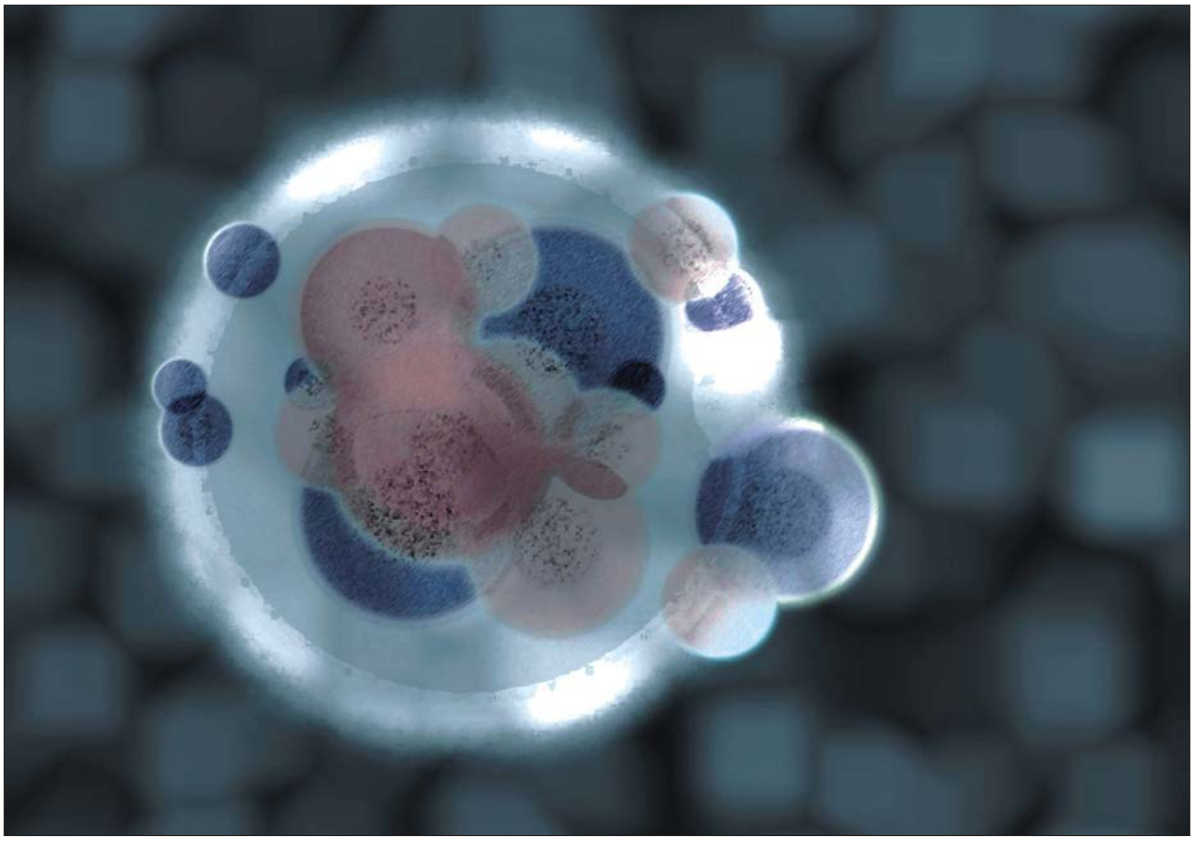

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 13 (5): 313-322, 2013
ISSN 1727-3048 / DOI: 10.3923/jbs.2013.313.322

Cardioprotective and Antioxidant Potential of *Scilla hyacinthina*

[1]K. Sakthivel, [1]S. Palani, [1]R. Selvaraj, [1]D.Venkadesan, [1]H. Sivasankari and [2]B. Senthil Kumar
[1]Deptartment of Biotechnology, Anna Bioresearch Foundation,
Arunai Engineering College, Tiruvannamalai, India
[2]Department of Zoology, Tiruvalluvar University, Vellore, Tamilnadu, India

Abstract: The present study were designed to scientifically evaluate the cardioprotective potential of ethanolic extract of *scilla hyacinthina* (Family: Hyacinthinae), a medicinal herb, on Doxorubicin (DOX) induced myocardial infarction (MI) 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^{-1} was selected for the present study as this dose offered significant alteration in biochemical parameters and moderate necrosis in heart. Effect of SHA oral treatment for 14 days at two doses (250 and 500 mg kg^{-1} b.wt) was evaluated against DOX-induced cardiac necrosis. Significant myocardial necrosis, depletion of endogenous antioxidants and biochemical parameters were observed in DOX-treated animals when compared with the normal animals. The pretreatment with SHA to DOX-induced rats significantly prevented the altered biochemical variation such as marker enzymes (SGPT, SGOT, CPK and LDH), lipid profile (LDL, VLDL, TGs, HDL and Total cholesterol) and antioxidant parameters (SOD, GSH, CAT, GSH-Px, MDA and GR) to near normal status. Serum urea, uric acid and alkaline phosphate, which increased on DOX administration, registered near normal values on pretreatment with SHA. Histology of pretreated with SHA to DOX-induced heart showed a significant recovery from cell damage. The present findings have demonstrated that the cardioprotective effects of SHA in DOX-induced oxidative damage may be due to an augmentation of the endogenous antioxidants and inhibition of lipid peroxidation of membrane.

