Research Article

PHYTOCONSTITUENTS ANALYSIS BY GC-MS, CARDIOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF *BUCHANANIA AXILLARIS* AGAINST DOXORUBICIN-INDUCED CARDIO TOXICITY IN ALBINO RATS

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ABSTRACT:

Buchanania axillaries(Anacardiaceae) is a traditional herbal medicine , the leaves are used to treat many diseases including cardiotoxicity. The present study was designed to scientifically evaluate the cardioprotective potential of the ethanol extract of *Buchanania axillaries*(BA), on Doxorubicin (DOX) induced *cardiotoxicity,* in albino rats. DOX is one of the most effective chemotherapeutic drugs in cancer; however, its incidence of cardiotoxicity compromises its therapeutic index. DOX-induced heart failure is thought to be caused by reduction/oxidation cycling of DOX to generate oxidative stress and cardiomyocyte cell death. A Doxorubicin dose of 20 mg/kg was selected for the present study as this dose offered significant alteration in biochemical parameters and moderate necrosis in heart. Effect of BA oral treatment for 14 days at two doses (250 mg and 500 mg/kg body weight) was evaluated against DOX-induced *cardiotoxicity*. Significant *cardiotoxicity*, depletion of endogenous antioxidants and biochemical parameters were observed in DOX-treated animals when compared with the normal animals. The Pretreatment of DOX- induced rats with BA significantly prevented the altered biochemical variation such as marker enzymes (SGPT, SGOT, CPK, ALP and LDH), lipid profile (LDL, VLDL, TGs, HDL and Total cholesterol), and antioxidant parameters (SOD, GSH, CAT, GPx, MDA, and GR) to near normal status. Serum urea, and uric acid which increased on DOX administration, registered near normal values on pretreatment with BA. Histology of Dox-induced heart of rats pretreated with BA showed a significant recovery from cell damage. The present findings have demonstrated that the cardioprotective effects of BA in DOX-induced oxidative damage may be due to an augmentation of the endogenous antioxidants and inhibition of lipid peroxidation of cell membrane.

KEY WORDS: cardioprotective, antioxidant, *Buchanania axillaris,* Doxorubicin, myocardial infarction.

INTRODUCTION:

Myocardial infarction (MI) is an acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand (1). This is most commonly due to occlusion of a coronary artery following the rupture of a vulnerable atherosclerotic plaque, which is an unstable collection of lipids and white blood cells in the wall of an artery. An increased risk of MI is associated with high levels of serum total cholesterol (2) low density lipoprotein (LDL) (3) and decreased levels of high density lipoprotein (HDL) (4). Oxidative stress produced by free radicals or reactive oxygen species (ROS), as evidenced by marked increase in production of lipid peroxidative products and transient inhibition of endogenous antioxidant defense such as superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) has been shown to underlie myocardial damage during MI (5,6,7). Minimizing myocardial necrosis

and improving heart function have been proved to be effective strategies to reduce the morbidity and mortality from myocardial infarction (8). Accordingly, antioxidants may decrease cellular injury and apoptosis through a radical-scavenging mechanism (9). Therapeutic intervention via suppression of free radical generation and/or augment action of endogenous antioxidant enzymes may attenuate myocardial dysfunction. An anthracycline anticancer drug, doxorubicin (DOX) is effective against malignancies such as leukemias, lymphomas and several solid tumors. However, dose-dependent cardiotoxic effects limit its practical therapeutic use. Thus DOX is reported to increase oxygen free radical activity (10) as well as induces the peroxidation of unsaturated lipids within the membranes (11). Although modern drugs are effective in preventing cardiovascular disorders, their use is often limited because of their side effects (12). Herbal drugs are prescribed widely, even when their biologically active compounds are unknown, because of their effectiveness, lesser side effects and relatively low cost (13). Now-a- days the usage of herbal drugs is gaining wider acceptance by the medical professional due to their positive contribution and influence on health and qualify of life. Therefore the search for indigenous cardioprotective herbal drugs is still continuing as part of scientific research. *Buchanania axillaries (Anacardiaceae*) is a traditional medicinal plant distributed in India and other Asian countries. *Its* leaf extract has been reported to possess anti-inflammatory and cardioprotective activity.(14). It is also reported to possess, the aerial parts is used to cure itch of the skin and to remove blemishes from the face. The kernels are used in Indian medicine as a brain tonic. The gum is antidiarhoeal used internally rheumatism (15). In addition the ethanolic extract of the aerial part showed CNS depressant activity in mice. Further the leaves are reported to be cooling digestive expectorant purgative depurative and aphrodisiac and are useful in hyperdipsia, burning sensation cough braonchitis, dyspepsia, leprosy and constipation. (16). Extensive phytochemical investigations carried out on BA revealed the presence of many chemical constituents including palmitic and linoleic acid such as n-hexadecanoic acid , 9,12-octadecadienoic acid (Z,Z)-, and oleic acid, which are considered significant for its Hypocholesterolemic property (17,18,19) However, no data is available on the cardioprotective and antioxidant properties of *Buchanania axillaries*(BA). Therefore, this study was designed to investigate the protective effects of the ethanol extract of *Buchanania axillaris* against DOX-induced cardiotoxicity in rats.

MATERIALS AND METHODS

Plant material

Leaves of *Buchanania axillaries* were collected identified and authenticated by a Botanist, Dr.C.Madhavachetty, Tirupathi university, Tirupathi, India. Voucher specimen (CAHC- 10/2010) was retained in the C.Abdul hakeem College, Melvisharam, Tamil Nadu, India.

