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Full Length Article

Protective effects of cupressuflavone against doxorubicin-induced hepatic damage in rats

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ABSTRACT

Doxorubicin (DOX) is a potent chemotherapeutic agent that is used in various sorts of malignancies. However, its uses are restricted owing to its deleterious effects on different organs including the liver. Cupressuflavone (CUP) is a plant-based flavonoid which is well known for its biological as well pharmacological potential. Our investigation aimed to evaluate the ameliorative potential of CUP against DOX provoked liver toxicity in rats. Twenty-four albino rats (*Rattus norvegicus*) were distributed into four distinct groups such as control, DOX (3 mg/kg), co-administrated DOX (3 mg/kg) + CUP (40 mg/kg) and CUP (40 mg/kg) only. DOX exposure reduced catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), Glutathione reductase (GSR), glutathione-S-transferase (GST) activities and glutathione (GSH) content while escalating the levels of reactive oxygen species (ROS) and malondialdehyde (MDA). Besides, the levels of ALT, AST and ALP were increased in response to DOX exposure. Furthermore, administration of DOX upregulated the levels of Interleukin-6 (IL-6), Nuclear factor kappa-B (NF-κB), Interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and the activity of cyclooxygenase-2 (COX-2). The treatment of DOX reduced the levels of Bcl-2 while escalating the levels of Caspase-3, Caspase-9 and Bax. Similarly, DOX intoxication instigated various histopathological disruptions in hepatic tissues of rats. However, supplementation of CUP notably ($p < 0.05$) restored abovementioned hepatic dysregulations owing to its anti-oxidative, anti-inflammatory as well as anti-apoptotic potential. Our results manifested that CUP could be used as hepatoprotective agent againt DOX induced liver damage in rats.

1. Introduction

Cancer is a profoundly lethal disease which is responsible for approximately 10 million deaths in 2020 and continues to pose substantial challenges to the health of humans (Sung et al., 2021). Various chemotherapeutic drugs are extensively used against metastatic cancer to improve the survivorship of cancer patients. Moreover, chemotherapeutic drugs exposure instigated various organ damages (Miller et al., 2019; Fitzmaurice et al., 2015). Among various chemotherapeutic agents, doxorubicin is a famous chemotherapeutic drug which is employed during the treatments of different malignancies such as lung, testicular, breast, thyroid as well as ovarian (Cortes-Funes and Coronado, 2007; Octavia et al., 2012; Chatterjee et al., 2010). However, the clinical uses of doxorubicin are restricted globally owing to its adverse effects on different organs (Jain and Rani, 2018). It is reported that DOX exposure escalates the levels inflammatory and oxidative biomarkers in hepatic, renal as well as cardiac tissues (Deepa and Varalakshmi, 2005).

It is documented that DOX treatment instigates hepatotoxicity via excessive generation of ROS which subsequently mediates different events of apoptosis, oxidative stress, as well as inflammation (AlAsmari et al., 2021). Furthermore, DOX exposure halts the normal functions of antioxidant enzymes as well as induces lipid peroxidation in hepatic cells (Al-Qahtani et al., 2022; Prasanna et al., 2020). The accumulation of DOX in hepatic tissues induces cytotoxicity via DNA damage, disrupting mitochondrial genes and metabolism as well as modulating apoptosis (Wang et al., 2022; Rawat et al., 2021). Moreover, El-Sayyad

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et al. (2009) elucidated that DOX induces histological disruptions such as localized necrosis, vacuolation of hepatocytes, hyperplasia of bile ducts, cellular edema as well as lymphocyte infiltration. It is revealed that DOX treatment elevates the levels of hepatic function biomarkers which indicates severe hepatic impairments (Hegazy et al., 2021). In hepatic tissues, DOX metabolizes to highly toxic compounds such as doxorubicinol which adversely affects the normal architecture of hepatic tissues (Camaggi et al., 1988).

