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Vitamin-E, Morin, Rutin, Quercetin prevents tissue biochemical changes induced by Doxorubicin in oxidative stress conditions: Effect on heart, liver and kidney homogenates

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ABSTRACT

Flavonoids in the diet useful for the control of stress which plays an essential role in control of the development of cardiomyopathy in doxorubicin treatment on cancer therapy. The present study represents the protective effect of vitamin –E, flavonoids morin, rutin and quercetin on heart, liver and kidney homogenate biochemical parameters such as protein, sugar, urea, blood urea nitrogen, creatinine and phosphorus. Treatment of experimental rabbits with flavonoids and vitamin-E for 28 days and two doses of doxorubicin on 29th and 30th day, than the animals were sacrificed. The tissues of heart, liver and kidneys were collected and tissue homogenates were prepared by using phosphate buffer saline solution. The homogenate examined for biochemical parameters protein, sugar, urea, creatinine and phosphorus by standard methods. The values were tested with Dunnette multiple significant test $P<0.01$ and $P<0.05$. The results of this study strongly indicate that flavonoids had the protective effect against doxorubicin induced oxidative stress in the experimental rabbits. This study also suggests that rabbits were good animal model for the experiments in the pharmacology and biochemistry.

Key words: Doxorubicin, cardiomyopathy, flavonoids, oxidative stress, tissue homogenate.

INTRODUCTION

The immense value of doxorubicin in treating a variety of solid and hematologic malignant conditions in unquestioned. Its usefulness is limited by its cardiotoxicity, an adverse effect of the drug that can preclude its use in some patients and limit the duration of its use in many others [1-6]. The deleterious effects of doxorubicin on the heart can be categorized into acute effects and a chronic cardiomyopathy. The overall prevalence of doxorubicin cardiomyopathy is 1.7 % to 6.8 % [6-13] and dependent on total dose. Because of its prominence in control of human cancers, several agents have been investigated in an attempt to reduce its toxicity [14-15]. Among these is vitamin-E [16-19] and flavonoids morin, rutin and quercetin. Supportive evidence indicates that vitamin-E does not obviously interfere with the effectiveness of doxorubicin as an antitumor agent [20-21]. Food is the major sources of antioxidants like vitamin C, vitamin E, selenium, and carotenoids that may help in providing protection against diseases by contributing, along with enzymes involved in scavenging of free radicals, to the total antioxidant defense system of the human body. Flavonoids are polyphenolic compounds present in the many plant derived foods. Many epidemiological studies have shown that flavonoid intake is inversely related to mortality from coronary heart disease and to the incidence of heart attacks. Recent studies have demonstrated that flavonoids found in fruits and vegetables may also act as antioxidants. Like alpha-tocopherol (vitamin E), flavonoids contain chemical structural elements that may be responsible for their antioxidant activities. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. Morin, rutin and quercetin by acting as antioxidants exhibited several beneficial effects, such as anti-inflammatory, antiallergic, antiviral as well as an anticancer activity. Quercetin, the most abundant dietary flavonol, is a potent antioxidant because it has all the right structural features for free radical scavenging activity. It is evident that the flavonoids play an important role in the various types of metabolic activities of life. They have also been suggested to play a protective role in liver diseases, cataracts, and cardiovascular diseases.

In this communication, we present evidence for a protective effect of vitamin-E, flavonoids in a comparatively small dosage against doxorubicin-induced acute toxicity in rabbits. Keeping in view of the above the present study was aimed to study the changes in the biochemical parameters in the tissue homogenates of heart, liver and kidney.

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 Doxorubicin [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, New Zealand white rabbits weighing 2.5 to 3.0 kg [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. All animals were kept under uniform managerial and standard hygienic conditions through the experimental period. All the rabbits were weighed and randomly housed, two animals in each cage. The cages were located in a well ventilated house and all the animals had free access to feed and water at all times. All animals were treated in accordance with the principles of laboratory animal care and the experimental protocol has been approved by the animal ethical committee of university.

Experimental Design

Thirty rabbits were randomly divided into five groups and six animals in each group. All the animals of group II-V were treated with a normal diet along with vitamin-E, morin, rutin and quercetin about 28 days orally. The group-I rabbits were fed on normal diet only which considered as Controls for the present study. On 29^{th} and 30^{th} day doxorubicin [10mg/ kg body weight] was administrated intra-venously for all groups of rabbits and sacrificed the animals on 31^{st} day.

