Effect Of Hesperidin and Rutin On Doxorubicin Induced Testicular Toxicity in Male Rats

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Abstract: The present study aims to investigate whether rutin and hesperidin could protect against acute doxorubicin (DXR)-induced testicular toxicity. Doxorubicin (DXR)– administered rats (25 mg/ kg; three times intraperitoneally/week for two weeks) were pretreated with rutin and/or hesperidin, (50 mg/ kg body weight) three times per week for three weeks and DXR 25mg/kg b.wt three times per week (at the last two weeks of the experiment). Results showed that DXR caused a decrease in free testosterone and FSH levels which were significantly increased as a result of pretreatment with rutin and/or hesperidin. LH level was increased significantly due to the pretreatment with the mixture of rutin and hesperidin. The pre-treatment of these animals with rutin, hesperidin, and/or both produced a potential increase of the lowered glutathione level in DXR administered control group. Also, glutathione –S-transferase and glutathione peroxidase activities had a significant elevation in all pretreated groups in corresponding to DXR treated group which exhibited a significant decrease as compared to normal control. Testis lipid peroxidation was increased significantly as a result of DXR administration while all the tested agents including rutin, hesperidin or their mixture induced a significant improvement of the elevated values.

Keywords: Doxorubicin; rutin; hesperidin; testicular toxicity; antioxidants

Introduction

Doxorubicin (DXR) is an anthracycline antibiotic used as an antitumor agent against human malignancies such as leukemia, lymphomas and many solid tumors (1). Though, previous investigations indicated that DXR has the ability to induce mutations and chromosomal aberrations in normal and malignant cells (2) in addition to a wide variety of toxic side effects on organs including liver (3), kidney (4), heart (5), brain (6) and testis (7). The collected evidence indicated that the side effects of doxorubicin were a type of oxidative stress related injury of the whole body (7,8). Though, other various mechanisms were involved, such as DNA binding, apoptosis, membrane composition differentiation and function alteration (7,9).

DXR was recognized to alter sperm development, production, structural integrity and motility rates in association with increased cellular apoptosis in spermatogonia and spermatocytes induction of spermatid micronuclei (7) and oligozoospermia (10). Also, previous studies registered the DXR effect on reproductive performance, testicular atrophy and spermatogenesis in mice and rats (11), beside the depletion of sperm motion and sperm count, and increased sperm morphological abnormalities (7).

Herbal drug therapy is considered a common practice adopted in traditional and alternative medicine and has been used in the treatment of many diseases from ancient times (12). This is done with the help of different herbal extracts and using different treatment methods. Rutin (quercetin rutinoside), as well as quercetin, is a glycoside of the flavonoid quercetin. Rutin is also an antioxidant, along with quercetin, acacetin, morin, hispidulin, hesperidin, and naringin; it was found to be the strongest (13). Dietary hesperidin exerts anticarcinogenic actions in the tongue, colon, esophagus, and urinary bladder in rat carcinogenesis models (14). Therefore, the present investigation was undertaken to test the
anticlastogenic or clastogenic effects of rutin and hesperidin on the testis tissue in rat, determine the protective effect of the components on the testicular dysfunction and testis oxidative stress induced by DXR and examine the dose dependency of these effects.

Material and Methods
Experimental animals:

Adult male albino rats weighing 120-150 g were used in the present study. The animals were obtained from the animal house in the Ophthalmology Research Center, Giza, Egypt. They were kept under observation for two weeks before the onset of the experiment to exclude any intercurrent infection. The animals were kept at room temperature and exposed to natural daily light-dark cycles. Rats were fed standard diet ad libitum and clean water was continuously available. All animal procedures are in accordance with the recommendations for the proper care and use of laboratory animals stated by the Canadian Council on Animal Care (15).

Chemicals:

Doxorubicin (Adriblastina® produced by Carlo Erba) was purchased from local pharmacy in the form of 10 mg/ampoule. Rutin and hesperidin were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used for the investigation were of analytical grade.