Nowadays, plant-based products have gained world attention owing to their pharmacological potential against various diseases (Atanasov et al., 2015). Numerous investigations revealed that plant-based flavonoids may be effective against DOX instigated hepatotoxicity (Mahmoudi et al., 2023). Cupressuflavone is a plant based biflavonoid which exhibits anti-inflammatory, analgesic, reno-protective, hepatoprotective as well as gastroprotective potential (Al-Sayed et al., 2018; Al-Sayed and Abdel-Daim, 2014; Koriem et al., 2015). The current investigation was designed to determine the attenuative ability of CUP against DOX instigated hepatic toxicity in rats.

2. Materials and methods

2.1. Chemicals

Both CUP (CAS NO. 101140-06-1, Purity: 98% (HPLC) and DOX (CAS NO. 25316-40-9, Purity: 98% (HPLC) were obtained from Sigma-Aldrich, Germany.

2.2. Animals

Twenty-four rats (albino) having weight (200 ± 20 g) and age (16–18 weeks) were used to conduct the experiment. The animal house of University of Agriculture, Faisalabad was used to conduct the experiment where the rats were provided optimal laboratory conditions (24 ± 2 °C temp and 55 ± 5% humidity) and kept in small rodent cages. The rats experienced 12 h of light alternative with 12 h of dark period. Rats were offered tap water and standard chaw feed (composition: Casein, refined groundnut oil, vitamin & salt mixture, and wheat flour). Furthermore, the experimental animals were handled as per the regulations of "European Union for Animal Care and Experimentation".

2.3. Experiment layout

Twenty-four rats (*Rattus norvegicus*) were apportioned into four distinct groups (n = 6) and each group received a different treatment. The group one was designated as a control group. The group 2nd received DOX (3 mg/kg). The group 3rd was treated with DOX (3 mg/kg) + CUP (40 mg/kg). The group 4th was administrated with CUP (40 mg/kg) only. Following the trial period (30 days), rats were exposed to anesthesia through administration of ketamine (60 mg/kg) and xylazine (5 mg/kg). Then the rats were decapitated, and samples were collected. Blood samples were drawn from trunk region using heparinized syringes and centrifuged at 3000 rpm to isolate the plasma. The isolated plasma was stored at −20 °C in a refrigerator. For histopathological examination, half part of liver was preserved in 10% formalin while the other half was enclosed in a zipper bag and stored at −80 °C for further biochemical examination. Homogenization of hepatic tissues was performed at 12000 rpm for 15 mints by using sodium phosphate buffer and homogenized mixture was used for further biochemical analysis.

2.4. Evaluation of biochemical profile

The methodology described by Aebi (1984) was used to evaluate CAT activity. The strategy elucidated by Rotruck et al. (1973) was used to quantify GPx activity. SOD activity was quantified by using the procedure of Kakkar et al. (1984). GSR activity was assessed by using the strategy designed by Carlberg and Mannerviek (1975). The technique elucidated by Hayashi et al. (2007) and Placer et al. (1966) was used to analyze the levels of ROS and MDA respectively. The strategy described by Couri and Abdel-Rahman (1979) was employed to quantify the activity of GST. The methodology ascribed by Sedlak and Lindsay (1968) was used to evaluate the GSH contents.

2.5. Evaluation of hepatic function markers

The levels of hepatic function markers including ALT (ab285264), AST (ab263883) and ALP (ab287823) were evaluated by using standard ELISA kits manufactured by Abcam, MA, USA. The methodology was carried out according to the guidelines provided in the manual book.

2.6. Evaluation of inflammatory indices

The standard ELISA kits manufactured by Abcam, MA, USA were employed to evaluate the levels of NF-κB (CSB-E13148r), TNF-α (CSB-E07379r), IL-6 (CSB-E04640r), IL-1β (CSB-E08055r) and COX-2 (CSB-E13399r). The assessment was carried out according to the guidelines of the manufacturer.

