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Research Article

Cardioprotective Effect of Mango and Kinnow Peel Extracts on Doxorubicin-induced Cardiotoxicity in Albino Rats

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Abstract: Different dose levels of mango (*Mangifera indica* L.) and kinnow (*Citrus reticulate* L.) peel extracts were administered to rats for 60 days and myocardial infarction was induced by administering doxorubicin (DOX) injection 2.5 mg/kg body weight (BW) intraperitoneal in six equal doses on alternate days from 50th to 60th day. Cardiac biomarkers lactate dehydrogenase (LDH), aspartate transaminase (AST), creatine kinase-MB fraction (CKMB), creatine phosphokinase (CPK), lipid profile (triglycerides, cholesterol, high density lipoprotein (HDL)) and low density lipoprotein (LDL), renal function activities (blood urea nitrogen, creatinine, uric acid) in serum and antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) in heart tissues were estimated. Histopathological studies were carried out for cardiac tissues of the studied rat groups. Mango and kinnow peel polyphenolic extracts at medium dose (i.e., 150 mg/kg BW) and high dose (i.e., 300 mg/kg BW) exhibited significant protection against doxorubicin induced myocardial infarction. Maximum cardioprotective activity was exhibited by groups pre-treated with 300 mg/kg of peel extracts, especially mango peel extracts, which maintained the membrane integrity of myocardial tissues, lowered the DOX-induced hyperlipidemia, nephrotoxicity and significantly restored the activity of cardiac endogenous antioxidant enzymes. Histopathological studies of cardiac tissues verifield the cardioprotective activity of both peels extracts at medium and high dose levels. Thus, mango and kinnow peel extracts have cardioprotective potential at medium and high dose levels.

Keywords: Polyphenols, peel extracts, cardioprotective activity, doxorubicin, histopathology

1. INTRODUCTION

Oxidative stress is the state that develops due to excessive production of free radicals and reactive oxygen species (ROS) in the body and is responsible for the pathogenesis of several chronic diseases such as atherosclerosis, diabetes mellitus, cardiovascular disease, cancer and neurodegenerative disorders [1-4]. Cardiovascular diseases are the leading cause of mortality in advanced and industrialized countries as well as increasing at alarming rate in developing countries. Coronary heart disease is one of the serious cardiovascular disorder results in high morbidity and mortality rate [5, 6]. Polyphenols are the plants antioxidants present especially in fruits and vegetables which impart a significant protective role on human health.

It has been reported that regular consumption of plant-derived foods containing polyphenols may limit the risk of coronary heart disease due to their antioxidant activity against free radicals and ROS [7, 8]. Cardiovascular health may be improved by dietary polyphenols through the regulation of platelet reactivity which have significant role in myocardial infarction venous thromboembolism.

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Decrease in platelet reactivity by polyphenols reduces the probability of blood clotting. Flavonoids such as quercetin, myricetin and kaempferol restrict platelet aggregation [9, 10].

Mango (*Mangifera indica* L.) is one of the popular tropical fruit whose peel constitutes about 15-20% of the mango fruit weight and is a rich source of cardioprotective polyphenols even higher than mango pulp [11]. Kinnow mandarin (*Citrus reticulate* L.) is the leading citrus fruit crop of Pakistan. Kinnow mandarin peel is about 35-40% of the fruit weight and is the major waste component after processing. Citrus peel is the rich source of phenolic compounds especially flavones, isoflavones, flavonones, flavonols and anthocyanidins [12]. Orange peels possess flavonones (naringenin and hesperitin), carotenoids, ascorbic acid which altogether contribute to protection against cardiovascular disease [13]. Pretreatments of rats with hesperidin lead to cardiac tissue protection from cardiotoxic effects of doxorubicin and thus hesperidin may be considered as cardio-protective agent [14].

Cardioprotective activity of plant polyphenols was assessed in animal models through chemically induced myocardial infarction. Doxorubicin or Adriamycin is an anthracycline drug used for the treatment of various malignancies such as solid tumors, lymphoma and leukemia [15]. However, its clinical use is now restricted due to dose-dependent cardiotoxicity leading to acute and chronic heart failure [16, 17]. Doxorubicin-induced cardiotoxicity has been mediated through various mechanisms including reactive oxygen species formation, mitochondrial DNA damage, cardiomycyte apoptosis, myofibrillar degeneration, inhibition of DNA and protein synthesis [18-20]. Injection of doxorubicin/adriamycin to animals such as rats leads to various morphological and metabolic disorders in cardiac tissues of experimental animals similar to human cardiomyopathy [21].

Keeping in view the above mentioned facts, the current study was designed to evaluate the cardioprotective activities of mango and kinnow peel polyphenolic extracts on doxorubicin induced cardiotoxicity in albino rats.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fruits of mango, Chaunsa variety, and kinnow mandarin were procured from the fruit market in Islamabad and taken to the Food Science and Product Development Institute (FSPDI) research laboratory, National Agricultural Research Center (NARC), Islamabad. Fruits were thoroughly washed under tap water. Fruit peels were removed, cut into small pieces, oven-dried at 50 °C for 48 hours, ground to fine powder in sample mill and packed in air-tight polyethylene zip bags.

2.2 Extraction of Polyphenols

Ultrasound-assisted extraction technique was employed for polyphenols extraction from mango and kinnow peel powders according to procedure depicted by Bimakr et al. [22] with minor variation. Peel powders samples were extracted with solvent 80% ethanol, sample to sovent ratio 1:20, at extraction temperature and time 45 °C and 60 minutes respectively in a sonicator set at 35 kHz frequency. Peel extracts were filtered, centrifuged, solvent evaporated by vacuum evaporator and microfiltered through 0.45 µm cellulose membrane filter, collected in amber glass bottles and refrigerated stored.

The total polyphenol content of mango and kinnow mandarin peel extracts was measured by the Folin-Ciocalteau method as described by Singleton et al. [23] and the absorbance was measured at 765 nm with UV-VIS Spectrophotometer (Agilent 8453, USA). Individual phenolic compounds in mango and kinnow peel extracts were quantified with high performance liquid chromatography (HPLC) according to the method described by Salvador et al. [24] with slight modifications. The analyses were conducted at a flow rate of 1 mL/min with the UV detector set at 280 nm for phenolic acids and 370 nm for flavonoids and sample injection volume 20 µL.

2.3 Experimental Conditions for Animals

Sprague Dawley strain albino rats of either sex weighing between 190-210 g were selected for the biological studies carried out at animal house,

National Institute of Health (NIH), Islamabad. Animals were housed in polypropylene cages with 12 hours light/dark cycle under environmental conditions of 25 ± 3 °C, relative humidity 50 ± 10% and had free access to feed and water *ad libitum*. The study protocol was approved by the institutional animal ethics committee, University of Sargodha, Pakistan.

2.4 Drugs and Chemicals

Doxorubicin (DOX) (Adriablastina, Pfizer) injections were purchased from a local pharmacy in Islamabad. Commercially available kits (DiaSys Diagnostic Systems GmbH, Germany) were used to estimate cardiac enzymes in serum, lipid profile and renal function activities. Other chemicals employed were of analytical grade.

2.5 Experimental Design

Animals were kept for one week acclimatization period and then randomly divided into 8 groups of 6 animals per group.

Group I: Negative control or normal control without any intervention; albino rats received standard feed and distilled water of for 60 days.

Group II: Positive control or DOX control group; rats received standard feed, distilled water for 60 days and DOX injection was administered 2.5 mg/kg body weight (BW) intraperitoneal in six equal doses on alternate days from 50th to 60th day.

Group III: Preventive group A; albino rats were pretreated with 75 mg/kg BW mango peel extract once daily by oral gavage for sixty days and DOX injection was administered 2.5 mg/kg intraperitoneal in six equal doses on alternate days from 50th to 60th day.

Group IV: Preventive group B; albino rats were pretreated with 150 mg/kg BW mango peel extract once daily by oral gavage for sixty days and DOX injection was administered 2.5 mg/kg intraperitoneal in six equal doses on alternate days from 50th to 60th day.