Doses and treatment:

The dose of doxorubicin (DXR) used in this study was 25 mg/kg body weight. This dose was previously reported to include an increase in the frequency of cell damage in mammalian systems (16,17). The animals were treated with doxorubicin by interaperitoneal route. The chosen dose of DXR was adjusted to 0.2ml/25g b.wt. in sterile water prior to use and was given three times per week for two weeks. Rutin and hesperidin doses were adjusted to 50mg/kg and were given three times per week for three weeks.

Experimental design:

Animals were divided into five groups comprising ten animals each:

1-Group 1 (normal control) is given the equivalent volume of the vehicle 1 (distilled water) daily for four weeks.

2-Group 2 (toxic) is given doxorubicin at dose level of 25mg/kg b.wt for three times per week for two weeks.

3-Group 3 (treated with rutin & doxorubicin) is given rutin at dose level of 50mg/kg b. wt.(18) three times per week for three weeks and DXR 25mg/kg b.wt three times per week (at the last two weeks of the experiment).

4-Group 4 (treated with hesperidin & doxorubicin) is given hesperidin at dose level of 50mg/kg b.wt. three times per week for three weeks and DXR 25mg/kg b.wt three times per week (at the last two weeks of the experiment).

5-Group 5 (treated with rutin + hesperidin & DXR) is given rutin and hesperidin at dose level of 50mg/kg b. wt (19) three times per week for three weeks and DXR 25mg/kg b.wt
Sampling:

Under diethyl ether anesthesia, six animals were sacrificed and blood sample was collected from jugular vein of each animal in a centrifuge tube and left to clot at room temperature for 45 minutes. Sera were separated by centrifugation at 3000 r.p.m. at 30°C for 15 minutes and kept frozen at -30°C for various physiological and biochemical analyses.

Testis from each animal was rapidly excised after dissection. One part was fixed in buffered formalin for 24 hrs, trimmed and then transferred into 70% alcohol for histopathological examination. 0.5g was homogenized in 5ml 0.9% NaCl (10% w/v) using teflon homogenizer (Glas-Col, Terre Haute, USA).

Biochemical analyses:

Serum LH was determined according to the method of (20) using reagent kits purchased from Monobind, INC (USA). FSH concentration in serum was determined according to the method of (21) using reagent kits purchased from Monobind, INC (USA). Testosterone concentration in serum was determined according to the method of (22) using reagent kits purchased from Biosource Company, (Belgium). Butyryl Cholinesterase activity in serum was determined according to the method of (23) using reagent kits purchased from Quimica Clinica Aplicada S. A. Company (Spain). Testis glutathione (GSH) was determined according to the method of (24). Testis lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) according to the method of (25). Testis peroxidase activity were assayed for the method of (26). Glutathione peroxidase activity in testis was assayed according to the chemical method of (27). Glutathione-S-transferase activity was determined according to (28).

Statistical analysis:

The data were analyzed using the one-way analysis of variance (ANOVA) (PC-STAT) (29) followed by LSD analysis to compare various groups with each other. Results were expressed as mean ± standard error (SE). F-probability, obtained from one-way ANOVA, expresses the effect between groups.

Results

Table 1 and 2 show the effect of the tested rutin, hesperidin and/or their mixture on the serum free testosterone, LH, FSH concentrations and cholinesterase activity of normal and doxorubicin-administered rats. Concerning serum enzymes related to testis function, the doxorubicin-administered rats exhibited significant decrease (p<0.01; LSD) of free testosterone, LH and FSH concentrations. The pre-treatment of these animals with rutin, hesperidin and/or their mixture produced a potential increase (p<0.01; LSD) of the lowered free testosterone level; which the percentage changes were 57.23%, 69.18% and 77.36 respectively as a result of rutin, hesperidin and their mixture as compared to DXR treated group (-51.67). The pre-treatment of these animals with rutin, hesperidin and/or their mixture produced a potential increase (p<0.01; LSD) of the LH level; the percentage changes were 50.00%, 37.50% and 31.25 respectively as a result of rutin, hesperidin and their mixture as compared to DXR administered group (-30.43).