2.7. Evaluation of apoptotic markers

The levels of Caspase-3 (CSB-E08857r), Caspase-9 (CSB-E08863r), Bax (CSB-EL002573RA) and Bcl-2 (CSB-E08854r) were quantified by using standard ELISA kits manufactured by Abcam, MA, USA according to the guidelines provided in the manual book.

2.8. Histomorphometry

Hepatic samples were taken for the assessment of histopathological dysregulations instigated by DOX. Hepatic tissues were fixed in 10% solution of formalin. Higher grades of ethanol were used to dehydrate the preserved tissues. Then tissues were fixed in paraffin wax. Paraffin blocks were sliced (4–5 μm) by using rotatory microtome. These thin pieces were placed on glass slides and stained by using hematoxylin-eosin and examined under light a microscope at 400X.

2.9. Statistical interpretation

The resulted values were illustrated as mean ± SE. Minitab (version v17) was used to apply Tukey test to carried out One-way ANOVA. The level of significance was considered at $p < 0.05$.

3. Results

3.1. Influence of CUP & DOX on biochemical profile

The mean values of biochemical profile are illustrated in Table 1. It was revealed that rats subjected to DOX showed notable ($p < 0.05$) reduction in CAT, GPx, SOD, GPx, GSR, GST activities and GSH content while escalating ROS and MDA levels as compared to the control group. Furthermore, CUP exposure remarkably ($p < 0.05$) upregulated the antioxidant enzymes activities while downregulating the levels of ROS and MDA. However, no discrepancy was observed among the values of CUP only and the control group.

3.2. Influence of CUP and DOX on hepatic function markers

The mean values of hepatic function markers were represented in Table 2. The rats administrated with DOX showed prominent ($p < 0.05$) increment in ALT, ALP and AST levels in compliance with the control group. Nonetheless, the combined exposure of CUP + DOX markedly ($p < 0.05$) reduced the levels of abovementioned liver function markers. The control and only CUP supplemented group showed no variation among the resulted values.

Table 1
Impact of CUP & DOX on biochemical profile.

Parameters	Groups			
	Control	DOX	DOX + CUP	CUP
CAT (U/mg protein)	12.88 ± 1.67[a]	5.14 ± 0.20[b]	10.597 ± 0.97[a]	12.84 ± 2.01[a]
SOD (U/mg protein)	9.53 ± 0.93[a]	3.81 ± 0.28[c]	7.14 ± 0.30[b]	9.79 ± 1.04[a]
GPx (U/mg protein)	34.02 ± 2.40[a]	8.95 ± 0.22[c]	23.59 ± 2.15[b]	34.60 ± 2.33[a]
GSR (nm NADPH oxidized/ min/mg tissue	7.85 ± 0.65[a]	2.57 ± 0.35[c]	5.94 ± 0.37[b]	7.92 ± 0.68[a]
GSH (μM/g tissue)	20.53 ± 0.84[a]	6.87 ± 0.91[c]	15.04 ± 1.13[b]	20.88 ± 1.19[a]
GST (nM/min/mg protein)	36.16 ± 2.34[a]	16.44 ± 1.15[c]	25.91 ± 2.55[b]	36.81 ± 2.67[a]
ROS (U/mg tissue)	1.32 ± 0.08[c]	9.11 ± 0.50[a]	2.55 ± 0.23[b]	1.28 ± 0.10[c]
MDA (nmol/mg protein)	0.82 ± 0.08[c]	7.43 ± 0.37[a]	1.71 ± 0.15[b]	0.78 ± 0.09[c]

Different superscripts on values showed discrepancies among other groups.

Table 2
Impact of CUP & DOX on the levels of hepatic function markers.

