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Doxorubicin Induced Hepatotoxicity, Apoptosis and Renal Dysfunction: Role of Hesperidin in Potentiation or Attenuation in Male Wistar Rats

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ABSTRACT

The common side effect of doxorubicin use in the treatment of neoplasms is cardio-hepatotoxicity and nephrotoxicity among others. Other treatment measures has been sort to alleviate the outrageous suffering of cancer patients. Hesperidin, a natural plant product from citrus plants has an antioxidant and protective property. The aim of the study was to find out the effect of hesperidin and captopril on doxorubicin induced liver and kidney injury as well as apoptotic activity. In this study, twenty five male Wistar rats weighing 160-180g were randomly selected into five groups (G1-G5) containing five rats each. G1 (control), received normal feed, G2

received 5mg/kg body weight of Doxorubicin(doxo) orally. G3 received 50mg/kg of hesperidin, G4 received 5mg/kg body weight of Doxorubicin(doxo)plus Hesp. While G5 received 5mg/kg body weight of Doxorubicin(doxo)plus Captopril(oral). Treatment lasted for 28 days. Blood samples were collected via cardiac puncture and centrifuged at 1000rpm for 10minutes. Results obtained showed elevated levels of malondialdehyde, Urea, creatinine and total proteins. Liver enzymes (AST, ALT, ALP, GGT), Caspase 3, Bax and Bcl2 were significantly ($p<0.05$) raised. Treatment with hesperidin and captopril respectively, significantly ($p<0.05$) decreased the parameters in all treatment groups when compared to doxo group. We conclude that hesperidin transiently attenuates apoptosis and ameliorates toxic effects of doxorubicin on the liver and kidney.

Keywords: Doxorubicin, hepatotoxicity, nephrotoxicity, hesperidin, apoptosis.

INTRODUCTION

The use of chemotherapy drugs in the treatment of different types of cancer has recorded great success over the. Doxorubicin (Dox), a member of the family of the anthracycline group of antibiotics, stands out as the commonest drug [1] used in oncology study. It is used to treat bone sarcomas, soft tissue, and cancers of the ovary, bladder, breast and thyroid, acute myeloblastic leukemia, Hogkin lymphoma, lymphoblastic leukemia and small cell lung cancer [2]. Despite the preference of the use of Doxo, research has associated it with some harzadous outcome to many body tissues. Apart from the heart being a primary target in doxo-induced toxicity, research has suggested that this toxicity may also affect other organs like the liver, kidney, and brain [3,4,5]. This effect is as a result of the generation of superoxide radicals during its activity causing oxidative stress that results in a breakdown of the antioxidants system [6,7]. These can cause lipid peroxidation and protein oxidation which may finally lead to tissue damage [8]. Evidences abound that anticancer agents including doxorubicin can trigger apoptotic cascade. essential in various toxicities, including hepatotoxicity and mitochondrial dysfunction [9, 10]by disrupting the hepatic antioxidant system [11,12]. Earlier reports have also demonstrated that Doxorubicin use in rats cause nephrotoxicity [13] which affects essentially glomerular capillary permeability and cause tubular atrophy [13] with elevated serum urea and creatinine in mice [15]. Hesperidin, a flavonoid plays a role in several processes including the antioxidant process, osmoregulation, cell plasma membrane stabilization, and detoxification, [16,17]. It protects against free radical-induced damage in biological systems such as the heart, liver, and kidney [18, 19] The aim of the present study was to demonstrate the protective effect of hesperidin in doxo-induced hepatotoxicity and renal toxicity as well as apoptotic biomarkers.

EXPERIMENTAL DESIGN

Twenty-five male Wistar rats weighing 120-170g were used in this study. They were randomly divided into five groups containing five rats each. The control group received normal feed and water. Group 2 received 5mg/kg body weight of doxorubicin orally, Group 3 received 50mg/kg of Hesperidin only while group 4 received 5mg/kg of Doxorubicin and 50mg/kg body weight of hesperidin dissolved in distilled water and administered orally. Group 5 received 50mg/kg of captopril and 50mg/kg of hesperidin(oral). Feeding and administration of drugs lasted for 28 days. Ethical approval for the study was obtained from the Faculty of Basic Medical Sciences, University of Cross River State, Research Ethical Committee approval number: UNICROSS/FBMS/NG/REC/2025/Vol 1/05

Blood Sample Collection

After anesthesia of the animal with sodium pentabarbitone, blood samples were collected by cardiac puncture into sterile plain bottles and allowed to stand for one hour for the blood to clot. The blood was centrifuged at 1000g for 10 minutes to obtain serum. The serum was collected into ependoff bottles and stored at -10°C till further use.

Determination of Malondialdehyde

The activity level of malondialdehyde (MDA) was determined spectrophotometrically using Thiobarbituric acid (TBA), as previously described by [20]. The sample MDA was allowed to react with TBA in acidic medium at 95 °C for 30 min to form thiobarbituric acid reactive product, followed by measuring the absorbance of the resultant pink product at 532 nm.

Determination of Urea and Creatinine

Serum Urea and creatinine levels were tested using commercially available kits in compliance with the manufacturer's instructions to determine level of kidney dysfunction (Bio diagnostic, Giza).