The pre-treatment of of doxorubicin-administered group with rutin, hesperidin and/or their mixture also produced a potential increase (p<0.01; LSD) of the FSH level; the percentage changes were 41.67%, 41.67% and 91.67 respectively as a result of rutin,
hesperidin and their mixture as compared to DXR control group (-45.45). Rutin, hesperidin or their mixture pretreatment produced a detectable but non-significant increase in the cholinesterase activity of doxorubicin-administered rats (p>0.05; LSD). With regards to ANOVA, it was found that the effect between groups on free testosterone, LH, and FSH level was very highly significant (p<0.001; F-prob.) throughout the experiment.

Table 3 shows the effect of the tested rutin, hesperidin and/or their mixture on the testis oxidative stress markers and antioxidant defense system of normal and doxorubicin-administered rats. The pre-treatment of DXR-administered rats with rutin, hesperidin or their mixture produced a potential increase (p<0.01; LSD) of the depleted glutathione level. Glutathione–S-transferase and glutathione peroxidase activities had a significant elevation in all pretreated groups in comparison with DXR administered group that exhibited a significant decrease in the activities of these enzymes. The pre-treatment of doxorubicin-exhibited rats with hesperidin produced a non-significant changes in the peroxidase level. Testis lipid peroxidation was increased significantly as a result of DXR administration while it was significantly decreased (p<0.05; LSD) in pre-treatment with either of rutin and hesperidin or their mixture recording percentages of -28.61, -42.01 and -37.89 respectively. One-way ANOVA revealed that the effect on the variables of oxidative stress and antioxidant system was of p<0.001 between groups.

Discussion

In consistence with our findings, it has been reported that animals treated with a single dose of doxorubicin showed low serum levels of free testosterone compared with the control group (7). Also, some investigators have reported testicular atrophy, sperm morphological abnormalities and disturbance in spermatogenesis as a result of doxorubicin administration (30). Previous studies attributed the effect of doxorubicin on reproductive performance to the destruction of meiotic and early spermatogenic stages (4). Moreover, Nambu and Kumamoto (1995) reported that spermatogenic disorder was generated by the impaired DNA synthesis in stem cells and Sertoli cell dysfunction, as a result of doxorubicin. Also, it was reported that the impaired sperm quality including morphological alterations was caused by prevention of spermatogenesis produced by doxorubicin (31). From all preceding data, the decrease of serum testosterone concentration was supposed to be the cause of diminished sperm noticed in the current study, as testosterone is the prime regulator of the spermatogenesis. Many previous investigations reported the ability of doxorubicin to react with electron rich areas of susceptible molecules such as nucleic acids and proteins (7). Therefore, doxorubicin was suggested to target rapidly dividing cells, disrupting cell growth, mitotic activity, differentiation, and elevate the ratio of dismutase to glutathione peroxidase and/or catalase which lead to increased H2O2 concentration (32, 33). It was suggested that H2O2 formed was considered the critical apoptotic trigger of doxorubicin via causing oxidative DNA damage (34). Also it was reported that meiotically dividing spermatogonia and spermatocytes as vulnerable targets of doxorubicin-induced apoptosis (35). In addition, Mishra and Bhiwgade (2007) reported that higher levels of H2O2 can be converted, in part, by Fenton reaction to OH· which may lead to lipid peroxidation and DNA cross-linking. These results of previous investigators could explain the present observations related to doxorubicin-induced oxidative stress via increasing lipid peroxidation through the impairment of SOD/ GPX and/or catalase ratio. It also gave reasons for damages and histopathological complications recorded herein.
With regards to testis, Diemer et al. (2003) has showed that H2O2 is a potent oxidant that could inhibit steroidogenesis in Leydig cells (36). Low serum levels of testosterone in the present work support this assumption of the inhibition of the testicular androgenesis in doxorubicin-injected group. Steroid hormones are a class of organic compounds including sex hormones (37). Steroidogenesis in the male rat is stimulated by hypothalamic gonadotropin releasing hormone (GnRH) acting to stimulate the production and release of pituitary luteinizing hormone (LH), which binds to LH receptors (LHR) on the surface of Leydig cells to upregulate androgen production (38). Longer-term regulation of androgen production by LH involves transcriptional regulation of genes important for the production of sex steroids such as enzymes and transport proteins. Testosterone is needed for the development of male reproductive tract, spermatogenesis, maintenance of secondary sex characteristics and other sexual parameters such as libido (39). Perturbations in the production of sex steroids can adversely impact reproduction by repairing sperm production among other effects (40). Toxic exposures cause hormonal and reproductive disorders (41,42). Oxidative stress-induced injury yielded damage to all major classes of macromolecules including lipids, consequently a reduction in sex hormones was resulted (43). This decrease of serum testosterone concentration was supposed to be the cause of diminished sperm noticed in the current study, as testosterone is the prime regulator of the spermatogenesis. Also, the significant decrease in haploid cells (spermatids and spermatozoa), as noticed in the present study, may be due to the inhibition of primary spermatocytes transformation to spermatids, the increased DNA damage in the spermatogonia and spermatocytes or the drop recorded in the primary spermatocytes as indicated by the present results. Numerous investigations indicated that, some of plant natural product extracts may protect from harmful oxygen species and free radicals on electrophiles intermediates of anticancer drugs, which damage DNA and other cell targets (16,44). Some of these protection studies have shown beneficial effects of these extracts against doxorubicin toxicity (2,45).