Preparation of tissue for biochemical assay:

Each heart, liver and kidney was quickly removed from the sacrificed, washed with ice cold saline solution, minced and homogenized in 50 mM phosphate buffer, pH 7.4 and centrifuged at 3000g for 15 min at 4^{0C}. The supernatant was used for all the assays.

Experimental Methods:

Protein contents in liver, heart and kidney homogenates were determined by the Folin-Ciocalteau Method [22]. Specific activity of the enzyme estimated in tissues homogenates were determined by dividing their enzyme activity with their respective protein content [mg protein].

The reducing sugars in liver, heart and kidney homogenates were estimated by Nelson and Somayogi method [23]. The protein content was precipitated by zinc hydroxide. The filtrate is heated with alkaline copper reagent and the reduced copper formed is treated with arsenomolybdate reagent resulting in the formation of violet colour which was read in spectrophotometer at 500nm. The intensity of the colour produced is proportional to the amount of reducing sugar present the tissues.

Urea in liver, heart and kidney tissue homogenates were determined by the diacetyl monoxime method [DAM]. Urea reacts with acidic diacetyl monoxime during a short heating period. The presence of thiosemicarbazide and cadmium ions in the reagent intensifies the pink colour and decreases its photosensitivity; the colour is measured at 540 nm [24].

Creatinine content in Liver, heart and kidney were determined by the alkaline picrate method. Creatinine reacts with alkaline picrate to form a yellow-red colour known as the Jaffe reaction. Many substances present chiefly within the cells contribute to a false high colour and therefore analysis of plasma or serum is preferable. [25].

Phosphorus is the chief mineral in the energy transportation in tissues. Thus phosphorus determination in tissues plays an important role. Phosphorus in liver, heart and kidney were determined by the Fiske and Subbarow method. The phosphate containing solutions are treated

with molybdic acid to produce phoshomolybdic acid which is reduced by the addition of 1, 2, 4-aminonaphtholsulphonic acid to giving a blue colour. This is read at 660 nm. [26].

Statistics:
The significance of differences between the control and the test groups was established by the Dunnette multiple comparison test with the significance level set at P< 0.05 and P<0.01.

RESULTS AND DISCUSSION

The present study aimed that the changes of biochemical parameters in the liver, heart and kidneys on the treatment of the anti cancer drug doxorubicin and the protection of the tissues by feeding vitamin-E and flavonoids morin, rutin and quercetin. The biochemistry of the tissues was the index of the metabolic disorders. The results were here as follows.

Table: 1. Mean ± SEM of Protein in mg/dL Vit-E, Morin, Rutin and Quercetin treatment against doxorubicin in tissue homogenates

Total Protein in mg/dl		After the treatment of the Doxorubicin	
Group Animals	**Liver**	**Heart**	**Kidney**
Control	282.33±5.32	201.33±18.79	125.00±17.73
Vitamin-E	312.33±5.32**	131.00±4.25**	115.00±3.54
Morin	216.00±2.83**	105.33±4.61**	142.33±1.78
Rutin	249.00±7.80**	108.33±1.08**	178.33±1.08**
Quercetin	96.40±2.55**	46.60±1.13**	51.98±1.23**

**In a row differ significantly at P<0.05 (Between weeks within treatment). **In a row differ significantly at P<0.01 (Between weeks within treatment)*

