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EFFECTS OF VITAMIN E, MORIN, RUTIN AND QUERCETIN AGAINST DOX INDUCED OXIDATIVE STRESS

[1]Raja Kumar Parabathina*,[2]E.Murlinath, [3]G.Kishore and [4]Kaza. Somasekhara Rao,

[1]Department of Biochemistry, NTR College of Veterinary Science, Gannavaram-521102, Krishna-District.Andhra Pradesh, India.
[2]Department of Physiology, NTR College of Veterinary Science, Gannavaram-521102, Krishna-District. Andhra Pradesh, India.
[3]Department of Biochemistry, ANU-Nuzvid Campus, Nuzvid-521201, Krishna-District. Andhra Pradesh, India.
[4]Professor of Inorganic Chemistry, Department of Chemistry,Nagaland University, Lumami HQr, Mokokchung Dt, NAGALAND -798627.

ABSTRACT : Doxorubicin (DOX) is a potent chemotherapeutic agent for the treatment of a variety of human malignancies. Increasing oxidative stress appears to play a major model to increase free radical producing and decrease activities of endogenous antioxidant enzymes. The present study, the development of oxidative stress was prevented by using natural antioxidant vitamin E (50 IU/kg body weight) and flavonoids morin, rutin and quercetin a poly phenolic compounds available in plants. The four weeks treatment of flavonoids (20mg/kg body weight) was affectively reduced the oxidative stress by DOX (10mg/kg body weight) on two dose treatment. The flavonoids maintained the biochemical parameters at optimum levels and they were significantly controlled by the flavonoids. The conclusion of this study is that the flavonoids are natural antioxidants, that they can ameliorate the biochemical markers. The authors suggest that the diet rich flavonoids can useful in the protection of certain diseases like cardiomyopathy (CAD, hypertension, heart failure and stroke), diabetes, cancers and oxidative stress etc., and this model can suggests that fields of Biochemistry and Pharmacology had good scope to evaluate the metabolic diseases and disorders.

Key words: Cardiomyopathy, Cancer, DOX, Flavonoids, Oxidative stress

INTRODUCTION

Doxorubicin (DOX) is a potential anti-cancer drug of all human malignancies. The dose related cardiomyopathy and congestive heart failure due to DOX has limited the use of this drug[1, 2]. The production of superoxide radical ions by oxidation-reduction cycling is critical in mediating the chronic cardio toxicity associated with the clinical use of DOX. Increased levels of oxygen species due to DOX have been observed directly by electron spin resonance spectroscopy[3,4] and indirectly by an increase in tissue malondialdehyde (MDA) which is a breakdown product of lipid peroxidation[5-7]. During DOX treatment serum enzymes SGOT, LDH, and CPK were increased significantly, particularly in the late stages of cardiac failure[1, 8-11] in mice[12,13], and rats[14, 15]. It is well documented that the major side effect, cardiomyopathy during DOX treatment in malignancies is due to oxidative stress. Cardiovascular diseases (coronary artery disease, hypertension, heart failure, and stroke) are the leading causes of death in human beings of modern days. Oxidative stress is the unifying mechanism for many cardiovascular risk factors like diabetes and obesity[16].

Oxidative damage causes a net stress on the normal body functions and may result in developing of many specific diseases[17.] Many free radicals exist in living systems are unstable and highly reactive, and disrupt the equilibrium of biological systems by damaging their major constituent molecules, leading eventually to cell death[17-20]. Free radicals can also react with serum LDL and resultant oxidized LDL damages the arterial wall[17.] To compensate for this, the cells have evolved both the enzymatic and non-enzymatic mechanisms to protect against oxidant's toxic effects. In heart GSHPx is extremely important because of its ability to use and remove organic and inorganic peroxides[6]. Administration of DOX resulted in a dose and time dependent decrease in myocardial glutathione enzymes activity in rats and mice[21-27]. Adjunct therapy of DOX along with antioxidants has become a practice to curb the oxidative damage by DOX in recent times.

