Layton, C. (2013). '*The hematoxylin and eosin, connective and mesenchymal tissues with their stains*. In: Suvarna SK, Layton C, Bancroft JD, editors. Bancroft's Theory and Practice of Histological Techniques'. 7th ed. Philadelphia, PA: Churchill Livingstone. p.p. 173-212.

Barakat, B.M., Ahmed, H.I., Bahr, H.I. et al. (2018). 'Protective effect of

boswellic acids against doxorubicin-induced hepatotoxicity: Impact on Nrf2/HO-1 defense pathway'. *Oxid. Med. Cell Longev.*, p.p. 8296451.

Ciftci, O., Ozcan, C., Kamisli, O. et al. (2015). 'Hesperidin, a Citrus flavonoid, has the ameliorative effects against experimental autoimmune encephalo-myelitis (EAE) in a C57BL/J6 mouse model'. *Neurochem. Res*.; 40(6), pp.1111–1120.

Cincin, Z., Kiran, B., Baran, Y., et al. (2018). 'Hesperidin promotes programmed cell death by downregulation of nongenomic estrogen receptor signalling pathway in endometrial cancer cells'. *Biomed. Pharmacother*., 103, p.p.335-345.

Cuadrado, A., Martin-Moldes, Z., and Ye, I. (2014). 'Lastres- Becker, Transcription factors NRF2 and NF-kappaB are coordinated effectors of the Rho family, GTP-binding protein RAC1 during inflammation'. *J. Biol. Chem*., 289 (22) p.p. 15244e15258.

Donia, T., Eldaly, S., Ali, E.M. (2019). 'Ameliorating oxidative stress and inflammation by Hesperidin and vitamin E in doxorubicin induced cardiomyopathy'. *Turkish Journal of Biochemistry*, 44(2), p.p. 207-217.

Dorak, M. (2018). 'Real-time PCR'. *Clin. Chem.*, 50, p.p.1680-1682.

Farsani, B.E., Karimi, S., Mansouri, E. (2018). 'Pravastatin attenuates testicular damage induced by doxorubicin a stereological and histopatological study'. J. Basic *Clin. Physiol. Pharmacol*., 30(1), pp.103-109.

Hozayen, W. G., Abou Seif, H. S., and Amin, S. (2014). 'Protective effects of ruitn and/or hesperidin against doxorubicin-induced hepatotoxicity'. *Int. J. Clin. Nutr*., *2*(1), p.p. 11-17.

Khodir, S., Alafify, A., Omar, E., et al. (2021). 'Protective potential of ginseng and/or coenzyme Q10 on doxorubicin-induced testicular and hepatic toxicity in rats'. *Open Access Macedonian Journal of Medical Sciences, 9*(A), p.p. 993-1005.

Li, C.J., Tsai, H.W., Chen, Y.L., et al. (2023). 'Cisplatin or Doxorubicin reduces cell viability via the PTPIVA3-JAK2-STAT3 cascade in hepatocellular carcinoma'. *Journal of Hepatocellular Carcinoma*, pp. 123-138.

Li. L., Xu, T., Huang, C., et al. (2014). 'NLRC5 mediates cytokine secretion in RAW264.7 macro-phages and modulated by the JAK2 /STAT3 pathway'. *Inflammation*, 37(3), p.p. 835-847.

Liu, Y., Dong, Y.H., Shen, W., et al. (2023). 'Platycodon grandiflorus poly-saccharide regulates colonic immunity through mesenteric lymphatic circulation to attenuate ulcerative colitis'. *Chin. J. Nat. Med.*, 20(4): p.p. 263-278.

Lu, Z., Xiong, W., Xiao, S., et al. (2020). 'Huanglian Jiedu Decoction ameliorates DSS-induced colitis in mice via the JAK2/STAT3 signaling pathway'. *Chin. Med.*, 15: p.p. 45.

Luedde, T. and Schwabe, R.F. (2010). 'NF-κB in the liver--linking injury, fibrosis and hepatocellular carcinoma'. Nat. Rev. Gastroenterol. Hepatol., 8(2) p.p.108-118..

