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Hematological and Serum Biochemical Evaluation of Doxorubicin Induced Toxicity in Wistar Male Rats and its Amelioration with Hesperidin

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ABSTRACT

Doxorubicin (DOX), an anthracycline antibiotic is used successfully to treat a variety of cancers. The present study was designed to evaluate protective role of hesperidin, a flavanone glycoside, against doxorubicin induced toxicity. For this purpose, 30 adult male Wister rats were divided into five different groups consisting six in each group. Normal saline was given to the group I as sham. Dose of 200 mg/kg of HES to the group III was given orally for 2 weeks. Group II was kept as DOX control (2.5 mg/kg body weight) four times in a week, intraperitoneally for 2 weeks. Group IV and V, were administered hesperidin low dose (100 mg/kg body weight) and high dose (200 mg/kg body weight), respectively, orally with DOX four times in a week over a period of two weeks. At the end of the experiment, hematological and biochemical parameters were performed in blood of rats. Results showed that DOX caused a marked rise in biochemical parameters such as serum creatinine kinase-mycocardial band, serum lactate dehydrogenase, alkaline phosphatase , troponins, serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase activities alongside an increase in serum total cholesterol and triglycerides level, and decrease in the values of hematological parameters such as total erythrocytes count, total leukocytes count, hemoglobin and pack cell volume. However, hesperidin low and high dose treated group IV and V, respectively exhibited significant ($P<0.05$) improvement in all the above parameters as compared group II indicating the protective role of hesperidin.

HIGHLIGHTS

- Studied hesperidin against doxorubicin induced toxicity.
- Hesperidin showed significant improvement.

Keywords: Doxorubicin, Hesperidin, Hematological, Biochemical, Wistar Rats

Doxorubicin is an antineoplastic anthracycline antibiotic class of drug obtained from *Streptomyces pecucetis var. caesius*. It is being widely used in treatment of various hematological and solid tumor malignancies including breast cancer, lung cancer, lymphoma, leukemia, multiple myeloma, and sarcoma (Rahimi *et al*., 2010). Despite having broad-spectrum anticancer action, doxorubicin's clinical value is constrained due to its low selectivity and severe adverse effects on tissues other than tumors (Yu *et al*., 2018). Doxorubicin therapy is associated with a variety of toxic effects, including dose-dependent cardiotoxicity

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(Marina *et al.,* 2002; Renu *et al*., 2018), hepatotoxicity (Liu *et al*., 2023), nephrotoxicity (Soltani *et al*., 2021), reproductive toxicity and neurotoxicity (Molehin *et al*., 2018; Orabi *et al*., 2021).

Doxorubicin-induced toxicity is caused by a variety of molecular mechanisms, including topoisomerase II inhibition, intercalation into the double strand of DNA, upregulation of the apoptotic pathway, and dysregulation of intracellular calcium levels. Oxidative stress is produced because of an imbalance between reactive oxygen species formed and intrinsic antioxidant systems (Nebigil and Desaubry, 2018; Sandamali *et al*., 2020). The dinucleotide enzyme nicotinamide adenine dinucleotide hydrogen (NADH) dehydrogenase transforms doxorubicin into the semiquinone radical, which then interacts with molecule oxygen to produce superoxide radical, which is then involved in the production of hydrogen peroxide and hydroxyl radicals. Additionally, the cardiomyocyte-specific enzyme known as (eNOS) reductase reduces doxorubicin to produce superoxide radicals (Octavia *et al*., 2012).

The effectiveness of doxorubicin in the treatment of cancer has been thoroughly proven after decade of clinical use. However, the doxorubicin associated organ toxicity is still a major problem that needs to be solved. The protective potential of natural chemicals derived from plant sources in prevention and treatment of doxorubicin-induced organ damage has attracted interest in recent years with the breakthroughs in modern pharmacology and molecular biology (Kumral *et al*., 2016).