Flavonoids are polyphenolic compounds present in the many plant derived foods. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Morin, rutin and quercetin by acting as antioxidants exhibited several beneficial effects, such as anti-inflammatory, antiallergic, antiviral as well as an anticancer activity. They have also been suggested to play a protective role in liver diseases, cataracts, and cardiovascular diseases. Quercetin acting as free radical scavengers where shown to exert a protective effect in reperfusion ischemic tissue damage[26-32].

The present study was aimed to know the ameliorative effects of flavonoids (morin, rutin, and quercetin) in DOX induced oxidative stress included to study biochemical changes in rats.

MATERIALS AND METHODS

Chemicals and reagent kits

The chemicals used in the present study were of analytical grade from E.Merck (India), SISCO Laboratories and Loba Chemicals and some chemicals were procured from Sd Fine Chemicals, Navi Mumbai, India. Vitamin E (Bio-E 400) was procured from the Dr.Reddy's Laboratories, Hyderabad and drug DOX (Doxopar-50) Parenteral drugs (India) limited, Indore, India. Some of the reagent kits were purchased from by the Laboratory of Ensure Biotech Pvt Limited, Hyderabad, and few reagent kits were purchased from the Transonic Bio-Medicals Limited, Solan (HP), was used for this study.

Experimental animals:

Thirty apparently healthy, white albino weighing 250 to 300 grams (about 3-6 months) were obtained from laboratory of small animal house, Department of Pharmacology, Dr.Pinnamaneni Siddhartha Institute of Medical Sciences and Research Foundation, Chinnoutapalli, Gannavaram, Krishna District, Andhra Pradesh, India. The animals were housed in the cages of departmental laboratory animal shed. All the animals were fed with control diet during a month acclimatization period.

Experimental Design:

30 rats were randomly divided into 5 groups and 6 in each group. Group I: Controls, normal feeding for 28 days and the 29th &30th day i.e. 2 days DOX 10 mg /kg body weight was given intra-venously. Group II: antioxidant vitamin E 50 IU/ kg body weight was given orally for 28 days and the 2 doses of DOX was administrated on 29th & 30th day. Group III, IV & V were fed orally with flavonoids morin, rutin and quercetin, 20mg/ kg body weight for 28 days and the DOX was administrated in 2 doses on 29th and 30th day as in group I and II rats. At weekly intervals blood samples were collected and analyzed for biochemical parameters. On 5th time i.e. after DOX treatment for two days, again blood samples were collected and analyzed for biochemical parameters.

METHODS

The biochemical parameters such as Protein profile such as total protein(Biuret method), albumin(BCG method), globulin, Lipid profile: total cholesterol (Cholesterol esterase-enzymatic method), triglycerides(Glycerol-3-phosphate oxidase- enzymatic method), HDL-cholesterol (Phosphotungstic acid method), LDL-cholesterol and VLDL-cholesterol(Friedwalds formula), Blood sugar (GOD-POD method), Urea (Berthelot method), Creatinine (alkaline picrate method), enzymes SGOT and SGPT (IFCC method), ALP (p-nitrophenyl phosphate method), minerals sodium (Modified Maruna and Trinders method), potassium (Turbidometric method), phosphorus (Fiske and Subbarow method).

Statistical Analysis

Graph Pad Instat Demo (Dataset1.ISD) software was used for the statistical analysis presented in the experiment. The experimental data were statistically analyzed using one-way analysis of variance (ANOVA), followed by Dunnett test for multiple comparisons versus control. Data were expressed as Mean±S.E.M. Differences were considered significant at P value of less than 0.01 and 0.05.

RESULTS AND DISCUSSION

It is evident that vit E increased total protein and albumin levels, in remaining all group total protein, albumin and globulin levels were decreased except in case of quercetin in which albumin and globulin were increased. According this study, the globulin levels were more compared with the albumin concentration, indicates that the rats had more immunopotency rather than the muscle formation (Table-1 and Fig-1).

