Ameliorative Potential of Rosuvastatin on Doxorubicin-induced Cardiotoxicity by Modulating Oxidative Damage in Rats

Sıçanlarda Oksidatif Hasar Modülasyonuyla Dokсорubisinin İndüklediği Kardiyotoksisite Üzerine Rosuvastatinin İyileştirici Potansiyeli

ABSTRACT

Objectives: The study aimed to explore the in vivo protective potential of rosvastatin (ROSS), an oral antihyperlipidemic drug against doxorubicin (DOXO) induced cardio toxicity in rats.

Materials and Methods: Cardiac toxicity was induced by DOXO injection (10 mg/kg, i.p.), once on the 20th day of the experiment. Except for the control rats, all were received DOXO and the study was continued for up to 21 days. The influence of ROSS on acute treatment was analyzed by quantification of cardiac marker enzymes such as creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH) and liver marker enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT) along with the measurement of in vivo antioxidants like superoxide dismutase and catalase. To observe histological changes of myocardial tissue hematoxylin and eosin staining were used.

Results: Acute administration of DOXO resulted in a marked rise of cardiac marker enzymes that confirms the myocardial damage compared to control animals whereas administration of ROSS (10 mg/kg, p.o.) resulted in the significant reduction of CK-MB, LDH levels (p<0.05) and AST, ALT levels to a remarkable extent. Moreover, ROSS administration significantly increased the activities of various in vivo antioxidants.

Conclusion: From the results, the acute administration of ROSS showed significant cardioprotective property, which was evidenced by a significant reduction of cardiac and liver marker enzymes along with significant improvement of in vivo antioxidant activities. Furthermore the results were supported with histopathological observations. Hence, it can be concluded that cardioprotective potential of ROSS may be through attenuation of oxidative stress by modulating oxidative damage in rats.

Key words: Cardioprotection, rosvastatin, doxorubicin, cardiotoxicity, cardiac markers, antioxidants

ÖZ

Amaç: Çalışma, sıçanlarda dokсорubisin (DOXO) indüklediği kardiyotoksisiteye karşı oral antihiperlipidemik bir ilaç olan rosvastatinin (ROSS) in vivo koruyucu potansiyelini araştırmayı amaçlamıştır. Kardiyak toksisite, deneyin 20. gününe bir kez DOXO enjeksiyonu (10 mg/kg, i.p.) ile indüklenmiştir. Kontrol sıçanları hariç tümüne DOXO verildi ve çalışmaya 21 güne kadar devam edilmiştir. Akut uygulamada ROSS’ın etkisi, süperoksit dismutaz ve katalaz gibi in vivo antioksidanların ölçümü ile birlikte kreatin kinaz-MB (CK-MB), laktat dehidrojenaz (LDH) gibi kardiyak

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INTRODUCTION

Incidence rates of cardiovascular disorders (CVDs) are increasing day by day around the globe, particularly developing countries like India, most mortality rates are due to CVDs despite several advances in medical treatments. Among all CVDs, ischemic heart disease (IHD) like angina pectoris, myocardial infarction (MI) is the most alarming clinical conditions and is the main principle cause of mortality even in developed countries also.1

According to the current status of estimation by 2030, around 23.6 million people will die from only CVDs. Moreover, MI is considered as one of the common forms of IHD leads to irreversible necrosis of cardiac myocytes or myocardial tissue damage due to the failure of vasoregulatory or auto regulatory mechanisms.2