Parameters	Groups			
	Control	DOX	DOX + CUP	CUP
ALT (U/L)	53.33 ± 2.65[c]	83.12 ± 1.93[a]	65.78 ± 2.80[b]	53.15 ± 2.44[c]
AST (U/L)	82.01 ± 3.39[bc]	193.59 ± 10.53[a]	97.59 ± 4.15[b]	81.26 ± 2.81[c]
ALP (U/L)	131.82 ± 3.59[c]	367.27 ± 7.39[a]	223.83 ± 10.05[b]	129.72 ± 3.88[c]

Different superscripts on values showed discrepancies among other groups.

3.3. Influence of CUP & DOX on inflammatory indices

The experimental values of inflammatory markers were showed in Table 3. It was noted that DOX treatment markedly ($p < 0.05$) upregulated the levels of NF-κB, TNF-α, IL-16, IL-1β and COX-2. Moreover, exposure to CUP notably ($p < 0.05$) downregulated the abovementioned inflammatory cytokines levels. Furthermore, the rats administrated with only CUP exhibited almost similar mean values as in the control group.

3.4. Influence of CUP and DOX on apoptotic markers

The mean values of apoptotic markers are illustrated in Table 4. The rats administrated with DOX markedly ($p < 0.05$) augmented the levels of Caspase-3, Caspase-9 and Bax while downregulating Bcl-2 levels. Conversely, co-treatment of CUP remarkably ($p < 0.05$) regulated the levels of abovementioned apoptotic proteins. However, the control and CUP alone exposed groups showed negligible discrepancy in their mean values.

Table 3
Impact of CUP & DOX on the levels of inflammatory indices.

Parameters	Groups			
	Control	DOX	DOX + CUP	CUP
NF-κB (ngg^{-1} tissue)	23.51 ± 2.22[c]	93.17 ± 2.25[a]	43.98 ± 2.23[b]	22.56 ± 2.17[c]
TNF-α (ngg^{-1} tissue)	11.87 ± 2.41[c]	53.51 ± 2.02[a]	23.51 ± 1.79[b]	11.77 ± 2.35[c]
IL-1β (ngg^{-1} tissue)	17.63 ± 1.78[c]	85.68 ± 1.50[a]	34.80 ± 1.32[b]	17.33 ± 2.01[c]
IL-6 (ngg^{-1} tissue)	14.05 ± 2.18[c]	72.65 ± 2.69[a]	28.96 ± 2.56[b]	13.88 ± 2.11[c]
COX-2 (ngg^{-1} tissue)	17.12 ± 2.06[c]	67.96 ± 3.21[a]	29.87 ± 2.41[b]	17.02 ± 2.13[c]

Different superscripts on values showed discrepancies among other groups.

Table 4
Impact of CUP & DOX on the levels of apoptotic biomarkers.

Parameters	Groups			
	Control	DOX	DOX + CUP	CUP
Bax (pg/mL)	1.44 ± 0.25[b]	9.65 ± 1.90[a]	3.36 ± 0.30[b]	1.42 ± 0.25[b]
Caspase-3 (pg/mL)	2.14 ± 0.18[c]	8.18 ± 0.33[a]	2.91 ± 0.12[b]	2.12 ± 0.18[c]
Caspase-9 (pg/mL)	3.81 ± 0.12[bc]	15.22 ± 0.80[a]	5.32 ± 0.89[b]	3.69 ± 0.13[c]
Bcl-2 (ng/mL)	16.56 ± 1.78[a]	5.39 ± 1.31[c]	12.16 ± 1.24[b]	16.85 ± 1.95[a]

Different superscripts on values showed discrepancies among other groups.

3.5. Influences of CUP & DOX on morphology of hepatic tissues

The control and CUP supplemented group exhibited normal architecture of hepatic tissues. However, administration of DOX substantially ($p < 0.05$) escalated the hepatic tissue damages including dilated sinusoid, nuclear dissolution, degenerated hepatocytes, inflammatory cell infiltration and degenerated lobules. However, administration of CUP substantially ($p < 0.05$) alleviated abovementioned histopathological alterations.