TABLE: 1. Concentration of protein profile in the groups of rats before and after the treatment of DOX (the before values are average values of 4th week treatment of vitamin-E and flavonoids morin, rutin and quercetin)

Components	Total Protein		Albumin		Globulin	
Groups	Before	After	Before	After	Before	After
Control	9.15±0.37	8.8±0.47	2.58±0.15	2.56±0.10	6.56±0.25	6.23±0.52
Vit-E	9.56±0.33	10.35±0.61	2.38±0.15	2.55±0.12	7.18±0.43	7.8±0.63
Morin	9.03±0.33	8.98±0.15	2.61±0.20	2.51±0.17	6.41±0.37	6.46±0.24
Rutin	9.36±0.51	9.01±0.32	2.48±0.16	2.43±0.15	6.88±0.57	6.58±0.46
Quercetin	7.08±0.56**	7.84±0.42	3.75±0.30**	3.93±0.36**	3.33±0.34**	3.91±0.28**

* In a row differ significantly at P<0.05, ** In a row differ significantly at P< 0.01

The lipid profile included in the study was total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol was shown various changes on the treatment of DOX and flavonoids in this study. Table-2 shows that morin and quercetin decreased total cholesterol where as increased levels were found in rutin, vit E and control group. Morin, rutin, vit E increased triglycerides and decreased in quercetin group but levels were maintained normal in DOX treated control group. Morin and quercetin decreased LDL levels where as vit E increased but no significant changes found in rutin and control group animals. Quercetin and control group animals showed decreased HDL levels and there is no significant change was found in morin, rutin and vit-E animals. VLDL levels were increased in morin, rutin and Vit E group but decreased in quercetin and maintained normal in control group. The overall study on lipid profile concentration indicates that pretreatment of flavonoids was able to reduce the cardiac toxicity by reducing the lipid profiles in the rats even after the treatment of DOX. Pre treatment of vitamin E, flavonoids showed the reduction in lipid profile with concomitantly increase in HDL-cholesterol was observed. Decrease in the blood lipid profiles and increase in the HDL-cholesterol levels in flavonoid treated groups is due to inhibition of hepatic cholesterol biosynthesis, increased fecal bile acid secretion and stimulation of receptor mediated catabolism of LDL Cholesterol and increase in the uptake of LDL-cholesterol from blood by liver [33] (Fig-2).

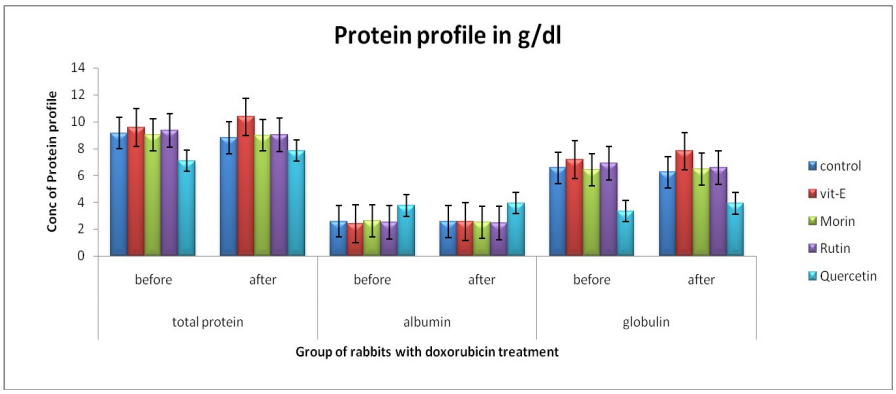

Fig: 1. Concentration of protein profile in the groups of rats before and after the treatment of DOX

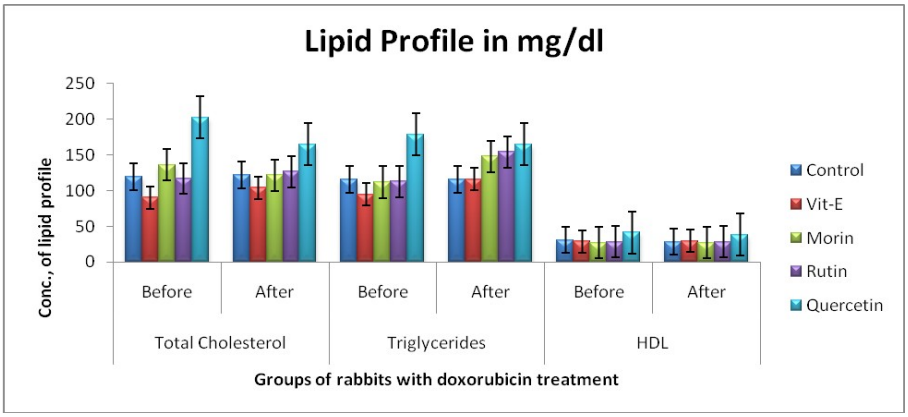

Fig: 2. Concentration of lipid profile in the groups of rats before and after the treatment of DOX