In addition to this, despite advances in the management of CVDs, MI remains the leading cause of mortality around the globe with high incidence rates around the age of 35 years was noticed in male patients who may be due to chronic stress, lack physical activity and lifestyle modifications, etc. 3,4 Hence, was noticed in male patients who may be due to chronic stress,*/i* globe with high incidence rates around the age of 35 years CVDs, MI remains the leading cause of mortality around the world. Despite advances in the management of CVDs, MI remains the leading cause of mortality around the globe with high incidence rates around the age of 35 years was noticed in male patients who may be due to chronic stress, lack physical activity and lifestyle modifications, etc.3,4 Hence, was noticed in male patients who may be due to chronic stress, */i* globe with high incidence rates around the age of 35 years. Dahasi, ROSS uygulaması, çeşitli in vivo antioksidan düzeylerini aktivitelerini önemli ölçüde artırır. Sonuçlardan, ROSS’ın akut uygulaması, kardiak ve karaciğer hasta enzimlerinde önemli bir azalmanın yanı sıra in vivo antioksidan aktivitelerinde önemli bir iyileşme ile kanlanan önemli kardiyoprotektif özellik göstermiştir. Ayrıca sonuçlar histopatolojik gözlemlerle desteklenmiştir. Bu nedenle, ROSS’un kardiyoprotektif potansiyelinin, sınırlardaki oksidatif hasarı modüle ederek oksidatif stresin azaltılması yoluyla olabileceği sonucuna varılabilir.

Anahtar kelimeler: Kardiyoproteksiyon, rosvastatin, dokorubisin, kardiyotoksisite, kardiyakギャルト, antioksidanlar

MATERIALS AND METHODS

Animal care and handling

Wistar albino rats with a weight range of 140-180 g were used and housed at 25±5°C until the acclimatization period of about one week at Sree Vidyakethan College of Pharmacy, A. Rangampet, Tirupati. All the animals were maintained under standard experimental conditions according to the guidelines of the Committee for control and supervision on experiments on Animals (CPCSEA) and the experimental protocols were duly approved by IAEC (Institutional Animal Ethics Committee-SVCP/IAEC/1-003/2019-20).

Drugs, chemicals, and instruments

DOXO was procured from Sigma-Aldrich, U.S.A whereas assay kits like LDH & creatine kinase-MB (CK-MB) were supplied by Crest Biosystems, Coral clinical systems, Goa, India. ROSS was received as a gift sample from Dr.N.N.Palei, Faculty of Pharmacy, Sree Vidyakethan College of Pharmacy. All biochemical estimation was done using a semi-automatic analyzer (Mispavitiva, Agappe Diagnostics, Kerala, India) following the methods and stepwise procedures described by the manufacturers.

Experimental design

Morphometric analysis

Body weight of all experimental animals was recorded at regular intervals, whereas relative organ weight was calculated post-experimental period after sacrificing the animals. Heart weight was measured after washing it in ice-cold saline after removal from the body, squeezing out the blood, and blotted on the filter paper.

Induction of cardiotoxicity

A total of 24 Albino rats (150±10 gm) were divided by following a random sampling technique into four groups of six animals each (n=6). All the experimental animals were treated as per the study design and the duration of the treatment was continued up to 21 days.

Group I - Rats were given normal saline (1 mL/kg, s.c),

Group II - Rats were given DOXO on 20th day (10 mg/kg, i.p.),
Group II - Animals were sacrificed and the vital organ like heart was harvested for preparation of PMS. In brief, the harvested heart was per fused with ice-cold normal saline solution and homogenized using a phosphate buffer (0.1 M, pH 7.4) and KCl (11.7%, w/v) solution. Then the homogenate was centrifuged (800 rpm for 5 min at 4°C) to separate the nuclear debris from the mixture. After that, the obtained supernatant was centrifuged at 10,500 rpm for 20 min at 4°C to obtain PMS. Collected PMS was used for analyzing for the presence of different enzymes related to MI, such as CK-MB fraction and LDH using an semi-automatic analyzer (Mispa-Viva-Agappe Diagnostics) with respective assay kits by following stepwise procedures given by the manufacturer.15-18

Estimation of marker enzymes using post mitochondrial supernatant (PMS)

Animals were sacrificed and the vital organ like heart was harvested for preparation of PMS. In brief, the harvested heart was per fused with ice-cold normal saline solution and homogenized using a phosphate buffer (0.1 M, pH 7.4) and KCl (11.7%, w/v) solution. Then the homogenate was centrifuged (800 rpm for 5 min at 4°C) to separate the nuclear debris from the mixture. After that, the obtained supernatant was centrifuged at 10,500 rpm for 20 min at 4°C to obtain PMS. Collected PMS was used for analyzing for the presence of different enzymes related to MI, such as CK-MB fraction and LDH using an semi-automatic analyzer (Mispa-Viva-Agappe Diagnostics) with respective assay kits by following stepwise procedures given by the manufacturer.15-18

Antioxidant activity

Collected PMS solution from the heart tissue was used for measuring superoxide dismutase (SOD)19 and catalase (CAT)20 following standard procedures.

