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RESEARCH ARTICLE

Efficacy of Herbal Mixture for the Treatment of Salbutamol Induced Myocardial Necrosis in Rabbits

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ABSTRACT

The current investigation was design to develop safer, efficacious and viable cardioprotective and antilipidemic herbal mixture to control cardiovascular diseases as new alternatives of synthetic drugs. Four medicinally valuable plants *Terminalia arjuna* (T), *Rauvolfia serpentina* (R), *Elettaria cardamomum* (C) and *Crataegus oxyacantha* (Cr) were selected for this study. Mixture of plants T, R, C, Cr was prepared with ratio 1:2:1:1 respectively. Both preventive and curative effects of plant mixture were studied. In preventive mode of treatment, rabbits were pretreated with plant mixture after that cardiotoxic compound was given. In curative mode of treatment, first cardiotoxicity was induced in rabbits then these cardio-intoxicated rabbits were treated with plant mixture. Cardiac marker enzymes, lipids profile and antioxidant enzymes were determined in experimental animals. Rabbits administrated with salbutamol (G-II) showed a significant ($P<0.05$) increase in cardiac marker enzymes, lipids and decrease in antioxidant enzymes as compared to positive control. However, pre and post administration of plant mixture appreciably restored their levels. Histopathological examination also confirmed the preventive and curative cardioprotective effects of plant mixture. As treatment of rabbits with plant mixture significantly ameliorated cardiotoxicity by bringing back myocardial biochemical parameters towards the normal levels. Therefore it can be optimally used in herbal preparations for the treatment of cardiovascular diseases with fewer side effects.

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INTRODUCTION

Modern green medicines have become a wave of hope that may find the biological friendly solution of health problems faced by the human beings. Since ages the use of medicinal plants has been the focus of complementary and alternative practitioners just because of their wisdom based observations. The recent trend of research on the green medicines has opened a new horizon in the field of alternative medicines that will sustain a lion share in health care system. Not to speaking of under developing countries like Pakistan, even advanced countries have not been able to control and provide the successful solution for the management and treatment of cardiovascular disorder. According to WHO estimate, 17.3 million people passed away from CVDs in 2008, this number will rise up to 23.6 million by 2030 which is an alarming situation (Cao *et al*., 2013). Myocardial infarction is happening at an epidemic proportion all over the world among them (Clarke *et al*., 2014). Contrary to the continuous efforts made all over the world to combat the heart diseases, rather to minimize the incidences, the number of cardiac patients are increasing therefore new drugs with new concepts is need of time. The available synthetic modern medicines only seem to be sufficient to cope with the rising cardiac problems but their side effects are taking their toll from their efficiency. Natural products from plants are green resources for invention of novel cardiovascular drugs with minimum side effects.

The therapeutic properties of plants have been widely explored in the current scientific advances all over the world owing to their powerful biological activities. Traditional remedial system documented several plants having cardioprotective properties. It revealed antioxidant, antibacterial, antihypertensive, and hypocholesterolemic properties of *Terminalia arjuna* due to its flavonoids, glycosides, minerals and tannins (Nema *et al*., 2012). *Rauvolfia serpentina* roots are reported to enclose almost

50 indole alkaloids (Deshmukh *et al*., 2012). *Elettaria cardamomum* seeds are documented to use for the treatment of cardiac disorders (Verma *et al*., 2009). *Crataegus oxyacantha* flowers, leaves and berries are reported to possess a diversity of flavonoid compounds (Rasmussen, 2011). Limited reports are available on mixture of medicinal plants. Plants when used in mixture show better potential because of synergetic effect and exhibit fewer side effects (Tende *et al*., 2015). It is therefore preferable to use herbal mixture instead of depending on single herb (Prince *et al*., 2008; Baber *et al*., 2012; Rajalakshmy *et al*., 2011).