TABLE: 2. Concentration of lipid profile in the groups of rats before and after the treatment of DOX (the before values are average values of 4th week treatment of vitamin-E and flavonoids morin, rutin and quercetin)

Components	Total Cholesterol		Triglycerides		HDL	
Groups	Before	After	Before	After	Before	After
Control	119.69±1.76	121.48±2.78	115.59±2.47	115.16±3.45	31.02±0.49	28.53±0.59
Vit-E	90.31±3.55**	104.04±1.90**	94.79±1.69**	115.95±2.85	28.67±0.63	29.53±0.47
Morin	136.09±2.85**	121.66±2.06	111.97±1.83	147.94± 2.77**	27.23±0.64	27.32±0.22
Rutin	116.74±1.84	126.45±2.34	112.91±2.08	154.1±2.72**	28.25±0.42	28.27±0.39
Quercetin	202.24±2.66**	164.79±2.33**	178.81±2.83**	164.94±5.41**	41.27±1.53**	38.51±1.16

* In a row differ significantly at P<0.05, ** In a row differ significantly at P< 0.01

In control group after the treatment of DOX for 2 days at the end of 4 weeks on normal diet, reducing sugar was decreased (Table-3). Vitamin E and morin treated groups were decreased the blood sugar concentration, but rutin and quercetin treated group rats increased the blood sugar concentration even after the treatment of DOX. Depletion in the reducing sugar due to DOX may be affected the release of insulin from the pancreas, or either due to hyper metabolic consumption of energy or diseases related to liver [34].(Fig-3).

TABLE: 3. Concentration of blood sugar in the groups of rats before and after the treatment of DOX (the before values are average values of 4th week treatment of vitamin-E and flavonoids morin, rutin and quercetin).

Components	Blood sugar	
Groups	Before	After
Control	71.4±2.99	62.11±2.23
Vit-E	71.28±2.07	68.48±3.22
Morin	68.21±2.70	66.35±1.80
Rutin	68.4±0.90	69.78±2.39
Quercetin	109.19±5.28**	118.74±4.97**

* In a row differ significantly at P<0.05, ** In a row differ significantly at P< 0.01

The serum SGOT and SGPT levels were decreased in group I-V but ALP levels were increased in group IV and V and decreased in group II and III but maintained normal in control group animals (Table-4). The decreased levels of SGOT and SGPT had no significant affect in the metabolism; whereas increased levels may lead to severe liver disorders like necrosis and myocardial infarction, which are indicators of poor quality protein in diets fed[35]. ALP levels were also maintained in normal range that indicates no significant affect on the rats. Even though the values were decreased in serum enzymes by DOX treatment had no specific affects, but the flavonoids were protected at optimum levels to maintain the normal range (Fig-4).

Quercetin decreased the nitrogenous profile but remaining groups increased blood urea, BUN and uric acid (Table-5 and Fig-5). Increased serum urea concentration may suggest an increase in activities of urea enzymes orninthine carboxyl transferase and orginase which may also indicate kidney damage[36]. The normal range of values obtained implied therefore that the dietary proteins of the CPB diets and the control were well utilized[37]. The urea levels were low because of low intake of protein or low catabolism of muscle proteins. The nitrogenous catabolic pathways may increase the formation uric acid is due to high protein intake or nucleotide catabolism.