Histopathological study

The collected heart tissue was washed with ice-cold normal saline and fixed in formalin (10% neutral) solution followed by embedding in paraffin. Then, it was sectioned (5 μm thickness) and stained with hematoxylin and eosin for histopathological examination under a light microscope.21

Statistical analysis

The statistical analysis was performed by ANOVA under One-Way classification followed by Dunnett’s test. *p<0.05 was considered statistically significant. Values are expressed as mean ± standard error of the mean.

RESULTS

Morphometric analysis

Morphometric analysis of all experimental animals is tabulated in Table 1. Results depict that the body weight of experimental animals was recorded on a weekly interval of drug treatment. From the results, the administration of DOXO had shown significant changes in the body weight of animals, whereas acute administration of ROSS has shown a remarkable increase (185.39±1.63, p<0.05) in body weight compared to control (171.29±1.36) and DOXO (164.45±2.49) treated group on day 21. However, heart weight and relative heart weight of experimental animals are summarized in Table 2. As per the results, heart weight followed by relative heart weight of rats received DOXO was statistically higher than control and ROSS received animals. But, treatment with ROSS significantly (p<0.05) inhibited these weight variation changes influenced by DOXO administration and the obtained results were significantly (p<0.05) comparable with control groups.

Cardiac marker enzymes

The results presented in Figure 1 and Table 3 depicts the effect of ROSS on serum CK-MB levels against DOXO-induced cardiotoxicity in rats. Results indicate that DOXO received group 2 animals showed a significant raise of CK-MB levels (p<0.05) compared to control animals. However, animals treated along with ROSS showed inhibitory action (**p<0.01) on the raising level of CK-MB induced by DOXO in rats. Similarly, the same trend was noticed in LDH levels too. The results of the effect of ROSS on serum LDH levels are presented in Figure 2 and Table 3. After injection of DOXO, we observe that the rats in group II animals showed a marked rise in LDH levels (p<0.05) compared with control groups. But the results from groups 3 and 4 show inhibitory action on the toxicity induced by DOXO in rats and the results were comparable with control animals.

Table 1. Effect of ROSS on morphometric analysis on DOXO induced cardiotoxicity

<table>
<thead>
<tr>
<th>Treatments (mg/kg, b.w)</th>
<th>Body weight (gr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>CON (1 mL/kg, s.c.)</td>
<td>143.39±1.67</td>
</tr>
<tr>
<td>DOXO (10 mg/kg, i.p.)</td>
<td>154.81±3.78</td>
</tr>
<tr>
<td>ROSS (10 mg/kg, p.o.)</td>
<td>152.68±2.62</td>
</tr>
<tr>
<td>ROSS (10 mg/kg, p.o.) + DOXO (10 mg/kg, i.p.)</td>
<td>145.73±2.45</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n=5: *p<0.05, when compared to the control group. SEM: Standard error of the mean. DOXO: Doxorubicin, ROSS: Rosuvastatin, CON: Control
Post mitochondrial supernatant

The results of the effect of ROSS on heart tissue homogenate CK-MB levels are shown in Figure 3 and Table 3. After the injection of DOXO, we observe that rats in group II animals showed a remarkable rise in CK-MB levels \( (p<0.05) \) in tissue homogenate compared with control groups. But the results from groups III and IV show the inhibitory action \( (p<0.05) \) on the toxicity induced by DOXO in rats and the results were comparable with control animals.

Liver marker enzymes

The effects of ROSS on serum AST, ALT levels are summarized in Table 4. As per the obtained results, DOXO received animals showed marked rise of both AST \( (240.34\pm5.53; ***p<0.001) \) and ALT \( (103.67\pm3.44; ***p<0.001) \) whereas acute administration of ROSS significantly reversed these biochemical alterations to a significant extent incase of AST \( (87.85\pm4.56; **p<0.01) \) and ALT \( (53.72\pm0.33**; **p<0.01) \) compared to DOXO and control animals.