Extraction

Leaves were cleaned with water and dried in the *Buchanania axillaries* until a constant weight was obtained. Then it was powdered using a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with ethanol (90% v/v) in Soxhlet apparatus at 60°C (20). The solvent was completely removed by rotary vacuum evaporator. The extract was freeze-dried and stored in a vacuum desiccator.

GC–MS analyses of ethanol extract of *Buchanania axillaries* for the identification of chemical composition

The identification of chemical composition of ethanol extract of BA was performed using a GC–MS spectrograph (Agilent 6890/Hewlett–Packard 5975) fitted with electron impact (EI) mode. The ethanol extract (2.0 mL) of BA was injected with a Hamilton syringe to the GC–MS manually for total ion chromatographic analysis in split mode. In quantitative analysis, selected ion monitoring (SIM) mode was employed during the GC MS analysis. SIM plot of the ion current resulting from very small mass range with only compounds of the selected mass were detected and plotted.

Experimental animals

Studies were carried out using Wistar albino rats (150–200 g), obtained from Institute of Veterinary Preventive Medicine (IVPM), Ranipet, Tamil Nadu, India. The animals were housed in polyacrylic cages (38 cm, 23 cm, 10 cm) and maintained under standard laboratory conditions (temperature 25-20°C) with dark/light cycle (12/12 h). The animals were fed with standard pellet diet (supplied by poultry research station, Nandhanam, India) and fresh water *ad libitum*. All the animals were acclimatized to lab conditions for a week before commencement of the experiment. All the procedures described were reviewed and approved by the University Animal Ethics Committee.

Experimental procedure

Animals were randomized and divided into five groups (1–5) of six animals each. Group 1 served as normal and the rats fed orally with normal saline (0.75 ml/animal, 5mL kg^{-1}) body weight daily for 14 days. Group II rats were treated similar to those in group 1. Rats of group 3 were treated with 500mg kg^{-1} body weight of the ethanol extract of BA for 14 days, respectively. Rats of group 4 and 5 are pretreated with 250 mg kg^{-1} and 500mg kg^{-1} of ethanol extract of BA body weight respectively.

Induction of experimental myocardial infarction

Doxorubicin was dissolved in sterile double distilled water and injected subcutaneously to rats (20 kg/kg) in group 2, 4 and 5 respectively after the last dose of the extract to induce *cardiotoxicity*

Isolation of working heart preparation:

The animals were anesthetized with chloroform after 72 h of DOX administration, then the heart was punctured with sterile syringe and blood was stored with EDTA is an anticoagulant agent and later excised. Cardiac muscle from lower third of the ventricle was visualized under light microscope and the remaining heart tissue was snap frozen in liquid nitrogen

Histopathological studies:

The hearts were removed, washed immediately with saline and then fixed in

10% buffered formalin. The hearts stored in 10% buffered formalin were embedded in paraffin, sections cut at 5 mm and stained with hematoxylin and eosin. These sections were then examined under a light microscope for histoarchitectural changes.

Biochemical analysis

Blood samples were collected into tubes pre-coated with EDTA by vein puncture at baseline and post intervention. Serum was separated by centrifuging for 10min 3000×*g* at 4 °C. The serum used for the assay of urea, and uric acid which were was estimated by the methods of [21] respectively. The activities of serum glutamate-pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) were determined spectrophotometrically by the method of [22]. The lactate dehydrogenase (LDH), creatine phosphokinase (CPK) and alkaline phosphatase (ALP) were determined by the methods of [23]. The levels of total cholesterol and triglycerides (TGs) were estimated by the methods of [24,25]. Serum high density lipoprotein (HDL) was determined according to the method of [26]. Serum low density lipoproteins (LDL) and very low density lipoproteins (VLDL) were calculated as VLDL=triglycerides/5 and LDL = total cholesterol − (HDL cholesterol + VLDL cholesterol) respectively.

Antioxidant assay

The heart was dissected, immediately washed in ice-cold saline and a homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged and the supernatant was used for the assay of antioxidant parameters. MDA content was measured according to an earlier method [27]. Superoxide dismutase (SOD) activity was determined according to [28]. CAT activity was determined from the rate of decomposition of H_2O_2 according to [29]. Glutathione peroxidase (GPx) activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN_3 according to [30]. Myocardial GSH content was estimated by the method of [31]. GR activity was determined according to the method described by [32].

Statistical analysis

The obtained results were obtained analyzed for statistical significance using one way ANOVA followed by Dunnet test statistical software for comparison with control group and DOX treated group. $P < 0.05$ was considered as significant.