4. Discussion

Liver is the primary site for the metabolization of various chemicals and is considered as secondary targeted organ of different toxic compounds (Liu et al., 2009). DOX is a potent chemotherapeutic drug but adversely affects the organ via excessive generation of ROS (Öz and İlhan, 2006). Previous investigations have revealed that natural compounds have potential to ameliorate DOX instigated hepatic damages (Kuzu et al., 2019). Therefore, our trail was conducted to investigate the palliative capability of CUP against DOX prompted hepatotoxicity via regulating oxidative stress, inflammatory indices, haptic function markers, apoptotic prolife as well as histopathological analysis of hepatic tissues.

In present investigation, DOX exposure remarkably ($p < 0.05$) reduced the antioxidant activities while augmenting the levels of ROS and MDA which demonstrating its potential to induce oxidative stress. Antioxidant enzymes have potential to counteract the production of ROS therefore considered as front-line barrier against oxidative stress (Ighodaro and Akinloye, 2018; Aboubakr et al., 2023). It is documented that free radicals interact with PUFAs in cell membrane and trigger lipid peroxidation which ultimately reduced the fluidity as well as disrupts the normal functions of plasma membrane (Raja and Ravindranadh, 2017; Akpinar et al., 2023). However, our findings revealed that supplementation of CUP substantially increased antioxidant enzymes activities while downregulating the levels of ROS and MDA owing to its ROS scavenging potential.

In the current experiment, DOX administration markedly escalated ALT, AST, & ALP levels. The higher levels of abovementioned hepatic markers exhibits hepatic impairments following the DOX exposure (Afsar et al., 2019). It is reported that DOX administration directly damages the hepatic tissues which elevated the levels of hepatic function enzymes in bloodstream (Sathesh et al., 2016). Nagai et al. (2016) also elaborated that administration of DOX augmented the levels of hepatic function biomarkers ultimately contributing to hepatotoxicity. Nevertheless, CUP treatment reduced the levels of abovementioned hepatic function markers in bloodstream which demonstrates its hepatoprotective potential.

The present study demonstrated that DOX treatment upregulated the levels inflammatory cytokines (NF-κB, TNF-α, IL-16, IL-1β & COX-2) in

hepatic tissues. The activation of cytosolic protein complex (NF-κB) mediated the release of inflammatory cytokines that ultimately prompt hepatic inflammation (Kim et al., 2019). Sauter et al. (2011) elucidated that DOX intoxication induces oxidative stress which triggers the release of inflammatory cytokines in bloodstream. Our findings revealed that CUP administration markedly decreased inflammatory markers levels in hepatic tissues which could be associated to its antioxidative potential against oxidative stress.

In the current investigation, DOX exposure upregulated the levels of pro-apoptotic (Caspase-3, Caspase-9 & Bax) while downregulating the anti-apoptotic (Bcl-2) markers. It is reported that oxidative stress is the primary factor underlying the apoptotic processes (Radi et al., 2014). Furthermore, oxidative stress impaired normal functions of mitochondrial membrane that ultimately released cytochrome C into the cytosol which mediates the activation of various pro-apoptotic markers (Waseem and Parvez, 2013). However, supplementation of CUP reduced the levels of pro-apoptotic while increasing the levels of anti-apoptotic markers. These findings demonstrated that CUP is potent anti-apoptotic compound against DOX instigated hepatic toxicity.

The results of our investigation revealed that DOX prompted various damages to hepatic tissues such as dilated sinusoids, degenerated nucleus, alterations in Kupffer cells as well as disrupted central venules. Ahmed et al. (2022) demonstrated that DOX exposure induces histopathological disruptions such as karyomegaly of hepatic nuclei, vacuolization in hepatic cells as well as necrosis in hepatic tissues of rats. However, CUP treatment significantly restored aforementioned histopathological disruptions in hepatic tissues of rats.