Antioxidant activity

The effects of ROSS on antioxidant levels are presented in Table 5, Figure 4, 5. The results reveal that SOD and CAT levels were reduced in PMS solution of heart tissue of DOXO treated group II animals. Administration of DOXO showed marked reduction in SOD \( (p<0.05) \) and CAT \( (p<0.05) \) at dose of

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**Table 2. Effect of ROSS on relative organ weight on DOXO-induced cardiotoxicity**

<table>
<thead>
<tr>
<th>Treatments (mg/kg, b.w)</th>
<th>Heart weight (mg)</th>
<th>Heart weight</th>
<th>Relative heart weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON (1 mL/kg, s.c.)</td>
<td>755±3.29</td>
<td>0.44±0.06</td>
<td></td>
</tr>
<tr>
<td>DOXO (10 mg/kg, i.p.)</td>
<td>908±2.53</td>
<td>0.55±0.08</td>
<td></td>
</tr>
<tr>
<td>ROSS (10 mg/kg, p.o.)</td>
<td>771±1.49</td>
<td>0.44±0.04</td>
<td></td>
</tr>
<tr>
<td>ROSS (10 mg/kg, p.o.) + DOXO (10 mg/kg, i.p.)</td>
<td>774±4.56</td>
<td>0.41±0.03</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n=5. SEM: Standard error of the mean, DOXO: Doxorubicin, ROSS: Rosuvastatin, CON: Control

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**Table 3. Effect of ROSS on creatine kinase-MB and lactate dehydrogenase levels on DOXO-induced cardio toxicity**

<table>
<thead>
<tr>
<th>Treatments (mg/kg, b.w)</th>
<th>Marker enzymes</th>
<th>CK-MB (U/L) (serum)</th>
<th>CK-MB (U/L) (heart tissue homogenate)</th>
<th>LDH (IU/L) (serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON (1 mL/kg, s.c.)</td>
<td></td>
<td>2912.05±4.59</td>
<td>222.05±2.47</td>
<td>1277±3.47</td>
</tr>
<tr>
<td>DOXO (10 mg/kg, i.p.)</td>
<td></td>
<td>3157.32±3.62</td>
<td>317.32±4.16</td>
<td>3560±5.29</td>
</tr>
<tr>
<td>ROSS (10 mg/kg, p.o.)</td>
<td></td>
<td>1973.37±7.48*</td>
<td>299.37±1.49</td>
<td>2541±4.58*</td>
</tr>
<tr>
<td>ROSS (10 mg/kg, p.o.) + DOXO (10 mg/kg, i.p.)</td>
<td></td>
<td>1091.62±2.39**</td>
<td>235.62±2.36*</td>
<td>2789±4.39*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n=5, *\( p<0.05 \), **\( p<0.01 \), when compared to control and toxic control group, ROSS: Rosuvastatin, CK-MB: Creatine kinase-MB, LDH: Lactate dehydrogenase, DOXO: Doxorubicin, ROSS: Rosuvastatin, SEM: Standard error of the mean, CON: Control

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Figure 1. Effect of ROSS on serum CK-MB levels on DOXO-induced cardiotoxicity in rats
ROSS: Rosuvastatin, CK-MB: Creatine kinase-MB, DOXO: Doxorubicin, CON: Control

Figure 2. Effect of ROSS on serum LDH levels on DOXO-induced cardiotoxicity in rats
ROSS: Rosuvastatin, DOXO: Doxorubicin, LDH: Lactate dehydrogenase
However, ROSS administration caused a reversal of depleted antioxidants to near normal levels, which indicated its protective and antioxidant capabilities.

**Histopathological study**

Histopathological observations of the cardiac tissues of all experimental groups are shown in Figure 6. From these results, cross-section of cardiac tissues of control animals showed normal myocardial architecture without inflammatory cell infiltration whereas necrotic cardiac tissue damage and like proliferated granulation tissues were seen in DOXO received animals. However, the administration of ROSS reversed these cellular changes to a remarkable significant extent with mild granulation tissue and restored normal cellular architecture, which reflects its protective potential against cardiotoxicity induced by DOXO.

