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Evaluation of cardiopreventive effects of *Ximenia americana* (Linn.) and *Pappea capensis* (Eckl. and Zeyh.) leaf aqueous extracts in rat models with myocardial infarction

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Abstract

Background Myocardial infarction is a significant health issue in both wealthy and underdeveloped nations. Globally, it is the leading cause of deaths among cardiovascular diseases. In 2012, myocardial infarction-related deaths were about 14.1 million out of 17.5 million cardiovascular disease-related deaths. Clinical management of myocardial infarction remains a challenge because most conventional drugs provide symptomatic relief only. In addition, conventional remedies are associated with numerous advese effects and arguably, in many cases are quite expensive. Hence, herbal remedies, which are widely available, with comparatively fewer side effects, and are affordable, provide a more attractive therapeutic alternative. This study aimed at determining cardiopreventive effects of aqueous leaf extracts of *X. americana* and *P. capensis*. Phytochemical screening was done using liquid chromatography-mass spectrometry. Wistar albino rats were employed to test for cardiopreventive effects of the extracts and were randomized into 6 groups of 5 animals each. Groups I, II, and III were normal, negative, and positive controls, respectively, and rats were given normal saline, salbutamol (7.5 mg/Kg bw), and propranolol, respectively. Groups IV, V, and VI rats were treated with extracts dose levels 50, 100, and 150 mg/Kg bw, respectively. Biochemical analysis was done to determine effects of the extracts on levels of serum cardiac troponin T, creatine kinase-MB, lactate dehydrogenase-1, and lipid profiles. Levels of oxidative stress markers were determined in the heart tissue.

Results The LC–MS analysis revealed different phytocompounds in the extracts, including flavonoids, phenolic acids, glycosides and tannins, which are known to confer cardioprotective activities. The extracts significantly prevented increase in cardiac troponin T, creatine kinase-MB, lactate dehydrogenase-1, total cholesterol, triglycerides, LDL, and MDA levels, as well as a significant increase in superoxide dismutase, catalase, glutathione peroxidase, and HDL levels.

Conclusions This study confirmed that *Ximenia americana* and *Pappea capensis* extracts have the potential to prevent myocardial infarction in rats. Generally, *P. capensis* extract showed better activity as compared to *X. americana* extract. The effects of the extracts could be attributable to the presence of various cardioactive phytocompounds. Therefore, these plants can be considered in the development of potent and safe cardiopreventive drugs.

Keywords Salbutamol, Propranolol, Myocardial infarction, Cardiopreventive, *Ximenia americana*, *Pappea capensis*

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Background

Cardiovascular diseases (CVDs) are a major cause of threat to life globally and an impediment to human development [1]. They cause about 17.9 million fatalities annually [2]. The CVDs-associated mortalities are predicted to reach 23.3 million by the year 2030 [3]. Among the CVDs, myocardial infarction (MI), commonly known as 'heart attack', is the leading cause of morbidity and mortality, contributing to approximately 85% of total CVD-related deaths [4]. According to estimates, 5% of persons over 75 years old have had MI at some point in their lives, either with little symptoms or none at all [5]. In 2012, MI caused about 6.7 million deaths globally [6]. Myocardial infarction is marked by damage to the heart's muscle due to a decreased or obstructed blood supply [7]. This condition is characterized by heartburn-like pain that radiates to the upper limb, shoulder, back or neck from the left side or center of the chest [8]. A patient with MI may also experience syncope, shortness of breath, sweating, palpitations, nausea, and fatigue [8]. However, some patients, especially women, may not present with chest pain, but only pain in the neck, upper limb or fatigue [9]. Complications of MI include ventricular aneurysms, arrhythmias, pericarditis, cardiogenic shock, and congestive heart failure [10].

High levels of LDL, low levels of HDL, hypercholesterolemia, diabetes, aging, high blood pressure, and obesity are the main risk factors for MI [11]. The underlying mechanism by which MI occur include the blockage of coronary artery, which is usually caused by atherosclerotic plaque, and to a lesser extent spasms in coronary artery as a result of emotional stress, extreme cold, and cocaine use, among others [12]. Cardiac troponin T and I (cTnT and I), creatine kinase MB (CK-MB) and lactate dehydrogenase-1 (LDH-1), lipid profiles, and oxidative stress markers are some of the specialized tests included in the blood workup for MI [13].

Prompt treatment of MI is highly recommended [14]. The drugs of choice include aspirin, administered with nitroglycerin and morphine, to alleviate pain, together with supplemental oxygen for patients presenting with difficulty breathing [15]. Furthermore, thrombolysis agents, percutaneous coronary intervention (PCI), and blood-thinning drugs, including heparin, are used in eradicating the clot [15]. In addition, following a MI, lifestyle modifications are advised along with long-term aspirin, beta-blocker, and statin medication [16]. However, these drugs are not only considered ineffective but also are associated with many side effects and are arguably expensive [17]. Therefore, other alternatives such as herbal medicines, which are widely available, effective, with minimal side effects, and arguably affordable, have been put into use and a few have been evaluated in the treatment of MI [18].

Various previous studies have confirmed efficacy of herbal plant extracts in treatment of MI. They include the leaf extract of *Carissa opaca* which was showed to confer cardioprotective effects against carbon tetrachloride (CCl_4) induced cardiotoxicity [19]. *Cinnamomum tamala* demonstrated cardioprotective activities in rats induced with myocardial infarction [20]. In addition, the presence of alkaloids, saponins, phenols, and flavonoids in an aqueous leaf extract of *Moringa oleifera* plant was linked to cardioprotective benefits, whereas *Mangifera indica* demonstrated strong in vivo cardioprotective effects in mice with isoproterenol-induced cardiotoxicity [21], 22. Moreover, *Trichopus zeylanicus* leaf extracts demonstrated significant cardioprotection in isoproterenol-induced cardiac injury in laboratory animals [23].

Ximenia americana belongs to the family Olacaceae [24]. The plant is commonly known as Sea Lemon or Yellow Plum plant [25]. It is a highly branched shrub, thorny, with slender twigs and alternating leaves [26]. This shrub mainly grows in tropical regions of Africa, Asia, Central America and South America [27]. On the other hand, *Pappea capensis* is a small, semi-deciduous tree that grows to about six meters high [28]. This tree is leafy, with a short trunk and low branches that spread into a rounded crown [29]. Its stem bark is pale to dark grey, smooth with horizontal marking, whereas the leaves are dull-green, wavy with spine-toothed edges and a rounded base [29]. The flowers of *P. capensis* are small, green-yellow in color with male at the tip and female at the base, whereas the fruits are edible and yellow-green in color [30].

Various previous toxicity studies done on *X. americana* and *P. capensis* showed that the plants are safe. Togbossi et al. [31] showed that *X. americana* hydroethanolic extract have neither acute nor sub-chronic toxicity in rats. In addition, Muhammad et al. [32] confirmed the safety of methanolic stem bark extract of *X. americana* in rats. On the other hand, a previous study done by Pendota et al. [33], showed that leaves extract of *P. capensis* were safe in African green (vero) monkey kidney cells.

The results of earlier investigations have demonstrated that *X. americana* and *P. capensis* leaf and stem bark extracts have outstanding antioxidant properties [33, 34]. The traditional use of antioxidants in the treatment of heart-related problems derives from their vital function in lowering oxidative stress, a mechanism by which MI occurs. Moreover, for a long time many communities, such as Ambeere, Ameru and Aembu in Kenya and Zulus in South Africa, have been using *X. americana* and *P. capensis* in the management of heart-related conditions

[35]. Despite the confirmed antioxidant activities and the traditional use of these plants, their efficacy in MI has not been scientifically evaluated. In light of this, the current study was developed to analyze the phytochemical profiles and determine preventive effects of *X. americana* and *P. capensis* leaf extracts in rats induced with MI in order to confirm their traditional use and to provide preliminary information for the development of potent and safe cardiopreventive drugs that can be used to treat MI.

Methods

Collection and preparation of plant materials

Fresh leaves of *X. americana* and *P. capensis* were collected from Nthawa village, Mbeere North Sub-County, Embu County, Kenya, with help of local herbalists (0°33′17″ N 37°36′18″ E and 0°33′17″ N 37°36′19″ E, for *X. americana* and *P. capensis,* respectively). The plant samples were collected during the dry season, in the month of September, when the phytochemical levels are known to be at the highest. Botanical identification of the plant samples was carried out by a qualified taxonomist and voucher specimens deposited to Herbarium of the National Museums of Kenya in Nairobi for future reference. The specimens were assigned voucher numbers DMG001 and DMG002 for *X. americana* and *P. capensis,* respectively. The collected fresh plant leaves were completely dried under room temperature, away from direct sunlight. The dry leaves were ground into a fine powder using an electric mill. The powder was stored in closed dry khaki bags under room temperature awaiting extraction.

Extraction

For each powdered plant material, 250 g was separately soaked in 2 L of distilled water, put in water bath and allowed to stand for 2 h at 60 °C [15]. The mixture was decanted and then filtered using Whatmann No.1 filter papers. The filtrate was dried in a freeze drier [36]. The resultant dry extracts from the two plants were separately packaged and stored in airtight containers at a temperature of − 4 °C awaiting use in the experiments [37].