Hesperidin (3,5,7-trihydroxy flavanone-7-rhamnoglucoside) is a bioflavonoid found rich in citrus fruits such as lemons, mandarins, oranges and grape fruits (Choi *et al*., 2022). It exhibits a wide range of pharmacologically useful qualities, including potent anti-lipid peroxidation activity in living membranes and effective free radical scavenging capabilities (Suarez *et al*., 1998). When compared to other naturally occurring and artificial antioxidants as ascorbic acid, -tocopherol, and butylated hydroxytoluene, hesperidin has the highest reducing ability, chelating activity on Fe^{2+}, hydrogen peroxide, and hydrogen radical scavenging activity (Hussein and Othman, 2011). Hesperidin may have various positive benefits, including those that are anti-allergic, anti-microbial, anticarcinogenic, and antithrombotic, according to studies. Hesperidin is a powerful natural antioxidant with other pharmacological qualities, such as anti-inflammatory, anti-carcinogenic, anti-microbial, and anti-viral activity, according to numerous research (Chen *et al*., 2010; Ansar *et al*., 2018; Liu *et al*., 2021).

MATERIALS AND METHODS

Chemicals

Doxorubicin was obtained locally from a government approved pharmaceutical outlet in Hyderabad, India and hesperidin was purchased from M/s. TCI (India) Pvt. Ltd. Hyderabad., India. All chemicals were procured from M/s. HiMEDIA Lab. Pvt. Ltd., India. All the chemicals used in the biochemical analysis were of analytical grade.

Animals

A total of thirty adult male Wistar rats, weighing around 200-220g, were procured from Jeeva Life Science, Hyderabad. The animals were housed in polypropylene cages with a 12-hour dark/light cycle at the department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Rajendranagar, Hyderabad animal house under hygienic conditions with ambient temperature of 22-24^{o}C. Rats were kept for one week to acclimate before the experiment began. Throughout the experiment, the animals were fed a commercially acceptable, sterile diet of pellets and provided with *ad libitum* reverse osmosis water. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) with approval no (05/26/CVSc, Hyd.IAEC).

Experimental design

All the animals were uniformly randomized into five groups with six male rats in each group:

Group I (Sham): Normal saline was given orally for 2 weeks.

Group II (DOX): Doxorubicin (2.5 mg/kg four times in a week) i.p route for 2 weeks.

Group III (HES): Hesperidin *per-se* (200 mg/kg daily dose oral route for 2 weeks).

Group IV (HLD): Low dose (treated with hesperidin @ 100 mg/kg body weight oral route for 2 weeks) + doxorubicin (2.5 mg/kg four times in a week for two weeks) i.p route.

Group V (HHD): High dose (treated with hesperidin @ 200 mg/kg body weight oral route for 2 weeks) + doxorubicin (2.5 mg/kg four times in a week for two weeks) i.p route.

On the 15th day of experiment, the rats were exposed to carbon dioxide (CO2) chamber for euthanasia. Blood samples were obtained from retro-orbital plexus into ethylene diamine tetra acetic acid (EDTA) vacutainers for the haematological parameters, and into serum vacutainers for the investigation of serum biochemical parameters. Blood serum samples were separated by centrifugation at 3000 RPM for 10 minutes and stored at -80^{0}C for analysis.

Hematological parameters

The hematological parameters include total erythrocytes count (TEC), total leukocytes count (TLC), hemoglobin (Hb) and pack cell volume (PCV) were analyzed using automated hematology analyzer.

Serum biochemical parameters analysis

The activity of serum lactate dehydrogenase (LDH), alkaline phosphatase (ALP), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and serum concentrations of total cholesterol and triglycerides were determined using their respective diagnostics kits (ERBA diagnostics Mannheim GmbH kits). CK-MB ELISA kit and car were purchased from the Krishgen Biosystems, Mumbai, India.

Troponins were estimated by employing the method of Bhaskar and Rao (2002). This method is based on the proteins react with copper at an alkaline pH to form copper-protein complex. The complex formed is treated with phosphomolybdic- phosphotungstic reagent (Folin-ciocalteu), which is measured at 660 nm.

STATISTICAL ANALYSIS

All the values were calculated as mean ± SE (n=6). The SPSS scientific software version 26.0 was used for statistical analysis, comparison among groups was analyzed by using one way analysis of variance (ANOVA) followed by duncan's test as post hoc analysis. The difference was considered statistically significant at ($P < 0.05$).

RESULTS

Effect of HES on hematological parameters in DOX-treated rats

The effect of HES on hematological parameters in DOX-treated rats are represented in table (1). The data obtained that DOX-treated group showed significant ($P<0.05$) decrease in the mean values total erythrocyte count (TEC),total leucocyte count (TLC), packed cell volume (PCV) and hemoglobin concentrations, when compared with sham. Furthermore, the values in treatment groups with HLD and HHD showed a significant ($P<0.05$) improvement in all of the hematological parameters as compared to doxorubicincontrol. There was no significant variation between the hesperidin *per-se* group and the sham.