5. Conclusions

Taken together, DOX administration mediated hepatotoxicity in rats via downregulating antioxidant enzymes activities while escalating the levels of oxidative stress in hepatic tissues. Furthermore, DOX exposure substantially elevated the levels of hepatic function markers, inflammatory indices, apoptotic profile as well as disrupted the normal architecture of hepatic tissues. Nevertheless, CUP treatment notably revoked abovementioned dysregulations via exhibiting antioxidative, anti-inflammatory as well as anti-apoptotic potential. However, further clinical trials are indispensable to assess the efficacy of CUP against hepatic toxicity in humans.

CRediT authorship contribution statement

Muhammad Faisal Hayat: Writing – original draft, Methodology, Investigation, Conceptualization. **Moazama Batool:** Writing – original draft, Methodology, Investigation, Formal analysis. **Hussain Ahmed:** Validation, Software, Resources, Formal analysis. **Rabia Azmat:** Writing – review & editing, Visualization, Validation, Conceptualization. **Mukhtar Ahmed:** Writing – review & editing, Visualization, Resources, Funding acquisition. **Mian Nadeem Riaz:** Visualization, Validation, Software, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Aboubakr, M., Elmahdy, A.M., Taima, S., Emam, M.A., Farag, A., Alkafafy, M., Said, A. M., Soliman, A., 2023. Protective effects of N acetylcysteine and vitamin E against acrylamide-induced neurotoxicity in rats. Pak. Vet. J. 43 (2), 262–268.
Aebi, H., 1984. [13] Catalase in vitro. In: Methods in Enzymology, 105. Academic press, pp. 121–126.
Afsar, T., Razak, S., Almajwal, A., 2019. Effect of Acacia hydaspica R. Parker extract on lipid peroxidation, antioxidant status, liver function test and histopathology in doxorubicin treated rats. Lipids Health Dis. 18 (1), 1–12.
Ahmed, O.M., Elkomy, M.H., Fahim, H.I., Ashour, M.B., Naguib, I.A., Alghamdi, B.S., Mahmoud, H.U.R., Ahmed, N.A., 2022. Rutin and quercetin counter doxorubicin-induced liver toxicity in Wistar rats via their modulatory effects on inflammation, oxidative stress, apoptosis, and Nrf2. Oxid. Med. Cell. Longev. 2022, 2710607.
Akpinar, D., Mercan, T., Demir, H., Ozdemir, S., Demir, C., Kavak, S., 2023. Protective Effects of Thymoquinone on Doxorubicin-induced Lipid Peroxidation and Antioxidant Enzyme Levels in Rat Peripheral Tissues. Pak. Vet. J. 43 (4), 29261.
AlAsmari, A.F., Alharbi, M., Alqahtani, F., Alasmari, F., AlSwayyed, M., Alzarea, S.I., Al-Alallah, I.A., Alghamdi, A., Hakami, H.M., Alyousef, M.K., Sari, Y., 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. Antioxid. 10 (12), 1998.
Al-Qahtani, W.H., Alshammari, G.M., Ajarem, J.S., Al-Zahrani, A.Y., Alzuwaydi, A., Eid, R., Yahya, M.A., 2022. Isoliquiritigenin prevents Doxorubicin-induced hepatic damage in rats by upregulating and activating SIRT1. Biomed. Pharmacother. 146, 112594.
Al-Sayed, E., Abdel-Daim, M.M., 2014. Protective role of Cupressuflavone from Cupressus macrocarpa against carbon tetrachloride-induced hepato-and nephrotoxicity in mice. Planta Med. 80 (18), 665–1671.
Al-Sayed, E., Gad, H.A., El-Shazly, M., Abdel-Daim, M.M., Nasser Singab, A., 2018. Anti-inflammatory and analgesic activities of cupressuflavone from Cupressus macrocarpa: impact on pro-inflammatory mediators. Drug Dev. Res. 79 (1), 22–28.