RESULTS:

Phytochemical analysis

The ethanol extract of BA was a complex mixture of many constituents and compounds which were identified in this plant by GC–MS (Table1). Phyto-constituents such as 1-Amino-2,6-dimethylpiperidine(1.52), 2-Octenoic acid, 4,5,7-trhydroxy(1.9), à-Methyl-D-manno-pyranoside (2.12) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (0.38) Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl- [Synonyms: à-Ionene] (2.94), D-Galactose, 6-deoxy-[Synonyms: D-Fucose] (6.06), 1,6-Anhydro-á-D-glucopyranose (Synonyms: levoglucosan) (5.85), Dodecanoic acid (0.83), Phosphonofluoridic acid, (1-methylethyl)-, cyclohexyl ester (0.50), (1R,3R,4R,5R)-(-)-Quinic

acid(26.04), Tetradecanoic acid (15.69), 3,7,11,15-Tetramethyl-2-hexadecen-1-zl(2.55), n-Hexadecanoic acid (11.44), Hexadecanoic acid, Octadecanoic acid ethyl ester(0.32), Phytol(1.85), 9,12-Octadecadienoic acid (Z,Z)-(7.70), Oleic Acid(1.89),4,8,12,16-etramethylheptadecan-4-olide (0.65), 1,2-Benzenedicarboxylic acid, diisooctyl ester (0.93), Phenol, 3-pentadecyl-(2.12), ç-Sitosterol (6.43), were identified in the ethanol extract of BA by relating to the corresponding peak area through coupled GC–MS (Fig 1).

Table 1.Phyto-Components identified in *the Buchannania axillaris -365* [GC MS study]

No.	RT	Name of the compound	Molecular	MW	Peak Area %	Compound Nature
1	4.31	1-Amino-2,6-dimethylpiperidine	$C_7H_{16}N_2$	128	1.52	Alkaloid
2	4.65	2-Octenoic acid, 4,5,7-trhydroxy	$C_8H_{14}O_5$	190	1.9	Hydroxy compound
3	5.14	à-Methyl-D-mannopyranoside	$C_7H_{14}O_6$	194	2.12	Sugar compound
4	5.75	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	0.38	Flavonoid compound
5	8.92	Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl- [Synonyms: à-Ionene]	$C_{13}H_{18}$	174	2.94	Naphthalene compound
6	9.21	D-Galactose, 6-deoxy- [Synonyms: D-Fucose]	$C_6H_{12}O_5$	164	6.06	Sugar compound
7	10.26	1,6-Anhydro-á-D-glucopyranose (Synonyms: levoglucosan)	$C_6H_{10}O_5$	162	5.85	Sugar moiety
8	10.91	Dodecanoic acid	$C_{12}H_{24}O_2$	200	0.83	Lauric acid
9	11.02	Phosphonofluoridic acid, (1-methylethyl)-, cyclohexyl ester	$C_9H_{18}FO_2P$	208	0.50	Fluro compound
10	12.14	(1R,3R,4R,5R)-(-)-Quinic acid	$C_7H_{12}O_6$	192	26.04	Quinic acid
11	13.38	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	15.69	Myristic acid
12	14.45	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	2.55	Terpene alcohol
13	16.22	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	11.44	Palmitic acid
14	16.49	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.88	Ester compound
15	18.44	Phytol	$C_{20}H_{40}O$	296	0.32	Diterpene
16	18.77	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	1.85	Linoleic acid
17	18.87	Oleic Acid	$C_{18}H_{34}O_2$	282	7.70	Oleic acid
18	19.17	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.89	Stearic acid
19	21.96	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324	0.65	Methyl compound
20	24.68	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	0.93	Plasticizer compound
21	27.11	Phenol, 3-pentadecyl-	$C_{21}H_{36}O$	304	2.12	Phenolic compound
22	29.55	ç-Sitosterol	$C_{29}H_{50}O$	414	6.43	Steroid

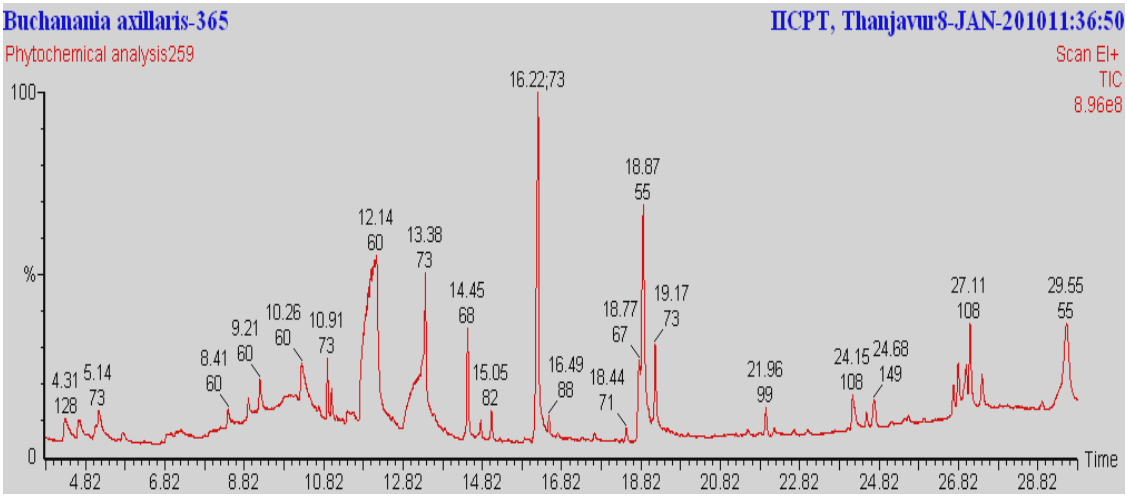


Fig 1: The chromatogram showing n-Hexadecanoic acid (16.49), 9, 12-Octadecadienoic acid (Z,Z)- (18.77), 4,8,12,16-Tetramethylheptadecan-4-olide(21.96),and ç-Sitosterol (29.55) peaks detected by GC-MS.