**Table 4. Effect of ROSS on liver marker enzymes on DOXO-induced cardiotoxicity**

<table>
<thead>
<tr>
<th>Treatments (mg/kg, b.w)</th>
<th>Liver marker enzymes</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AST (IU/mL)</td>
<td>ALT (IU/mL)</td>
<td></td>
</tr>
<tr>
<td>CON (1 mL/kg, s.c.)</td>
<td>64.23±3.45</td>
<td>43.48±2.57</td>
<td></td>
</tr>
<tr>
<td>DOXO (10 mg/kg, i.p.)</td>
<td>240.34±5.53***</td>
<td>103.67±3.44***</td>
<td></td>
</tr>
<tr>
<td>ROSS (10 mg/kg, p.o.)</td>
<td>131.67±3.49*</td>
<td>73.43±1.29*</td>
<td></td>
</tr>
<tr>
<td>ROSS (10 mg/kg, p.o.) + DOXO (10 mg/kg, i.p.)</td>
<td>87.85±4.56**</td>
<td>53.72±0.33**</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n=5, *p<0.05, **p<0.01, when compared to control and toxic control group. ROSS: Rosuvastatin, CK-MB: Creatine kinase-MB, DOXO: Doxorubicin, ROSS: Rosuvastatin, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, SEM: Standard error of the mean, CON: Control

**Table 5. Effect of ROSS on Antioxidant levels on DOXO-induced cardiotoxicity**

<table>
<thead>
<tr>
<th>Treatments (mg/kg, b.w)</th>
<th>Antioxidant enzymes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SOD (µg/mg of protein)</td>
<td>CAT (U/mg of protein)</td>
</tr>
<tr>
<td>CON (1 mL/kg, s.c.)</td>
<td>45.86±1.49</td>
<td>1.37±0.69</td>
</tr>
<tr>
<td>DOXO (10 mg/kg, i.p.)</td>
<td>32.6±2.32</td>
<td>0.77±0.54</td>
</tr>
<tr>
<td>ROSS (10 mg/kg, p.o.)</td>
<td>58.7±1.56*</td>
<td>1.41±0.48*</td>
</tr>
<tr>
<td>ROSS (10 mg/kg, p.o.) + DOXO (10 mg/kg, i.p.)</td>
<td>60.9±1.58*</td>
<td>1.67±0.53*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n=5, *p<0.05, when compared to control and toxic control group. ROSS: Rosuvastatin, SOD: Superoxide dismutase, CAT: Catalase
myocardial tissue damage which was reflected in the study. and ALT into extra cellular fluid and in serum which indicates the causing it to release cytosolic enzymes such as CK-MB, LDH, AST, antioxidative properties. Furthermore, according to diverse pharmacological effects, including anti-inflammatory and antioxidative properties.23,24 Furthermore, according to pharmacological profile of ROSS. Hence, the study elucidated have more gap to support the scientific background of diverse pharmacological effects, including anti-inflammatory and antioxidative properties.23,24 Furthermore, according to studies, ROSS reduces oxidative stress by mediating many antioxidant enzymes, including decreased NADPH oxidase, suppression of endothelial nitric oxide synthase uncoupling, up regulation of antioxidant enzymatic defense mechanisms, and inhibition of hydrogen peroxide-induced DNA harm.25,26 But the experimental background found to be fertile or seem to have more gap to support the scientific background of diverse pharmacological profile of ROSS. Hence, the study elucidated the possible in vivo ameliorative influence of ROSS in DOXO-induced cardiotoxicity in rats.

DOXO is a well-known cardiotoxic agent capable of inducing cardiac injury in experimental animals and it is widely used model to induce cardiotoxicity.27 DOXO destroys the myocardial cells, causing it to release cytosolic enzymes such as CK-MB, LDH, AST, and ALT into extra cellular fluid and in serum which indicates the myocardial tissue damage which was reflected in the study.