Atanasov, A.G., Waltenberger, B., Pferschy-Wenzig, E.M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E.H., Rollinger, J.M., 2015. Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol. Adv. 33 (8), 1582–1614.
Camaggi, C.M., Comparsi, R., Strocchi, E., Testoni, F., Angelelli, B., Pannuti, F., 1988. Epirubicin and doxorubicin comparative metabolism and pharmacokinetics: a cross-over study. Cancer Chemother. Pharmacol. 21, 221–228.
Carlberg, I., Mannerviek, B., 1975. Glutathione reductase levels in rat Peristen, P. and Maragoudakis, ME (1994) Evidence that nitric oxide is a brain. J. Biol. Chem. 250, 5475–5480.
Chatterjee, K., Zhang, J., Honbo, N., Karliner, J.S., 2010. Doxorubicin cardiomyopathy. Cardiol. 115 (2), 155–162.
Cortes-Funes, H., Coronado, C., 2007. Role of anthracyclines in the era of targeted therapy. Cardiovasc. Toxicol. 7, 56–60.
Couri, D., Abdel-Rahman, M.S., 1979. Effect of chlorine dioxide and metabolites on glutathione dependent system in rat, mouse and chicken blood. J. Environ. Pathol. Toxicol. Oncol. 3 (1–2), 451–460.
Deepa, P.R., Varalakshmi, P., 2005. Biochemical evaluation of the inflammatory changes in cardiac, hepatic and renal tissues of adriamycin-administered rats and the modulatory role of exogenous heparin-derivative treatment. Chem. Biol. Interact. 156 (2–3), 93–100.
El-Sayyad, H.I., Ismail, M.F., Shalaby, F.M., Abou-El-Magd, R.F., Gaur, R.L., Fernando, A., Raj, M.H., Ouhtit, A., 2009. Histopathological effects of cisplatin, doxorubicin and 5-flurouracil (5-FU) on the liver of male albino rats. Int. J. Biol. Sci. 5 (5), 466.
Fitzmaurice, C., Dicker, D., Pain, A., Hamavid, H., Moradi-Lakeh, M., MacIntyre, M.F., Allen, C., Hansen, G., Woodbrook, R., Wolfe, C., Hamadeh, R.R., 2015. The global burden of cancer 2013. JAMA Oncol. 1 (4), 505–527.
Hayashi, I., Morishita, Y., Imai, K., Nakamura, M., Nakachi, K., Hayashi, T., 2007. High-throughput spectrophotometric assay of reactive oxygen species in serum. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 631 (1), 55–61.
Hegazy, Y., Turner, M., Fettig, D., 2021. S2674 A subtle case of doxorubicin-induced hepatotoxicity. Am. J. Gastroenterol. 116, 1121.
Ighodaro, O.M., Akinloye, O.A., 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. Alexandria J. Med. 54 (4), 287–293.
Jain, A., Rani, V., 2018. Assessment of herb-drug synergy to combat doxorubicin induced cardiotoxicity. Life Sci. 205, 97–106.
Kakkar, P., Das, B., Viswanathan, P.N., 1984. A modified spectrophotometric assay of superoxide dismutase. 21(2), 130–132.
Kim, S.E., Kawaguchi, K., Hayashi, H., Furusho, K., Maruyama, M., 2019. Remission effects of dietary soybean isoflavones on DSS-induced murine colitis and an LPS-activated macrophage cell line. J. Nutr. 11 (8), 1746.
Koriem, K.M., Gad, I.B., Nasiry, Z.K., 2015. Protective effect of Cupressus sempervirens extract against indomethacin-induced gastric ulcer in rats. Interdiscip. Toxicol. 8 (1), 25.
Kuzu, M., Yıldırım, S., Kandemir, F.M., Küçükler, S., Çağlayan, C., Türk, E., Dörtbudak, M.B., 2019. Protective effect of morin on doxorubicin-induced hepatorenal toxicity in rats. Chem. Biol. Interact. 308, 89–100.
Liu, J., Qu, W., Kadiiska, M.B., 2009. Role of oxidative stress in cadmium toxicity and carcinogenesis. Toxicol. Appl. Pharmacol. 238 (3), 209–214.