Effects of BA extract on serum urea and uric acid concentrations:

Serum urea, uric acid and alkaline phosphatase concentrations were significantly increased in DOX-treated animals (group 2) compared to normal (group1), indicating the induction of severe cardiotoxicity. Treatment of DOX-administered rats (Groups 4 and 5) with BA significantly lowered concentrations of serum urea, uric acid, and alkaline phosphatase compared with treatment with DOX alone (Fig 2). BA (500mg/kg) extract alone treated rats did not show any significant effect on serum urea, uric acid and alkaline phosphatase concentrations (group 3).

Effect of BA extract on serum markers during DOX induced cardiotoxicity:

The serum markers indicating myocardial injury; LDH, CPK, ALP, SGPT and SGOT were significantly ($p < 0.01$) elevated in the DOX-only treated group compared to normal group (Fig 3 and 4). Pretreatment with BA (250 and 500 mg/kg body weight) group (group 4 and 5) significantly reduced their levels when compared with DOX-only treated group (group 2). BA (500mg/kg) extract alone treated did not show any significant effect on serum maker enzymes (group 3).

Effect of BA extract on lipid profile during DOX induced cardiotoxicity:

DOX treated rats showed significant increase in the levels of serum total cholesterol, TGs, LDL and VLDL and the level of HDL were significantly decreased when compared to the normal rats (group 1). Pretreatment with BA (250 and 500 mg kg−1 day−1 for 14 days) to DOX-treated rats significantly prevented the altered levels of serum total cholesterol, TGs, LDL, and VLDL (Fig 5 and 6). But it has increased the serum HDL concentration when compared to normal rats (group 1). BA (500mg/kg) extract alone treated did not show any significant effect on lipid level in the blood (group 3).

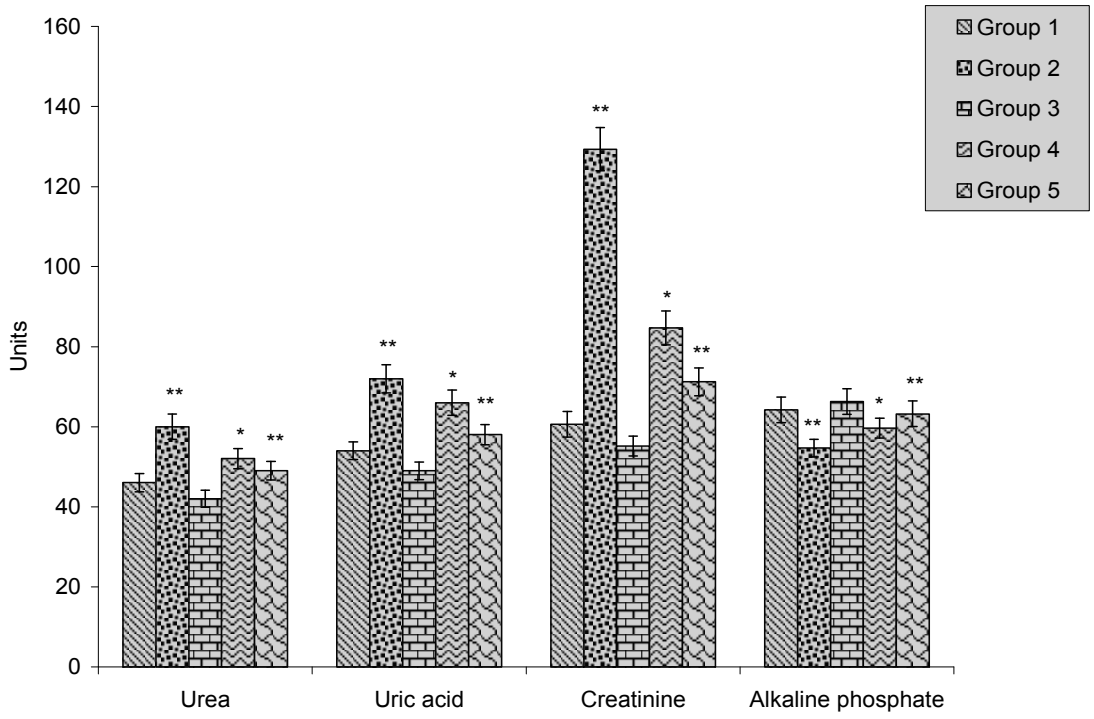


Fig 2: Effect of ethanol extract of BA on urea (mg/dl), uric acid (mg/dl), GR (nmol of NADPH oxidized/min/100mg protein), and alkaline phosphatase (mg/dl) in DOX intoxicated rats. Values are near ± S.D (n=6) **P<0.01, *P<0.05 respectively.