Group V: Preventive group C; albino rats were pretreated with 300 mg/kg BW mango peel extract once daily by oral gavage for sixty days and DOX injection was administered 2.5 mg/kg intraperitoneal in six equal doses on alternate days from 50th to 60th day.

Group VI: Preventive group D; rats were pretreated with 75 mg/kg BW kinnow peel extract once daily by oral gavage for sixty days and DOX injection was administered 2.5 mg/kg intraperitoneal in six equal doses on alternate days from 50th to 60th day.

Group VII: Preventive group E; rats were pretreated with 150 mg/kg BW kinnow peel extract once daily by oral gavage for sixty days and DOX injection was administered 2.5 mg/kg intraperitoneal in six equal doses on alternate days from 50th to 60th day.

Group VIII: Preventive group F; albino rats were pretreated with 300 mg/kg BW kinnow peel extract once daily by oral gavage for sixty days and DOX injection was administered 2.5 mg/kg intraperitoneal in six equal doses on alternate days from 50th to 60th day.

2.6 Biochemical Assessment

After 48 hours of last doxorubicin injection dose, the animals were anaesthetized with chloroform and blood was collected by cardiac puncture in blood collection tubes. Serum was separated by centrifugation at 4000 rpm for 10 min and used for biochemical studies [25].

2.7 Estimation of Cardiac Enzymes in Serum

Serum was analyzed for various enzyme biomarkers related to myocardial infarction like lactate dehydrogenase (LDH), aspartate transaminase (AST), creatine kinase-MB fraction (CKMB), creatine phosphokinase (CPK) according to procedures described by Thomas [26] and Rosalki [27] with commercially available kits (DiaSys Diagnostic Systems GmbH, Germany) by using Microlab Chemistry Analyzer (300 1x, Merck).

2.8 Evaluation of Lipids and Lipoprotein Profile

Serum triglycerides [28], total cholesterol [29], high density lipoprotein (HDL) [30] and low density lipoprotein (LDL) [31] were evaluated by using Microlab Chemistry Analyzer with commercially available kits (DiaSys Diagnostic Systems GmbH, Germany).

2.9 Estimation of Renal Function Profile

Blood urea nitrogen (BUN), creatinine and uric acid were analyzed in accordance with the methods described by First [32] with commercially available kits (DiaSys Diagnostic Systems GmbH, Germany) by using Microlab Chemistry Analyzer (300 1x, Merck).

2.10 Assessment of Antioxidant Enzymes in Heart Tissues

After blood collection by cardiac puncture, the albino rats were slaughtered and heart tissues were removed, washed with ice-cold saline and dried with filter paper. Heart tissues were diced and homogenized in 0.05 M ice-cold phosphate buffer. Homogenate was centrifuged at 4000 rpm for 10 min at 4 °C. The supernatant was collected and utilized for the determination of antioxidant enzymes.

2.10.1 Superoxide Dismutase (SOD) Assay

Superoxide Dismutase activity was analyzed according to method described by Kono [33]. SOD activity was determined by observing the rate of inhibition of NBT reduction by superoxide radicals generated by the auto-oxidation of hydroxylamine hydrochloride. Briefly, 1.3 mL of EDTA solution (0.1 mM EDTA containing 50 mM sodium carbonate, pH 10.0) was mixed with 0.5 mL NBT (90 µM) and 0.1 mL Triton-X (0.6%). Then 0.1 mL hydroxylamine hydrochloride (20 mM, pH 6.0) was added and the rate of NBT reduction was observed for one minute at 560 nm. Then 0.1 mL enzyme sample was added to cuvette and the enzyme activity was calculated. SOD activity was expressed as units per mg protein change in optical density per minute where one unit of enzyme is the SOD amount required to inhibit 50% rate of reaction.

2.10.2 Catalase (CAT) Assay

Catalase activity was determined according to the method of Aebi [34] by using 0.1 mL enzyme sample, 1 mL hydrogen peroxide (2 mM) and phosphate buffer (0.01 M, 1 mL, pH 7.0). Catalase activity was analyzed by measuring the decrease in absorbance at 240 nm for 3 min by UV-Vis spectrophotometer and expressed as units per mg protein.

2.10.3 Glutathione Peroxidase (GPx) Assay

Glutathione peroxidase was estimated by the method described by Rotruck et al. [35]. Briefly, the assay mixture consisted of 0.5 mL phosphate buffer(0.4 M, pH 7.0), 0.1 mL sodium azide (10 mM), 0.2 mL reduced glutathione (4 mM), 0.2 mL enzyme tissue(supernatant) and 0.1 mL hydrogen peroxide (0.2 mM). The contents were incubated at 37°C for 10 min, the reaction was stopped by adding 0.5 mL 10 % trichloroacetic acid and centrifuged at 3000 rpm for 5 min. The glutathione in the supernatant was quantified by using 0.5 mL Ellman's reagent (19.8 mg 5,5'-dithiobisnitrobenzoic acid "DTNB" in 50 mL phosphate buffer, pH 7.6) and absorbance was noted after 5 min at 412 nm by UV-Vis spectrophotometer and expressed as µg of GSH consumed/min/mg of protein.

2.11 Histopathological Studies

Heart tissues were fixed in 10 % formalin and embedded in paraffin. Paraffin blocks were prepared, cut at 5µm thickness, mounted on glass slides, deparaffinized and stained with hematoxlin and eosin for histopathological studies through light microscope [36]. The histopathological parameters to be studied were necrosis, loss of myofibril, cytoplasmic vacuole formation, edema, mitochondrial swelling and leukocyte infiltration.

3. RESULTS

Solvent ethanol categorized under GRAS (Generally Recognized as Safe) at 80% concentration level was employed for the extraction of polyphenols from mango and kinnow peels. Maximum polyphenols were extracted in mango peels (67.58± 0.21 mg GAE/g of extract) as compared to kinnow peel extracts (29.75 ± 0.23 mg GAE/g of extract) as evident in Table 1.

Peel extracts phenolic compounds identified and quantified through HPLC included phenolic acids, i.e., gallic acid, chlorogenic acid, ferulic acid, coumaric acid, caffeic acid and flavonoids catechin, epicatechin, hesperidin, naringenin, quercetin, kaempferol, mangiferin, myrecetin and rutin according to retention time and their peaks

Table 1. Total polyphenol content of mango and kinnow peels extracts.

Peel Extract	mg GAE/g extract
Mango peel	67.58±0.21a*
Kinnow peel	29.75±0.23b

**Values are mean ± standard error of triplicate analyses. Different letters denote a significant difference at P<0.05*

Table 2. HPLC quantification of polyphenols in mango and kinnow peel extracts.

Phenolic compound	Mango Peel Extract (µg/g)	Kinnow Peel Extract (µg/g)
Gallic acid	91.00±0.67[a*]	54.13±1.12[b*]
Chlorogenic acid	28.90±0.44[a]	20.52±0.82[b]
Ferulic acid	115.65±2.25[a]	65.21±1.16[b]
Coumaric acid	188.97±4.13[a]	27.29±0.44[b]
Caffeic acid	N.D.	2.43±0.30[a]
Catechins	67.41±1.28[a]	49.46±1.03[b]
Epicatechins	152.13±1.48[a]	18.62±0.54[b]
Magniferin	112.18±1.54[a]	N.D.
Hesperidin	N.D.	92.94±1.23[a]
Naringenin	N.D.	N.D.
Quercetin	30.46±0.60[a]	23.71±0.50[b]
Myrecetin	11.08±0.42[a]	N.D.
Rutin	80.24±2.29[a]	N.D.
Kaempferol	42.56±0.62[a]	16.85±0.41[b]
Total	**920.58+5.60[a]**	**371.16±6.79[b]**

All values are means of three replications
** Means followed by same letter do not differ significantly (P < 0.05)*
N.D. Not detected

spectral characteristics against those of standards. Mangiferin, rutin and myrectin were identified only in mango peel extracts while caffeic acid, hesperidin and naringenin were detected only in kinnow peel extracts (Table 2). Results revealed that mango and kinnow peel extracts phenolics varied considerably as function of solvent concentration level. Mango peel extracts showed comparatively higher quantity of phenolic compounds than kinnow peel extracts. Among the phenolic compounds, coumaric acid was the most abundant phenolic acid in mango peel extracts whereas ferulic acid and hesperidin were the most abundant in kinnow mandarin peel extracts. Gallic acid, catechin and epicatechin were the other phenolic compounds present in high concentration.