Effect of HES on biochemical parameters in DOX-treated rats

The mean values of CK-MB, troponin, LDH, SGPT, SGOT and ALP activities in blood serum of different groups are presented in table 2 and Fig. 1 (A-F). The level of all

Table 1: Effect of hesperidin on hematological parameters (TEC, TLC, Hb and PCV) in DOX-treated rats. (DOX; doxorubicin, HES; hesperidin, HLD; low dose hesperidin, HHD; high dose hesperidin)

Parameters	Sham	DOX	HES	HLD	HHD
TEC	8.14 ± 0.10^{a}	4.37 ± 0.12^{c}	8.02 ± 0.11^{a}	5.84 ± 0.13^{b}	7.03 ± 0.19^{d}
TLC	9.14 ± 0.19^{a}	5.25 ± 0.13^{c}	9.20 ± 0.24^{a}	6.35 ± 0.13^{b}	7.63 ± 0.19^{d}
Hb	14.80 ± 0.34^{a}	8.52 ± 0.18^{c}	14.03 ± 0.21^{a}	9.96 ± 0.20^{b}	10.43 ± 0.19^{b}
PCV	47.80 ± 1.13^{a}	35.90 ± 1.30^{c}	46.91 ± 1.14^{a}	41.05 ± 1.17^{b}	42.35 ± 1.19^{b}

Table 2: Effect of hesperidin on serum biochemical markers (serum CK-MB, troponins, LDH, SGPT, SGOT and ALP) in DOX-treated rats. (DOX; doxorubicin, HES; hesperidin, HLD; low dose hesperidin, HHD; high dose hesperidin)

Parameters	Sham	DOX	HES	HLD	HHD
CK-MB(IU/L)	66.95 ± 0.60^{a}	249.00 ± 4.80^{c}	69.30 ± 1.22^{a}	179.97 ± 2.80^{b}	112.74 ± 1.62^{d}
Troponins (mg/dl)	8.21 ± 0.89^{a}	25.92 ± 0.67^{c}	8.55 ± 0.86^{a}	18.01 ± 0.57^{b}	11.60 ± 0.34^{d}
LDH(IU/L)	605.00 ± 6.34^{a}	1899.66 ± 7.07^{c}	613.00 ± 18.13^{a}	1501.33 ± 6.80^{b}	1097.83 ± 22.16^{d}
SGOT(IU/L)	84.78 ± 1.54^{a}	167.15 ± 3.01^{c}	83.01 ± 1.62^{a}	137.73 ± 1.15^{b}	134.40 ± 2.05^{b}
SGPT(IU/L)	110.58 ± 4.67^{a}	206.46 ± 3.29^{c}	108.50 ± 4.43^{a}	170.33 ± 2.84^{b}	161.66 ± 1.55^{b}
ALP(IU/L)	111.03 ± 4.65^{a}	239.30 ± 3.70^{c}	114.00 ± 3.50^{a}	204.25 ± 3.33^{b}	165.61 ± 5.22^{d}

Table 3: Effect of hesperidin on serum lipids profile (cholesterol and triglycerides) in doxorubicin-treated rats

Parameters	Sham	DOX	HES	HLD	HHD
cholesterol (mg/dl)	69.90 ± 3.40^{a}	136.75 ± 2.30^{c}	71.68 ± 4.60^{a}	109.95 ± 3.92^{b}	100.81 ± 1.24^{b}
Triglycerides (mg/dl)	105.41 ± 3.04^{a}	209.95 ± 7.34^{c}	102.38 ± 3.04^{a}	172.80 ± 2.90^{b}	139.10 ± 1.51^{d}

activities biochemical parameters significantly ($P<0.05$) increased in DOX-treated control group when comparison to sham group. The effect of HES, in the treatment groups HLD and HHD showed a significant ($P<0.05$) decrease in values of CK-MB, troponin, LDH, SGPT, SGOT and ALP activities with compared to doxorubicin-treated group in a dose-dependent manner.

Effect of HES on serum lipid profile in DOX-treated rats

In this study, DOX induced toxicity was reflected by significant ($P<0.05$) elevation in the serum lipid profile values such as total cholesterol and triglycerides with compared to sham group. In the treatment groups HLD and HHD showed significantly ($P<0.05$) decrease values than DOX- treated group. Whereas, hesperidin *per-se* group showed no change in the mean values of serum lipid profile as compared to sham group on 15th day of the experiment (Table 3 and Fig. 2).