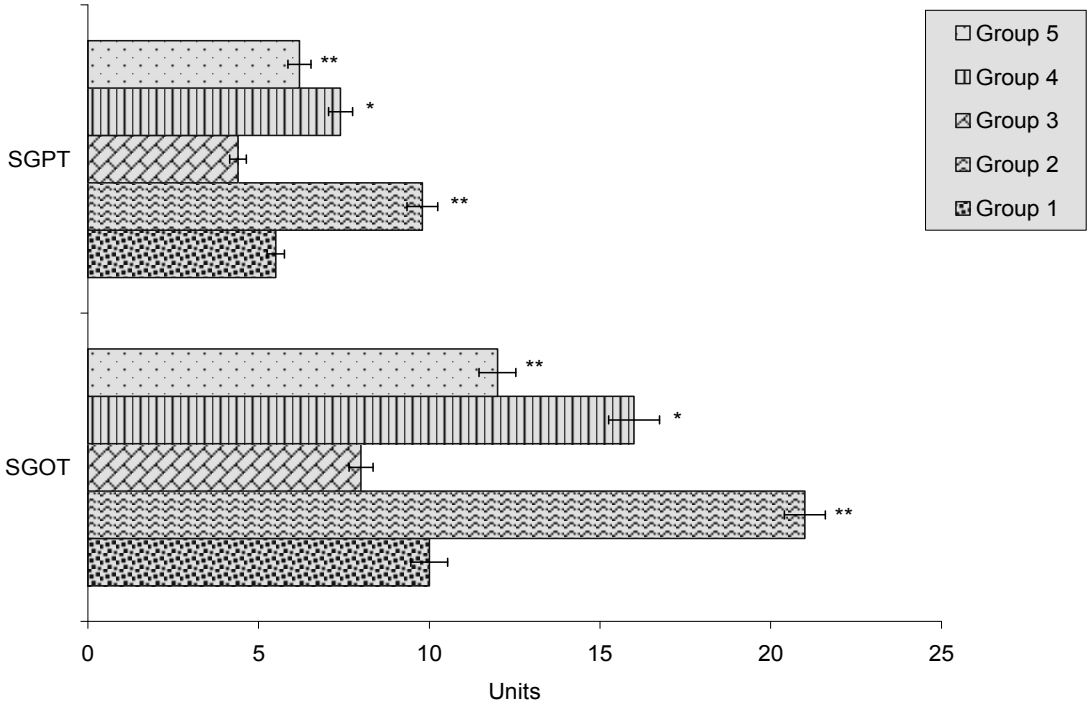


Fig 3: Effect of ethanol extract of BA on SGOT (IU/L) and SGPT (IU/L) in DOX intoxicated rats. Values are near ± S.D (n=6) **P<0.01, *P<0.05 respectively.

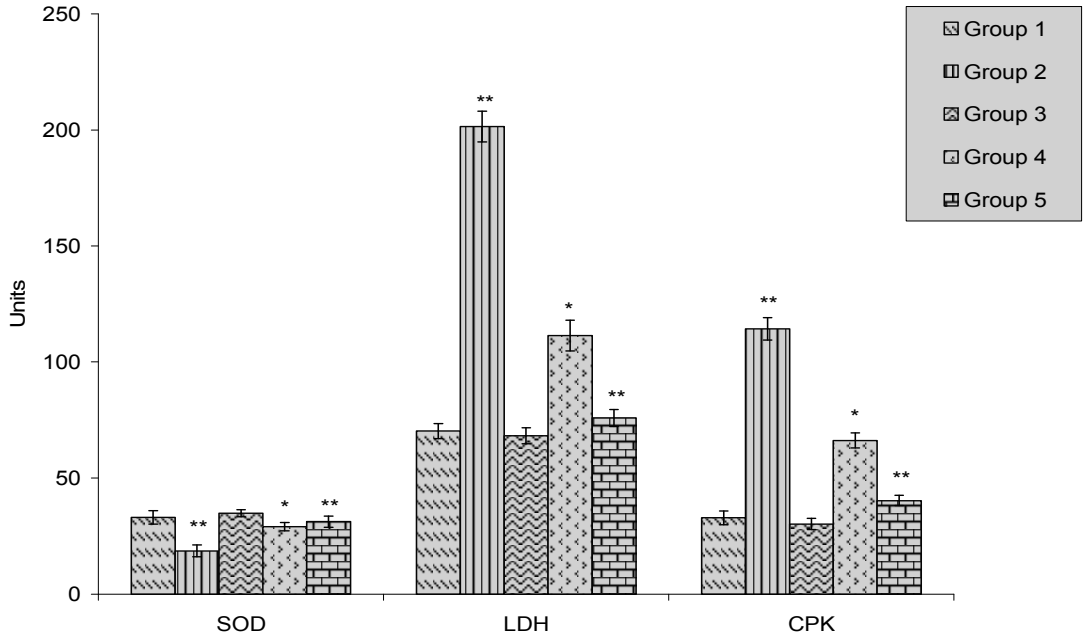


Fig 4: Effect of ethanol extract of BA on SOD (U/mg protein), LDH (IU/L) and CPK (IU/L) in DOX intoxicated rats. Values are near ± S.D (n=6) **P<0.01, *P<0.05 respectively.