Liquid chromatography—mass spectrometry (LC–MS)

LC–MS machine used had the following features: a quaternary LC pump (Model 1200) coupled to Agilent MSD 6120-Single quadruple MS with electrospray source (Palo Alto, CA), and ChemStation software (Hewlett-Packard) control system. Agilent technology 1200 infinite series, Zorbax SB C_{18} column, 2.1 × 50 mm, 1.8 µm (Phenomenex, Torrance, CA) was used to carry out reversed-phase liquid chromatography with gradient program of 0 min, 5% B; 0–5 min, 5–50% B; 5–10 min, 50–80% B; 10–15 min, 80–100% B; 15–25 min 100% B; 25–30 min 5% B; 30–35 min 5% B (Water; Acetonitrile). One ml/min flow rate was held constant. The injection volume was 20.0 µL.

Data was acquired in a full-scan positive-ion mode using a 100 to 1500 *m/z* scan range. The dwell time for each ion was 50 ms. Other parameters of the mass spectrometer included: capillary voltage of 3.0 kV, cone voltage of 70 V, extract voltage of 5 V, RF voltage of 0.5 V, source temperature of 110 °C, nitrogen gas temperature for desolvation of 380 °C, and nitrogen gas flow for desolvation of 400 L/h.

The identities of present phytochemicals were proposed based on their general fragmentation pattern and using reference spectra published by library–MS databases of National Institute of Standards and Technology (NIST).

Experimental animals

This study employed rats (Wistar albino) of either sex, aged between 10 and 11 weeks and weighing 250 ± 10 g. The animals were housed in propylene cages and put under standard laboratory conditions, including 25 °C room temperature and 12 h day followed by 12 h night cycle. The animals were fed on pellets and water supplied ad libitum [38]. Ethical guidelines on experimental animal handling were adhered to throughout the study [39]. Institutional Animal Ethics Committee of Kenyatta University reviewed and approved the protocols used in the experiments (Approval number PKUA/006/006).

Induction of myocardial infarction

Salbutamol, acquired from Dawa pharmaceuticals, was used to induce cardiac injury. A dose of 7.5 mg Kg^{-1} bw^{-1} was administered intraperitoneally once a day for 2 consecutive days [40]. Salbutamol (4-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxmethyl) phenol) is a β-adrenergic receptor agonist that, when given in high doses, causes high oxidative stress of the heart muscle leading to necrosis and infarct formation [41]. The presence of MI was marked by high serum levels of cardiac specific biomarkers, including cTnT, CK-MB and LDH-1 among rats treated with salbutamol only (negative group).

Experimental design

Randomized controlled experimental design was used in the present study. Rats were randomized into six groups of five each. Group I (normal control) rats were given the vehicle only (oral 0.9% w/v normal saline) and MI not induced. Group II (negative control) comprised rats treated with 0.9% w/v normal saline and MI induced intraperioteally with salbutamol on days 15 and

16. Group III consisted of positive control group of rats, orally treated with the standard drug (propranolol 10 mg/Kg bw) and MI induced on days 15 and 16. Groups IV, V and VI rats were orally treated with plant extracts at dosages of 50, 100 and 150 mg/kg bw, respectively, following which MI was induced on days 15 and 16 (Table 1). Throughout the study, food and water was provided to all animals ad libitum.

On 17th day, animals were weighed, euthanized using overdose of isoflurane in a vacuum glass desiccator and then sacrificed. Each animal's heart was carefully removed, weighed and recorded. Blood for biochemical assessment was taken by cardiac puncture. All blood samples were collected in well labelled vacutainer tubes and centrifuged in order to get serum used in biochemical assessment [15].

Determination of relative heart weight

Following euthanization, the heart of each animal was carefully removed, blood squeezed out and then washed using cold normal saline. The heart weight was recorded using Mettler PJ 3000 weighing balance and used in computing relative heart weight [42].

Determination of cardiac specific biomarkers

Cardiac specific biomarkers analysed include cTnT, CKMB and LDH-1. Cobas E 411-Roche was used in determination of serum cTnT, whereas Cobas Integra 400 Plus was used in analysis of CK-MB and LDH-1 [15].

Determination of lipid profiles

The lipid profiles analysed include total cholesterol, triglycerides, low density lipoprotein (LDL) and high density lipoprotein (HDL). The locally available kits were used to carry out the analysis in a chemistry autoanalyzer (Mindray BS 120) [15].

Determination of oxidative stress markers

The heart tissue was homogenized in a homogenizer and then centrifuged to obtain supernatant that was used to determine the levels of cardiac antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). Malondialdehyde (MDA) levels were also determined from the same supernatant [15].

Determination of catalase activity

Spectrophotometric method described by Aebi was used to assay for CAT levels [43]. First, 3 ml assay sample was obtained from a mixture of 0.063% H_2O_2 in 0.1 M KPB pH 7.4 and 10 µl of tissue supernatant. The decrease in absorbance at 240 nM was recorded for 1 min and enzyme activity expressed as millimoles of decomposed H_2O_2 per minute per milligram of protein using a molar extinction coefficient of 43.6 M^{-1} cm^{-1}. The catalase enzyme level was expressed as units per milligram protein.

Determination superoxide dismutase activity

This was done in line with the method described by Marklund and Marklund [44]. The first step involved obtaining 3 ml aliquot of assay sample from a mixture of 50 mM Tris–HCl buffer (pH 8.2), 1 mM diethylene triamine penta acetic acid, 45 µl of 10 mM pyrogallol in 10 mM HCl and 10 µl of tissue supernatant. The rate of inhibition of pyrogallol auto-oxidation after the addition of enzyme extract was recorded at a wavelength of 420 nm.

The amount of enzyme required to give 50% inhibition of pyrogallol auto-oxidation was considered as one unit of enzyme activity and was expressed in units per milligram protein. Bovine serum albumin (BSA) was used as standard and protein content in the tissue supernatant estimated using standard protocol [45].

Determination glutathione peroxidase activity

The protocol developed by Paglia and Valentine [46] was used to determine GPx activity. The procedure involved adding 750 µl of KPB (0.1 M, pH 7), 60 µl NADPH (2.25 mM in 0.1% NaHCO3), 15 µl of glutathione

Table 1 Treatment Protocol for the Evaluation of Cardiopreventive Effects of Aqueous Leaf Extracts of *X. americana* and *P. capensis* in Rats with Myocardial Infarction

Group	Treatment
I (normal control)	Vehicle (0.9% w/v normal saline)
II (negative control)	Vehicle + 7.5 mg Kg^{-1} bw^{-1} Salbutamol
III (positive control)	10 mg Kg^{-1} bw^{-1} Propranolol + 7.5 mg Kg^{-1} bw^{-1} Salbutamol
IV (experimental A)	50 mg Kg^{-1} bw^{-1} Extract + 7.5 mg Kg^{-1} bw^{-1} Salbutamol
V (experimental B)	100 mg Kg^{-1} bw^{-1} Extract + 7.5 mg Kg^{-1} bw^{-1} Salbutamol
VI (experimental C)	150 mg Kg^{-1} bw^{-1} Extract + 7.5 mg Kg^{-1} bw^{-1} Salbutamol

reductase (7.1 μ ml^{-1}) and 25 μl GSH (11.52 mg ml^{-1}) in a 1 ml cuvette. Approximately 100 μl of 1.5 mM H_2O_2 and 50 μl of supernatant were added to initiate the reaction. A spectrophotometer was used to observe decrease in absorbance for 1 min at a wavelength 340 nm. The enzyme activity was expressed as millimoles of NADPH oxidized per minute using molar extinction coefficient of 6.22 × 103 $mmol^{-1}$ cm^{-1}. Glutathione peroxidase level was expressed in units/mg protein [15].

Determination lipid peroxidation

Malondialdehyde (MDA) is the end product of lipid peroxidation in a tissue. The levels of malondialdehyde were determined according to the procedure described by Wills with few modifications [47]. Approximately 200 μL aliquot of tissue supernatant was mixed with 2 ml of thiobarbituric acid (TBA)–trichloroacetic acid (TCA) reagent (0.375 and 15%, respectively) and the volume was made up to 3 ml with distilled water. The mixture was boiled on a water bath at 95 °C for 20 min and the solution cooled under tap water. The reaction product (TBA–MDA complex) was extracted by adding 3 ml of n-butanol to the solution. The spectrophotometer (UV-6100 UV/VIS 190–1100 nM/1.8 nM, China) was used to measure absorbance of the pink colored extract in n-butanol at a wavelength of 532 nm. The amount of MDA was computed using a Molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and results expressed in nmoles of MDA formed per gram of used tissue [15].