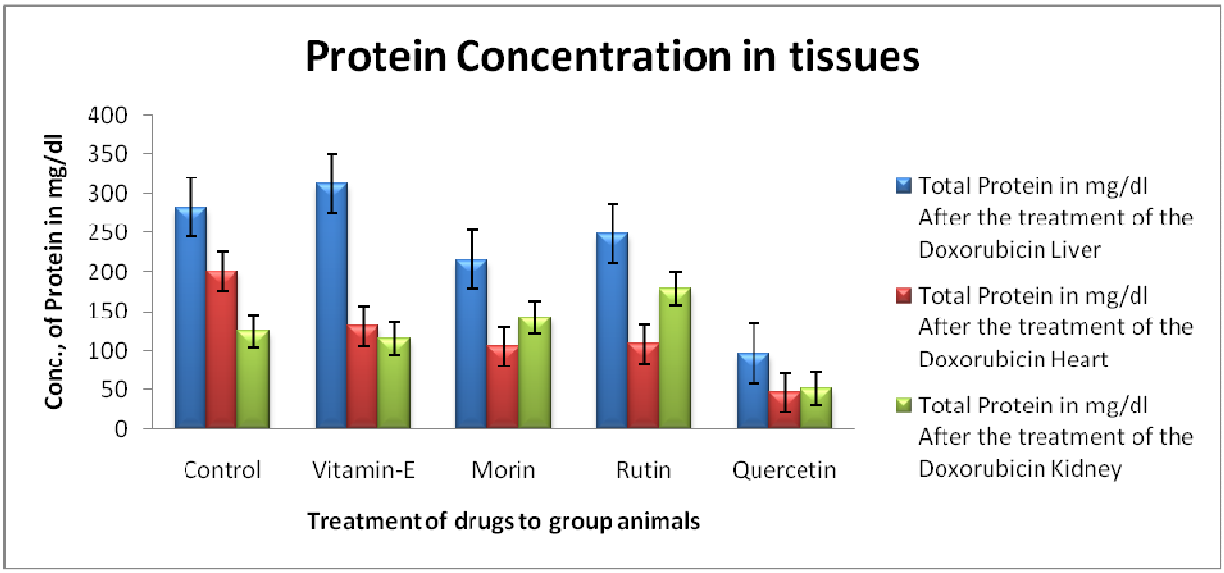

Fig: 1. Mean ± SEM of Protein in mg/dL Vit-E, Morin, Rutin and Quercetin treatment against doxorubicin in tissue homogenates

The total protein concentration in mg/dl of liver, heart and kidney were shown in table 1 and figure 1. The concentration values were significantly (P<0.05 & P<0.01) varied in all the tissues

and groups. In the tissues of liver, heart and kidneys, shows an significant change of the total protein concentration was observed in all the groups. The values were representing that the doxorubicin shows effects on the heart tissue which may an indication that control group rabbits had the increased protein whereas the reaming group animals had lower protein when compared with the control group but the quercetin treated group animals had very little protein in tissue.

The reducing sugar concentration in mg/dl of liver, heart and kidney were shown in table 2 and figure 2. The concentration values were significantly varied in all the tissues and groups. In liver, heart and kidney, a show that a significant change of the reducing sugar concentration in all the groups. The results of reducing sugar in control group tissues had variation but the vitamin-E and flavonoids treated groups were maintained the sugar values constantly. This study shows that reducing sugar concentration was decreased on doxorubicin treatment, where as the sugar value was maintained in the flavonoids and vitamin-E treated groups. It is evidence for the flavonoids were useful for maintenance of the energy metabolism in tissues.

Table: 2. Mean ± SEM of Reducing sugar in mg/dL Vit-E, Morin, Rutin and Quercetin treatment against doxorubicin in tissue homogenates

Reducing Sugar in mg/dl	After the treatment of the Doxorubicin		
Group Animals	**Liver**	**Heart**	**Kidney**
Control	22.08±1.35	24.68±0.22	32.42±1.82
Vitamin-E	30.60±0.42**	30.15±0.10	33.78±0.86
Morin	38.44±1.10**	28.36±2.38	37.09±2.06
Rutin	31.77±1.25**	34.19±0.57**	25.75±0.53*
Quercetin	16.98±0.94*	30.89±2.31*	23.35±2.11**

**In a row differ significantly at P<0.05 (Between weeks within treatment). **In a row differ significantly at P<0.01 (Between weeks within treatment)*