Bulk extraction of polyphenols was carried out from mango and kinnow peels by employing 80% ethanol for the prepation of polyphenolic extract doses to albino rats in order to evaluate cardioprotective activities of polyphenolic extracts.

3.1 Effect of Mango and Kinnow Peel Extracts on Serum Cardiac Markers

Serum cardiac markers (LDH, CK-MB, CPK and AST) in albino rats of each group were presented in Fig. 3. DOX administration to albino rats significantly increased serum cardiac marker. LSD-test revealed significant difference between serum enzyme levels of control and DOX group for all cardiac markers.

As regards LDH (Fig. 1A), doxorubicin administration significantly elevated the serum LDH level (342.33 ± 11.97 IU/L) in DOX group rats as compared to normal control group (182.83±13.05 IU/L). Pre-treatment with peel extracts significantly minimize the effect of doxorubicin administration on serum cardiac biomarkers level in different treatment groups. However, the changes were more pronounced at low dose of peel extracts especially kinnow peel extracts (304.67 ± 6.22 IU/L) and were non-significantly different from DOX group. Rats group pre-treated with high dose of mango peel extract had minimum elevation of doxorubicin induced LDH level (209.17 ± 5.51 IU/L) and were non-significantly different from control group. In case of CK-MB (Fig. 1B), significant increase in enzyme level of DOX group (52.50 ± 4.39 IU/L) was observed as compared to control (19.18 ± 3.83 IU/L). For pre-treatment groups, highest and lowest elevation in CK-MB level in serum were observed in kinnow peel extract low dose (46.50± 4.16 IU/L) and mango peel extract high dose (28.33± 2.13 IU/L) respectively. Cardiac biomarker CPK level in serum ranged from137.33±11.72 IU/L (control group) to 281.50±8.78 IU/L (DOX group) (Fig. 1C). Pre-treatment with mango peel extract high dose resulted in maximum protective effect due to minimum increase in CPK level (171.67±6.98 IU/L) as compared to other pre-treatment groups which might be due to activity of mango peel

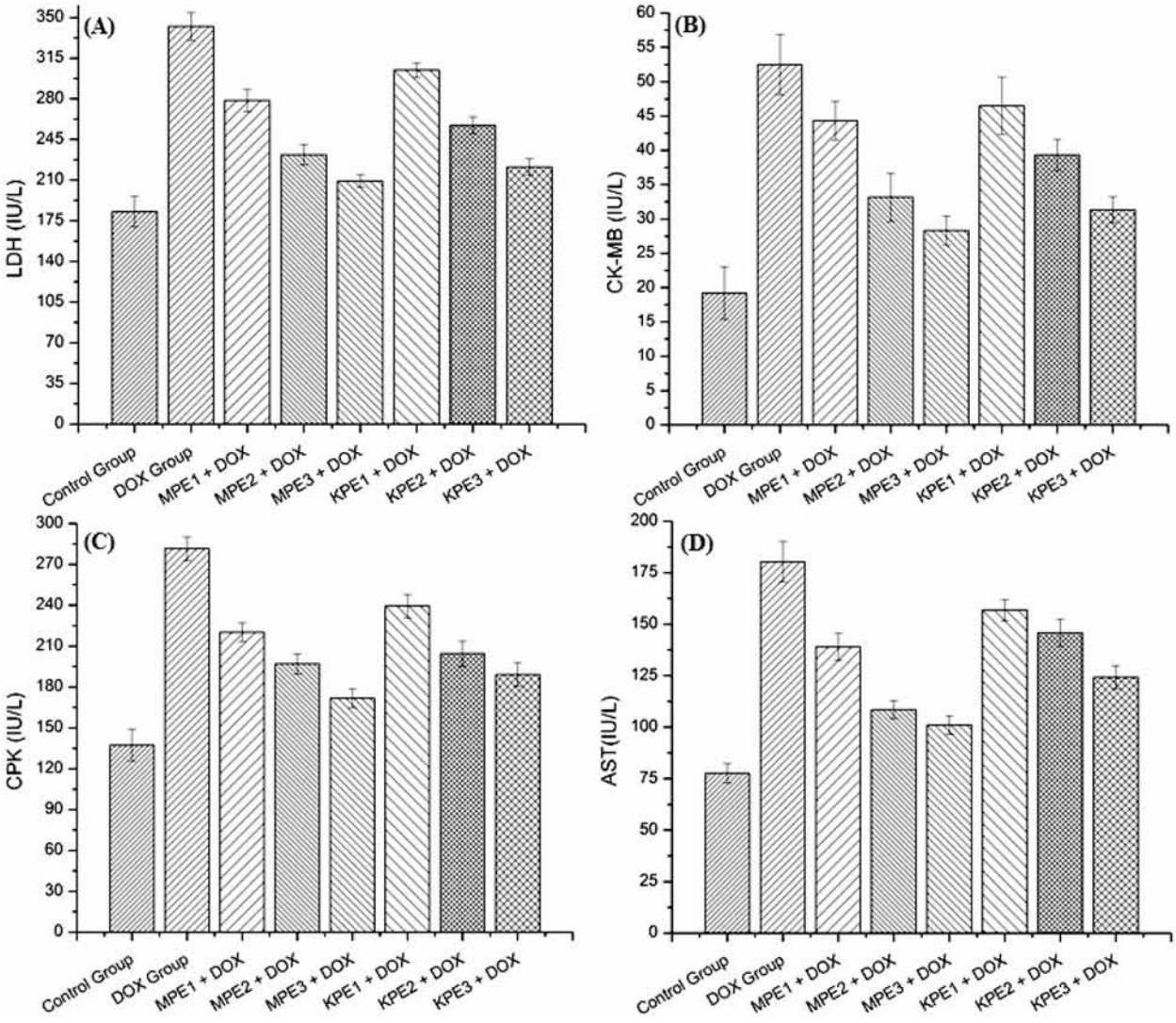

Fig. 1. Cardioprotective effect of mango and kinnow peel extracts on serum cardiac markers in doxorubicin induced cardiotoxicity in rats. LDH: Lactate dehydrogenase; CKMB: Creatine kinase-MB fraction; CPK: Creatine phosphokinase; AST: Aspartate transaminase; DOX: Doxorubicin; MPE1: Mango peel extract (75 mg/kg body weight); MPE2: Mango peel extract (150 mg/kg body weight); MPE3: Mango peel extract (300 mg/kg body weight); KPE1: Kinnow peel extract (75 mg/kg body weight); KPE2: Kinnow peel extract (150 mg/kg body weight); KPE3: Kinnow peel extract (300 mg/kg body weight).

extract on maintaining the membrane integrity thus restricting the enzymes leakage. As regards AST enzymes (Fig. 1D), doxorubicin administration significantly elevated the AST level in DOX group (180.33±9.81 IU/L) than control group (77.67±4.74 IU/L). Pre-treatment with peel extracts especially at medium and high doses efficiently hindered the AST secretions from cardiac tissues into the blood, therefore decreased doxorubicin induced AST levels.