Key words: Cardioprotective, antioxidant, *Scilla hyacinthina,* 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 (De Bono and Boon, 1992). An increased risk of MI is associated with high levels of serum total cholesterol (Grundy, 1986) and Low Density Lipoprotein (LDL) (Brown and Goldstein, 1986) and decreased levels of High Density Lipoprotein (HDL) (Castelli *et al*., 1986). 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 (Loper *et al*., 1991; Padmanabhan and Prince, 2006; Zhou *et al*., 2008). Minimizing myocardial necrosis and improving heart function have been proved to be effective strategies to reduce the morbidity and mortality from myocardial infarction (Kloner and Rezkalla, 2004). Accordingly, antioxidants may decrease cellular injury and apoptosis through a radical-scavenging mechanism (Angeloni *et al*., 2007). Therapeutic intervention via suppression of free radical generation and/or augment endogenous antioxidant enzymes may attenuate myocardial dysfunction. An anthracycline anticancer drug, doxorubicin 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 (Lee *et al*., 1991) as well as induces the peroxidation of unsaturated lipids within the membranes (Myers *et al*., 1977).

Although modern drugs are effective in preventing cardiovascular disorders, their use is often limited because of their side effects (Rajadurai and Prince, 2005). Herbal drugs are prescribed widely, even when their biologically active compounds are unknown, because of their effectiveness, lesser side effects and relatively low cost (Kumar *et al*., 2008). Now-a- days, the usage

Corresponding Author: K. Sakthivel, Department of Biotechnology, Anna Bioresearch Foundation, Arunai Engineering College, Tiruvannamalai, Tamilnadu, India

of herbal drugs is gaining greater acceptance from the medical and public profession due to their positive contribution and influence on health and qualify of life. So, search for indigenous cardioprotective herbal drugs is still continuing as part of scientific research.

Scilla is a genus of bulb-forming perennial herbs in the Hyacinthaceae family, a preparation of powdered bulbs extracted in ethanol, is an ingredient in cough medicines and cardiac surgery. The leaf and fruits of *Scilla hyacinthina* (Roth.) Macbr, are reported to possess anticancer, diuretic, expectorant and cardio stimulant activity (Sudhersan and Padmanabhan, 1993). Extensive phytochemical investigations carried out on SHA 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 Hypocholesterolemic property (Kurian *et al*., 2010; Lee *et al*., 2005; Mohamed *et al*., 2009). All these factors and lack of scientific data on the cardiac activities led to the investigation of the cardioprotective potential of SHA, ethanolic extract in rat models Therefore, the present study was designed to investigate the effects of SHA on DOX-induced myocardial necrosis in rats.

MATERIALS AND METHODS

Kurian

Preparation of *Scilla hyacinthina* extract: Leaves of *Scilla hyacinthina* (Roth.) Macbr. Was collected and identified and authenticated (AECBT 10/2010) by a Botanist, Dr.C.Madhavachetty, Tirupathi university, Tirupathi, India. Materials were cleaned with water and dried in the shade until a constant weight was obtained. It was extracted with 95% ethanol in a Soxhlet extractor. Extracts were concentrated; the percentage yield for ethanol extract was 7.9% and for pharmacological studies, since the ethanol extract was not soluble in water, it is suspended in 5% guni acacia.

GC-MS analyses of ethanol extract of SHA for the identification of chemical composition: The identification of chemical composition of ethanol extract of SHA 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 SHA 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.

Induction of experimental myocardial infarction: Doxorubicin was dissolved in sterile double distilled water and injected subcutaneously to rats (20 mg kg^{-1}) after last dose of the extract to induce experimental MI (Singh *et al*., 2008).

Experimental animals: Studies were carried out using Wistar albino rats (150-200 g), obtained from Indian Veterinary Preventive Medicine (IVPM), Ranipet, Tamilnadu, India. The animals were housed in polyacrylic cages (38, 23, 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's Ethical Committee.

Experimental procedure:

- **Group 1:** (Normal). Saline (0.75 ml/animal), orally for 14 days
- **Group 2:** (Drug control). Saline (0.75 mL $animal^{-1}$)+ DOX 20 mg kg^{-1}, single intraperitoneal injection after 14th day
- **Group 3:** (Extract control). SHA (500 mg kg^{-1}), orally for 14 days
- **Group 4:** SHA (250 mg kg^{-1}), orally for 14 days+ DOX (20 mg kg^{-1}) single intraperitoneal injection after 14th day
- **Group 5:** SHA (500 mg kg^{-1}), orally for 14 days+ DOX (20 mg kg^{-1}) single intraperitoneal injection after 14th day

Isolation of working heart preparation: The animals were anesthetized with chloroform after 72 h of DOX administration and then heart was punctured with sterile syringe and blood was stored with EDTA which is an anticoagulant agent and was excised out. 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: Since it has been ascertained that DOX-induced myocardial lesions are focal and uniformly scattered throughout the whole organ, isolated trial preparation were used instead of the whole heart owing to the greater stability of response and reproducibility of the results. 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 sample were collected into tubes pre-coated with EDTA by vein puncture at baseline and post intervention. Samples were prepared by centrifuging for 10 min 3000×g at 4°C. Plasma, buffy coat and red blood cell sub-fractions were collected and stored at -80°C until further analysis. The serum used for the assay of marker enzymes as well as urea, uric acid, was estimated by the methods of Caraway (1963) and respectively. The activities of Serum Glutamate-pyruvate Transaminase (SGPT) and Serum Glutamate Oxaloacetate Transaminase (SGOT) in serum were determined spectrophotometrically by the method of Mohun and Cook (1957).The Lactate Dehydrogenase (LDH) and Creatine Phosphokinase (CPK) were determined by the method of King (1965) and by the method of Okinaka *et al.* (1961), respectively. The levels of total cholesterol and triglycerides (TGs) were estimated by the methods of Zlatkis *et al.* (1953) and Foster and Dunn (1973). Serum High Density Lipoprotein (HDL) was determined according to the method of Wilson and Spiger (1973). 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. 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 Zhang (1992). Superoxide dismutase (SOD) activity was determined according to Rai *et al.* (2006). CAT activity was determined from the rate of decomposition of H_2O_2 according to Bergmeyer *et al.* (1974). Glutathione peroxidase (GSH-Px) 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 Hafeman *et al.* (1974). GSH reductase activity was assayed according to Carlberg and Mannervik (1975) and Mohandas *et al.* (1984). GR activity was determined according to the method described by Staal *et al.* (1969).