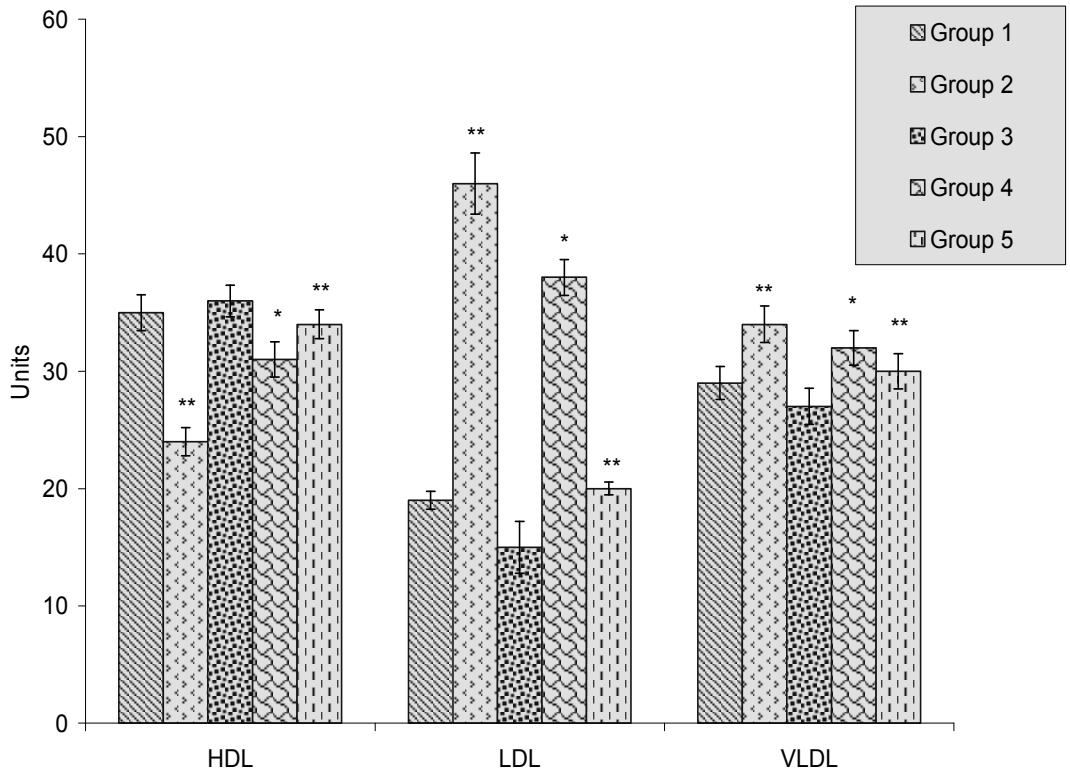


Fig 5: Effect of ethanol extract of BA on serum HDL (mg/dl), LDL (mg/dl), and VLDL (mg/dl) in DOX intoxicated rats. Values are near ± S.D (n=6) **P<0.01, *P<0.05 respectively.

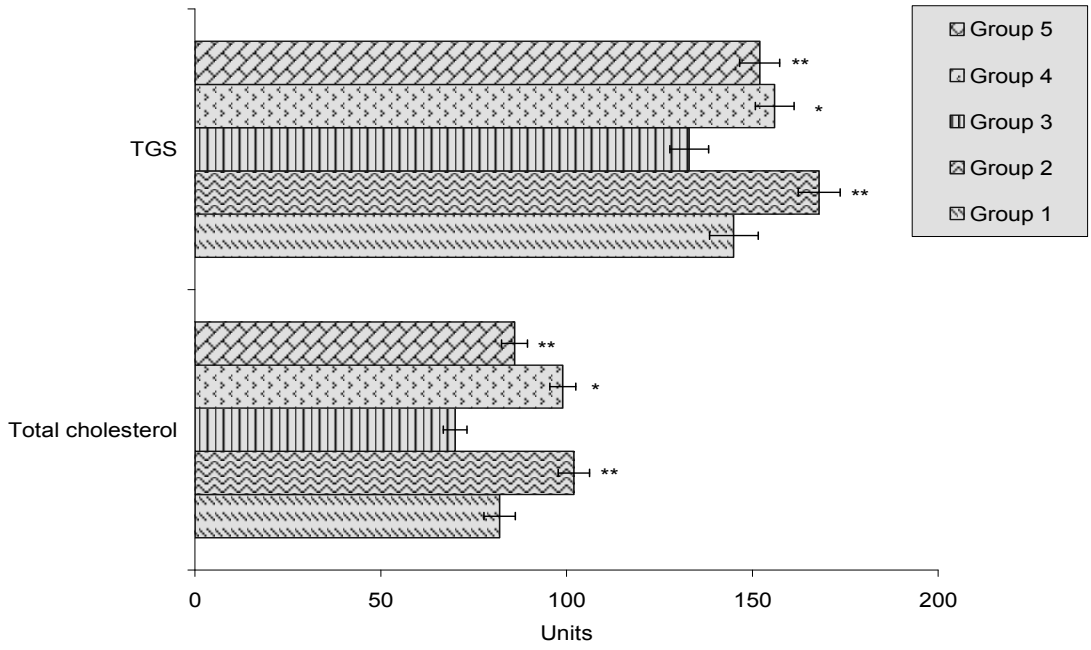


Fig 6: Effect of ethanol extract BA on serum TGs (mg/dl) and total cholesterol (mg/dl) in DOX intoxicated rats. Values are near ± S.D (n=6) **P<0.01, *P<0.05 respectively.