Data processing and statistical analysis

Data was first entered into an Excel spreadsheet, organized, and then exported, for analysis, to statistical software (version 19 of Minitab software, NC, USA). The collected data conformed to basic assumptions of parametric data. Descriptive statistical results were expressed as Mean ± Standard Error of Mean (SEM). Inferential statistical analysis was done using one-way ANOVA, whereas Tukey's post hoc test was used for pairwise mean comparisons and separation. The differences were considered to be statistically significant if $p \leq 0.05$. The results were presented in form of tables and graphs.

Results

Phytochemical screening

The LC–MS chromatogram of aqueous leaf extract of *X. americana* showed presence of a variety of phytocompounds with corresponding peaks at different retention times (Fig. 1). About 20 phytocompounds were identified, belonging to the class of flavonoids, tannins, phenolic acids, glycosides, fatty acids and phytosterol. From the analysis, flavonoids were the most abundant (epicatechin, gallocatechin, quercitrin, kaempferol-G-O-arabinopyranoside, kaempferol -G-l-rhamnoside, quercetin-G-glucosyl-rhamnosyl, quercetin-3 glucoside and quercetin-3-0-Beta-D-glucoside) with a relative abundance of 59%, followed by tannins (gallotannin and ellagitannin) at 37.39%, then phenolic acids (caffeic, ellagic, gallic, m-coumaric and protocatechuic acids) with relative abundance of 1.79%, glycosides (quercetin-3,O-alpha-L-rhamnoside, quercetin rhamnoside and quercetin-G-L rhamnoside), 1.2%, fatty acid (tariric acid) 0.36%, and lastly phytosterol (beta-sitosterol) with 0.28%.

Similarly, LC–MS chromatogram of *P. capensis* aqueous leaf extract revealed different phytocompounds with corresponding peaks at various retention times (Fig. 2). There were about 24 phytocompounds identified, including flavonoids, glycosides and phenolic acids. Flavonoids (hesperidin, quercitrin, epicatechin, myricetin, quercetin-3 glucoside, rhamnetin, kaempferol-3-0-arabinopyranoside, quercetin-3-O-beta-arabinopyranosyl, quercetin-3-O-beta-D-L-glucosyl, quercetin-3-O-beta-D-L-glucoside and quercetin-3-O-beta-D-G-glucoside) accounted for the highest relative abundance at 56.12%, followed by glycosides (quercetin rhamnoside, kaempferol hexoside and quercetin-3-0-alpha-L-rhamnoside) at 31.47%. Phenolic acids (3,4,5-trimethoxycinnamic, caffeic, chlorogenic, ellagic, ferulic, gallic, gentisic, isoferulic, p-coumaric and protocatechuic acids) showed the least relative abundance at 6.72%.

Effects of aqueous leaf extracts of *X. americana* and *P. capensis* on relative heart weight in rats

The aqueous leaf extracts of *X. americana* and *P. capensis* significantly prevented increase in relative heart weight among rats treated with the extracts as compared with negative control rats ($p < 0.05$; Fig. 3). Generally, the extracts prevented increase of relative heart weight in a dose-related trend (Fig. 3). The effects of the two plant extracts were comparable at the corresponding dose levels ($p > 0.05$; Fig. 3).

Effects of aqueous leaf extracts of *X. americana* and *P. capensis* on Serum cardiac troponin T levels in rats

The current study results established that *X. americana* and *P. capensis* leaf aqueous extracts significantly ($p < 0.05$) prevented increase of serum cTnT among extracts-treated MI rats as compared with levels in negative control rats (Fig. 4). Generally, the two extracts inhibited raise of serum cTnT levels in a dose-dependent manner (Fig. 4). Findings of the present study showed that *P. capensis* extract dose level of 50 mg/Kg bw was significantly more effective in preventing cTnT levels increase as compared to the corresponding *X. americana* extract dose level ($p < 0.05$; Fig. 4). However, the effects of

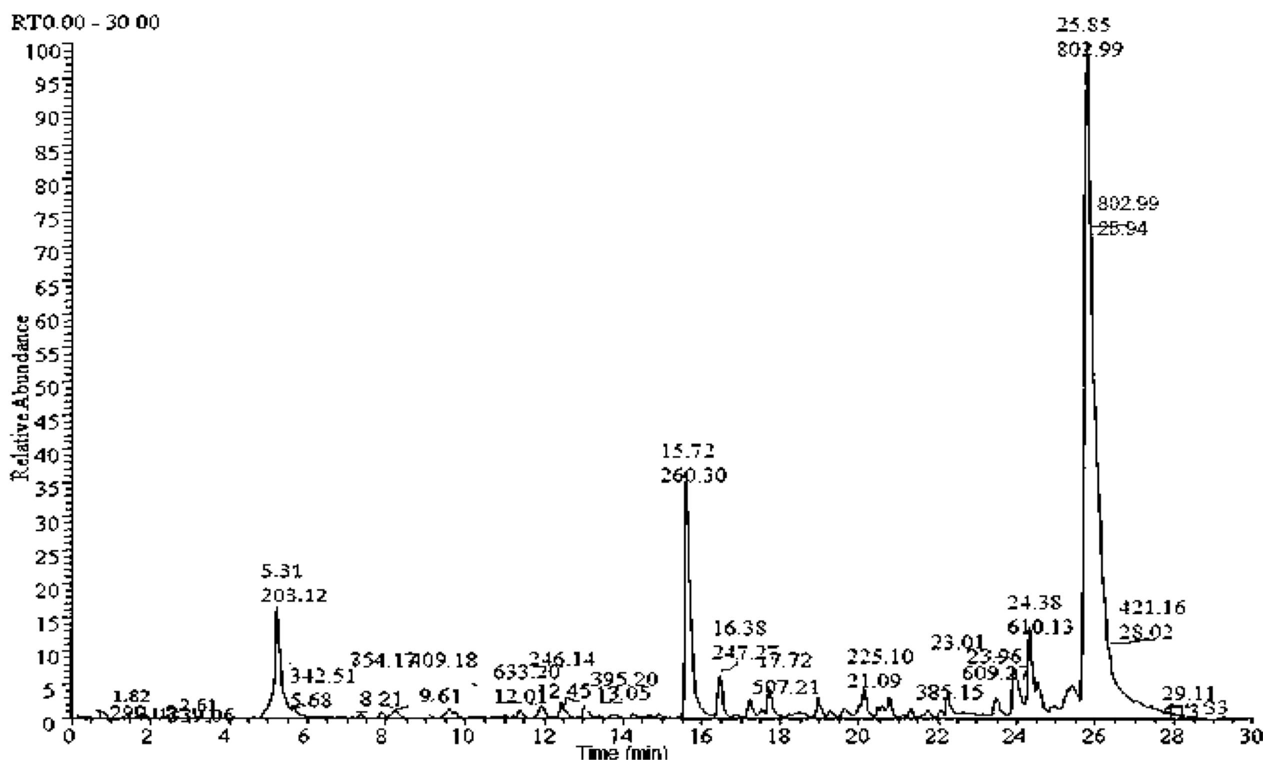


Fig. 1 shows LC–MS chromatogram for aqueous leaf extracts of *Ximenia americana*. The peaks represent phytocompounds with different retention times. The identities of phytocompounds were proposed based on their general fragmentation pattern and using reference spectra published by the library–MS databases of the National Institute of Standards and Technology (NIST). Twenty phytocompounds were successfully identified, including flavonoids (epicatechin, gallocatechin, quercitrin, kaempferol-G-O-arabinopyranoside, kaempferol-G-l-rhamnoside, quercetin-G-glucosyl-rhamnosyl, quercetin-3 glucoside, and quercetin-3-0-Beta-D-glucoside), tannins (gallotannin and ellagitannin), phenolic acids (protocatechuic acid, gallic acid, caffeic acid, ellagic acid, and m-coumaric acid), glycosides (quercetin-3, O-alpha-L-rhamnoside, quercetin rhamnoside, and quercetin-G-L rhamnoside), a fatty acid (tariric acid) and a phytosterol (beta-sitosterol)

the two plant extracts were not significantly different at dose levels of 100 and 150 mg/Kg bw ($p > 0.05$; Fig. 4).

Effects of aqueous leaf extracts of *X. americana* and *P. capensis* on creatine kinase-MB (CK-MB) Levels in rats

The current study confirmed that the aqueous leaf extracts of *X. americana* and *P. capensis* significantly ($p < 0.05$) prevented increase in serum CK-MB levels among extracts-treated MI rats as compared with levels revealed by negative control animals (Fig. 5). In comparison, the findings of this study showed that *P. capensis* extract dose level of 50 mg/Kg bw significantly inhibited increase in serum CK-MB levels as compared to the corresponding dose level of *X. americana* extract ($p < 0.05$; Fig. 5). However, the effects of the two plant extracts were not significantly different at dose levels of 100 and 150 mg/Kg bw ($p > 0.05$; Fig. 5).

Effects of aqueous leaf extracts of *X. americana* and *P. capensis* on serum lactate dehydrogenase-1 levels (LDH-1) in rats

The present study showed that the aqueous leaf extracts of *P. capensis* and *X. americana* significantly prevented increase in serum LDH-1 in extracts-treated MI rats as compared to the negative control rats ($p < 0.05$; Fig. 6). In comparison, the the three extract dose levels of *P. capensis* were found to be significantly more effective as compared to the corresponding extract dose levels of *X. americana* ($p < 0.05$; Fig. 6).