DISCUSSION

Doxorubicin is an anthracycline antibiotic that was isolated from the *Streptomyces peucetius* species. It is used successfully to treat a variety of cancers, including solid tumours and haematological malignancies. Doxorubicin's anticancer properties are based on its ability to interfere with DNA replication by intercalating between nitrogenous bases and inhibiting the activity of the topoisomerase II enzyme (Prasanna *et al*., 2020). Doxorubicin's clinical use is severely constrained because of its significant adverse effects such as, cumulative dose-related cardiotoxicity (Renu *et al*., 2018). In cancer cells, it causes DNA damage to have an antitumor effect, but in non-cancerous cells, it can cause oxidative stress and build up free radicals, which can cause lipid peroxidation and apoptosis in organs that are not the target (Asensio-Lopez *et al*., 2017; Song*et al*., 2019).

Strategies aiming to prevent toxic side effects of doxorubicin as well as to improve the antioxidant and anti-inflammatory defense in the body by using safe and natural molecules is essential. Hesperidin, a flavonoid found in citrus fruits like lemons, oranges, and mandarins that is part of the *Rutaceae* family, is one of the many natural antioxidants that has been shown to have a wide range of pharmacological activities, including anti-inflammatory, antioxidant, and scavenging of reactive oxygen species formation associated organoprotective effect (heart, liver, brain, and kidney) (Hozayen *et al*., 2014; Syahputra *et al*., 2022) which makes it as an effective approach towards protective effect against doxorubicin induced toxicity.

Based on the aforementioned information, the current study examined the haematological parameters, biochemical parameters, and serum lipid profile studies in Wistar rats to determine the dose-dependent ameliorative efficacy of hesperidin at doses of 100 and 200 mg/kg body weight in doxorubicin-induced organ toxicity.