This study has been designed and executed with objective to develop safer, efficacious and viable herbal mixture to control cardiovascular disorder as new alternatives of synthetic drugs. Four medicinally important plants including *Terminalia arjuna*, *Rauvolfia serpentina*, *Elettaria cardamomum* and *Crataegus oxyacantha* has been used to prepare a mixture with ratio 1:2:1:1 respectively, in order to enhance their cardioprotective action and to dispose of unwanted effects.

MATERIALS AND METHODS

Plant material and preparation of mixture: Four medicinally important plants (*Terminalia arjuna*-bark; *Rauvolfia serpentina*-roots; *Elettaria cardamomum*-fruit; *Crataegus oxyacantha*-fruit) were selected. Methanolic extracts were prepared to elicit bioactive constituents of plants. Methanolic extract of *Crataegus oxyacantha* was purchased from market. Other plants parts were air dried and grinded to powdered form. Powdered plant material was elicited with methanol in a reflux apparatus for 1 hour (plant versus solvent 1:10) (Aslam *et al*., 2012).

Different mixtures of these plants were prepared by varying their ratio and assessed for antioxidant potential (in-vitro). However, plant mixture T, R, C, Cr with ratio 1:2:1:1 exhibited maximum antiradical activity. Thereby, this plant mixture was chosen for the estimation of cardioprotective potential in animal model. This plant mixture was administrated to rabbits in the experimental period for the determination of cardioprotective potential. Both preventive and curative effects of plant mixture were studied by its pre and post administration.

Evaluation of cardioprotective activity: Male albino rabbits (n=21) of age 10-12 months weighing 1-1.5 kg were chosen for the evaluation of cardioprotective activity. The animals were housed in a well-ventilated animal house under standard conditions of environment with free access of water and diet *ad libitum*, under a 12h light/dark cycle. They were kept for one week adaptation period before the commencement of the experiment.

Biochemical assessment: Serum separated from blood samples was used for analysis of cardiac marker enzymes (creatine kinase-MB fraction CK-MB, aspartate transaminase AST, lactate dehydrogenase LDH, and alanine transaminase ALT) and lipids (triglycerides TG, low density lipoprotein cholesterol LDL-C, high density lipoprotein cholesterol HDL-C and total cholesterol TC) with commercially obtainable kits. Antioxidant Enzymes

Experimental design: After *acclimatization* period, animals were alienated into 7 groups. Every group consists of 3 rabbits.

Group No.	Group name	Treatment
I	Positive control	Standard diet only.
II	Negative control	Salbutamol only.
III	Baseline	Plant mixture 100mg/kg for 3 days.
IV	Preventive	Plant mixture 100mg/kg (21 days) afterward salbutamol 60mg/kg (3 days).
V	Curative	Salbutamol 60mg/kg (3 days) afterward plant mixture 500mg/kg (5 days).
VI	Herbal formulation curative	Salbutamol 60mg/kg (3 days) afterward herbal formulation (gemmo b-up, 500mg/kg bw.) for 5 days.
VII	Synthetic drug Curative	Salbutamol 60mg/kg (3 days) afterward Captopril (25 mg/kg), Amlodipine Besylate (5 mg/kg) and Atenolol (25mg/kg) for 5 days.

Catalase (CAT), Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) were estimated in heart tissues (Hameed *et al*., 2008).

Histopathological Examination: Hearts sections were cut at 5 µm and stained with hematoxylin and eosin and observed in a light microscope for histoarchitectural alterations.

Statistical analysis: All assessments were performed in triplicate and results were presented as mean±SE. Data were analyzed by one way ANOVA followed by Tukey's multiple comparison tests. $P<0.05$ was regarded as significant.

RESULTS

Preventive effect of plant mixture on cardiac markers and lipid profile: Table 1 presented the preventive effect of plant mixture on the levels of cardiac marker enzymes and lipid profile. Cardiotoxicity induced with salbutamol (G-II) exhibited a significant ($P<0.05$) increase in the level of cardiac enzymes (ALT, AST, Ck-MB, LDH) and lipids (TG, TC and LDL-C) whereas significant decrease ($P<0.05$) in the level of HDL-C (good cholesterol) as compared to positive control (G-I).