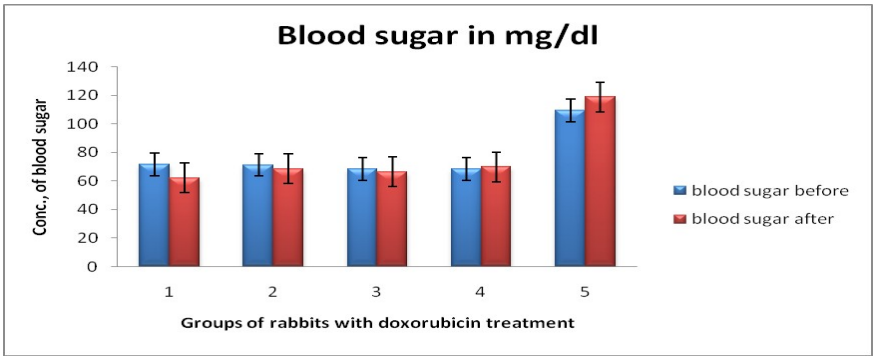

Fig: 3. Concentration of blood sugar in the groups of rats before and after the treatment of DOX

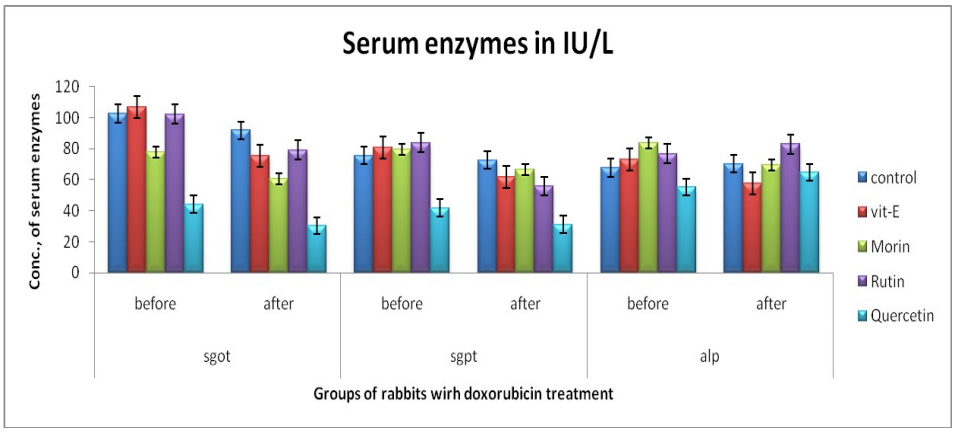

Fig: 4. Concentration of serum enzymes in the groups of rats before and after the treatment of DOX

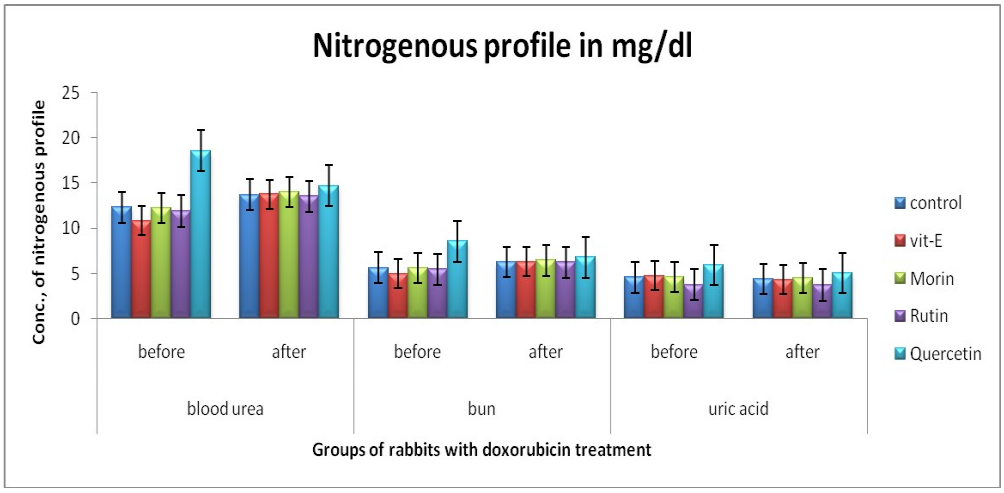

Fig: 5. Concentration of nitrogenous profile in the groups of rats before and after the treatment of DOX

TABLE: 4. Concentration of serum enzymes in the groups of rats before and after the treatment of DOX (the before values are average values of 4th week treatment of vitamin-E and flavonoids morin, rutin and quercetin)