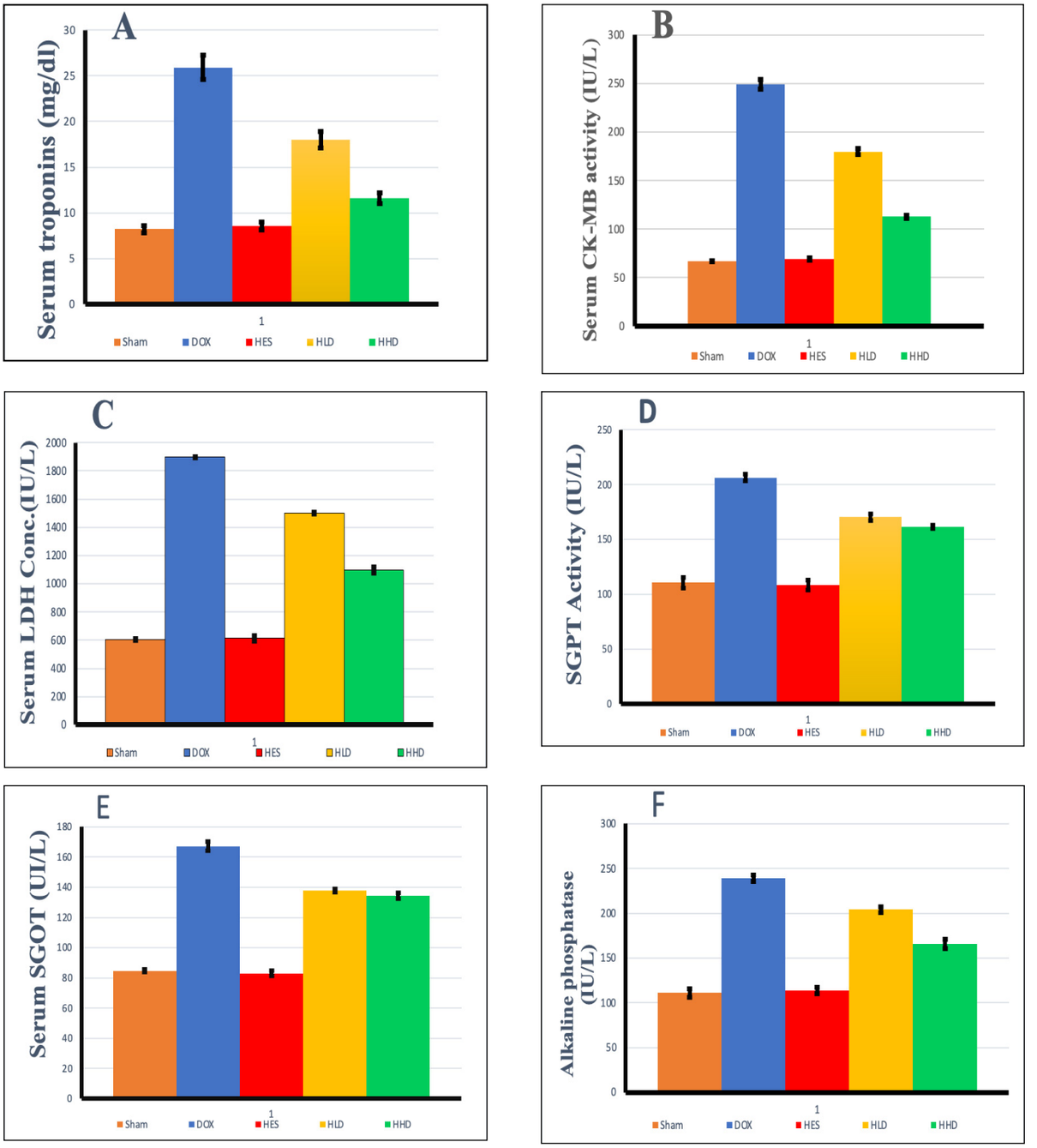

Fig. 1 (A-F): Effect of hesperidin on the serum biochemical markers in doxorubicin-treated rats **(A)** serum troponions, **(B)** Serum CK-MB activity, **(C)** Serum LDH activity, **(D)** Serum SGPT activity, **(E)** Serum SGOT activity, **(F)** Alkaline phosphatase. Sham; normal, DOX; doxorubicin, HES; hesperidin, HLD; low dose hesperidin, HHD; high dose hesperidin. Data are presented as mean ± SE (n=6 animals in each group). All the values with different letters within the same Fig. are significantly different using one way ANOVA followed by duncan's test ($p < 0.05$).

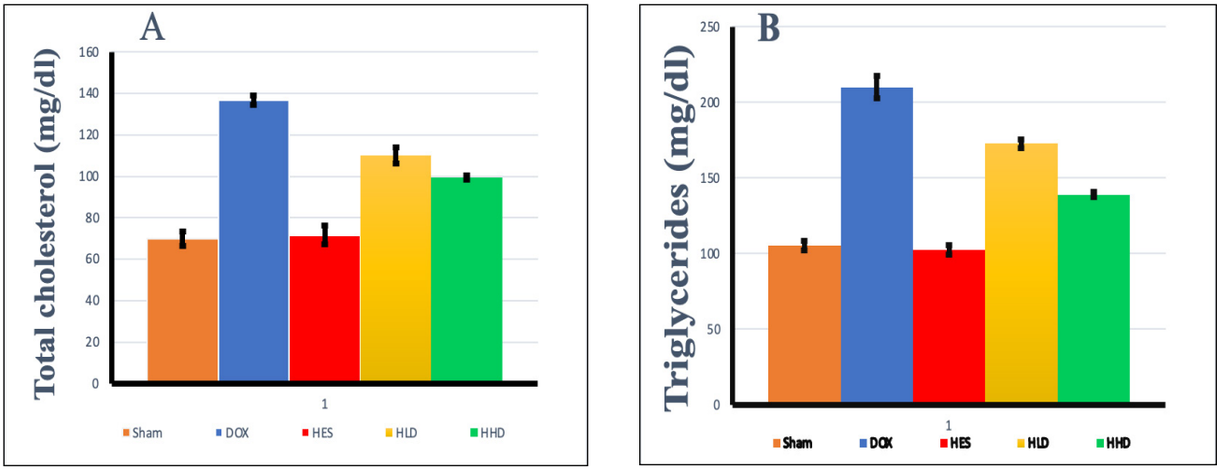

Fig. 2 (A and B): Effect of hesperidin on serum lipid profile **(A)** total cholesterol, **(B)** triglycerides in blood serum. Sham; normal, DOX; doxorubicin, HES; hesperidin, HLD; low dose hesperidin, HHD; high dose hesperidin. Values were presented as mean ± SE (n= 6 animals in each group); values with non identical letters (a, b, c and d) within the same Fig. are significantly different using one way ANOVA followed by duncan's test ($p < 0.05$).