Baseline changes: Administration of plant mixture for 21 days to trial animals did not alter the level of cardiac enzymes and lipids as compared to positive control (G-I). A minor change in group III levels could be seen due to other components of the extract. These variations can be avoided by removing the other ineffectual constituents of the extract. These results were statistically insignificant ($P>0.05$) as compared to positive control.

Curative effect of plant mixture on cardiac markers and lipid profile: Table 2 and 3 depicted the curative effects of plant mixture on the levels of cardiac markers and lipid profile. Salbutamol induction to trial animals (G-II) significantly increased the level of all cardiac enzymes and altered the level of lipids due to its cardiotoxic effects. Lipids level increased by the administration of salbutamol except HDL (good cholesterol). HDL-C level decreased from its normal level in G-II (negative control). However, after treatment with plant mixture (G-V) significantly ($P<0.05$) restored the level of cardiac markers and lipids towards normal in dose dependent way. Plant mixture

Table 1: Preventive cardioprotective potential of plant mixture on cardiac marker enzymes and lipids in different experimental groups

Parameters	Concentration (mg/dL) in different groups			
	G-I	G-II	G-III	G-IV
ALT	44.1±0.9[a]	197.2±1.13[b]	44.15±0.68[a]	48.3±1.09[c]
AST	39.2±0.3[a]	165.4±0.51[b]	39.11±0.5[a]	66.2±1.7[c]
CK-MB	44.39±0.5[a]	88.7±1.34[b]	44.4±0.34[a]	50.4±0.5[c]
LDH	433.1±1.1[a]	834±1.64[b]	433.5±0.56[a]	453.8±2.9[c]
TG	184.9±1.34[a]	338.1±2.8[b]	185.1±0.2[a]	200.1±2.3[c]
TC	69.1±1.11[a]	185.4±1.5[b]	69.7±0.12[a]	83.6±2.1[c]
LDL-C	33.4±0.11[a]	69.5±0.45[b]	33.8±0.41[a]	35±2.21[a]
HDL-C	31.5±0.05[a]	22.2±0.21[b]	32.0±0.3[a]	37±1.19[c]

Values (mean±SE) bearing different superscript differ significantly (P<0.05). G-1 (positive control) received normal diet, G-II (negative control) received three doses of salbutamol 60mg/kg, G-III (baseline group) received plant mixture (100mg/kg) for 21 days and G-IV (preventive group) received plant mixture 100mg/kg (21 days) afterward salbutamol 60mg/kg (3 days). ALT (alanine transaminase), AST (aspartate transaminase), CK-MB (creatine kinase-MB fraction), LDH (lactate dehydrogenase), TG (triglycerides), TC (total cholesterol) LDL-C (low density lipoprotein cholesterol) and HDL-C (high density lipoprotein cholesterol).

exhibited curative results comparable to both standards (G-VI and VII).

Effect of plant mixture on antioxidant enzymes: Table 4 showed significant (P<0.05) decline in the level of all antioxidant enzymes in salbutamol treated animals (G-II) as compared to untreated animals (G-I). Baseline group (G-III) which was only treated with plant mixture exhibited noteworthy increase in antioxidant enzymes activities. Pretreatment with plant mixture (G-IV) significantly (P<0.05) protected against cardiac injury and prevented decrease in the level of antioxidant enzymes. Only superoxide dismutase level was slightly decreased from its normal level. Catalase and Glutathione peroxidase levels were higher than their normal levels. Post treatment of cardio intoxicated rabbits (G-V) with plant mixture restored and increased the level of all antioxidant enzymes as compared to positive control. Standard herbal formulation (G-VI) also not only restored the level of antioxidant enzymes but raised their levels from normal values. However, synthetic drug curative (G-VII) was unable to restore SOD level.