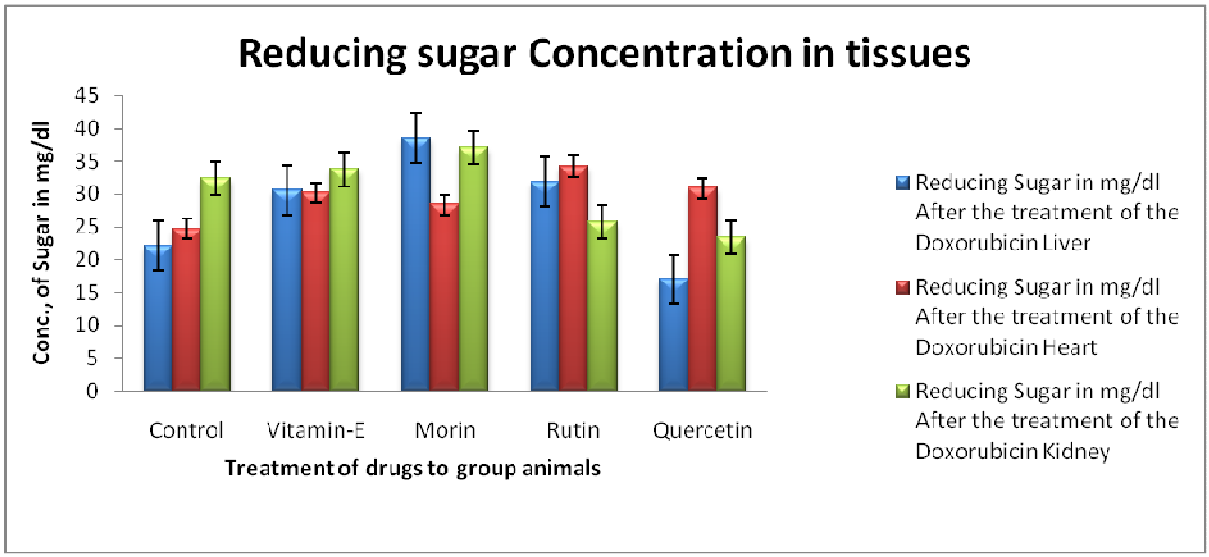

Fig: 2. Mean ± SEM of Reducing sugar in mg/dL Vit-E, Morin, Rutin and Quercetin treatment against doxorubicin in tissue homogenates

The Urea concentration in mg/dl of liver, heart and kidney were shown in table 3 and figure 3. The concentration of urea was significantly differed in all the tissues and all the groups. The tissues of liver, heart and kidneys were varied in the formation of urea based on the protein

degradation in their tissues. The rutin treated animals had high protein breakdown indicates that urea concentration was increased. Here, an interesting issue of urea metabolism in liver indicates that higher concentration of urea in all group rabbits is an evidence for the protein degradation is the effect of the doxorubicin. The heart and kidney tissues were also had the effect of doxorubicin but the flavonoids treated groups shown mixed values of urea concentration.

Table: 3. Mean ± SEM of Urea in mg/dL Vit-E, Morin, Rutin and Quercetin treatment against doxorubicin in tissue homogenates

Urea in mg/dl	**After the treatment of the Doxorubicin**		
Group Animals	**Liver**	**Heart**	**Kidney**
Control	58.50±0.75	45.25±1.91	64.88±0.75
Vitamin-E	49.72±2.07**	47.53±0.51	53.36±1.37**
Morin	73.01±0.47**	46.65±0.10	75.04±1.09**
Rutin	67.28±0.56**	64.68±0.61**	70.42±1.35*
Quercetin	53.68±1.86	62.18±1.05**	71.00±0.82**

In a row differ significantly at P<0.05 (Between weeks within treatment). In a row differ significantly at P<0.01 (Between weeks within treatment)*