3.2 Effect of Mango and Kinnow Peel Extracts on Serum Lipid Profile

Serum lipid profile (cholesterol, triglycerides, HDL, LDL) of different rat groups studied showed significant variations between normal and Dox-treated group for all parameters (Fig. 2). As regards cholesterol level (Fig. 2A), a significant elevation was observed in serum cholesterol level (115.33 ± 5.46 mg/dL) in DOX group as compared to control group without intervention (61.17 ±

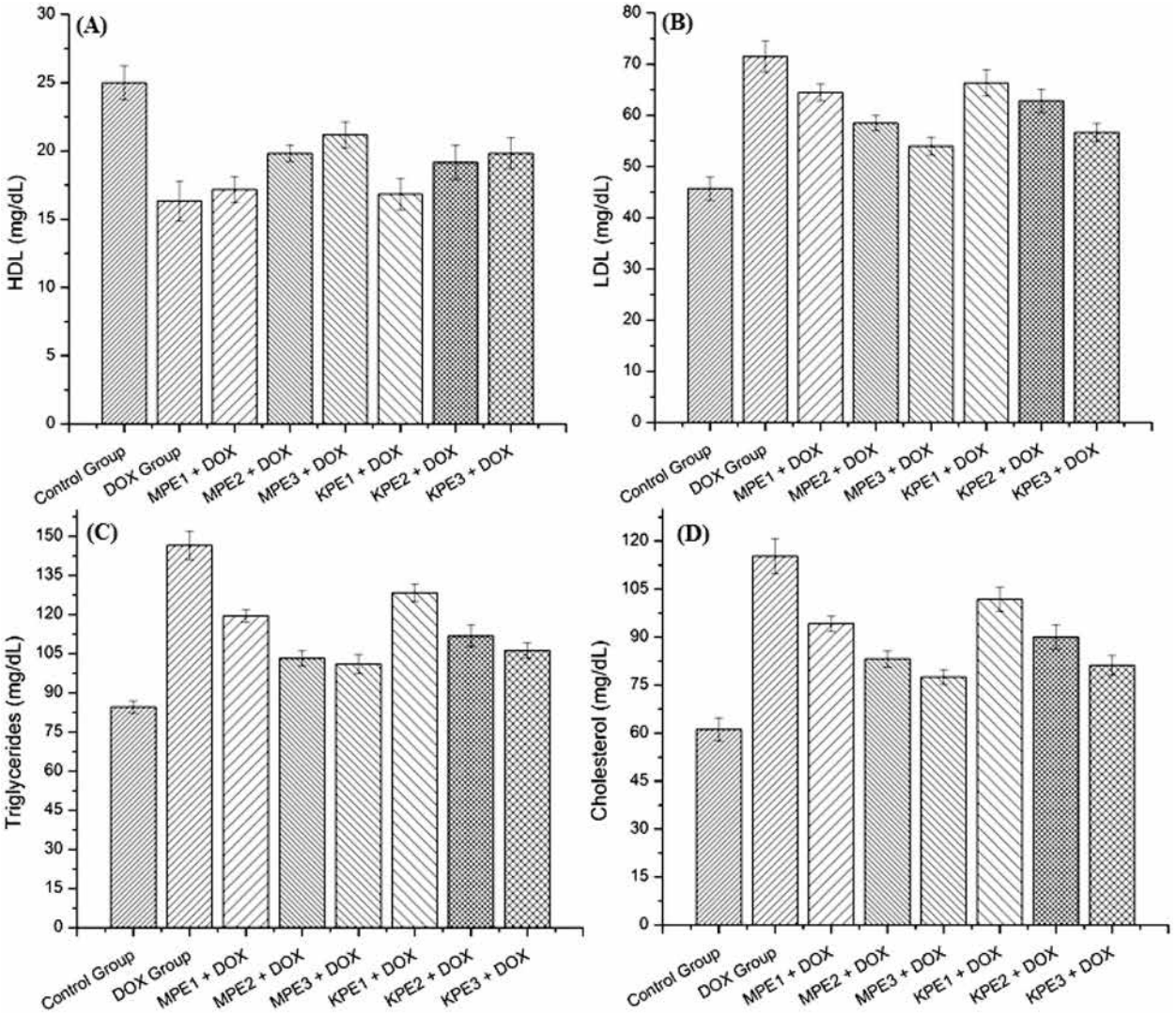

Fig. 2. Cardioprotective effect of mango and kinnow peel extracts on serum lipid profile in doxorubicin-induced cardiotoxicity in rats. HDL: High density lipoprotein; LDL: Low density lipoprotein; DOX: Doxorubicin; MPE1: Mango peel extract (75 mg/kg body weight); MPE2: Mango peel extract (150 mg/kg body weight); MPE3: Mango peel extract (300 mg/kg body weight); KPE1: Kinnow peel extract (75 mg/kg body weight); KPE2: Kinnow peel extract (150 mg/kg body weight); KPE3: Kinnow peel extract (300 mg/kg body weight).

3.57 mg/dL). Pre-treatment with peel extracts significantly minimize the effect of doxorubicin administration on serum cholesterol level in different treatment groups. Preventive effect of peel extracts against doxorubicin induced cholesterol level was pronounced at medium and higher doses. Maximum preventive effect (77.50 ± 2.29 mg/dL) was exhibited by mango peel extract high dose (300 mg/kg) while minimum preventive effect (101.83 ± 3.74 mg/dL) was observed in kinnow peel extract low dose (75 mg/kg) and was non-significant to DOX group. Thus the results showed that peel extracts especially at high dose levels were effective against DOX-induced hyperlipidemia.

In case of triglycerides (Fig. 2B), a significant increase (146.50 ± 5.46 mg/dL) was recorded in the level of serum triglycerides in rats of DOX group than normal control group (84.50 ± 2.36 mg/dL). Pre-treatment with mango and kinnow peel extracts at different dose levels significantly minimize the effect of doxorubicin administration on serum triglycerides concentration. However, preventive effect of peel extracts low dose was least as compared to medium and high dose levels. Highest preventive effect (103.17 ±2.91 mg/dL)

was exhibited by mango peel extracts medium dose i.e. 75 mg/kg and was non-significantly different to dose 300 mg/kg of mango peel extract (101.00 ± 3.66 mg/dL).

During the current study, administration of doxorubicin elevated serum LDL level in DOX treated group (71.50 ± 3.06 mg/dL) as compared to control group (45.67 ± 2.28 mg/dL) (Fig. 2C). There was an elevation in the LDL mobilization from the blood into myocardial membranes, leading to abnormally high deposition of cholesterol in the myocardium. Pre-treatment of rats with peel extracts medium and high dose significantly reduced the level of LDL. Maximum preventive activity (54.00 ± 1.73 mg/dL) was determined in mango peel extracts high dose (300 mg/kg) while minimum preventive activity (66.33 ± 2.55 mg/dL) was recorded in kinnow peel low dose (75 mg/kg) that was non-significantly different from DOX group.

A significant decline in HDL level (Fig. 2D) was estimated in doxorubicin treated group (16.33±1.45 mg/dL) than control group (25.00 ± 1.24 mg/dL). Pre-treatment of rats with peel extracts elevated the HDL level but the increase was non-significant except for 300 mg/kg dose level of mango peel extract (21.17 ± 0.95 mg/dL).

3.3 Effect of Mango and Kinnow Peel Extracts on Serum Renal Function Activities

Renal function activities parameters studied in albino rats include BUN, creatinine and uric acid. DOX administration to albino rats significantly increased their renal function activities parameters and induced kidney disorders. As evident by LSD-test, there were significant differences between control and DOX treated groups (Fig. 3). Pre-treatment with different doses of peel extracts decreased the DOX-induced renal disorders in the dose-dependent manner. As regards BUN level (Fig. 3A), a significant increase was observed in p DOX treated group (48.00 ± 3.24 mg/dL) as compared to control group without intervention (17.50 ± 1.73 mg/dL). Pre-treatment with peel extracts significantly minimize the effect of doxorubicin administration on blood urea level in different treatment groups. Preventive effect of peel extracts against doxorubicin induced elevated BUN level was pronounced at medium and higher doses. Maximum preventive effect (31.00 ± 2.14 mg/dL) was exhibited by mango peel extract high dose (300 mg/kg) while minimum preventive effect (42.33 ± 2.42 mg/dL) was observed in mango peel extract low dose (75 mg/kg) and was non-significant to DOX group. Thus the results showed that peel extracts especially at high dose levels were effective against DOX-induced BUN elevated levels.