Figure 6. Histopathological observations of different experimental animals using hematoxylin and eosin staining (CON: Control animals showed normal myocardial cellular architecture, DOXO: Doxorubicin received groups showed noticeable necrotic cellular tissue damage, ROSS: Rosuvastatin treated groups; DOXO + ROSS: Doxorubicin plus rosuvastatin treated groups showed significant reversal of necrotic tissue damage caused by doxorubicin)

DISCUSSION

MI is an acute necrotic disorder of the heart and one of the most commonly diagnosed forms of CVD in industrialized nations like India. It is a common and life-threatening manifestation of IHD leads to mortality.22 MI is the technical name for a heart attack, occurring due to myocardial ischemia resulting from irreversible myocardial tissue necrosis because of complete occlusion of blood vessel (coronary artery) supplying blood to the myocardium. Therefore, the development of cardioprotective agents to improve myocardial function is of great clinical importance.

ROSS has shown significant organ protective properties with diverse pharmacological effects, including anti-inflammatory and antioxidative properties.23,24 Furthermore, according to studies, ROSS reduces oxidative stress by mediating many antioxidant enzymes, including decreased NADPH oxidase, suppression of endothelial nitric oxide synthase uncoupling, up regulation of antioxidant enzymatic defense mechanisms, and inhibition of hydrogen peroxide-induced DNA harm.25,26 But the experimental background found to be fertile or seem to have more gap to support the scientific background of diverse pharmacological profile of ROSS. Hence, the study elucidated the possible in vivo ameliorative influence of ROSS in DOXO-induced cardiotoxicity in rats.

Results of this study indicate that, DOXO caused significant myocardial tissue damage, as indicated by a marked raise in the levels of cardiac injury markers, oxidative stress, heart weight, and histological changes in myocardial tissue, etc.

The same trend was noticed in heart tissue homogenate of DOXO treated animals compared with the control animals demonstrating the necrotic damage of the myocardial membrane by DOXO. Whereas ROSS-treated groups at doses of 10 mg/kg alone and in combination demonstrated a significant decline in CK-MB and LDH levels (p<0.05), which ultimately reflects its protective activity against DOXO-induced cardiotoxicity in rats. However, in the pathogenesis of various cardiovascular diseases, reports from earlier studies indicated the involvement of reactive oxygen species and the reduction of antioxidants. In this connection, it is well known that SOD is one of the important antioxidant enzymes to control many pathological progress in vast disease and disorders. In this study, increased levels of SOD to a significant extent (p<0.05), which in turn reflects the protective role of ROSS. The same trend was observed in CAT too. CAT is also considered as one of the vital enzymes made of hemeprotein and used to scavenge the formed ROS, prevents the tissue damage against free radicals.28,29 Additionally, histopathological reports further corroborated the obtained results of the present investigations. As per the results, the administration of ROSS reversed cellular changes caused by DOXO to a significant extent and restored the normal cellular architecture, which reflects its protective potential against cardiotoxicity induced by DOXO.

CONCLUSION

Results from this study demonstrate that ROSS has shown promising potent cardioprotective effects on DOXO-induced cardiotoxicity in rats, as evidenced by the significant reduction of cardiac marker enzymes like CK-MB and LDH both in serum and in heart tissue homogenate along with significant reduction of liver marker enzymes like ALT, AST along with significant beneficial effects were seen in morphometric analysis. However, it is well recognized that various radical scavengers, such as CAT, SOD, and GPX, as well as GSH, serve as the first line of defense against oxidative damage, including cardiotoxicity. Reactive oxygen species can cause oxidative damage due to the lack of an appropriate endogenous antioxidant defense mechanism. The same were reflected in this study, which clearly indicated the diminishment of antioxidants such as SOD, CAT in DOXO treated animals. Thus, from the results, oxidative damage was clearly involved in DOXO-induced cardiotoxicity. However, the treatment of ROSS remarkably restored DOXO-induced cardiotoxicity by up-regulating the antioxidants. Thus, results from in vivo antioxidant study reflect that administration of ROSS significantly raised the level of SOD and CAT along with significant reversal of necrotic tissue damage caused by DOXO. Hence, it can be concluded that the cardioprotective potential of ROSS may be due to attenuation of cardiac, liver marker enzymes and oxidative stress by modulating oxidative damage by up-regulating the antioxidant defense mechanism.
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REFERENCES