Serum Indices of Hepatotoxicity

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), GGT colorimetric activity assay, and LDH Assay Kits were used to estimate AST, ALT, GGT, ALP and LDH levels according to manufacturer protocol (Bio diagnostic, Giza) using double beam spectrophotometer (Shimadzu, Japan).

Determination of Biomarkers: Caspase-3; Bax, Bcl2

ELISA kits were used for the determination of caspase-3 activity, Bax and Bcl2 according to the manufacturers description. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 ± 2 nm. The concentration of Rat biomarkers in the samples were calculated by comparing the OD of the samples to the standard curve. The OD value is proportional to the concentration of Rat biomarkers.

HISTOLOGICAL STUDY

The Liver & kidney were sectioned and were fixed in 10% neutral formalin for histological examination. After tissue sectioning (5µm thick), they were treated in paraffin and stained with standard Hemotoxylin and Eosin (H&E). Samples were examined by light microscope using the Spot Advanced Software (V.3.2.4; Diagnostic Instruments, Sterling Heights, USA). The Sections were digitally photographed using a Spot Insight Color 3.2.0 diagnostic camera.

STATISTICAL ANALYSIS

All statistical analyses were performed using Graph pad prism version 5.0 software. Data obtained were expressed as mean ± SEM. All statistical analysis was performed using the one way analysis of variance followed by Bonferroni's multiple comparison test. The results were considered statistically significant at the level of $P<0.05$.

DISCUSSION

One of the major limitations of doxorubicin in the treatment of cancer as reported by most researchers is its side effect on non target organs [21,22].

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In this study, we examined the possible apoptotic process involving damage to the liver and the kidney as well as the co-administration with hesperidin and captopril respectively.

In our study we recorded a significant increase in serum malondealdehyde (MDA), alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alanine phosphatase (ALP) levels and gamma glutamyl transferase (GGT) in the doxorubicin treated group. Usually, elevated levels of liver enzymes in serum is used clinically as an indicator for hepatic cell injury [23,24]. This changes in membrane permeability leading to altered serum liver enzyme concentration is suggested to be the result of lipid peroxidation and doxorubicin induced oxidative stress as indicated by the elevated level of Malondealdehyde and histological changes of the liver showing cirrhotic tissues and the kidney with acute tubular pyknosis. This results agrees with earlier reported work by [25,26,27].

Similarly, from our study we noted some changes in kidney injury biomarkers. Urea and creatinine concentration were significantly increased following the use of doxorubicin. This is consistent with the some experimental reports that demonstrated doxorubicin assaults to vital organs via induced oxidative stress and production of reactive oxygen species, apoptosis and a resultant decrease in natural antioxidant status [12,28-30]. Part from the nephrotoxicity observed in this study, it has been reported that doxorubicin causes damage to the glomerular podocytes, causing proteinuria and nephropathy, and can result in renal failure. [31, 12]. Despite its toxic effect, co-administration with hesperidin ameliorated the kidney damage. This is supported by the histological studies that showed reversed glomerular damage.

Furthermore, our study demonstrated that the use of doxorubicin induced elevated apoptotic enzymes like capase-3 and Bax while the B-cell Lymphoma-2(Bcl2) protein component was reduced. These results agrees with earlier reported activity of doxorubicin and its crucial mechanisms in its apoptotic process. Raised Caspase 3 and Bax levels are associated with mitochondrial membrane impairment that leads to the formation of cytochrome C and its flow into the cytosol and eventually triggers apoptosis [32]. This activity is highly linked to overproduction of ROS. Excessive Bax formation overrides the physiological relevance of Bcl2 and therefore accelerates programmed cell death [33]. Bcl-2 is found at the mitochondrial membrane and being an anti-apoptotic protein. it helps to stabilize the mitochondrial integrity and also inhibit cytochrome c release into the cytosol.

Generally speaking, co-administration with hesperidin and captopril greatly reduced cytotoxicity caused by doxorubicin and hence protected the cells against injuries and damage. Hesperidin is natural compound (a biflavanoid) with a strong antioxidant capacity known for its various pharmacological actions, including anti-inflammatory, antioxidant, and anticancer effects [34]. The possible reversal actions of the toxic conditions of doxorubicin may be attributed to the high antioxidant property of the plant natural product. This result was further consolidated by the histological presentations showing a no pathogenic condition in hesperidin and captopril supplementation.

CONCLUSION

In this study, DOX treatment potentiates hepatotoxicity, kidney damage and apoptosis. Hesperidin transiently reduces apoptosis and attenuates toxic effects of doxorubicin on the liver and kidney.

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Authors Contribution:

Ada Akwari wrote the manuscript, Esther George proof read the manuscript and did most of the laboratory work, Moses Nyirenda did data analysis and edited the manuscript, Ukula Obia did histology and interpretation, Etah Etah Nkanu conceptualized the work and revised the article for intellectual content Reuben Mbuki did the final proof reading of the manuscript before publication.

Consent for Publication

All authors agreed that the work be published. The work described is an original article that has not been published and is not under consideration for publication elsewhere.

Ethics approval and consent to participate

All authors were in agreement to participate in the study and to have the data published. All participants signed a written informed consent, which was revised by the local Ethics Committee, which then approved the study protocol

Conflict of interest: The authors report no conflict of interest.

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