Data regarding creatinine level revealed significant elevation in DOX group (1.328 ± 0.038 mg/dL) than normal control group (0.715 ± 0.035 mg/dL) (Fig. 3B). Preventive effect of peel extracts against doxorubicin induced elevated ctreatinine level was maximum (0.982 ± 0.027 mg/dL) at mango peel extract high dose (300 mg/kg) while low dose levels (75 mg/kg) peel extracts though prevented the creatinine elevation but the prevention was non-significant with the least preventive effect (1.220 ± 0.025 mg/dL) for low dose mango peel extract.

In case of serum uric acid (Fig. 3C), a significant increase was recorded in DOX group (8.75 ± 0.045 mg/dL) as compared to control group without intervention (3.59 ± 0.036 mg/dL). Pre-treatment with peel extracts significantly minimize the effect of doxorubicin administration on creatinine level in different treatment groups. Preventive effect of peel extracts against doxorubicin induced elevated creatinine level was pronounced at medium and higher doses. Low dose mango and kinnow peel extracts were non-significant to each other but were significantly different from other peel extracts. Maximum preventive effect (6.47 ± 0.076 mg/dL) was exhibited by mango peel extract high dose (300 mg/kg) while minimum preventive effect (8.16±0.045 mg/dL) was observed in kinnow peel extract low dose (75 mg/kg).

3.4 Effect of Mango and Kinnow Peel Extracts on Antioxidant Enzymes in Heart Tissues

During the study, DOX administration to albino rats significantly decreased the myocardial antioxidants superoxide SOD, CAT and GPx. Pre-treatment with peel extracts significantly minimize the effect of DOX administration on cardiac antioxidant level in different treatment groups (Fig. 4). As regards

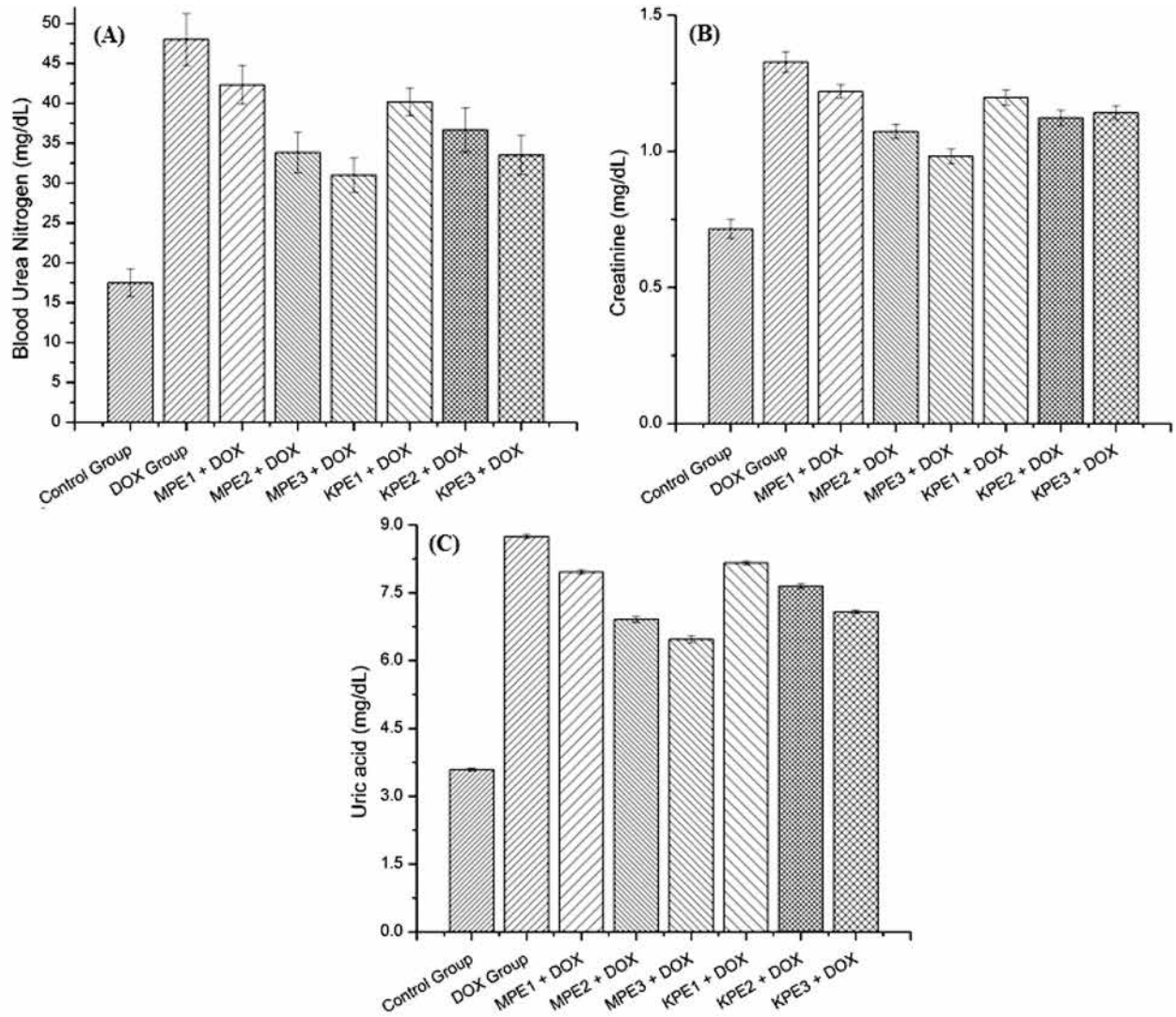

Fig. 3. Effect of mango and kinnow peel extracts on serum renal function parameters in doxorubicin-induced cardiotoxicity in rats. DOX: Doxorubicin,; MPE1: Mango peel extract (75 mg/kg body weight); MPE2: Mango peel extract (150 mg/kg body weight); MPE3: Mango peel extract (300 mg/kg body weight); KPE1: Kinnow peel extract (75 mg/kg body weight); KPE2: Kinnow peel extract (150 mg/kg body weight); KPE3: Kinnow peel extract (300 mg/kg body weight).

SOD activity (Fig. 4A), a significant decrease was observed in DOX group (3.13 ± 0.47units/mg protein) as compared to normal control group (6.95 ± 0.61 units/mg protein). The decreased activity of SOD may be due to accumulation of superoxide anions in the myocardial tissues which are detrimental for myocardium. Pre-treatment with peel extracts followed by DOX administration significantly minimize the effect of doxorubicin induced cardiotoxicity on cardiac SOD level in different treatment groups. Maximum preventive effect (5.15 ± 0.66 units/mg protein) was exhibited by mango peel extract high dose (300 mg/kg) whereas minimum preventive effect (3.40 ± 0.35 units/mg protein) was observed in kinnow mandarin peel extract low dose (75 mg/kg) and was non-significantly different to DOX group as well as mango peel low dose.