Effects of aqueous leaf extracts of *X. americana* and *P. capensis* on lipid profiles in rats

The present study showed that the aqueous leaf extracts of *X. americana* and *P. capensis* significantly prevented ($p < 0.05$) raise in triglycerides, total cholesterol and LDL concentrations among extracts-treated MI rats as compared with the levels in the negative control rats (Figs. 7a–c). Moreover, this study showed that the two plant extracts significantly improved ($p < 0.05$) levels of

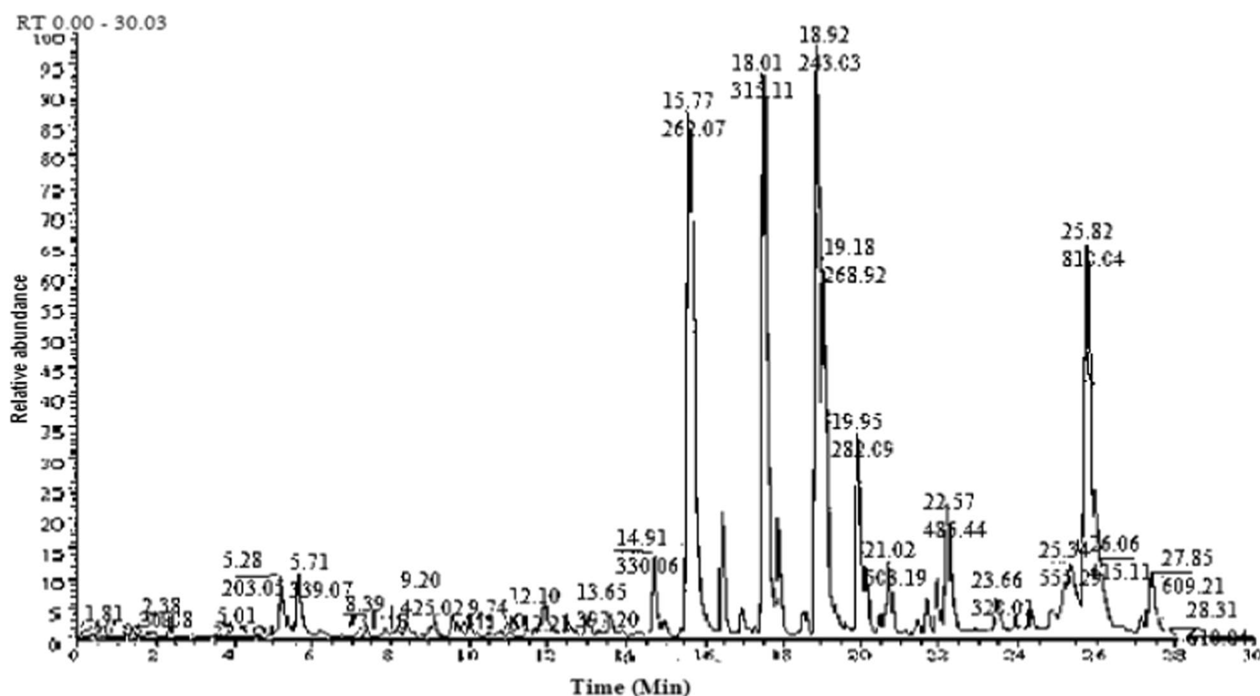


Fig. 2 shows LC–MS chromatogram for aqueous leaf extracts of *Pappea capensis*. The peaks represent phytocompounds with different retention times. The identities phytocompounds were proposed based on their general fragmentation pattern and using reference spectra published by the library–MS databases of the National Institute of Standards and Technology (NIST). Twenty-four phytocompounds were successfully identified, including flavonoids (hesperidin, quercitrin, epicatechin, myricetin, quercetin-3 glucoside, rhamnetin, kaempferol-3-0-arabinopyranoside, quer cetin-3-O-beta-arabinopyranosyl, quercetin-3-O-beta-D-L-glucosyl, quercetin-3-O-beta-D-L-glucoside and quercetin-3-O-beta-D-G-glucoside), glycosides (quercetin rhamnoside, kaempferol hexoside and quercetin-3-0-alpha-L-rhamnoside), and phenolic acids (protocatechuic acid, gallic acid, caffeic acid, p-coumaric acid, ferulic acid, 3,4,5,trimethoxycinnamic acid, ellagic acid, chlorogenic acid, gentisic acid and isoferulic acid)

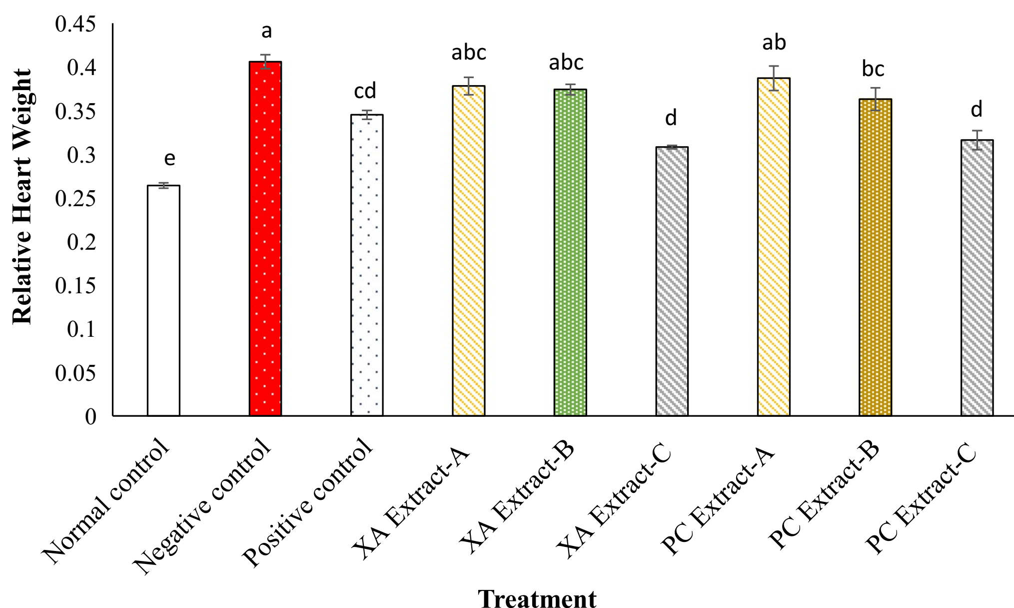


Fig. 3 shows a comparison of the effects of different treatments on the relative heart weight of rats. Each bar represents the mean of five rats used in each treatment (n = 5). The bars with the same alphabet are not significantly different ($p \leq 0.05$). XA = *Ximenia americana*, PC = *Pappea capensis*, Extract-A = 50 mg/Kg bw, Extract-B = 100 mg/Kg bw, Extract-C = 150 mg/Kg bw

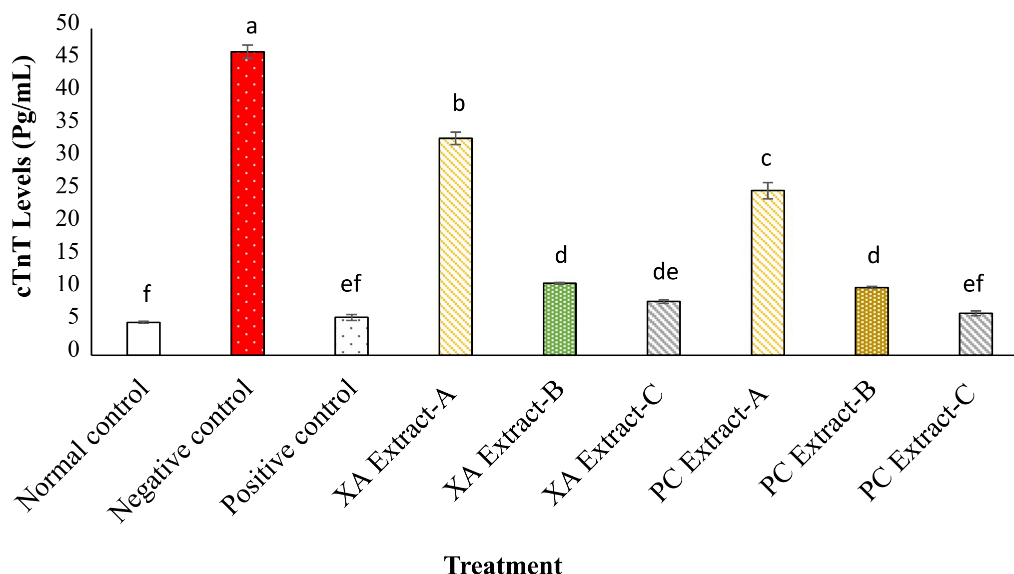


Fig. 4 shows a comparison of the effects of different treatments on serum cardiac troponin T (cTnT) levels in rats. Each bar represents the mean of five rats used in each treatment (n = 5). The bars with the same alphabet are not significantly different ($p \leq 0.05$). XA = *Ximenia americana*, PC = *Pappea capensis*, Extract-A = 50 mg/Kg bw, Extract-B = 100 mg/Kg bw, Extract-C = 150 mg/Kg bw