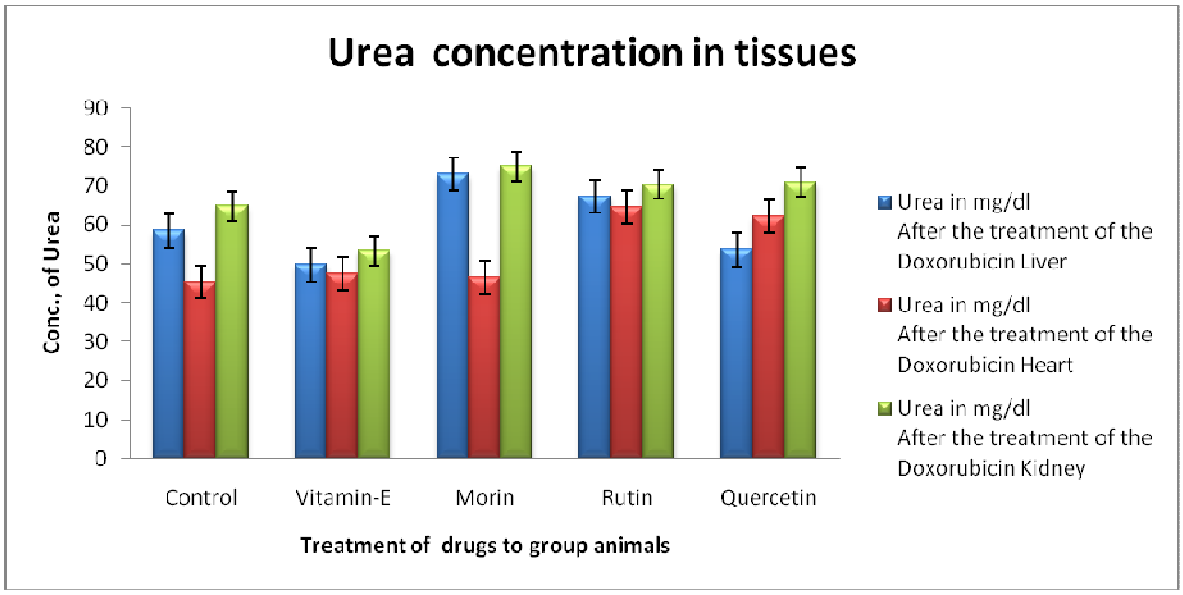

Fig: 3. Mean ± SEM of Urea in mg/dL Vit-E, Morin, Rutin and Quercetin treatment against doxorubicin in tissue homogenates

The creatinine concentration in mg/dl of liver, heart and kidney were shown in table 4 and figure 4. The creatinine concentration of liver was shown as a higher value when compared with the tissues of heart and kidney. According to the serum creatinine values the values obtained in the tissues were more because of high muscle protein breakdown or muscle activity of rabbits. Quercetin treated animals were only maintained lower concentrations of creatinine in all the tissues, whereas remains were had higher creatinine. This indicates that the doxorubicin action was controlled by quercetin.

Table: 4. Mean ± SEM of Creatinine in mg/dL Vit-E, Morin, Rutin and Quercetin treatment against doxorubicin in tissue homogenates

Creatinine in mg/dl	After the treatment of the Doxorubicin		
Group Animals	**Liver**	**Heart**	**Kidney**
Control	5.12±0.05	3.49±0.03	3.84±0.07
Vitamin-E	4.54±0.16	3.59±0.04	3.38±0.04*
Morin	3.81±0.08**	3.47±0.09	3.62±0.06
Rutin	4.56±0.07	4.26±0.07**	3.87±0.09
Quercetin	2.45±0.26**	1.61±0.06**	2.55±0.17**

***In a row differ significantly at P<0.05 (Between weeks within treatment). ****In a row differ significantly at P<0.01 (Between weeks within treatment).*

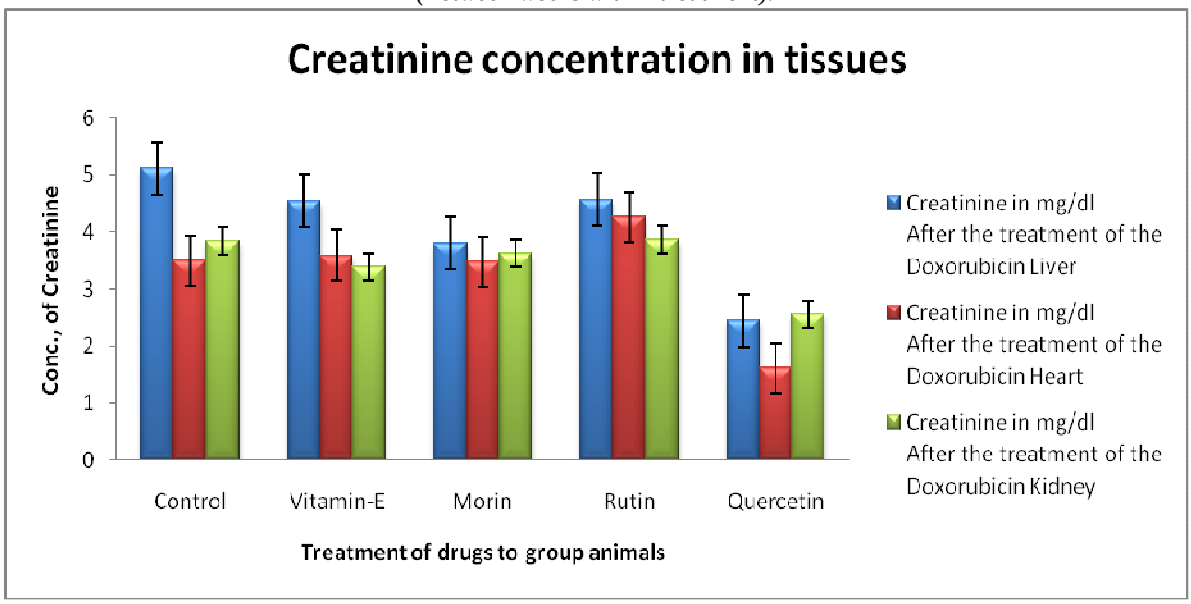

Fig: 4. Mean ± SEM of Creatinine in mg/dL Vit-E, Morin, Rutin and Quercetin treatment against doxorubicin in tissue homogenates