Mahmoudi, F., Arasteh, O., Elyasi, S., 2023. Preventive and therapeutic use of herbal compounds against doxorubicin induced hepatotoxicity: a comprehensive review. Naunyn. Schmiedebergs. Arch. Pharmacol. 1–23.
Miller, K.D., Nogueira, L., Mariotto, A.B., Rowland, J.H., Yabroff, K.R., Alfano, C.M., Jemal, A., Kramer, J.L., Siegel, R.L., 2019. Cancer treatment and survivorship statistics, 2019. CA Cancer J. Clin. 69 (5), 363–385.
Nagai, K., Fukuno, S., Oda, A., Konishi, H., 2016. Protective effects of taurine on doxorubicin-induced acute hepatotoxicity through suppression of oxidative stress and apoptotic responses. Anticancer Drugs 27 (1), 17–23.
Octavia, Y., Tocchetti, C.G., Gabrielson, K.L., Janssens, S., Crijns, H.J., Moens, A.L., 2012. Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. J. Mol. Cell. Cardiol. 52 (6), 1213–1225.
Öz, E., İlhan, M.N., 2006. Effects of melatonin in reducing the toxic effects of doxorubicin. Mol. Cell. Biochem. 286, 11–15.
Placer, Z.A., Cushman, L.L., Johnson, B.C., 1966. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal. Biochem. 16 (2), 359–364.
Prasanna, P.L., Renu, K., Gopalakrishnan, A.V., 2020. New molecular and biochemical insights of doxorubicin-induced hepatotoxicity. Life Sci. 250, 117599.
Radi, E., Formichi, P., Battisti, C., Federico, A., 2014. Apoptosis and oxidative stress in neurodegenerative diseases. J. Alzheimers Dis. 42 (s3), 125–S152.
Raja, S., Ravindranadh, K., 2017. In vivo antioxidant activity of Limnophila heterophylla and Michelia champaca. Int. J. Pharm. Pharm. Sci. 241–246.
Rawat, P.S., Jaiswal, A., Khurana, A., Bhatti, J.S., Navik, U., 2021. Doxorubicin-induced cardiotoxicity: an update on the molecular mechanism and novel therapeutic strategies for effective management. Biomed. Pharmacother. 139, 111708.
Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G., Hoekstra, W., 1973. Selenium: biochemical role as a component of glutathione peroxidase. J. Sci. 179 (4073), 588–590.
Sathesh, K., Sharmila, S., Premkumar, T., Palanisamy, K., Jagan, S., Devaki, T., 2016. Protective effect of umbelliferone against doxorubicin induced cardiotoxicity in Wistar albino rats. Sci. Technol. Stud. 2, 90–98.
Sauter, K.A., Wood, L.J., Wong, J., Iordanov, M., Magun, B.E., 2011. Doxorubicin and daunorubicin induce processing and release of interleukin-1β through activation of the NLRP3 inflammasome: progress at a snail's pace. Cancer Biol. Ther. 11 (12), 1008–1016.
Sedlak, J., Lindsay, R.H., 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem. 25, 192–205.
Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F., 2021. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 71 (3), 209–249.
Wang, Y.C., Wang, L.T., Hung, T.I., Hong, Y.R., Chen, C.H., Ho, C.J., Wang, C., 2022. Severe cellular stress drives apoptosis through a dual control mechanism independently of p53. Cell Death Dis. 8 (1), 282.
Waseem, M., Parvez, S., 2013. Mitochondrial dysfunction mediated cisplatin induced toxicity: modulatory role of curcumin. Food Chem. Toxicol. 53, 334–342.