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

RESULTS

Phytochemical analysis: The ethanol extract of SHA was a complex mixture of many constituents and compounds were identified in this plant by GC-MS (Table 1). Phytoconstituents such as n-Hexadecanoic acid (13.97%), 9,12-Octadecadienoic acid (Z,Z)-(5.36%), Oleic Acid (12.65%), 2-Pyrazoline, 5-hydroxy-3-methyl-5-trifluoromethyl-1-(3,4-dimethylphenoxyacetyl)- (21.01%), 4H-1,3-Dithiin, 2,2,6-trimethyl-4-(2,2,6-trimethyl-4H-1,3-dithiin-4-ylidene)- (16.35%), 2-Thiophenecarboxaldehyde, 5-ethynyl-, (2,4-dinitrophenyl) hydrazone (11.09%), 4H-1-Benzopyran-4-one, 5,7-dihydroxy-2-(3,4,5-trimethoxyphenyl)- (8.46%), Octadecanoic acid (4.64%), 1-Octanol, 3,7-dimethyl-(0.87%), 1-Heptanol, 2-propyl-(0.20%), 2-Isopropyl-5-methyl-1-heptanol (0.95%), 1-

Table 1: Chemical composition of ethanolic SHA leaf extract by GCMS

RT	Name of the compound	Molecular formula	MW	Peak area (%)	Compound nature
3.60	Glycerin	$C_3H_8O_3$	92	1.74	Alcohol
4.78	1-Octanol, 3,7-dimethyl- [Synonyms: Dihydrocitronellol]	$C_{10}H_{22}O$	158	0.87	Terpene alcohol
6.93	Decane, 2,3,5,8-tetramethyl-	$C_{14}H_{30}$	198	0.44	Alkane
7.47	1-Heptanol, 2-propyl-	$C_{10}H_{22}O$	158	0.20	Alcohol
7.88	2-Isopropyl-5-methyl-1-heptanol	$C_{11}H_{24}O$	172	0.95	Alcohol
9.04	Diphenylmethane	$C_{13}H_{12}$	168	0.09	Aromaticcompound
9.89	1-Octanol, 2-butyl-	$C_{12}H_{26}O$	186	0.28	Alcoholcompound
16.19	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	13.97	Palmitic acid
16.49	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.49	Ester compound
18.45	Phytol	$C_{20}H_{40}O$	296	0.30	Diterpene
18.78	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	5.36	Linoleic acid
18.86	Oleic Acid	$C_{18}H_{34}O_2$	282	12.65	Oleic acid
19.18	Octadecanoic acid	$C_{18}H_{36}O_2$	284	4.64	Stearic acid
23.25	4H-1-Benzopyran-4-one, 5,7-dihydroxy-2-(3,4,5-trimethoxyphenyl)- [Synonyms: Flavone, 5,7-dihydroxy-3',4',5'-trimethoxy-]	$C_{18}H_{16}O_7$	344	8.46	Flavonoidcompound
24.72	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	1.12	Plasticizercompound
30.61	2-Thiophenecarboxaldehyde, 5-ethynyl-, (2,4-dinitrophenyl)hydrazine	$C_{13}H_8N_4O_4S$	316	11.09	Sulphur compound
31.40	4H-1,3-Dithiin, 2,2,6-trimethyl-4-(2,2,6-trimethyl-4H-1,3-dithiin-4-ylidene)-	$C_{14}H_{20}S_4$	316	16.35	Sulphur compound
32.39	2-Pyrazoline, 5-hydroxy-3-methyl-5-trifluoromethyl-1-(3,4-dimethylphenoxyacetyl)-	$C_{15}H_{17}F_3N_2O_3$	330	21.01	Alkaloid