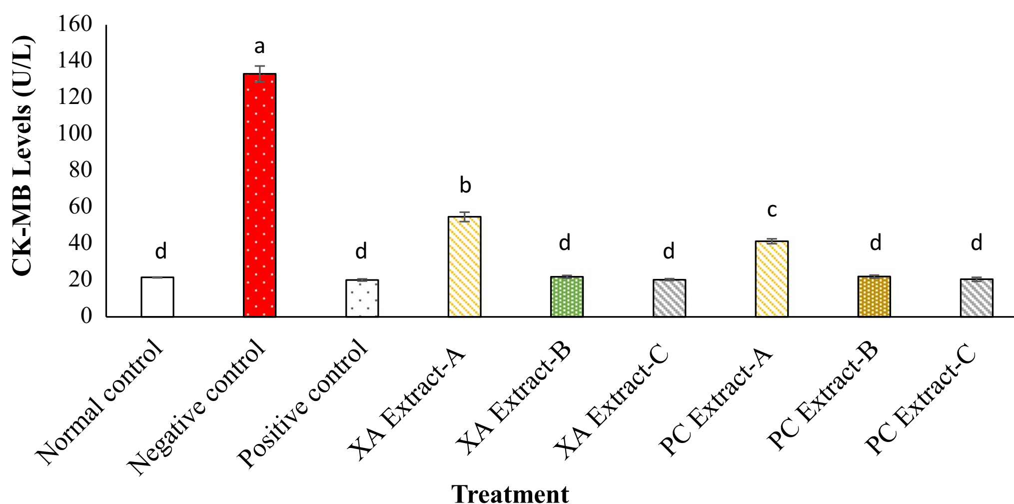


Fig. 5 shows a comparison of the effects of different treatments on serum creatine kinase MB (CK-MB) levels in rats. Each bar represents the mean of five rats used in each treatment (n = 5). The bars with the same alphabet are not significantly different ($p \leq 0.05$). XA = *Ximenia americana*, PC = *Pappea capensis*, Extract-A = 50 mg/Kg bw, Extract-B = 100 mg/Kg bw, Extract-C = 150 mg/Kg bw

HDL in extracts-treated rats as compared to rats in the negative control group ($p < 0.05$; Fig. 7d). In general, the two extracts exerted dose-dependent effects (Figs. 7a–d).

In comparison, the current study revealed that, at 50 and 100 mg/Kg bw, the two plant extracts exhibited comparable effects on T-cholesterol ($p > 0.05$; Fig. 7a). However, *P. capensis* extract showed significantly greater effects on T-cholesterol levels, at dose of 150 mg/Kg bw as compared to the corresponding dose level of *X. americana* extract ($p < 0.05$; Fig. 7a). Overall, on triglyceride levels, the current study findings showed that *P. capensis* extract had significantly greater effects as compared to *X. americana* extract ($p < 0.05$; Fig. 7b). On LDL levels, *P. capensis* showed significantly greater activity at extract dose levels of 50 and 100 mg/Kg bw as compared with the corresponding extract dose levels of *X. americana* ($p < 0.05$; Fig. 7c). However, at extract dose level of 100 mg/Kg bw, the effects of the two plant

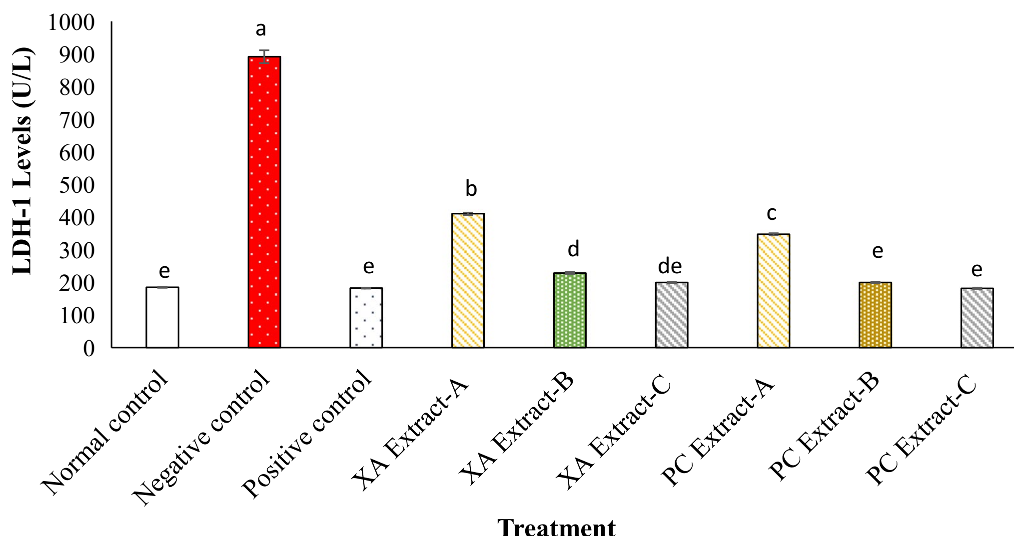


Fig. 6 shows a comparison of the effects of different treatments on serum lactate dehydrogenase-1 (LDH-1) levels in rats. Each bar represents the mean of five rats used in each treatment (n = 5). The bars with the same alphabet are not significantly different ($p \leq 0.05$). XA = *Ximenia americana,* PC = *Pappea capensis,* Extract-A = 50 mg/Kg bw, Extract-B = 100 mg/Kg bw, Extract-C = 150 mg/Kg bw

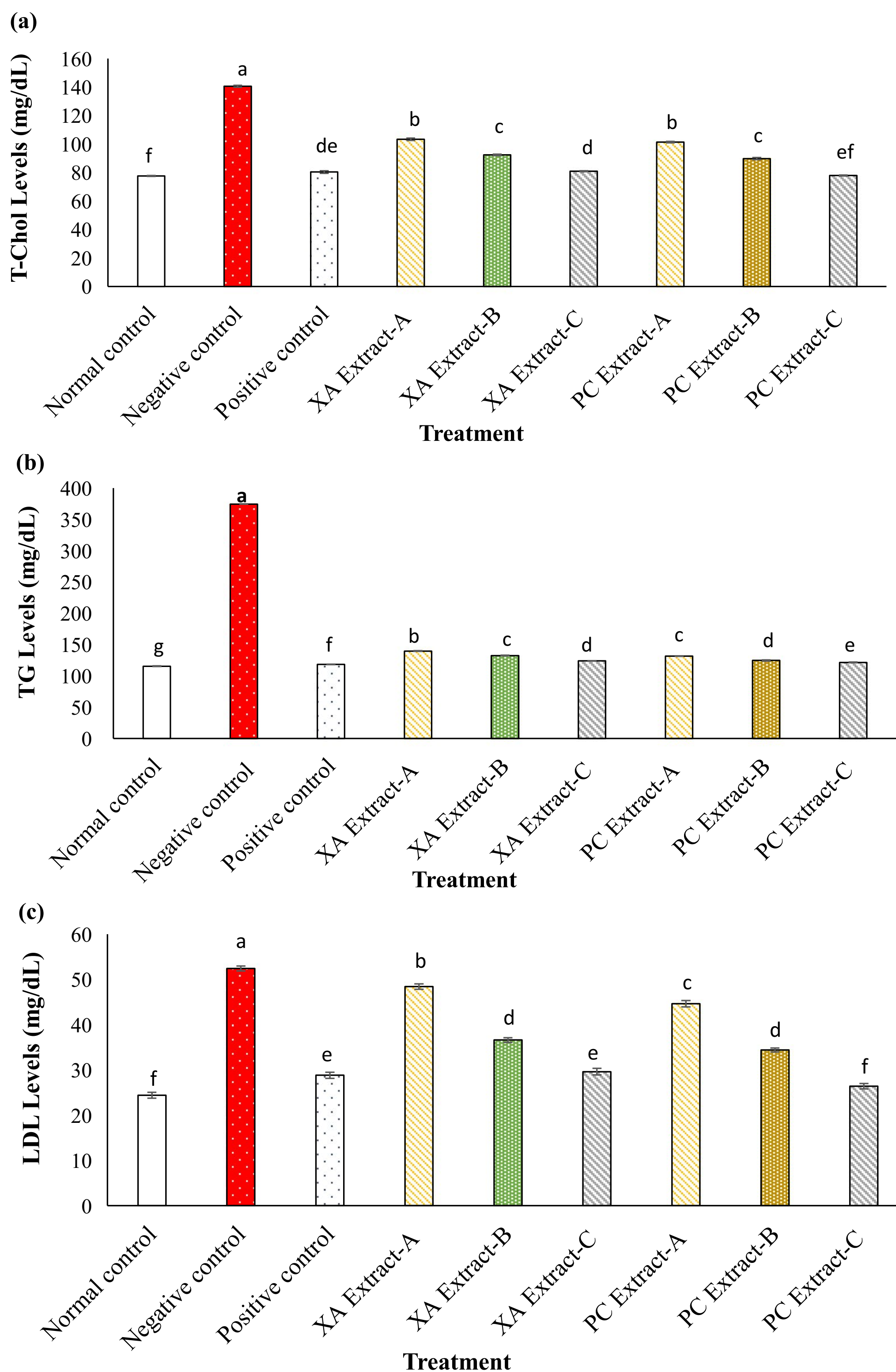
extracts on LDL were comparable ($p > 0.05$; Fig. 7c). On HDL levels, the two extracts caused comparable ($p > 0.05$) effects at the corresponding dose levels of 50 and 100 mg/Kg bw (Fig. 7d). The *P. capensis* extract showed significantly greater effects on HDL at dose of 150 mg/Kg bw as compared to the corresponding dose level of *X. americana* extract ($p < 0.05$; Fig. 7d).