Histopathological examination: Histopathological examination of positive control (G-I) showed obvious integrity of myocardial cell membrane and intact cardiomyocytes (Fig.1a). No Inflammatory cell penetration was observed. Whereas negative control (G-II) showed cardiomyolysis, congestion and severe necrotic changes in heart (Fig.1b). The nuclei were found to be hyperchromatic and pyknotic. However, pre and post treatment rabbits with plant mixture prevented and cured cardiac injury. They showed less degree of necrosis and decreased infiltration of inflammatory cell. Preventive group (G-IV) rabbit's histoarchitecture was almost similar to that of G-I (Fig.1c). Photomicrograph of curative group (G-V) showed minor muscle damage.

DISCUSSION

Chemically provoked myocardial necrosis is a well-recognized typical model to examine the valuable outcomes of numerous drugs/medicinal plants on cardiac dysfunction (Ahsan *et al*., 2014; Sahreen *et al*., 2014). Salbutamol (Sal) is an artificial catecholamine and β_2-adrenergic agonist. Catecholamines are significant controllers of myocardial contractility and metabolism. Their excess quantity leads to ischemia, hypoxia and necrosis as a result of myocardial hyperactivity and coronary hypertension (Jahan *et al*., 2012; Yousefi *et al*., 2013; Beaulah *et al*., 2014). Salbutamol brings about severe myocardium stress and necrosis of the heart muscles due to its structural similarity with isoproterenol. Cardiac marker enzymes measurement particularly Creatine kinase (Ck-MB), Alanine transaminase (ALT), lactate dehydrogenase (LDH) and Aspartate transaminase (AST) act as diagnostic feature to determine the severity

Table 2: Curative effect of plant combination on the level of cardiac marker enzymes in different experimental groups

Enzymes	Experimental days	Enzymes concentration (1U/L) in different groups Experimental Groups				
		G-I	G-II	G-V	G-VI	G-VII
ALT	1	44.3±1.01[a]	197.2±1.13[b]	123.2±1.1[c]	169.50±1.8[d]	141.66±1.2[e]
	2	44.6±1.31[a]	196.5±1.89[b]	119.4±1.95[c]	106.5±1.44[c]	137.4±1.6[e]
	3	43.9±1.21[a]	198.25±1.9[b]	103.3±1.11[c]	103.5±1.76[c]	106.5±1.9[c]
	4	44.7±0.87[a]	195.6±1.3[b]	101.1±0.9[c]	93.40±1.34[c]	104.66±0.9[c]
	5	44.1±0.9[a]	197.5±1.6[b]	62.3±0.3f	61.50±0.78[f]	64.60±1.67[f]
AST	1	45.3±0.22[a]	165.4±0.51[b]	91.33±1.89[c]	87.70±0.65[c]	140.0±2.5[d]
	2	45.6±0.27[a]	166.1±1.89[b]	82.33±1.21[c]	56.90±0.49[e]	97.66±1.99[c]
	3	45.2±1.1[a]	165.8±1.76[b]	87.33±1.5[c]	53.40±0.89[e]	88.33±1.24[c]
	4	45.1±0.9[a]	165.1±1.11[b]	58.33±1.3[e]	46.20±0.19a	47.0±0.76a
	5	45.9±0.1[a]	164.9±1.21[b]	44.2±0.33[a]	44.50±0.7[a]	46.6±0.66a
CK-MB	1	44.5±0.23[a]	88.7±1.34[b]	70.1±1.55[c]	74.50±1.3[c]	59.8±0.11[d]
	2	43.4±0.12[a]	88.8±1.65[b]	64.66±1.78[c]	64.50±1.7[c]	51.0±0.78[d]
	3	44.7±0.5[a]	89.9±1.56[b]	53.33±0.74[d]	61.40±0.98[c]	47.66±0.98a
	4	45.0±0.7[a]	88.7±1.01[b]	48.33±0.9[a]	59.40±0.46[d]	45.33±0.37[d]
	5	44.1±0.8[a]	89.1±0.87[b]	45.4±0.43[a]	48.23±0.33[a]	44.33±0.56a
LDH	1	433.5±1.1[a]	834±1.64[b]	763.66±1.2[c]	810.5±1.97[b]	613.9±0.31[d]
	2	432.9±1.4[a]	835.1±2.4[b]	738.5±2.25[c]	571.5±1.20[e]	546.66±0.5[e]
	3	434.4±1.6[a]	834.7±3.7[b]	688.1±2.9[d]	534.6±1.54[e]	533.33±1.2[e]
	4	433.1±1.4[a]	835.6±6.9[b]	566.3±1.67[e]	488.5±1.88[a]	468.33±0.89[f]
	5	432.7±1.2[a]	835.3±1.43[b]	435.4±1.99[a]	429.4±1.93[a]	495.8±0.76[f]