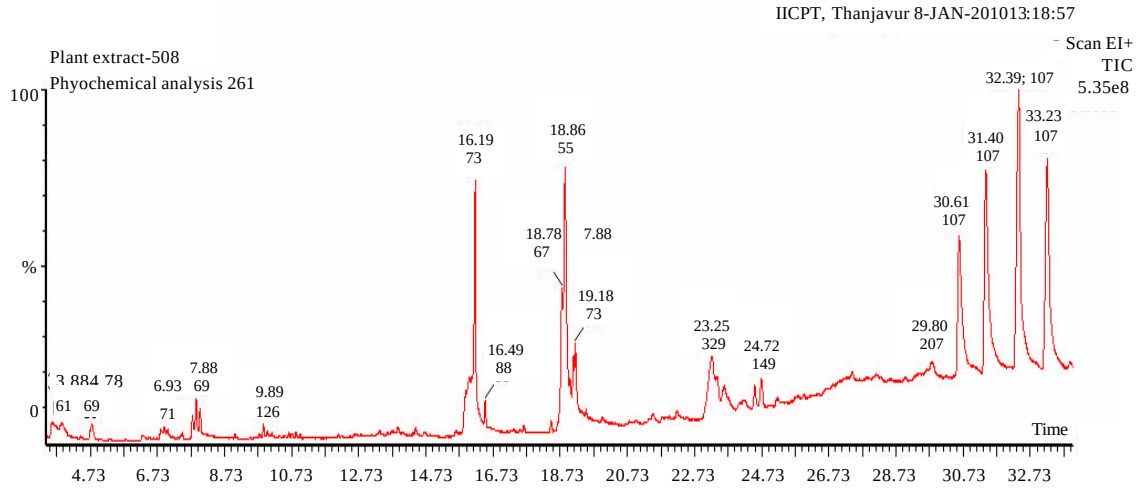

Fig. 1: GCMS analysis of ethanolic SHA leaf extract

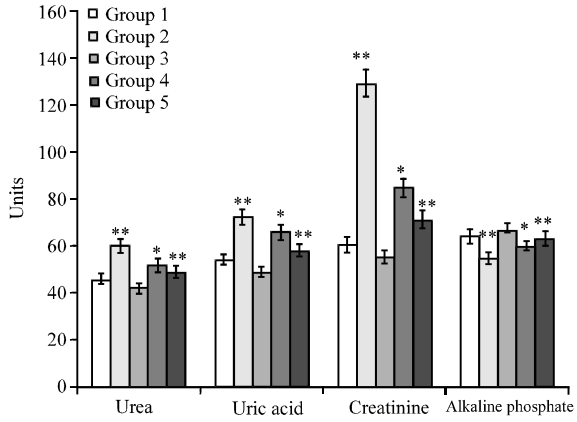

Fig. 2: Effect of ethanolic extract SHA on Urea (mg dL^{-1}), Uric acid (mg dL^{-1}), GR (nmol of NADPH oxidized/min/100 mg protein) and alkaline phosphate (mg dL^{-1}) in DOX intoxicated rats, Values are Mean±S.D (n = 6) **p<0.01, **p<0.05, respectively

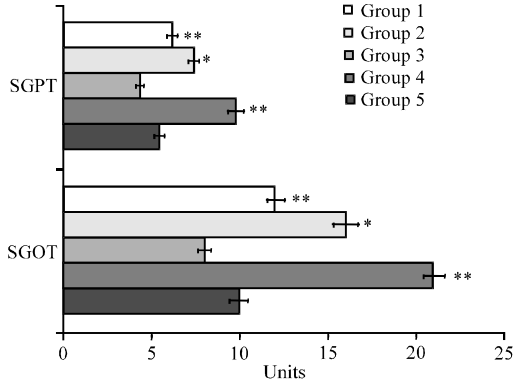

Fig. 3: Effect of ethanolic extract SHA on SGOT (IU L^{-1}) and SGPT (IU L^{-1}) in DOX intoxicated rats, Values are Mean±S.D (n = 6) *,**Significant at p<0.05 and 0.01, respectively

Octanol, 2-butyl- (0.28), Hexadecanoic acid, ethyl ester (0.49%) and Phytol (0.30%), were identified in the ethanol extract of SHA by relating to the corresponding peak area through coupled GC-MS (Fig. 1).

Effects of SHA extract on serum urea, Uric acid and Alkaline Phosphate concentrations: Serum urea, uric acid and alkaline phosphate concentrations were significantly increased in DOX-treated animals (group 2) compared with normal (group 1), indicating the induction of severe cardiotoxicity. Treatment with SHA of DOX-administered rats significantly (groups 4 and 5) lowered concentrations of serum urea, uric aid and alkaline phosphate compared with treatment with DOX alone (Fig. 2).

Effect of SHA extract on serum markers during DOX induced cardiotoxicity: The serum markers indicating myocardial injury; LDH, CPK, SGPT and SGOT were significantly (p<0.01) elevated in the DOX-only treated group compared with normal and SHA-only treated group (Fig. 3 and 4). Pretreatment with SHA (250 and 500 mg kg^{-1} b.wt) group (group 4 and 5) significantly reduced their levels as compared with DOX-only treated group (group 2).

Effect of SHA 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 SHA (250 and 500 mg kg^{-1} day-1 for 14 days) to DOX-treated rats

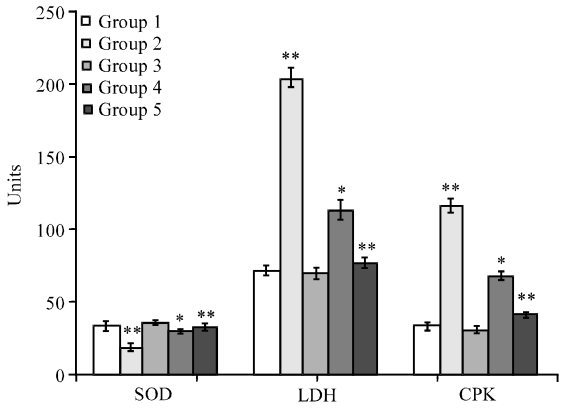

Fig. 4: Effect of ethanolic extract SHA on SOD (U mg^{-1} protein), LDH (IU L^{-1}) and CPK (IU L^{-1}) in DOX intoxicated rats, Values are Mean±S.D (n = 6)

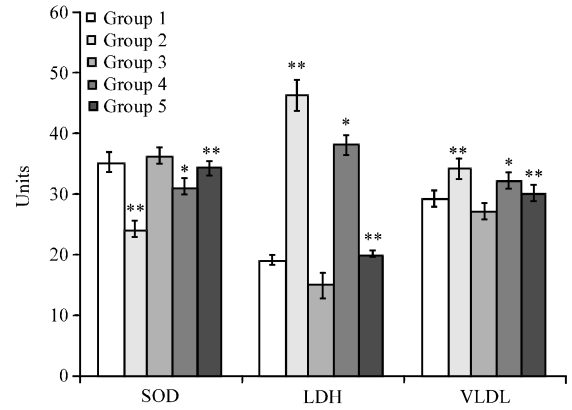

Fig. 5: Effect of ethanolic extract SHA on serum HDL (mg dL^{-1}), LDL (mg dL^{-1}) and VLDL (mg dL^{-1}) in DOX intoxicated rats, Values are Mean±S.D (n = 6)

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).