Components	SGOT		SGPT		ALP	
Groups	Before	After	Before	After	Before	After
Control	102.71±2.14	91.8±3.65	75.62±2.85	72.54±2.93	67.6±1.82	70.21±1.35
Vit-E	106.86±2.17	75.35±2.61**	80.56±1.16	61.68±1.74*	72.85±2.12	57.56±1.37**
Morin	77.85±2.34**	60.43±2.06**	79.31±3.03	66.35±2.44	83.59±1.74**	69.39±1.64
Rutin	102.28±3.21	79.2±2.32*	83.81±1.65*	55.77±2.88**	76.71±2.40*	82.86±2.26**
Quercetin	44.13±2.53**	30.41±3.15**	41.85±1.19**	31.25±1.68**	55.36±1.06**	64.78±1.54

* In a row differ significantly at P<0.05, ** In a row differ significantly at P< 0.01

TABLE: 5. Concentration of nitrogenous profile in the groups of rats before and after the treatment of DOX (the before values are average values of 4th week treatment of vitamin-E and flavonoids morin, rutin and quercetin)

Components	Blood Urea		BUN		Uric acid	
Groups	Before	After	Before	After	Before	After
Control	12.31±0.37	13.73±0.72	5.66±0.17	6.31±0.33	4.57±0.24	4.41±0.24
Vit-E	10.86±0.72	13.75±0.52	4.99±0.33	6.32±0.24	4.76±0.41	4.31±0.33
Morin	12.25±0.59	14±0.85	5.63±0.27	6.44±0.39	4.61±0.16	4.49±0.20
Rutin	11.91±0.78	13.55±0.70	5.48±0.35	6.23±0.32	3.76±0.13	3.72±0.26
Quercetin	18.6±0.97**	14.72±0.93	8.55±0.44**	6.77±0.42	5.97±0.81	5.06±0.83

* In a row differ significantly at P<0.05, ** In a row differ significantly at P< 0.01

TABLE: 6. Concentration of minerals in the groups of rats before and after the treatment of DOX (the before values are average values of 4th week treatment of vitamin-E and flavonoids morin, rutin and quercetin)

Components	Sodium		Potassium		Phosphorus	
Groups	Before	After	Before	After	Before	After
Control	151.65±3.05	135.03±3.95	6.56±0.64	6.66±0.27	6.13±0.30	6.02±0.16
Vit-E	136.32±2.00**	116.31±1.16**	6.72±0.21	6.97±0.56	6.53±0.35	5.91±0.31
Morin	131.7±1.55**	124.45±1.99*	6.07±0.46	8.01±0.75	6.56±0.24	6.27±0.38
Rutin	146.31±2.04	168.4±2.08**	6.87±0.56	6.58±0.47	6.08±0.40	6.03±0.25
Quercetin	142.78±2.38*	176.63±3.86**	4.32±0.33**	3.31±0.15**	5.75±0.39	6.36±0.25

* In a row differ significantly at P<0.05, ** In a row differ significantly at P< 0.01

Table-6 shows the sodium levels were decreased in I-III and increased in IV and V (fig-6). Potassium levels increased in group III (Fig-7), decreased in group V and maintained normal in group I, II, IV. Phosphorus levels were normal during entire experimental period in group I, III and IV and mount in group V but significantly decreased in group II (Fig-8). Flavonoids significantly increased sodium concentration due to increase the transport mechanism of the free radicals which were formed on DOX treatment or reduction in the water reabsorption in kidneys. The increase in potassium may be due to decrease in the reabsorption of water at kidneys or renal failure by DOX. The decreased levels of potassium in quercetin treated group may be due to malabsorption syndrome. This study indicates that the DOX-induced oxidative stress had significantly ameliorated by the flavonoids by maintaining the phosphorus concentration. DOX induced oxidative stress was confirmed in by the specific changes occurred in the biochemistry. The changes in biochemical parameters due to DOX treatment in rats did not alter significantly. Interestingly the rats of group III, IV and V which were provided the flavonoids (morin, rutin and quercetin) along with diet showed remarkable antioxidant changes indicating the amelioration of oxidative stress induced by DOX there by cardiomyopathy.