Values (mean±SE) bearing different superscript differ significantly (P<0.05). G-1(positive control) received normal diet, G-II (negative control) received three doses of salbutamol 60mg/kg, G-III (baseline group) received plant mixture (100mg/kg) for 21 days and G-V (curative) received salbutamol 60mg/kg (3 days) afterward plant mixture 500mg/kg (5 days), G-VI (herbal formulation) received salbutamol 60mg/kg (3 days) afterward herbal formulation 500mg/kg (5 days), G-VII (synthetic drug) received salbutamol 60mg/kg (3 days) afterward captopril (25 mg/kg), amlodipine besylate (5 mg/kg) and atenolol (25mg/kg) for 5 days. ALT (alanine transaminase), AST (aspartate transaminase), CK-MB (creatine kinase-MB fraction) and LDH (lactate dehydrogenase).

Table 3: Curative effect of plant mixture on lipid profile) in different experimental groups

Lipids	Experimental days	Lipids concentration (mg/dL) in different groups Experimental Groups				
		G-I	G-II	G-V	G-VI	G-VII
TG	1	184.3±1.67[a]	338.1±2.8[b]	280.1±1.65[c]	290.8±1.23[c]	344.5±2.5[b]
	2	184.1±1.1[a]	339.4±1.54[b]	272.5±1.78[c]	206.5±2.33[c]	310.3±2.8[b]
	3	183.6±1.4[a]	338.6±1.87[b]	219.5±1.21[c]	187.1±1.88[a]	273.6±1.26[c]
	4	185.2±1.1[a]	338.3±1.22[b]	193.4±1.34[a]	185.3±1.35[a]	244.7±1.66[c]
	5	183.8±1.9[a]	340.2±2.1[b]	181.1±0.43[a]	183.5±1.66[a]	229.1±2.3[c]
TC	1	69.4±0.9[a]	185.4±1.5[b]	129.6± 1.5[c]	125.5±0.77[c]	135.6±1.4[c]
	2	68.3±1.5[a]	187.3±1.35[b]	104.3±1.11[c]	101.2±0.94[c]	105.3±1.9[c]
	3	68.1±0.6[a]	188.2±1.97[b]	77.6±1.45[d]	86.5±1.39[c]	91.3±1.1[c]
	4	68.9±0.45[a]	187.1±1.43[b]	73.3±0.77[d]	74.6±1.49[d]	89.9±0.9[c]
	5	69.1±0.32[a]	188.6±1.6[b]	68.1±0.89[a]	69.6±0.55[a]	84.2±1.2[c]
LDL-C	1	33.1±0.44[a]	69.5±0.45[b]	63.94±0.24[b]	59.13±0.65[c]	48.7±0.26[c]
	2	33.7±0.67[a]	67.3±0.47[b]	57.4±0.11[c]	44.5±0.47[c]	47.5±0.46[c]
	3	33.6±0.34[a]	68.7±0.21[b]	51.3±0.34[c]	39.3±0.66[c]	36.6±0.87[a]
	4	34.9±0.69[a]	67.5±1.43[b]	34.2±0.25[a]	35.5±0.57[a]	35.7±0.5[a]
	5	33.1±0.54[a]	68.3±0.98[b]	32.3±1.45[a]	34.2±0.43[a]	35.3 ±0.5[a]
HDL-C	1	30.4±0.21[a]	22.2±0.21[b]	23.9±0.45[b]	19.2±0.23[b]	21.4±0.3[b]
	2	31.5±0.27[a]	22.5±0.34[b]	25.6±0.56[b]	23.1±0.41[b]	23.1±0.4[b]
	3	30.1±0.5[a]	22.9±0.45[b]	25.5±0.65[b]	27.3±0.33[a]	27.3±0.1[a]
	4	31.6±0.4[a]	22.4±0.1[b]	29.1±0.21[a]	28.1±0.11[a]	29.7±0.3[a]
	5	30.4±0.11[a]	22.2±0.33[b]	32.3±0.81[a]	30.5±0.41[a]	40.6±0.41[c]