Effect of SHA extract on antioxidant parameters: DOX- induced rats (group 2) had exhibited a significant ($p<0.01$) decrease in activities of CAT, SOD, GSH-Px and GSH level as compared to normal rats (group 1). Although SHA (250 and 500 mg kg day^{-1}) dose dependently counteracted the deleterious effect of DOX by increasing the content of these antioxidants, significance could be achieved with 500 mg kg day^{-1} dose of SHA only ($p<0.01$) (Fig. 7). Administration of SHA alone (500 mg day^{-1}) (group 3) did not show significant changes in antioxidants as compared to normal rats. There is significant reduce in the GR during the SHA administration to the DOX induced heart when compared to the normal (group 1).

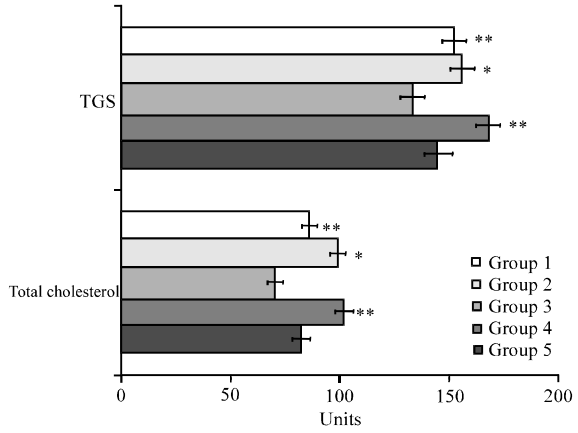

Fig. 6: Effect of ethanolic extract SHA on serum TGs (mg dL^{-1}) and total cholesterol (mg dL^{-1}) in DOX intoxicated rats, Values are Mean±S.D (n = 6) *,**significant at $p<0.05$ and 0.01

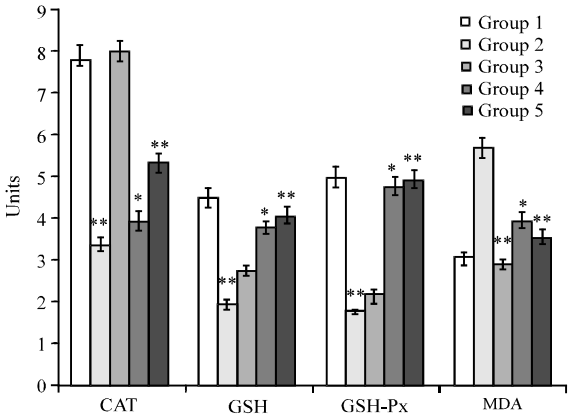

Fig. 7: Effect of ethanolic extract SHA on CAT (µmol of H_2O_2 consumed min mg^{-1} protein), GSH (mM g^{-1} tissue), GSH-Px (µg of GSH oxidized min mg^{-1} protein) and MDA (nmol g^{-1} tissue) in DOX intoxicated rats, Values are Mean±S.D (n = 6)

Cardiac lipid peroxidation assessed by MDA production did not rise in the pretreated rats after DOX administration when compared with DOX - induced 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. 8). Histology of the rat from DOX-induced animals (group 2) revealed the cytoplasmic vacuole formation and myofibrillar loss, which is typical finding in DOX induced cardiomyopathy. Heart myocardial lesions were significantly reduced in animals that received SHA treatment (group 4 and 5).