Thus, this study concludes that the flavonoids morin, rutin and quercetin can ameliorate the oxidative stress induced cardiomyopathy. Dietary intervention of flavonoids may be a good practice to protect myocardium in the DOX treatment of cancers or malignancies. Flavonoids like quercetin, rutin and morin could be good adjunct molecules in DOX therapy. Further investigations are necessary to prove clinical efficacy and use of flavonoids in amelioration of DOX oxidative stress leading to cardiomyopathy.

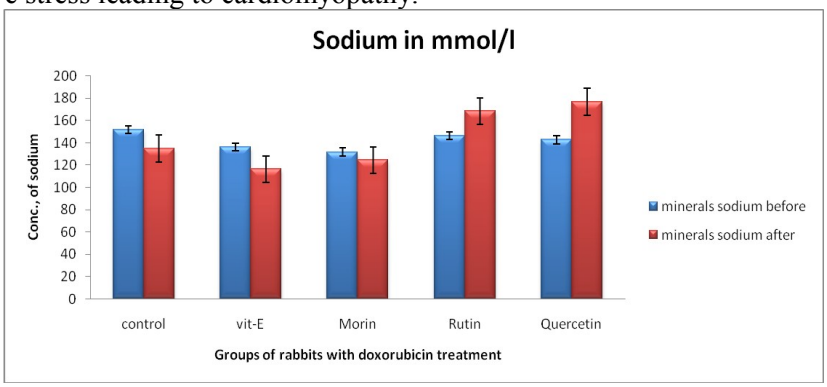

Fig: 6. Concentration of Sodium in the groups of rats before and after the treatment of DOX

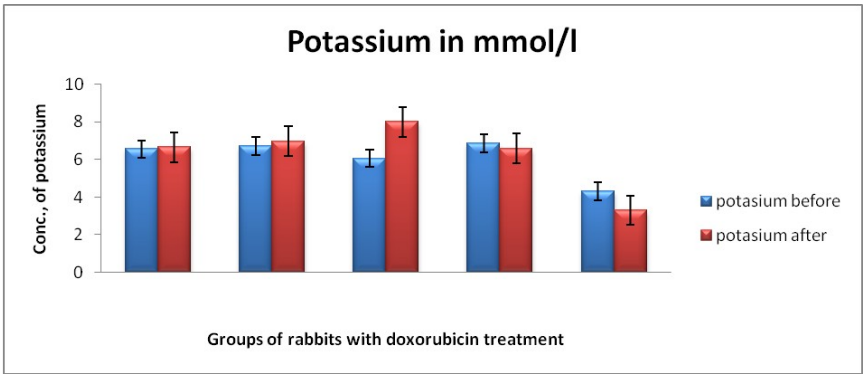

Fig: 7. Concentration of Potassium in the groups of rats before and after the treatment of DOX

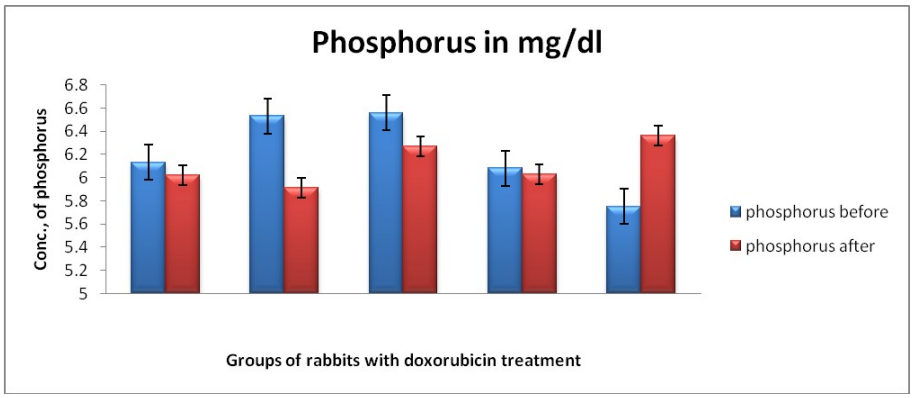

Fig: 8. Concentration of Phosphorus in the groups of rats before and after the treatment of DOX

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