Effects of aqueous leaf extracts of *X. americana* and *P. capensis* on levels of cardiac oxidative stress markers in rats

The current study showed that the *X. americana* and *P. capensis* leaf aqueous extracts significantly prevented ($p < 0.05$) reduction of SOD, CAT and GPs levels in extract-treated rats as compared with the levels recorded in the negative control rats (Figs. 8a–c). Additionally, the studied extracts significantly prevented ($p < 0.05$) lipid peroxidation in extract-treated rats as compared with levels noted in the negative control rats (Fig. 8d). Moreover, it was was found out that the effects of the extracts were dose-dependent (Figs. 8a–d). In comparison, *P. capensis* extract at 100 mg/Kg bw caused significantly greater rise in SOD levels as compared to the corresponding *X. americana* extract dose level ($p < 0.05$; Fig. 8a). Nevertheless, effects of the two extracts were not significantly different ($p > 0.05$) at 50 and 150 mg/Kg bw (Fig. 8a). On the other hand, the current study results indicated that *P. capensis* extract at 50 mg/Kg bw more effectively improved CAT levels as compared to the corresponding *X. americana* extract dose level ($p < 0.05$; Fig. 8b). Notably, activity of the two extracts were not significantly different at dose levels of 100 and 150 mg/Kg bw ($p > 0.05$; Fig. 8b). Moreover, this study established that *P. capensis* extract at 50 and 100 mg/Kg bw caused significantly greater effects on GPx as compared to equivalent *X. americana* extract dose ($p < 0.05$; Fig. 8c). However, effects of the two extracts were not significantly different at 150 mg/Kg bw ($p > 0.05$; Fig. 8c). Further, *P. capensis* extract dose level of 50 mg/Kg bw was significantly more

(See figure on next page.)

Fig. 7 **a** shows a comparison of the effects of different treatments on serum total cholesterol (T-Chol) levels in rats. Each bar represents the mean of five rats used in each treatment (n = 5). The bars with the same alphabet are not significantly different ($p \leq 0.05$). XA = *Ximenia americana,* PC = *Pappea capensis,* Extract-A = 50 mg/Kg bw, Extract-B = 100 mg/Kg bw, Extract-C = 150 mg/Kg bw. **b** shows a comparison of the effects of different treatments on serum triglyceride (TG) levels in rats. Each bar represents the mean of five rats used in each treatment (n = 5). The bars with the same alphabet are not significantly different ($p \leq 0.05$). XA = *Ximenia americana,* PC = *Pappea capensis,* Extract-A = 50 mg/Kg bw, Extract-B = 100 mg/Kg bw, Extract-C = 150 mg/Kg bw. **c** shows a comparison of the effects of different treatments on serum low-density lipoprotein (LDL) levels in rats. Each bar represents the mean of five rats used in each treatment (n = 5). The bars with the same alphabet are not significantly different ($p \leq 0.05$). XA = *Ximenia americana,* PC = *Pappea capensis,* Extract-A = 50 mg/Kg bw, Extract-B = 100 mg/Kg bw, Extract-C = 150 mg/Kg bw. **d** shows a comparison of the effects of different treatments on serum high-density lipoprotein (HDL) levels in rats. Each bar represents the mean of five rats used in each treatment (n = 5). The bars with the same alphabet are not significantly different ($p \leq 0.05$). XA = *Ximenia americana,* PC = *Pappea capensis,* Extract-A = 50 mg/Kg bw, Extract-B = 100 mg/Kg bw, Extract-C = 150 mg/Kg bw



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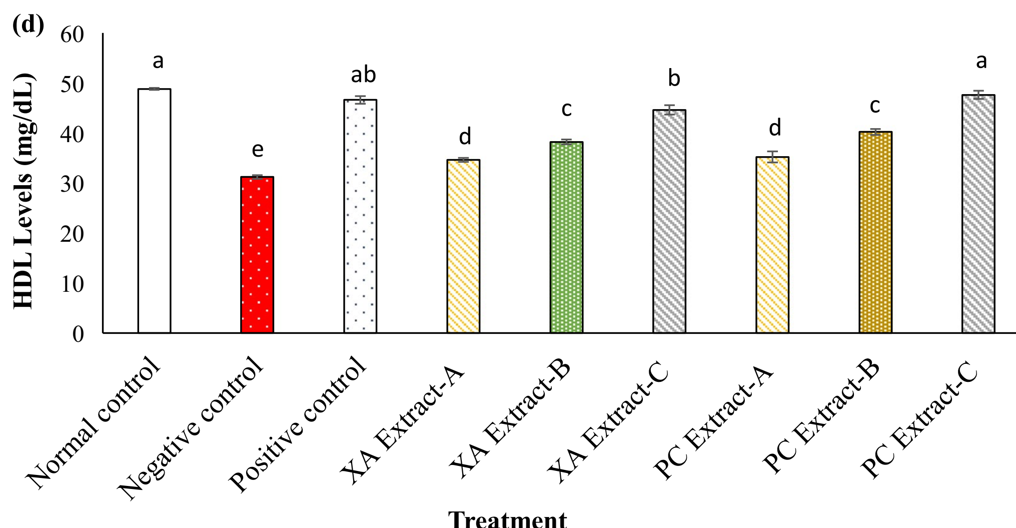

Fig. 7 continued

effective in prevention of lipid peroxidation as compared to the corresponding *X. americana* extract dose level ($p < 0.05$; Fig. 8d). However, effects of the two extracts were comparable at 100 and 150 mg/Kg bw dose levels ($p > 0.05$; Fig. 8d).

Discussion

In the present study, cardiopreventive activities of aqueous leaf extracts of *X. americana* and *P. capensis* in MI rats were determined. Salbutamol was used to induce myocardial injury in rats. It was noted that salbutamol caused increase in relative heart weight and cTnT, CK-MB, LDH-1, T-cholesterol, triglycerides, LDL-c and MDA levels, as well as decreased SOD, CAT, GPx and HDL-c levels in salbutamol-treated rats.

Salbutamol is a beta adrenergic receptor agonist with positive inotropic and chronotropic effects on myocardium, leading to increased heart rate and force of myocardial contraction [48]. In addition, salbutamol induces oxidative stress that subsequently causes cardiac injury. The oxidative stress is caused by accumulation of free radicals, which accumulate because the rate of production is higher than the rate of scavenging [49]; [50]. Free radicals also cause necrosis of heart muscle cells by lipid peroxidation [51]. Moreover, the inflammatory response triggered by free radicals, further damage the heart muscle tissue, leading to loss of cardiac function, increased workload, heart remodelling, as well as cardiac hypertrophy [52].

In the present study, relative heart weight was determined as first aspect of measuring myocardial damage. The increased relative heart weight among salbutmol-treated rats could have been caused by increased vascular hemorrhage, edema, and necrosis of cardiac tissue, followed by inflammatory cell invasion on damaged tissues, that lead to hypertrophy [53]. The heart muscle mass increased as the heart remodelled so as to compensate for the lost function [54]. These findings are in agreement with a previous study that evaluated effects of *Atractylodes macrocephala* on heart weight index of rats induced with chronic heart failure [55]. Generally, the studied extracts showed dose-dependent effects on