Table: 5. Mean ± SEM of Phosphorus in mg/dL Vit-E, Morin, Rutin and Quercetin treatment against doxorubicin in tissue homogenates

Phosphorus in mg/dl	After the treatment of the Doxorubicin		
Group Animals	**Liver**	**Heart**	**Kidney**
Control	6.56±0.28	6.44±0.29	8.10±0.43
Vitamin-E	7.10±0.07	6.25±0.21	7.62±0.34
Morin	7.05±0.67	6.00±0.06	8.55±0.33
Rutin	6.94±0.24	6.36±0.35	7.79±1.24
Quercetin	7.52±0.25	6.77±0.03	8.25±0.21

***In a row differ significantly at P<0.05 (Between weeks within treatment). ****In a row differ significantly at P<0.01 (Between weeks within treatment)*

The Phosphorus concentration in mg/dl of liver, heart and kidney were shown in table 5 and figure 5. The concentration of phosphorus was maintained significantly in all the tissues and groups. This indicates that the rabbits were fighting against the doxorubicin effects on their energy metabolism. This study concludes that the falvonoids may have protective effects against

the treatment of the doxorubicin. The cancer patients advised to have flavonoids rich diet may helpful for the maintenance of the biochemistry of the tissues for proper functioning of the metabolic activities of the tissues. The rabbits were good experimental models for the studies of pharmacology and biochemistry.

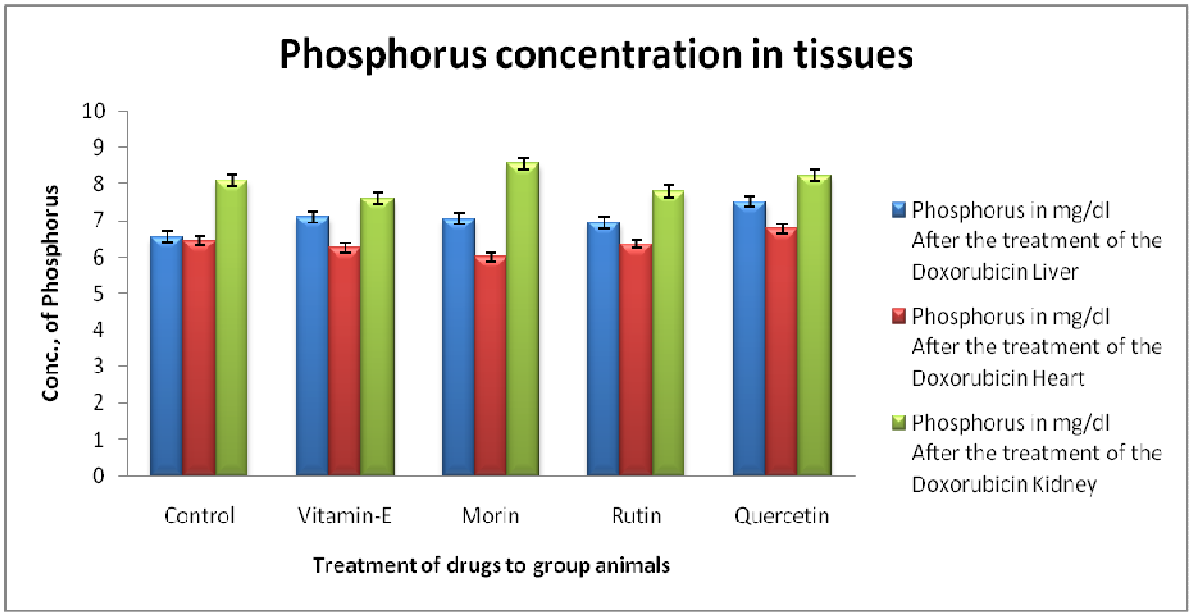

Fig: 5. Mean ± SEM of Phosphorus in mg/dL Vit-E, Morin, Rutin and Quercetin treatment against doxorubicin in tissue homogenates

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