Ma, T., Kandhare, A.D., Mukherjee-Kandhare, A.A. et al. (2019). 'Plant flavonoid ameliorates doxorubicin-induced cardiotoxicity in experimental rats: The decisive role of caspase-3, COXII, cTn-I, iNOs and TNF-α'. *Mol. Biol. Rep*., 46(1), pp.105-118.

Mahmoud, A.M., Mohammed, H.M., Khadrawy, S.M et al. (2017). 'Hesperidin protects against chemically induced hepatocarcinogenesis via modulation of Nrf2/ARE/HO-1, PPARγ and TGF-β1/Smad3 signaling, and amelioration of oxidative stress and inflammation'. *Chemico-Biological Interactions*, 277, p.p.146-158.

Malekinejad, H., Janbaz-Acyabar, H., Razi. M. et al. (2012). 'Preventive and protective effects of silymarin on doxorubicin- induced testicular damages correlate with changes in c-myc gene expression'. *Phytomedicine.*, 19(12), p.p.1077-1084.

Markham, M.J., Wachter, K., Agarwal, N., et al. Clinical cancer advances (2020). 'Annual report on progress against cancer from the American society of clinical oncology'. *J. Clin. Oncol.*; 38(10), p.p.1081-1101.

Monroe, R.K. and Halvorsen, S.W. (2006). 'Mercury abolishes neurotrophic factor– stimulated Jak-STAT signaling in nerve cells by oxidative stress'. *Toxicol. Sci*.; 94(1):p.p.129-138.

Neilan, T.G., Blake, S.L., Ichinose, F. et al. (2007). 'Disruption of nitric oxide synthase 3 protects against the cardiac injury, dysfunction, and mortality induced by doxorubicin'. *Circulation*; 116(5), pp.506-514.

Roy, J., Azamthulla, M., and Mukkerjee, D., (2020). 'Hesperidin and diosmin-a novel drugs'. *International Journal of Pharmacy Research & Technology*. 10(2), pp.25-33.

Tabeshpour, J., Hosseinzadeh, H., Hashemzaei, M., et al. (2020). 'A review of the hepatoprotective effects of hesperidin, a flavanon glycoside in citrus fruits, against natural and chemical toxicities'. *DARU Journal of Pharmaceutical Sciences, 28*, pp. 305-317.

Turk, E., Kandemir, F. M., Yildirim, S., et al. (2019). 'Protective effect of hesperidin on sodium arsenite-induced nephrotoxicity and hepatotoxicity in rats'. *Biological Trace Element Research*, 189, p.p. 95-108.

Walker, J.G., Smith, M.D. (2005). 'The Jak-STAT pathway in rheumatoid arthritis'. *J. Rheumatol.,* 32: p.p. 650–1653.

Wang, Y., Yu, H., Zhang, J. et al. (2015). 'Hesperidin inhibits HeLa cell proliferation through apoptosis mediated by endoplasmic reticulum stress pathways and cell cycle arrest'. *BMC Cancer*, 15, p.p. 682.

Wei, D., Ci, X., Chu, X., et al. (2012). 'Hesperidin suppresses ovalbumin-induced airway inflammation in a mouse allergic asthma model'. *Inflammation*, 35, p.p.114–121.

Yang, L., Quanwei, S., Xuefei, X., et al. (2024). 'Platycodon grandiflorus polysaccharides combined with hesperidin exerted the synergistic effect of relieving ulcerative colitis in mice by modulating PI3K/AKT and JAK2/STAT3 signaling

pathways'. *Chin. J. Nat. Med.*, 22 (0), p.p. 1-20.

Yeh, Y.C., Liu, T.J., Wang, L.C. et al. (2009). 'A standardized extract of *Ginkgo biloba* suppresses doxorubicin-induced oxidative stress and p53-mediated mitochondrial apoptosis in rat testes'. *Br. J. Pharmacol*., 156(1), pp.48-61.