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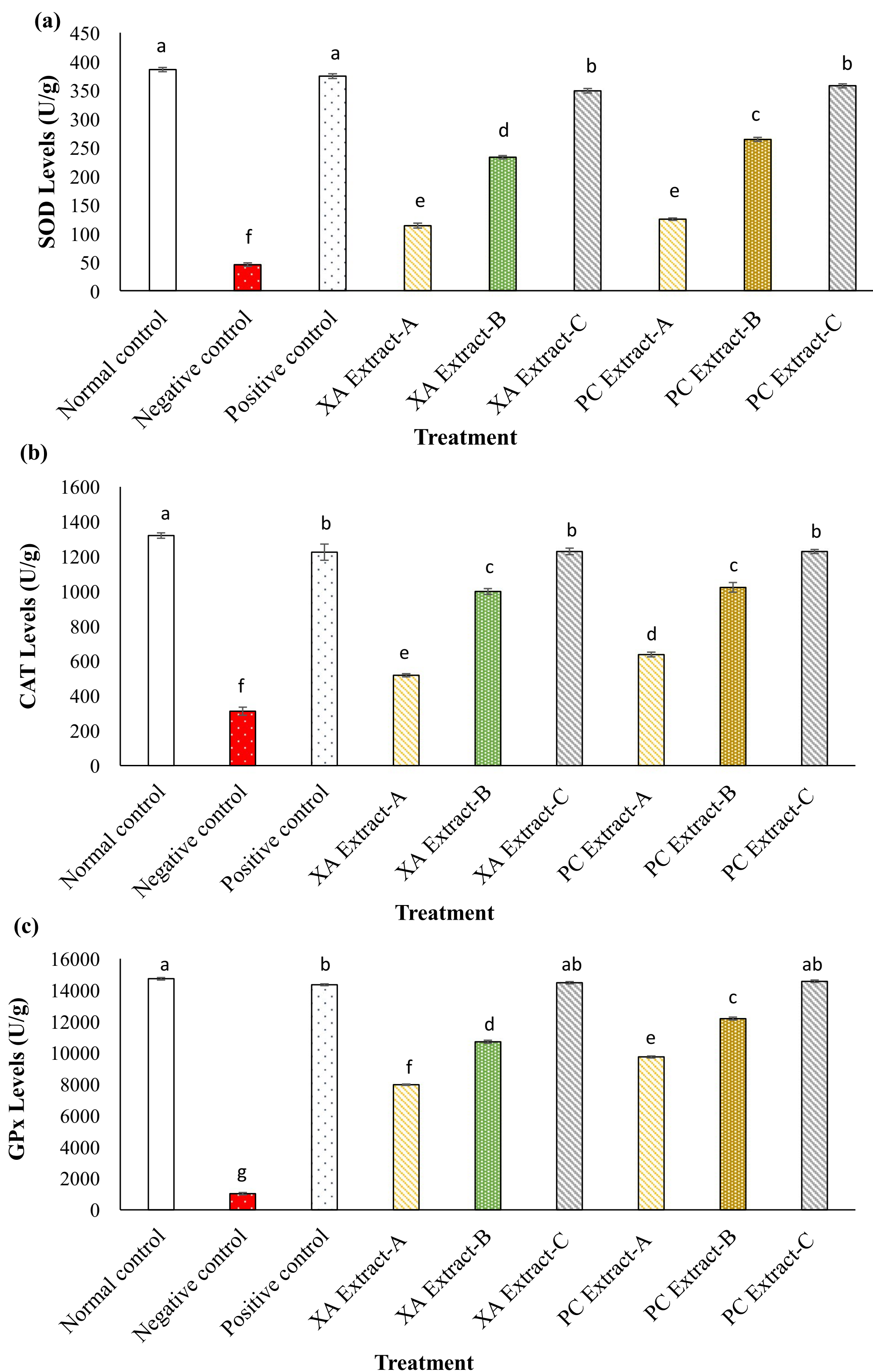
Fig. 8 **a** shows a comparison of the effects of different treatments on superoxide dismutase (SOD) levels in rats. Each bar represents the mean of five rats used in each treatment (n = 5). The bars with the same alphabet are not significantly different ($p \leq 0.05$). XA = *Ximenia americana,* PC = *Pappea capensis,* Extract-A = 50 mg/Kg bw, Extract-B = 100 mg/Kg bw, Extract-C = 150 mg/Kg bw. **b** shows a comparison of the effects of different treatments on catalase (CAT) levels in rats. Each bar represents the mean of five rats used in each treatment (n = 5). The bars with the same alphabet are not significantly different ($p \leq 0.05$). XA = *Ximenia americana,* PC = *Pappea capensis,* Extract-A = 50 mg/Kg bw, Extract-B = 100 mg/Kg bw, Extract-C = 150 mg/Kg bw. **c** shows a comparison of the effects of different treatments on glutathione peroxidase (GPx) levels in rats. Each bar represents the mean of five rats used in each treatment (n = 5). The bars with the same alphabet are not significantly different ($p \leq 0.05$). XA = *Ximenia americana,* PC = *Pappea capensis,* Extract-A = 50 mg/Kg bw, Extract-B = 100 mg/Kg bw, Extract-C = 150 mg/Kg bw. **d** shows a comparison of the effects of different treatments on malondialdehyde (MDA) levels in rats. Each bar represents the mean of five rats used in each treatment (n = 5). The bars with the same alphabet are not significantly different ($p \leq 0.05$). XA = *Ximenia americana,* PC = *Pappea capensis,* Extract-A = 50 mg/Kg bw, Extract-B = 100 mg/Kg bw, Extract-C = 150 mg/Kg bw



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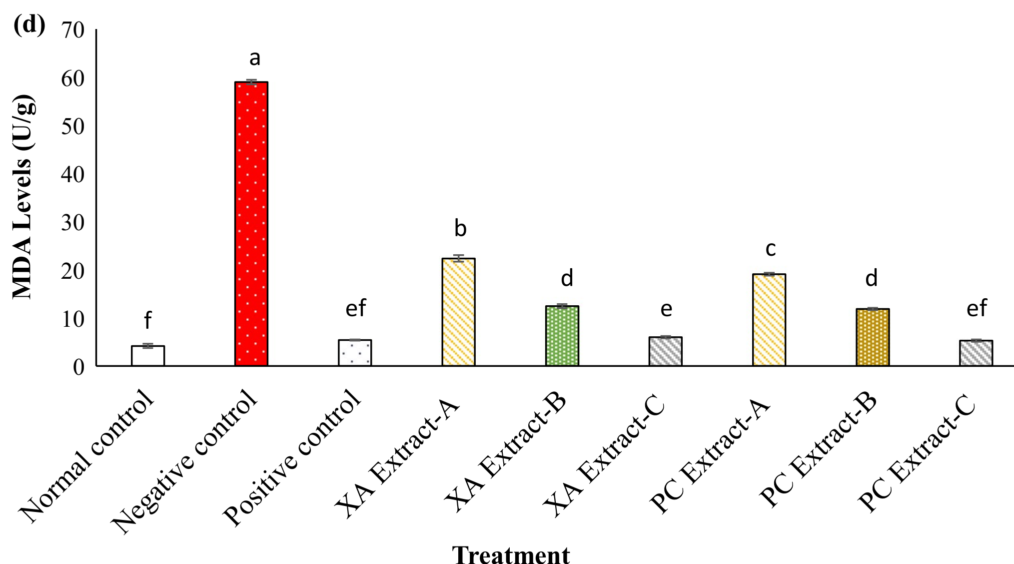

Fig. 8 continued

relative heart weight. The dose-dependence behaviour is similar to the findings reported by Li et al., [56] who determined the cardioprotective effects of garcinol in isoproterenol-induced MI and heart failure rats. The effects of the extracts on relative heart weight could be due to presence of various phytocompounds that prevented cell damage by oxidative stress, including catechins that are known to prevent oxidative stress by direct scavenging of reactive oxygen species, as well as by chelation of metal ions and promotion of antioxidants and phase II detoxification enzymes synthesis [57]. In addition, the extacts contained kaempferol derivatives that confer excellent anti-inflammatory activities by inhibiting gene expression for COX-2 and lipo-oxygenase enzymes [58]. Moreover, protocatechuic acid, also present in the extracts, has been associated with anti-apoptotic and pro-survival activities of heart muscle tissue [59].

Moreover, cTnT, CK-MB and LDH-1 levels were determined as a second aspect of evaluating cardiac injury. These are intracelllular proteins predominantly found in cardiomyocytes, and therefore, used as cardiac function biomarkers [60]. High serum levels of these biomarkers is an indication of cardiac cell necrosis. In recent years, various guidelines have reccommended use of cTnT, CK-MB and LDH-1 in clinical diagnosis of MI [22]. In the current study, the efficacy of the extracts in MI was confirmed by reduced cTnT, CK-MB and LDH-1 levels among extracts-treated rats as compared to the negative control rats. These findings are in agreement with a previous study on the effects of aqueous leaf extract of *Moringa oleifera* in bromate-induced MI Wistar albino rats [61].

The effects of the extracts could be attributable to the presence of cardioactive phytochemicals, including phenolic acids, flavonoids, cardiac glycosides and tannins. Phenolic acids such as protocatechuic acid, gallic acid, caffeic acid, ellagic acid and coumaric acid are known to confer various benefits in the treatment of cardiovascular diseases [62]. The efficacy of phenolic compounds in cardiovascular pathologies is due to their ability to prevent oxidative stress by chelation, reduction and scavenging on free radicals [63]. In addition, the phenolic acids mount anti-inflammatory activities by way of cell membrane stabilization, inhibition of proteinase and prevention of albumin denaturation [64]. Moreover, ellagic acid, a phenolic acid, is a potent anti-inflammatory agent that inhibits the production of nitric oxide, IL-1β, TNF-α, COX-2, NF-κB and MDA, as well as upregulating gene expression for glutathione and IL-10 [65]. These findings are also in agreement with those of a study by Nayagam et al., [66] on the effects of *Caesalpinia bonducella* (Linn.) extracts on doxorubicin-induced myocardial infarction in albino rats.