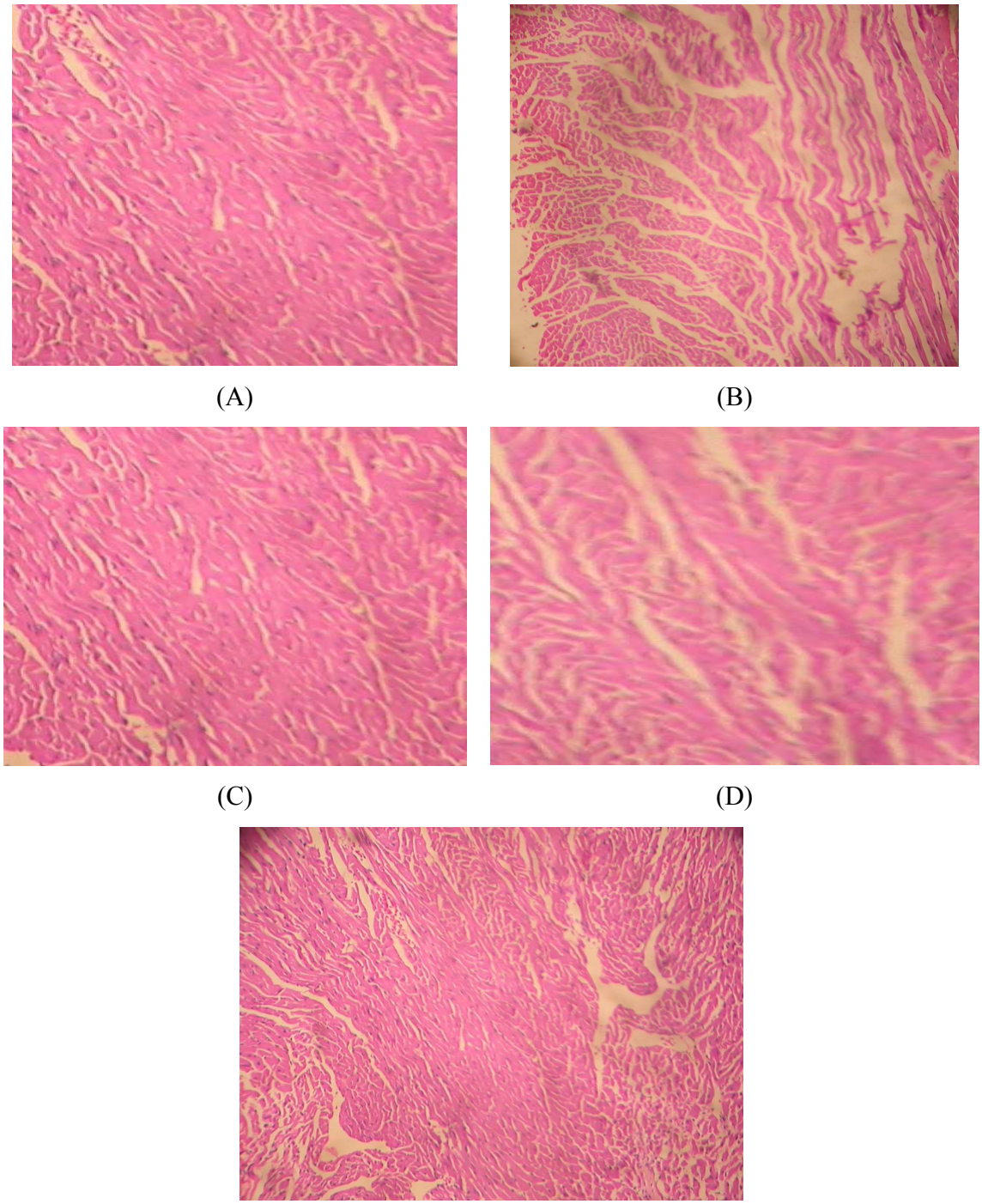
Effect of BA extract on antioxidant parameters:

DOX - induced rats (group 2) exhibited a significant (P<0.01) decrease in activities of CAT, SOD, GPx and GSH level when compared to normal rats (group 1). Although BA (250 and 500mg/kg/day) dose dependently counteracted the deleterious effect of DOX by increasing the content of these antioxidants, significance could be achieved with 500mg/kg/day dose of BA only (P<0.01) (Fig 7).

Administration of BA alone (500mg/day) (group 3) did not show significant changes in antioxidants as compared to normal rats. There was a significant reduction in the GR during the BA administration to the DOX induced heart when compared to the normal (group 1). Cardiac lipid peroxidation assessed by MDA production did not rise in the pretreated rats after DOX administration to compared with DOX – induced cardiotoxic rats (Fig 7).

Histopathological findings

Cardiotoxicity induced by DOX (group 2) was further assessed using H and E stain. The heart from normal group (group 1) showed a regular cell distribution and normal myocardium morphology (Fig 8A). Histology of the rat from DOX-induced animals (group 2) revealed the cytoplasmic vacuole formation and myofibrillar loss, which is the typical finding in DOX-induced cardiotoxicity (Fig. 8B). In BA 250 mg/kg/day + DOX group, there was less edema and myonecrosis with less inflammatory cells (Fig. 8D). The BA 500 mg/kg/day+ DOX- treated heart tissue shows mild edema but no infarction (Fig. 8E). BA (500 mg/kg) administration alone did not lead to any histopathological changes in the myocardium (Fig. 8C).