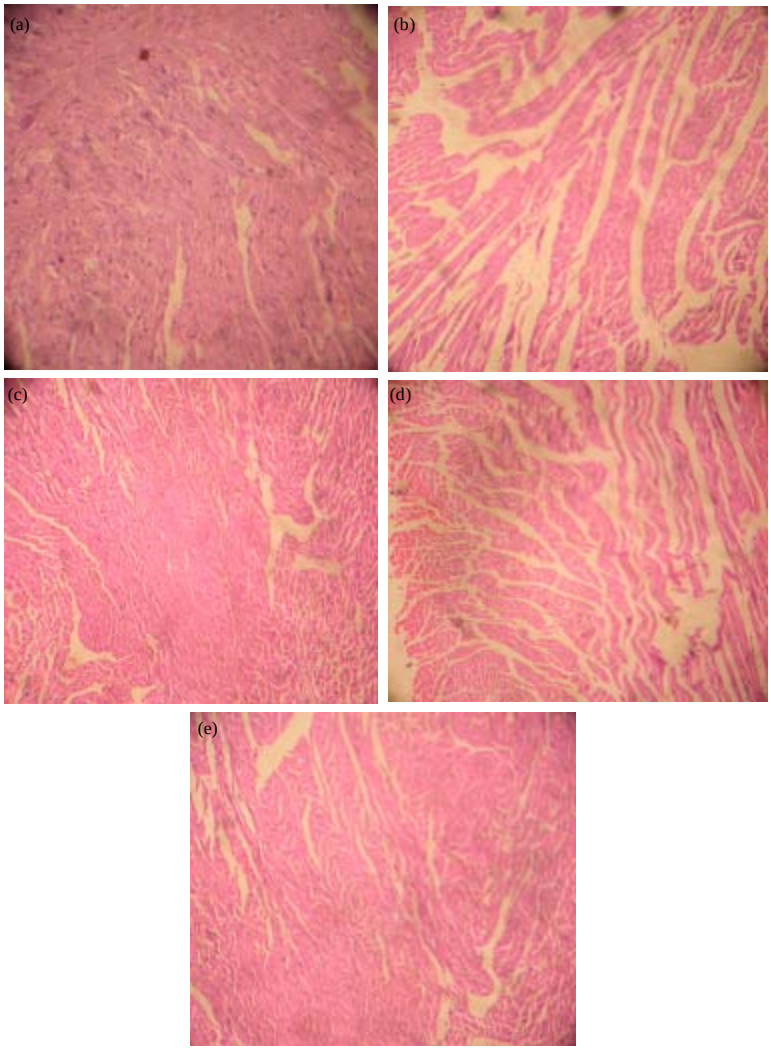

Fig. 8(a-e): Cardioprotective effect of *Scilla hyacinthina extract*. Histopathological observations (heart sections stained with Hematoxy lin-Eosin, magnification-100x), (a) Normal, (b) DOX, (c) Extract 250 mg kg^{-1}+DOX and (d-e) Extract 500 mg kg^{-1}+DOX

DISCUSSION

Cardiotoxicity caused by treatment with Doxorubicin can be life-threatening and may occur even years after completion of therapy (Kapusta *et al*., 2000). The current study entails the cardioprotective potential of the ethanolic extract of SHA against DOX-induced cardiotoxicity. Serum CPK, SGPT, SGOT 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 (Mathew *et al*., 1985). Activities of these enzymes in serum decreased in SHA pretreated DOX induced group probably due to the protective effect of SHA on myocardium, which had reduced the extent of myocardial damage induced by DOX and thereby restricting 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 (Julicher *et al*., 1985; Singal and Iliskovic, 1998; Van Acker *et al*., 1997; Venditti *et al*., 1998). Oxygen radical generation affects the heart because doxorubicin and its toxic metabolite

doxorubicinol accumulate in cardiac tissue that has low antioxidant levels (De Jong *et al*., 1991).

Cardioprotective activity of SHA 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 (Hemnani and Parihar, 1998). Lipid peroxidation is known to cause cellular damage and is primarily responsible for ROS-induced organ damage (Halliwell and Gutteridge (1989). Our studies have shown that DOX-induced MI considerable increased in lipid peroxidation, which was significantly prevented by SHA pretreatment.

Redox cycling of DOX generates superoxide free radicals (Hrdina *et al*., 2000) due to conversion of quinone to semi-quinone moiety, whereas SOD enzyme dismutase 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 SHA reversed the SOD activity in 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 (Herman, 1991). 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 (Mukherjee *et al*., 2003). 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 SHA. 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 (Li and Singal, 2000).

Uric acid is considered to be a risk factor in the development of MI (Upston *et al*., 1999). We observed significance increase in the level of plasma uric acid 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 (Weir *et al*., 2003). This could be one of the reasons for the elevated levels of plasma uric acid in DOX induced rats.

GSH levels were also lowered significantly in DOX-treated animals, while pretreatment with SHA showed significant increase in GSH levels in DOX-treated animals at doses of 250 and 500 mg kg^{-1} in rats. Catalase activity was increased after DOX treatment and pretreatment of SHA further increased its activity significantly at 250 and 500 mg kg^{-1} dose levels. The increase in catalase activity in DOX-treated animals could be indicative of enhanced oxidative stress due to an adaptive myocardial mechanism.

Myocardial infarction (MI) 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 (Mathew *et al*., 1981). 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 (Kumari *et al*., 1990) and also reported that the decreased levels of phospholipids in DOX-treated rats might be due to the enhanced membrane degradation. Pretreatment with SHA 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 (Paritha and Devi, 1997). Studies have shown that high levels of LDL cholesterolhave a positive correlation with MI, whereas high levels of HDL cholesterol have a negative correlation with MI (Buring *et al*., 1992). These findings indicate the promising role of SHA as a cardioprotective agent against DOX-induced cardiotoxicity.

CONCLUSION

The present study shows that the administration of ethanolic extract of SHA has cardioprotective potential against Dox-induced cardiotoxicity. It provides experimental evidence that SHA augmented the myocardial antioxidant enzymes level, preserved histoarchitecture and improved cardiac performance following DOX administration. This cardioprotective activity of SHA 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 ethanolic extract of SHA may provide new directions for identification of cardioprotectives, which could be given concomitantly during Dox treatment.

REFERENCES

Angeloni, C., J.P.E. Spencer, E. Leoncini, P.L. Biagi and S. Hrelia, 2007. Role of quercetin and its *in vivo* metabolites in protecting H9c2 cells against oxidative stress. Biochimie, 89: 73-82.

Bergmeyer, H.U., K. Gowehn and H. Grassel, 1974. Enzymes as Biochemical Reagents. In: Methods of Enzymatic Analysis, Bergmeyer, H.U. and K. Gawehn (Eds.). 2nd Edn., Vol. 4. Verlag Chemie, Weinheim, Germany, ISBN-13: 9783527255986, pp: 38-39.

Brown, M.S. and J.L. Goldstein, 1986. A receptor-mediated pathway for cholesterol homeostasis. Science, 232: 34-47.