Endogenous enzyme catalase converts hydrogen peroxide (H_2O_2) into water and oxygen. Data regarding catalase activity (Fig. 4B) revealed significant decline in DOX group (19.78 ± 1.37 units/mg protein) than normal group (33.42 ± 0.84 units/mg protein). The decline in catalase

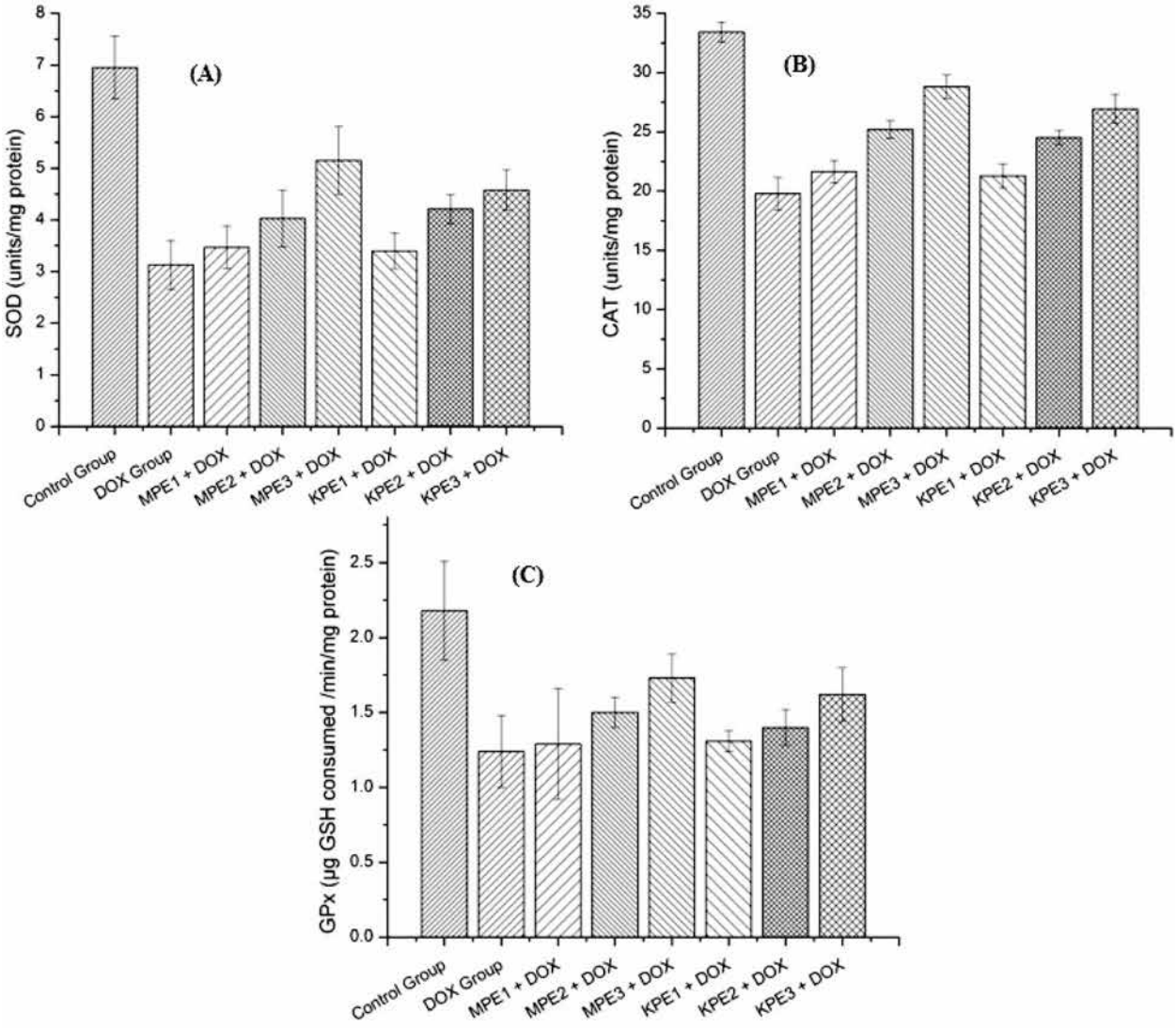

Fig. 4. Effect of different doses of mango and kinnow peel extracts on cardiac tissues antioxidant enzymes in doxorubicin-induced cardiotoxicity in rats. SOD: Superoxide Dismutase; CAT: Catalase; GPx: Glutathione peroxidase; DOX: Doxorubicin; MPE1: Mango peel extract (75 mg/kg body weight); MPE2: Mango peel extract (150 mg/kg body weight); MPE3: Mango peel extract (300 mg/kg body weight); KPE1: Kinnow peel extract (75 mg/kg body weight); KPE2: Kinnow peel extract (150 mg/kg body weight); KPE3: Kinnow peel extract (300 mg/kg body weight).

activity might be due to inactivation of superoxide dismutase by excessive superoxide anions leading to further inactivation of hydrogen peroxide scavenging catalase enzyme. Preventive effect of peel extracts against DOX induced decline in catalase level was maximum (28.82 ± 1.00 units/mg protein) at 300 mg/kg dose level of mango peel extract whereas kinnow peel extract low dose, i.e.,75 mg/kg exhibited lowest preventive effect (21.27 ± 1.01 units/mg protein).

Glutathione peroxidase reduces hydrogen peroxide, peroxides as well as peroxynitrite and thus plays a significant role in neutralizing the oxidative stress. DOX administration to albino rats significantly decreased the GPx activity in DOX treated group (1.24 ± 0.24µg GSH consumed /min/mg protein) as compared to normal group (2.18 ± 0.33 µg GSH consumed /min/mg protein) (Fig. 4C). Pre-treatment of albino rats with different doses of mango and kinnow peel extracts especially medium and high dose levels exhibited elevation in the GPx activity. Highest preventive effect (1.73 ± 0.16 µg GSH consumed /min/mg protein) was observed in groups pretreated with 300 mg/kg dose of mango

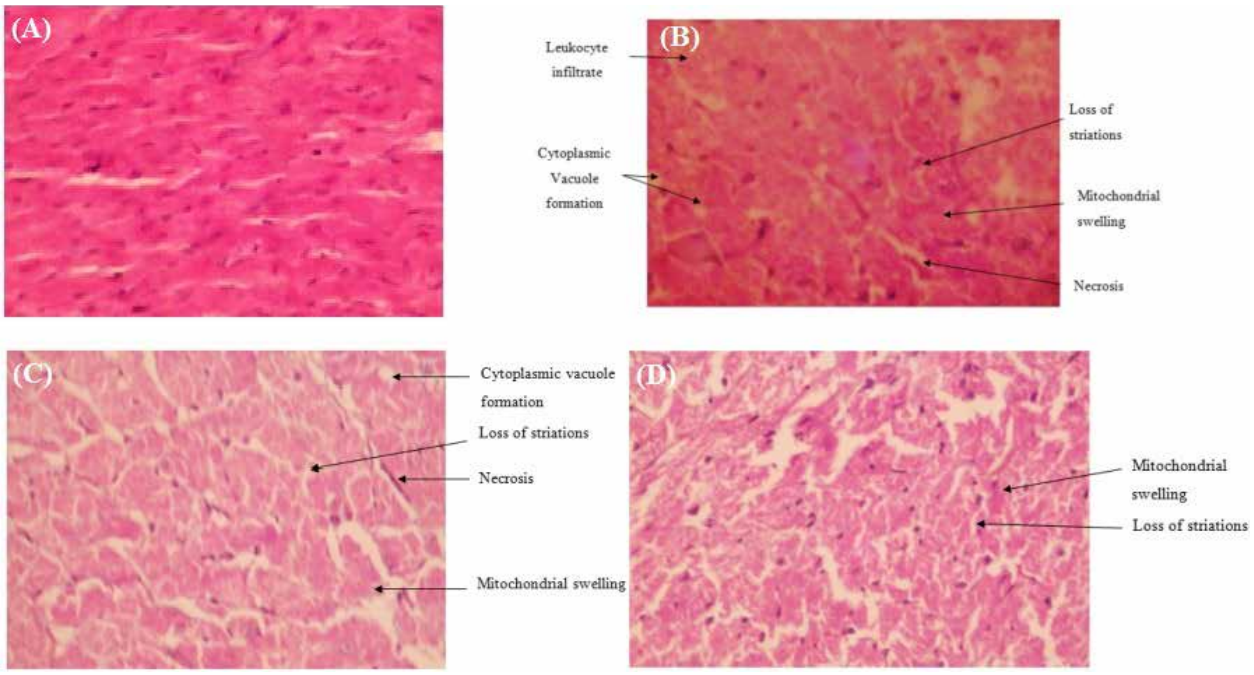

Fig. 5. (A) Photomicrograph of normal control without intervention rat heart tissue showing normal myocardium. **(B)** Photomicrograph of DOX group rat heart tissue showing marked degeneration as loss of striation, mitochondrial swelling, leukocyte infiltrate, cytoplasmic vacuole formation, necrosis and edema. **(C)** Photomicrograph of rat heart tissue pretreated with mango peel extract (75 mg/kg B.W.) + DOX treated rat heart tissue showing moderate to severe changes as mitochondrial swelling, loss of striations, cytoplasmic vacuole formation and necrosis. **(D)** Photomicrograph of rat heart tissue pretreated with mango peel extract (150 mg/kg B.W.) + DOX treated rat heart tissue showing moderate changes as mitochondrial swelling and loss of striations.