On the other hand, treatment of doxorubicin-intoxicated rats, with either rutin and/or hesperidin have proved efficacy towards improving testis histological lesions and oxidative stress via increasing serum testosterone level, elevating antioxidant enzymes like GST, glutathione peroxidase, peroxidase and glutathione-S-transferase and non enzyme molecules like decreasing LPX level. In contrary, Choudhary and Bhatt (1996) studied the chicory root suspension effects at threshold and sublethal dose levels i.e. 4.5 and 8.7 gm / kg on the fertility of mice (46). They reported that degenerated gametogenic cells (fitted with disquamous and cellular debris) of seminiferous tubules of testis with diminution of serum testosterone concentration indicating impairment of spermatogenesis in mice. In the present study, treatment of doxorubicin-intoxicated rats, with either rutin and/or hesperidin significantly ameliorated the histological deterioration of the testis & the biochemical changes. This amendment activity may be attributed in part to the improvement effect of the either rutin and/or hesperidin on the lipid peroxidation and/or antioxidant system (oxidative stress). Both quercetin and rutin are used in many countries as medications for blood vessel protection and are ingredients of numerous multivitamin preparations and herbal remedies (47). It can combine with cations, supplying nutrients from the soil to the cells in plants (47). In humans, it attaches to the iron ion Fe2+, preventing it from binding to hydrogen peroxide, which would otherwise create a highly-reactive free-radical that may damage cells. It is also an antioxidant. Rutin is also an antioxidant, along with quercetin, acacetin, morin, hispidulin, hesperidin, and naringin; it was found to be the strongest (13). Dietary hesperidin exerts anti-carcinogenic actions in the tongue, colon, esophagus, and urinary bladder in rat carcinogenesis models (14,48). The superoxide radical (O$_2^-$) is a highly toxic species
generated in numerous biological reactions. In fact, superoxide, the one-electron reduced form of molecular oxygen, is a precursor of the more reactive oxygen species, such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, that have the potential of reacting with biological macromolecules, thereby inducing tissue damages (49).

The present study shows that doxorubicin administration caused a marked decrease in peroxidase, glutathione peroxidase activities as well as glutathione-S- transferase activity. On the other hand, doxorubicin administration caused a marked increase in lipid peroxidation level. Inversely, doxorubicin pretreated with rutin and/or hesperidin shows a potential amelioration (increase) in glutathione level, peroxidase, glutathione peroxidase and glutathione-S- transferase activities, while records a significant reduction in lipid peroxidation level. These results are in agreement with several authors. Patel et al. (2010) reported that oxidative stress leads to lipid peroxidation, which is the result of an interaction between free radicals of diverse origin and unsaturated fatty acids typically in membrane lipids. The net result of these events translates into accumulation of a variety of toxic lipid peroxides and malondialdehyde (MDA) (3). The level of tissue MDA is reported to be a reliable marker of lipid peroxidation. Oxidative stress has been implicated as a factor that contributes to various forms of cell death, including as a specific inducer of apoptosis. Doxorubicin continues to be one of the most widely used anticancer agents in the clinic despite its dose-limiting side-effects. Many of doxorubicin’s dose-limiting toxicities occur due to its generation of toxic oxygen species, resulting in oxidative stress. Some clinical observations have suggested that doxorubicin may have greater toxicity in older patients (50). The studies presented here compare basal and doxorubicin-induced antioxidant enzyme activities in brain, heart, kidney and liver tissues of Fisher 344 rats of different ages to determine whether differences in these enzymes can account for the age-dependent differences observed in doxorubicin-induced toxicity (50).