Buring, J.E., G.T. O'Connor, S.Z. Goldhaber, B. Rosner and P.N. Herbert *et al*., 1992. Decreased HDL2 and HDL3 cholesterol, Apo A-I and Apo A-II and increased risk of myocardial infarction. Circulation, 85: 22-29.

Caraway, W.T., 1963. Uric Acid. In: Standard Methods of Clinical Chemistry, Seligson, D. (Ed.). Vol. 4, Academic Press, New York, pp: 239-247.

Carlberg, I. and B. Mannervik, 1975. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J. Biol. Chem., 250: 5475-5480.

Castelli, W.P., R.J. Garrison, P.W. Wilson, R.D. Abbott, S. Kalousdian and W.B. Kannel, 1986. Incidence of coronary heart disease and lipoprotein cholesterol levels. The framingham study. JAMA, 256: 2835-2838.

De Bono, D.P. and N.A. Boon, 1992. Diseases of the Cardiovascular System. In: Davidson`s Principle of Practise of Medicine, Edwards, C.R.W. and I.A. Boucheir (Eds.). Churchill Livingstone, Hong Kong, pp: 249-340.

De Jong, J., W.S. Guerand, P.R. Schoofs, A. Bast and W.J.F. van der Vijgh, 1991. Simple and sensitive quantification of anthracyclines in mouse atrial tissue using high-performance liquid chromatography and fluorescence detection. J. Chromatogr. B: Biomed. Sci. Appl., 570: 209-216.

Foster, L.B. and R.T. Dunn, 1973. Stable reagents for determination of serum triglycerides by a colorimetric Hantzsch condensation method. Clin. Chem., 19: 338-340.

Grundy, S.M., 1986. Cholesterol and coronary heart disease: A new era. J. Am. Med. Assoc., 256: 2849-2858.

Hafeman, D.G., R.A. Sunde and W.G. Hoekstra, 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J. Nutr., 104: 580-587.

Halliwell, B. and J.M.C. Gutteridge, 1989. Free Radicals in Biology and Medicine. 2nd Edn., Clarendon Press, Oxford.

Hemnani, T. and M.S. Parihar, 1998. Reactive oxygen species and oxidative DNA damage. Indian J. Physiol. Pharmacol., 42: 440-452.

Herman, D., 1991. The aging process: Major risk factor for disease and death. Proc. Natl. Acad. Sci. USA., 88: 5360-5363.

Hrdina, R., V. Gersl, I. Klimtova, T. Simunek, J. Mach and M. Adamcova, 2000. Anthracycline-induced cardiotoxicity. Acta Medica, 43: 75-82.

Julicher, R.H., A. van der Laarse, L. Sterrenberg, C.H. Bloys van Treslong, A. Bast and J. Noordhoek, 1985. The involvement of an oxidative mechanism in the adrianıycin induced toxicity in neonatal rat heart cell cultures. Res. Commun. Chem. Pathol. Pharmacol., 47: 35-47.

Kapusta, L., J.M. Thijssen, J. Groot-Loonen, T. Antonius, J. Mulder and O. Daniels, 2000. Tissue Doppler imaging in detection of myocardial dysfunction in survivors of childhood cancer treated with anthracyclines. Ultrasound Med. Biol., 26: 1099-1108.

King, J., 1965. The Dehydrogenases or Oxidoreductases-Lactate Dehydrogenase. In: Practical Clinical Enzymology, King, J. (Ed.). Van Nostrand Company Ltd., London, UK., pp: 83-93.

Kloner, R.A. and S.H. Rezkalla, 2004. Cardiac protection during acute myocardial infarction: Where do we stand in 2004? J. Am. Coll. Cardiol., 22: 276-286.

Kumar, K.E., S.K. Mastan, N. Sreekanth, G. Chaitanya, G. Sumalatha and P.V. Krishna, 2008. Hypoglycemic and antihyperglycemic activity of aqueous extract of *Diospyros peregrina* fruits in normal and alloxaninduced diabetic rabbits. Pharmacologyonline, 3: 250-256.

Kumari, S.S., A. Verghese, D. Muraleedharan and V.P. Menon, 1990. Protective action of aspirin in experimental myocardial infarction induced by isoproterenol in rats and its effect on lipid peroxidation. Indian J. Exp. Biol., 28: 480-485.

Kurian, G.A., R.S.S. Srivats, R. Gomathi, M.M. Shabi and J. Paddikkala, 2010. Interpretation of inotropic effect exhibited by *Desmodium gangeticum* chloroform root extract through GSMS and atomic mass spectroscopy: evaluation of its anti ischemia reperfusion property in isolated rat heart. Asian J. Biochem., 5: 23-32.

Lee, H.K., Y.M. Choi, D.O. Noh and H.J. Suh, 2005. Antioxidant effect of Korean traditional lotus liquor (Yunyupju). Food Sci. Technol., 40: 709-715.

Lee, V., A.K. Randhawa and P.K. Singal, 1991. Adriamycin-induced myocardial dysfunction *In vitro* is mediated by free radicals. Am. J. Physiol., 261: H989-H995.

Li, T. and P.K. Singal, 2000. Adriamycin-induced early changes in myocardial antioxidant enzymes and their modulation by probucol. Circulation, 102: 2105-2110.

Loper, J., J. Goy, L. Rozensztajn, O. Bedu and P. Moisson, 1991. Lipid peroxidation and protective enzymes during myocardial infarction. Clin. Chim. Acta., 196: 119-126.