(A) (B)

(C) (D)

(E)

Fig 8: Cardioprotective effect of *Buchanania axillaris extract*. Histopathological observations (heart sections stained with Hematoxylin-Eosin, magnification-100x) (A) Normal, (B) DOX, (C)BA alone (500mg/kg), (D) Extract 250 mg/kg + DOX (E) Extract 500 mg/kg + DOX

DISCUSSION

Cardiotoxicity caused by treatment with Doxorubicin can be life-threatening and may occur even years after completion of therapy [33]. The current study assesses the cardioprotective potential of the ethanol extract of BA against DOX-induced cardiotoxicity. Uric acid is considered to be a risk factor in the development of *cardiotoxicity* [34]. We observed a significant increase in the level of plasma uricacid in DOX induced rats which could be due to increased free radical production by DOX. In hypoxic tissue, ATP depletion occurs which leads to accumulation of hypoxanthine when tissues are disturbed, the enzyme Xanthine Dehydrogenase is converted to Xanthine Oxidase by the oxidation of essential SH groups. Xanthine Oxidase catalyzes the conversion of Hypoxanthine to Xanthine, Uric acid to super oxide [35]. This could be one of the reasons for the elevated levels of plasma uric acid in DOX induced rats. It is found that urea inhibits the lactate dehydrogenase activities of the crystalline ox-heart, rabbit-muscle enzymes and of human heart and human liver extracts to a much greater extent with 2-oxobutyrate as substrate than with pyruvate. It also said to be reducing the serum LDH activity to about 50% (36).

Cardiotoxicity is also associated with altered lipid metabolism. The increased concentration of cholesterol could be due to a decrease in HDL, since HDL is known to be involved in the transport of cholesterol from tissues to the liver for its catabolism [37]. In this context, we have observed decreased levels of HDL in DOX-treated rats. The observed increase in TGs might be due to a decrease in the activity of lipoprotein lipase, resulting in decreased uptake of TGs from the circulation [38]. Pretreatment with BA decreases the concentration of total cholesterol, TGs, VLDL and increases the concentration of HDL in heart of DOX-induced rats. These changes in lipid levels might be due to enhanced lipid biosynthesis by cardiac cyclic adenosine monophosphate[39]. Studies have shown that high levels of LDL cholesterol have a positive correlation with *cardiotoxicity*, whereas high levels of HDL cholesterol have a negative correlation with *cardiotoxicity*,[40]. Serum CPK, SGPT, SGOT, ALP and LDH are well known markers of myocardial infarction. When myocardial cells are damaged or destroyed due to deficient oxygen supply or glucose, the cardiac membrane becomes permeable or may rupture which results in leakage of enzymes. These enzymes enter into the blood stream thus increasing their concentration in the serum[40]. Activities of these enzymes in serum decreased in the BA pretreated DOX induced group probably due to the protective effect of BA on myocardium, which would have reduced the extent of myocardial damage induced by DOX and thereby restriced the leakage of these enzymes from myocardium. It is widely accepted that oxygen-free radicals generated during Doxorubicin redox cycling are responsible for the damage that doxorubicin causes to the heart [41,42,43,44]. Oxygen radical generation affects the heart because doxorubicin and its toxic metabolite doxorubicinol accumulate

in cardiac tissue that has low antioxidant levels [45].

Cardioprotective activity of BA is supported by increased myocardial antioxidant enzyme activity and decreased extent of lipid peroxidation. The most abundant ROS generated in living cells are superoxide anion and its derivatives, particularly highly reactive and damaging hydroxyl radical, which induces peroxidation of cell membrane lipids [46]. Lipid peroxidation is known to cause cellular damage and is primarily responsible for ROS-induced organ damage [47]. Our studies have shown that in DOX-induced *cardiotoxicity*,there was considerable increased in lipid peroxidation, which was significantly prevented by BA pretreatment.

Redox cycling of DOX generates superoxide free radicals [48] due to conversion of quinone to semi-quinone moiety, whereas SOD enzyme dismutates this free radical to hydrogen peroxide. In this respect, any increase in SOD activity of the organ appears to be beneficial in the event of increased free-radical generation. Our studies showed that the activity of SOD was significantly decreased in DOX-treated animals and the pretreatment with BA reversed the SOD activity in a dose-dependent manner. However, it has been reported that a rise in SOD activity, without a concomitant rise in the activity of catalase/GSH might be detrimental [49]. This is due to the fact that SOD generates hydrogen peroxide as a metabolite, which is cytotoxic and needs to be scavenged by catalase/GSH. Thus a simultaneous increase in catalase/GSH activity is essential for an overall beneficial effect of increase in SOD activity (50). Inhibition of DOX-induced oxidative stress and tissue injury might be due to an increase in GSH, myocardial SOD and catalase activities, following the pretreatment of BA. The observed increase in catalase activity in DOX-treated animals supports the above hypothesis that this increase is possibly required to overcome excessive oxidative stress [51]. GSH levels were also lowered significantly in DOX-treated animals, while pretreatment with BA showed significant increase in GSH levels in DOX-treated animals at doses of 250 mg/kg and 500 mg/kg in rats. Catalase activity was increased after DOX treatment and pretreatment with BA further increased its activity significantly at 250 mg/kg and 500mg/kg dose levels. The increase in catalase activity in DOX-treated animals could be indicative of enhanced oxidative stress due to an adaptive myocardial mechanism. These findings indicate the promising role of BA as a cardioprotective agent against DOX-induced cardiotoxicity.

CONCLUSION

The present study shows that the administration of ethanol extract of BA has cardioprotective potential against DOX-induced cardiotoxicity. It provides experimental evidence that BA augmented the myocardial antioxidant enzymes level, preserved histoarchitecture and improved cardiac performance following DOX administration. This cardioprotective activity of BA might be due to the synergetic effect of chemical compounds present in them making them good sources for the production of a cardioprotective

herbal medicine. The identification of molecules with cardioprotective potential from this ethanol extract of BA may provide new directions for identification of cardioprotectives, which could be given concomitantly during Dox treatment.

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