Values (mean±SE) bearing different superscript differ significantly (P<0.05). TG (triglycerides), TC (total cholesterol) LDL-C (low density lipoprotein cholesterol) and HDL-C (high density lipoprotein cholesterol). Groups remain the same as mentioned in footnote of Table 2.

Table: 4: Antioxidant Enzymes (Heart Tissue) in different Experimental Groups

Experimental groups	Antioxidant enzymes level (Unit/g of wt.)		
	SOD	CAT	GPx
G-I	92.95±0.55[a]	400.00±2.75[a]	800±2.45a
G-II	45.84±0.45[b]	58.00±0.21[b]	680±4.78[b]
G-III	97.48±0.56[a]	580.00±1.67[c]	2800±3.77[c]
G-IV	82.43±0.98[a]	480.00±1.55[d]	1000±2.83[d]
G-V	91.68±1.22[a]	603.00±1.89[e]	1100±1.45[d]
G-VI	98.84±1.42[a]	266.67±1.11[f]	2000±2.91[e]
G-VII	51.64± 0.33[b]	933.33±3.15[g]	1200±3.4[d]

Values (mean±SE) bearing different superscript in a column differ significantly (P<0.05). SOD (Superoxide Dismutase), CAT (Catalase) and GPx (Glutathione Peroxidase). Groups remain the same as mentioned in footnote of Table 2.

of myocardial infarction (Dianita *et al*., 2015). They reflect pathological alteration in myocardium. These enzymes leakage from cardiomyocytes into the blood stream occurs as a result of collapse of cellular and sub-cellular compartments (Jaffe *et al*., 2006). Appreciably prominent levels of these marker enzymes have been reported in chemically provoked myocardial injury. Quantity of enzymes released depends upon degree of cellular damage (Alla *et al*., 2007; Azmat *et al*., 2012; Khan *et al*., 2014).

In this study, increased levels of cardiac enzymes (CK-MB, AST, ALT, and LDH) and lipids (TG, TC, LDL-C) except HDL-C were observed in G-II (negative control). Increase in cardiac enzymes was due to unnecessary formation of free radicals which activate membrane permeability variations, causing the failure of functions and integrity of myocardial membranes. Disruption of cardiac myocytes can lead to rise in cardiac enzymes in the serum (Barman *et al*., 2013). Increase in lipids levels was due to increase in adenylate cyclase action leading to enhanced cAMP production, which in order direct to increased lipid accumulation in myocardium. Enhanced cholesterol level might be caused by HDL decrease as, it is recognized that HDL restrain the uptake of LDL by the arterial wall and assists the transfer of cholesterol from tissue to liver where it is catabolized and discharged from body. High triglycerides level was because of the restriction of protein lipase activity and so their transportation into the circulation (Adi *et al*., 2013).