Mathew, S., V.P. Menon and P.A. Kurup, 1981. Changes in myocardial and aortic lipids, lipolytic activity and fecal excretion of sterols and bile acids in isoproterenol-induced myocardial infarction in rats. Indian J. Biochem. Biophys., 18: 131-133.

Mathew, S., P.V. Menon and P.A. Kurup, 1985. Effect of administration of vitamin A, ascorbic acid and nicotinamide adenine dinucleotide + flavin adenine dinucleotide on severity of myocardial infarction induced by isoproterenol in rats. Indian J. Exp. Biol., 23: 500-504.

Mohamed, H., M. Ons, E.T. Yosra, S. Rayda, G. Neji and N. Moncef, 2009. Chemical composition and antioxidant and radical-scavenging activities of *Periploca laevigata* root bark extracts. J. Sci. Food Agric., 89: 897-905.

Mohandas, J., J.J. Marshall, G.G. Duggin, J.S. Horvath and D. Tiller, 1984. Differential distribution of glutathione related enzymes in rabbit kidney: Possible implications in analgesic neuropathy. Cancer Res., 44: 5086-5091.

Mohun, A.F. and I.J. Cook, 1957. Simple methods for measuring serum levels of the glutamic-oxalacetic and glutamic-pyruvic transaminases in routine laboratories. J. Clin. Pathol., 10: 394-399.

Mukherjee, S., S.K. Banerjee, M. Maulik, A.K. Dinda, K.K. Talwar and S.K. Maulik, 2003. Protection against acute adriamycin-induced cardiotoxicity by garlic: Role of endogenous antioxidants and inhibition of TNF-κ expression. BMC Pharmacol., Vol. 3. 10.1186/1471-2210-3-16

Myers, C.E., W.P. McGuire, R.H. Liss, I. Ifrim, K. Grotzinger and R.C. Young, 1977. Adriamycin: The role of lipid peroxidation in cardiac toxicity and tumor response. Science, 197: 165-167.

Okinaka, S., H. Kumogai and S. Ebashi., 1961. Serum creatine phosphokinase activity in progressive muscular dystrophy and muscular disease. Arch. Neurol., 4: 520-525.

Padmanabhan, M. and P.S.M. Prince, 2006. Preventive effect of S-allylcysteine on lipid peroxides and antioxidants in normal and isoproterenol-induced cardiotoxicity in rats: A histopathological study. Toxicology, 224: 128-137.

Paritha, I.A. and C.S. Devi, 1997. Effect of á-tocopherol on isoproterenol-induced changes in lipid and lipoprotein profile in rats. Indian J. Pharmacol., 29: 399-404.

Rai, S., A. Wahile, K. Mukherjee, B.P. Saha and P.K. Mukherjee, 2006. Antioxidant activity of *Nelumbo nucifera* (sacred lotus) seeds. J. Ethnopharmacol., 104: 322-327.

Rajadurai, M. and P.S.M. Prince, 2005. Comparative effects of *Aegle marmelos* extract and alpha-tocopherol on serum lipids, lipid peroxides and cardiac enzyme levels in rats with isoproterenol-induced myocardial infarction. Singapore Med. J., 46: 78-81.

Singal, P.K. and N. Iliskovic, 1998. Doxorubicin-induced cardiomyopathy. N. Engl. J. Med., 339: 900-905.

Singh, G., A.T. Singh, A. Abraham, B. Bhat and A. Mukherjee *et al*., 2008. Protective effects of *Terminalia arjuna* against Doxorubicin-induced cardiotoxicity. J. Ethnopharmacol., 117: 123-129.

Staal, G.E.J., J. Visser and C. Veeger, 1969. Purification and properties of glutathione reductase of human erythrocytes. Biochim. Biophys. Acta (BBA)-Enzymology, 185: 39-48.

Sudhersan, C. and D. Padmanabhan, 1993. *In vitro* propagation of *Scilla hyacinthina* (Roth.) Macbr. Curr. Sci., 64: 710-710.

Upston, J.M., A.C. Terentis and R. Stocker, 1999. Tocopherol-mediated peroxidation of lipoproteins: Implications for vitamin E as a potential antiatherogenic supplement. FASEB J., 13: 977-994.

Van Acker, S.A., E. Boven, K. Kuiper, D.J. van den Berg and J.A. Grimbergen *et al*., 1997. Monohydroxyethylrutoside, a dose-dependent cardioprotective agent, does not affect the antitumor activity of doxorubicin. Clin. Cancer Res., 3: 1747-1754.

Venditti, P., M. Balestrieri, T. De Leo and S. Di Meo, 1998. Free radical involvement in doxorubicin-induced electrophysiological alterations in rat papillary muscle fibres. Cardiovasc. Res., 38: 695-702.

Weir, C.J., S.W. Muir, M.R. Walters and K.R. Lees, 2003. Serum urate as an independent predictor of poor outcome and future vascular events after acute stroke. Stroke, 34: 1951-1956.

Wilson, D.E. and M.J. Spiger, 1973. A dual precipitation method for quantitative plasma lipoprotein measurement without ultracentrifugation. J. Lab. Clin. Med., 82: 473-482.

Zhang, X.Z., 1992. Crop Physiology Research Methods. China Agricultural Press, Beijing, China.

Zhou, R., Q. Xu, P. Zheng, L. Yan, J. Zheng and G. Dai, 2008. Cardioprotective effect of fluvastatin on isoproterenol-induced myocardial infarction in rat. Eur. J. Pharmacol., 586: 244-250.

Zlatkis, A., B. Zak and A.J. Boyle, 1953. A new method for the direct determination of serum cholesterol. J. Lab. Clin. Med., 41: 486-492.