Moreover, other phytocompounds present in the extracts, including kaempferol, quercetin and catechin derivatives, are likely to have further contributed to the cardiopreventive effects of the extracts. These phytocompounds possess excellent antioxidant and anti-inflammatory properties, making them useful in treatment of MI [67–69]. Catechins modulate apoptosis by regulating genes expression for anti- and pro-apoptosis [70]. In addition, catechins confer anti-inflammatory effects by regulating different isoforms of nitric oxide synthase [70]. On the other hand, quercetins reduce oxidative stress

by inhibition of xanthine oxidase and NADPH oxidase, blockage of Fenton reaction and by direct scavenging of reactive oxygen species. Moreover, quercetins cause vasodilatory effects by inhibition of endothelin-1 receptors, increasing NO stimulation and induction of the large-conductance calcium-activated potassium channels [71]. Quercetin is a powerful anti-inflammatory compound that inhibits activities of TNF-α in extracellular signal-related kinase, c-Jun NH2-terminal kinase and c-Jun and nuclear factor-κB [72]. Additionally, tannins, such as gallotannin and ellagitannin, prevent MI by reducing accumulation of free radicals and inflammatory mediators [73].

It is also believed that reduced serum cTnT, CK-MB and LDH-1 levels among extracts-treated rats was linked to inhibition of lipid peroxidation by the extracts. This prevented necrosis of the cardiomyocytes thereby preventing leakage of the three biomarkers into the blood. Protocatechuic acid, a phytocompound present in the studied extracts, is known to prevent lipid peroxidation by scavenging hydrogen peroxide, superoxide and by promoting glutathione peroxidase synthesis [74]. These findings are comparable to those of a study by Balea et al. [75], that evaluated cardioprotective, antioxidant and polyphenolic profile of Fetească Neagră Cultivar pomace extracts.

In addition, the current study determined lipid profile as a third aspect of determining effects of the studied extracts on cardiac injury. High serum total cholesterol, triglycerides and LDL levels show increased risk of MI, whereas high HDL levels show decreased MI risk [76]. Salbutamol causes catecholamine-like oxidative stress that leads to increased serum total cholesterol, triglycerides and LDL levels in rats. Increased serum total cholesterol, triglycerides and LDL levels are as a result of direct activation of lipolysis in the adipocytes, as well as hydrolysis of phospholipid membranes by oxidative stress [77]. In the body, serum LDL transport cholesterol to arteries, where it is deposited on walls of the vessels, thereby forming atherosclerotic plaques [78]. These plaques break-off to form clots, which may dislodge to form embolus that occludes blood vessels [79]. When this happens in coronary arteries, there is impaired blood supply to the heart muscle, leading to MI [80]. On the other hand, HDL transports cholesterol to the liver, where it is broken down and excreted from the body hence preventing development of atherosclerotic plaques [81]. This prevents MI from occuring. Therefore, the effects of the studied extracts could have been due to their role in the regulation of lipid metabolism.

In addition, the anti-lipidaemic effects of the extracts could be associated with the antioxidative potential of constituent phytochemicals, that are known to stabilize cell membranes by neutralizing free radicals [82]. These phytochemicals include phenolic acids, phytosterols, flavonoids and tannins [83]. In addition, gallic acid, a phenolic acid present in both extracts, has cytoprotective and chelating effects [84]. Moreover, caffeic acid confers antioxidative effects by inhibition of Fenton-induced oxidative damage, iron metal chelation and direct free radical scavenging [85]. Additionally, the anti-lipidaemic effects of the extracts could have been due to anti-inflammatory activities of phytocompounds, such as coumaric acid that suppresses infiltration of inflammatory cells and reduces expression of inflammatory mediators, including TNF-α and IL-6 [86].

Generally, *P. capensis* extract exhibited greater anti-lipidaemic effects as compared to *X. americana* extract. This could be due the presence of higher number of phytochemicals in *P. capensis* extract, including gentisic acid, chlorogenic acid and quercetin derivatives. Gentisic acid is an aspirin metabolite that inhibits LDL oxidation and formation of lipid hydroperoxides [87]. On the other hand, chlorogenic acic confers hypocholesterolemic effects by activation of hepatic lipase via upregulation of peroxisome proliferation-activated receptor α mRNA [88]. Moreover, quercetin and its derivatives regulate lipid metabolism by downregulating expression of genes for sterol regulatory-element binding proteins (SREBPs) and low-density lipoprotein receptor (LDLR) protein [89]. These findings are in agreement with Rani et al., [90] who compared phytochemical profile, antibacterial and antioxidant effects of *Calotropis procera* and *Calotropis gigantea*.

Moreover, in the current study, levels of cardiac antioxidant enzymes (SOD, CAT and GPx) and MDA were measured as a fourth aspect of determining effects of *X. americana* and *P. capensis* extracts against MI [91]. These antioxidant enzymes alleviate oxidative stress by stabilizing free radicals through reduction of radical energies or by donation of electrons [92]. On the other hand, MDA, a product of lipid peroxidation, is formed as a result of free radical-induced degradation of polyunsaturated fatty acids [93]. MDA is a useful marker of oxidative stress [93]. Its formation is inhibited by SOD that catalyzes the dismutation of highly reactive superoxide anion to less reactive hydrogen peroxide and a stable oxygen molecule [94]. Hydrogen peroxide is further degraded by the action of catalase enzyme, whereas hydrogen and lipid peroxides are neutralized by GPx [95].

In the present study, the extracts maintained high levels of SOD, CAT and GPx, and low levels of MDA. The effects of the axtracts could be attributed to presence of different phytochemicals, including tannins, protocatechuic acid, quercitin and kaempferol derivatives. Tannins, such as gallotannin, possess many hydroxyl

molecules and other functional groups that scavenge free radicals [96]. Moreover, protocatechuic acid causes upregulation of genes expression for CAT, SOD, and GPx enzymes [97]. Additionally, quercitin and kaempferol derivatives have been associated with increased synthesis of SOD and GPx enzymes, as well as decreased MDA levels [98]. On the other hand, coumarin and sitosterol indirectly alleviate oxidative stress by activating nuclear factor erythroid 2-related factor 2 (Nrf2), which in turn upregulate synthesis of various antioxidant enzymes, including CAT, SOD and GPx. [99].

At doses levels of 50 and 100 mg/Kg bw, *P. capensis* extract generally showed greater antioxidant activities as compared to *X. americana* extract. This could be due to the presence of epicatechins and more quercetin derivatives*.* Various quercetin derivatives have demostrated significant effects in upregulation of genes expression for CAT and SOD synthesis [100]. In addition, epicatechins are associated with potent activities in upregulating gene expression for anti-inflammatory mediators and antioxidants synthesis [101].

In general, it is believed that the cardioprotective effects of the studied extracts were caused by the presence of various phytocompounds. The phytocompounds present are believed to possess ability to scavenge free radicals, upregulate gene expression for various antioxidant enzymes, inhibit inflammatory mediators, chelate metals, block beta adrenoceptors, among others. This stabilized cell membranes of cardiomyocytes in extracts-treated rats thereby reducing leakage of cell's components into the blood, including cTnT, CK-MB, LDH-1 and lipids.

Conclusions

Based on the findings of the current study, it can be concluded the aqueous leaf extracts of *X. americana* and *P. capensis* contain various cardioactive phytochemicals. Moreover, the extracts have potent in vivo cardiopreventive activities in salbutamol-induced MI rats. However, further studies are necessary to isolate and bioassay for activities of individual phytochemical present in the extracts.

Abbreviations

CAT Catalase
CCl_4 Carbon tetrachloride
CK-MB Creatine Kinase-MB
COX Cyclooxygenase
cTnT Cardiac troponin T
CVDs Cardiovascular diseases
GPx Glutathione peroxidase
H_2O_2 Hydrogen peroxide
HDL High-density lipoprotein
IL Interleukin
LC–MS Liquid chromatography–mass spectrometry
LDH Lactate dehydrogenase
LDL Low-density lipoprotein
MDA Malondialdehyde
MI Myocardial infarction
NACOSTI National Commission for Science, Technology and Innovation
NADPH Nicotinamide adenine dinucleotide phosphate
NF-κB Nuclear factor kappa B
TNF Tumor necrosis factor

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Author contributions

DMG, PM, and MPN wrote and revised the manuscript. DMG performed the statistical analysis. PM and MPN developed the study design. DMG, PM, and MPN contributed equally to data collection. All authors read and approved the final manuscript.

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Availability of data and materials

The corresponding author can provide the data that were utilized to support the study's conclusions upon request.

Declarations

Ethics approval and consent to participate

The present study was approved by the Institutional Animal Ethics Committee of Kenyatta University (PKUA/006/006).

Consent for publication

All authors have consented to the publication of this article.

Competing interests

The authors declare that there are no competing interests.

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