However, pre and post treatment with plant mixture prevented increase in the levels of cardiac biomarkers and lipids (G-VI & V). Plant mixture rendered the myocytes less leaky by preventing myocardial membrane destruction and disorganization ensuing to reduced lipid peroxidation. This effect on cardiac biomarkers could be due to protective or membrane-stabilizing effect of plant mixture on the myocardium, secured the cardiac injury, and thus limiting the escape of these enzymes. Preventive effect of plant mixture might be due to increase in antioxidant enzymes by the administration of plants which are first line of defense against free radical injuries. The lipid lowering effect of plant mixture may be due to restriction of hepatic cholesterol biosynthesis, greater fecal bile acid discharge and activation of receptor mediated catabolism of LDL cholesterol and rise in the uptake of LDL by the liver from blood. Plant mixture may activate the production of HDL or increase the activity of the protein lipase. These findings were in concord with previous researcher (Prince *et al*., 2008; Hamid *et al*., 2013; Gomathi *et al*., 2014: Shatoor *et al*., 2014).

Antioxidant enzymes such as glutathione peroxidase, catalase and superoxide dismutase are first line of defense against oxidative stress. Excess amount of free radicals lead to decline in the activities of antioxidant enzymes (Eshaghi *et al*., 2012). In this study, significantly low level of antioxidant enzymes in G-II might be due to the excess formation of free radicals by salbutamol which reduced the level of these detoxifying enzymes. Plant mixture is noticed to generate a prominent effect on functional revival and improvement in the tissue defense antioxidant network. Along with enzymes and lipid study, histopathological examination also proved the remedial effects of plant mixture.

Conclusion: Treatment of rabbits with plant mixture significantly ameliorated cardiotoxicity by restoration of biochemical parameters towards the normal levels. Phytochemical constituents and antioxidants might be

responsible for the cardio-protection. As plant mixture effectively cured salbutamol induced myocardial necrosis, thus it can be optimally used in herbal preparations to form novel herbal drugs for the treatment of cardiovascular diseases.

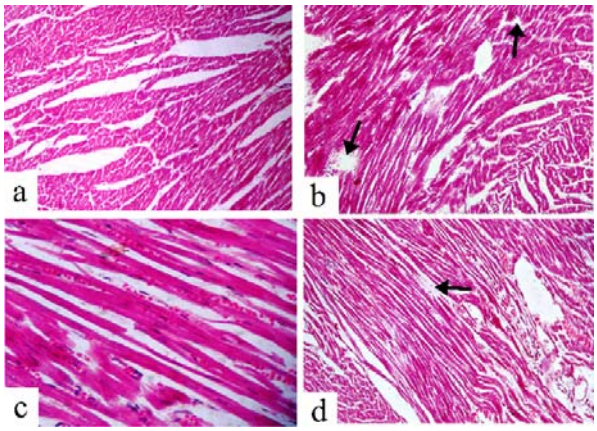

Fig. 1: Photomicrograph of rabbit's heart (a, b & d: 100X, c: 50X) after experimental period (24 days) (Hematoxylin-Eosin stained). a) G-I (positive control) exhibited all the myocardiocytes intact and prominent nucleus, b) G-II (negative control) exhibited cardiomyolysis, congestion and severe necrotic changes in heart (arrow), c) G-IV (preventive group) exhibited normal architecture of myocardiocytes and d) G-V (curative group) showed a mild muscle damage (arrow). H & E Stain.

Authors' contribution: NJ, KR and KMK conceived the idea and designed the project. SA performed research work, wrote the protocol and draft of the manuscript. NJ managed the laboratory facilities and funds to conduct the research and approved the final manuscript. KR and KMK provided guidance and KR provided laboratory facilities regarding clinical analysis. All authors read and approved the final manuscript.

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