Doxorubicin-induced haematological changes were investigated, and the findings showed a significantly lower total erythrocyte count, total leukocyte count, haemoglobin, and PCV in the doxorubicin control (group II) compared to sham. This could be explained by the fact that doxorubicin suppresses bone marrow by targeting proliferative stem cells (Farag *et al*., 2021). In comparison to the sham and hesperidin control groups, the drop was also seen in the HLD and HHD groups. However, when compared to the doxorubicin control (group II), a significant rise in the haematological indices was seen in both hesperidin treatment groups. These findings are similar with the studies of Bashandy *et al*. (2017).

Myocardium produces a high concentration of diagnostic marker enzymes such lactate dehydrogenase (LDH) and CK-MB into the extracellular fluid. When metabolically harmed. The enzyme LDH is responsible for converting lactate and NAD into pyruvate and NADH. According to Mohammed and Safwat (2016), changes in plasma membrane integrity and permeability are reflected in the enhanced CK-MB activity of serum CK-MB isoenzyme. A sensitive and specific biomarker of myocardial necrosis, cardiac troponin (cTn) is a cell structural protein that is specific to myocardial tissue (Adamcova *et al*., 2019). The most trustworthy biomarkers for cardiotoxicity are thought to be LDH activity, CK-MB levels, and levels of cTn released from cardiac myocytes since they are proportionate to cardiac damage (Saad *et al*., 2020).

In the present study it was observed that a significant increase in the activity of the CK-MB, LDH and troponins levels in doxorubicin treated rats suggesting myocardial injury in the group II. Similar findings were recorded by Adamcova *et al*. (2019), Birari *et al*. (2020) and Saad *et al.* (2020). However, hesperidin-treated groups normalised the aforementioned cardiac biomarkers, with HHD *i.e*. 200 mg/kg body weight, having a greater impact. These results are consistent with the research by Kuzu *et al*.(2021). Furthermore, it is well known that the heart is more vulnerable to damage caused by reactive oxygen species as a result of increased oxidative metabolism and inadequate antioxidant defence mechanisms. As a result, an increase in the levels of cardiac biomarkers is expected when doxorubicin is administered in several doses. However, the investigation identified hesperidin's heart protective function.

Doxorubicin harm affected not only myocardial function but also hepatic function. Any increase in the enzymes SGOT, SGPT, and ALP is a sign of liver injury. The leaking of these enzymes with an increase in serum and a decrease at the cellular level is caused by damage to the hepatocytes with altered membrane permeability and transport function (Mohan *et al*., 2010).

In the current study, the activity of SGOT, SGPT and ALP were elevated in the doxorubicin control (group II) when compared to sham. These results were in accordance with the report of Prasanna *et al.* (2020) and Badmus *et al*. (2022).However, the treatment groups with HLD and HHD significantly decreased the activity of these hepatic biomarkers compared to group II. The findings are in tune with the findings of Caglayan *et al.* (2021). The findings indicated that doxorubicin therapy caused hepatocellular damage and that hesperidin administration greatly increased these markers' activity, demonstrating the protective effect of hesperidin in doxorubicin-induced hepatotoxicity.

The precursor to corticosteroids, bile acid, vitamin D, and sex steroids is cholesterol. Triglycerides and cholesterol are lipid derivatives that have a significant impact on the development of complications for cardiovascular disease and liver damage.

In the current experiment, the activity of lipid profile such as total cholesterol and triglycerides were significantly elevated in doxorubicin control (group II) when compared to sham and hesperidin *per-se*. These observations are in accordance with the reports of Akter *et al*. (2022) and Soliman *et al*. (2023). When hesperidin was administered, both treatment groups HLD and HHD significantly reduced their total cholesterol and triglycerides. This contrasted with group II that had received DOX treatment, demonstrating that hesperidin has antioxidant and anti-inflammatory effects. The findings of the current investigation are consistent with those of Aboraya *et al*. (2022).

CONCLUSION

In conclusion, the results of the present study demonstrated that administration of doxorubicin induced organ toxicity due to the over generation of ROS and the related to the inflammation and oxidative damage. The results of present work point out that ameliorative effect against doxorubicin induced organ toxicity with hesperidin in a dose dependent manner *via* modulation of oxidative damage and inflammation due to antioxidant and anti-inflammatory properties of hesperidin in rats.

DATA AVAILABILITY

All relevant data are within the paper and its supporting information files.

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