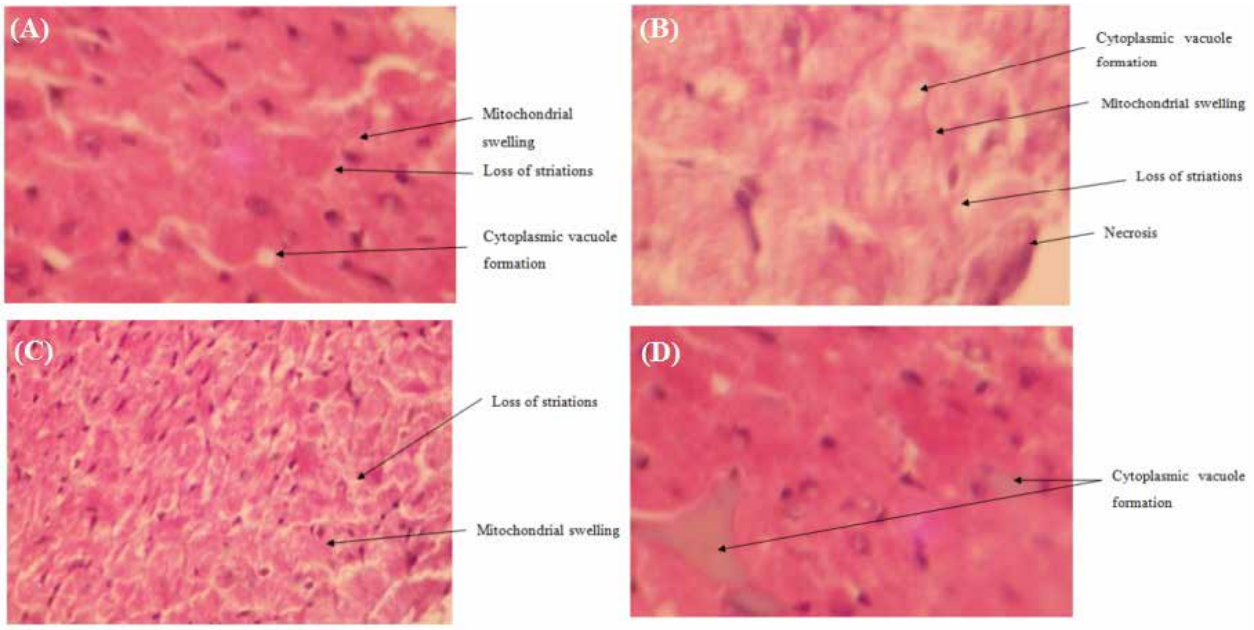

Fig. 6. (A) Photomicrograph of rat heart tissue pretreated with mango peel extract (300 mg/kg B.W.) + DOX treated rat heart tissue showing slight change as cytoplasmic vacuole formation. **(B)** Photomicrograph of rat heart tissue pretreated with kinnow peel extract (75 mg/kg B.W.) + DOX treated rat heart tissue showing severe changes as cytoplasmic vacuole formation, mitochondrial swelling, loss of striation, onset of necrosis and edema. **(C)** Photomicrograph of rat heart tissue pretreated with kinnow peel extract (150 mg/kg B.W.) + DOX treated rat heart tissue showing moderate changes as mitochondrial swelling loss of striations. **(D)** Photomicrograph of rat heart tissue pretreated with kinnow peel extract (300 mg/kg B.W.) + DOX treated rat heart tissue showing slight change as cytoplasmic vacuole formation.

peel extract which was non-significant to rats group pre-treated with 300 mg/kg dose of kinnow peel extract (1.62 ± 0.18 µg GSH consumed /min/mg protein but was significantly different from DOX group.

3.5 Effect of Mango and Kinnow Peel Extracts on Cardiac Histopathology

Histopathological evaluation of cardiac tissue on negative control or normal control group showed a normal mycordium with well-preserved cytoplasm, myocardial fibers of uniform configurations, no inflammatory cell infiltrates and regular morphology of myocardial cell membrane (Fig. 5A). Marked degeneration and cardiomyopathy occurred in myocardium of positive control or DOX administered group as evident by mitochondrial swelling, cytoplasmic vacuole formation, loss of striation, leukocyte infiltration and edema (Fig. 5B). Pretreatment with mango peel extract 75 mg/kg + DOX group showed moderate to severe changes in myocardium such as swelling of mitochondria, cytoplasmic vacuole formation, loss of striations and necrosis (Fig. 5C). Rats group pretreated with 150 mg/kg mango peel polyphenolic extract for 60 days + DOX administration indicated moderate changes in myocardium like mitochondrial swelling, loss of striation (Fig. 5D). However, rats group pretreated with 300 mg/kg mango peel phenolic extract for 60 days + DOX resulted in least changes in myocardium such as cytoplasmic vacuole formation and inhibited DOX induced cardiac damage (Fig. 6A). As regards cardiac histopathology of albino rats pretreated with kinnow mandarin peel extract, 75 mg/kg kinnow peel extract dose for 60 days + DOX was ineffective against doxorubicin induced cardiotoxicity and exhibited severe changes in myocardium similar to DOX group like cytoplasmic vacuole formation, mitochondrial swelling, loss of striation, edema and necrosis (Fig. 6B). Pretreatment with 150 mg/kg kinnow peel extract + DOX resulted in moderate changes to myocardium of rats such as loss of striation and mitochondrial swelling (Fig. 6C). Albino rats group pretreated with 300 mg/kg kinnow mandarin phenolic extract for 60 days + DOX showed slight changes in myocardium like cytoplasmic vacuole formation (Fig. 6D).

4. DISCUSSION

During the current study, the cardioprotective activities of mango and kinnow peel polyphenolic extracts were evaluated. Serum cardiac markers such as LDH, CK-MB, CPK and AST are the significant indicators of deviation in normal cardiac activity. Lactate dehydrogenase (LDH) is the enzyme expressed widely in body tissues especially in cardiac muscles that catalyzes the interconversion of pyruvate and lactate. Since LDH is released during tissue damage, it is considered as biomarker of cardiac injury. Another enzyme creatine phosphokinase is significant for muscle functions, generally present in cardiac muscles which are released into bloodstream after cardiac injury. CK-MB is another enzyme specifically found in heart muscles and considered as biomarker of myocardial infarction. Aspartate transminase (AST) catalyzes the amino groups intermolecular transfer and thus considered as a vital enzyme in metabolic reactions. Significantly higher concentrations of AST are present in heart and liver tissues. Upon injury to heart or liver, these enzymes are released into blood. Elevated concentration of AST is an important diagnostic test for myocardial infarction. During the study, DOX group exhibited significant elevation in the levels of cardiac marker enzymes which indicated severe myocardial damage. Low dose of peel extracts had non-significant effect on serum cardiac markers. Pre-treatment with medium (150 mg/kg BW) and high dose (300 mg/kg BW) of polyphenolic peel extracts exhibited cardioprotective activity as evident by restricted cardiac enzyme concentration in serum. Maximum cardioprotective activity was exhibited by groups pre-treated with high dose (300 mg/kg) of peel extracts especially mango peel extracts that maintained the membrane integrity of myocardial tissues. The cardioprotective potential of mango and kinnow mandarin peel extracts might be attributed to the presence of antioxidant phenolic compounds with free radical scavenging activity. Prabhu et al. [37] investigated the cardioprotective effect of mango xanthone mangiferin in rats and concluded that mangiferin protected the experimental myocardial infarction due to its antioxidant, free radical scavenging, immunomodulatory, antilipidperoxidative and

cardiotonic characteristics. Results were comparable with the investigations of Abdel-Raheem and Abdel-Ghany [14] who reported that hesperidin, a citrus bioflavonoid protected cardiac tissues against cardiotoxic effects of doxorubicin owing to its free radical scavenging activities.

Lipids play a significant role in the pathogenesis of cardiovascular disease, not only by inducing hyperlipidemia and subsequent development of atherosclerosis but also by modifying the cellular membrane structure, composition and stability. A high concentration of lipids in blood accelerates atherosclerosis and is considered as major risk factor in myocardial infarction [38]. Doxorubicin administration to albino rats significantly elevated the lipids level in blood and induced hyperlipidemia. Pre-treatment with different doses of peel extracts reduced the DOX-induced hyperlipidemia in the dose-dependent manner. Experimental hyperglyceridemia in doxorubicin administered rats might be due to decline in the lipoprotein lipase activity in the myocardium leading to lesser uptake of triglycerides from the blood circulation [39]. Low density lipoprotein (LDL) is a kind of lipoprotein that carries triglycerides and cholesterol from the liver to the exterior tissues and facilitates the movement of cholesterol and fat within the blood stream as well as modulates cholesterol synthesis. They are often considered bad cholesterol due to the fact that their elevated levels may pose serious cardiovascular disorders. High density lipoprotein (HDL) is a type of lipoproteins that remove lipids such as cholesterol, triglycerides and phospholipids from the cells, from atheroma within arteries and transport it back to liver for re-utilization or excretion and thus considered as good cholesterol. The HDL- cholesterol protective role may be ascribed to its antioxidant, antithrombotic characteristics as well as its role in reverse cholesterol transport [40]. The increase in the level of total cholesterol, triglycerides, LDLs and decrease in HDLs in the doxorubicin group revealed that doxorubicin interfered with the biosynthesis of lipids. Pre-treatment of mango and kinnow peel extracts at medium (150 mg/kg B.W.) and high dose (300 mg/kg) levels indicated a decline in serum lipid profile with concurrently elevation in HDLs. Therefore, peel extracts especially mango peel polyphenolic extracts showed a strong lipid lowering chemotherapeutic agent. Lipid lowering activity of peel extracts might be due to restriction of cholesterol biosynthesis, enhanced fecal bile acid secretion, catabolism of LDL cholesterol and enhanced uptake of LDL from blood by the liver [41]. Flavonoids may assist in uptake of oxidatively modified LDL by scavenging mechanism [42]. The findings of current study exhibiting doxorubicin induced hyperlipidemia and were in line with the earlier investigations [38, 43].

The BUN is a measure of nitrogen concentration in the blood that originates from urea and is considered as renal function marker, though not as significant marker as creatinine due to the fact that external factors such as dehydration and diet influenced blood urea levels. Serum creatinine is a byproduct of muscle metabolism, removed from the blood by the kidneys and since it is excreted unaltered by the kidneys, it is considered as the vital renal health indicator. Creatinine blood level elevates in case of malfunctioning of kidneys or deficient filtration in the kidneys. Uric acid is the breakdown product of purine nucleotides, elevated concentrations in the blood may lead to gout, kidney stones, hypertension, obesity and other medical disorders. Due to low excretion by the kidneys, serum uric acid concentration may be elevated and high concentration of uric acid in blood above normal is known as hyperuricemia which is also considered as risk factor for cardiovascular disorders [44, 45]. During the study, elevated levels of BUN, creatinine and uric acid in DOX or positive control group indicated that DOX interfered with the functioning of renal organs. Similar nephrotoxicity induced by DOX administration in albino rats was earlier reported by Shafik et al. [46]. Pre-treatment of mango and kinnow peel extracts especially at high dose (300 mg/kg) levels exhibited a reduction in serum renal parameters and ameliorated the nephrotoxicity which might be due to extracts phenolic compounds with free radical scavenging activity.

In biological systems free radicals are generated frequently but nature has provided within cells the reducing mechanism that neutralizes free radicals. Oxidative stress is the condition induced due to change in normal redox state which is ameliorated by

the endogenous enzymes like superoxide dismutase, glutathione peroxidase and catalase. Superoxide dismutase converts reactive oxygen species into hydrogen peroxide (H_2O_2) which is then converted into H_2O by catalase or glutathione peroxidase. During Ischemia, the endogenous antioxidant system is destroyed and hydrogen peroxide is converted into hydroxyl radical [41]. During the current study, endogenous antioxidant enzymes SOD, CAT and GPx activities were significantly lower in doxorubicin administered group which might be attributed to reduce availability of their substrates. These enzymes play a significant role in alleviating the ROS induced mycordial injury and constitute the first line of defense against oxidative injury. Generation of highly reactive free radicals restricted the antioxidant enzymes activities [46]. Restriction in antioxidant enzymes activities may result in elevated production of free radicals and hydrogen peroxide which ultimately forms hydroxyl radical(OH•) leading to number of detrimental reactions [41]. Pre-treatment with mango and kinnow peel extracts especially at 300 mg/kg dose level inhibited the doxorubicin-induced cardiotoxicity which might be due to peel extracts polyphenols with antioxidant activity. Low dose of peel extracts had non-significant effect on cardiac antioxidant enzymes. Mango peel extracts exhibited relatively higher cardioprotective activity due to prevention of antioxidant enzymes activity depletion in the rat myocardium. The elevation in heart tissues antioxidant enzymes might be due to cellular adaptive mechanism that leads to more synthesis of these antioxidant enzymes as well as ascribed to free radical scavenging activity of phenolic compounds present in the extracts [47]. Results were in line with the earlier investigations of Abdel-Raheem and Abdel-Ghany [14] who reported that polyphenol flavonoid hesperidin enhanced the superoxide dismutase activity and glutathione levels in cardiac tissues and attenuated the doxorubicin induced cardiotoxicity. Similarly, Bhupati et al. [25] reported *Vitis vinifera* (black grapes) significantly elevated the cardiac antioxidant enzymes SOD, CAT and GSH in doxorubicin-induced oxidative stress in rats due to free radical scavenging activity of polyphenols and flavonoids present in the extracts.

Histopathology studies of different groups cardiac tissues exhibited that peel extract dose levels 150 mg/kg and 300 mg/kg provided cardio-protection to albino rats myocardium against DOX induced cardiotoxicity. However, pretreatment with peel extracts 300 mg/kg BW for 60 days exhibited maximum cardio-protection as evident by mild histopathological changes as compared to DOX group cardiac histopathology. Mango peel extract exhibited comparatively higher cardioprotective activity than kinnow mandarin peel extract. The higher cardioprotective activity of mango peel extracts revealed by histopathological investigations might be due to free radical scavenging and protective activity of phenolic compound mangiferin present in mango peel extracts. The histopathological changes observed in the myocardium of doxorubicin administered rat group were similar to those earlier reported [48] Results were in agreement with the findings of Prabhu et al. [37] that mangiferin, a xanthone polyphenol pretreatment with 10mg/100g BW for 28 days revealed maximum protection evident by least histopathological changes as compared to isoproterenol induced myocardial infarction in rats. Similarly, Abdel-Raheem and Abdel-Ghany [14] observed that pretreatment with hesperidin flavonoid 200 mg/kg to albino rats protected the myocardium against DOX induced cardiotoxicity revealed by normal myocardium with no inflammatory cells infiltration alleviated the edema and blood vessels congestion. Likewise, phenolic compound gallic acid exhibited the cardioprotective properties by alleviating the myocardial damage to myocardial tissues induced by DOX [49].

5. CONCLUSIONS

Results of the current study indicated the cardioprotective effect of mango and kinnow peel polyphenolic extracts. Pretreatment of albino rats with mango and kinnow peel polyphenolic extracts significantly reduced the DOX-induced damage, i.e., elevation in cardiac enzymes, lipids, renal function parameters and decrease in myocardial enzymes as well as ameliorated the myocardial injury. Mango peel extracts exhibited comparatively more cardioprotective activity than kinnow peel extracts.

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