Based on the prementioned results and publications, it is worth mentioning that rutin and hesperidin have potent anti-oxidant activities which in turn may protect against doxorubicin-induced testis lesions.

In the present study, cholinesterase activity in cerebral hemispheres was depressed in DXR administered rats as compared with normal ones. The treatment of these rats with rutin and/or hesperidin induced an increase of the suppressed enzyme activity. In our opinion, the increase of acetyl cholinesterase activity in the treated rats may be secondary to the increased synthesis and discharge of acetylcholine which is hydrolyzed by this enzyme. The present results demonstrated that rutin and hesperidin play important role in the protection against doxorubicin induced testicular toxicity, by amelioration in the levels of (testosterone, LH and FSH) in addition to the amelioration in the levels of cholinesterase.

**Conclusion**

In conclusion, pretreatment with rutin and hesperidin may protect the testis from testicular dysfunction caused by doxorubicin. These ameliorative effects may mediated at least in part via improvement of the oxidative stress and anti-oxidant defense system. Clinical testing is necessary to assess safety, benefits and hazards of these plant extracts in human beings to seek the approval of their concerned authorities of their use.

**Acknowledgement**

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References


3-Patel N.; Joseph, C.; Corcoran, G. B. and Ray, S. D. Silimarin modulates doxorubicin-induced oxidative stress, Bcl-xL and p53 expression while preventing apoptotic and necrotic cell death in the liver. Toxicology and applied pharmacology 2010;ISSN: 1096-0333; Nlm Unique ID: 0416575.


Table 1: Effects of rutin and/or hesperidin on serum testosterone, LH levels, FSH levels and cholinesterase activity in doxorubicin-administered rats.

<table>
<thead>
<tr>
<th>Parameters/Treatments</th>
<th>Free Testosterone (pg/ml)</th>
<th>% change</th>
<th>LH (mIU/ml)</th>
<th>% change</th>
<th>FSH (mIU/ml)</th>
<th>% change</th>
<th>Cholinesterase (U/L)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Normal</td>
<td>3.291 ± 0.002 a</td>
<td>-</td>
<td>0.230 ± 0.008 a</td>
<td>-</td>
<td>0.220 ± 0.006 a</td>
<td>-</td>
<td>4.530 ± 0.18 a</td>
<td>-</td>
</tr>
<tr>
<td>G2 Doxorubicin</td>
<td>1.590 ± 0.004 b</td>
<td>-51.67</td>
<td>0.160 ± 0.006 a,b</td>
<td>-30.43</td>
<td>0.120 ± 0.006 a</td>
<td>-45.45</td>
<td>2.860 ± 0.15 a</td>
<td>-36.87</td>
</tr>
<tr>
<td>G3 Rutin + Doxorubicin</td>
<td>2.501 ± 0.006 c</td>
<td>57.23</td>
<td>0.240 ± 0.01 a,b</td>
<td>50.00</td>
<td>0.170 ± 0.007 b</td>
<td>41.67</td>
<td>3.830 ± 0.53 a</td>
<td>33.92</td>
</tr>
<tr>
<td>G4 Hesperidin + Doxorubicin</td>
<td>2.690 ± 0.006 d</td>
<td>69.18</td>
<td>0.220 ± 0.006 b</td>
<td>37.50</td>
<td>0.170 ± 0.007 b</td>
<td>41.67</td>
<td>3.120 ± 2.44 a</td>
<td>9.09</td>
</tr>
<tr>
<td>G5 Rutin + Hesperidin + Doxorubicin</td>
<td>2.821 ± 0.008 e</td>
<td>77.36</td>
<td>0.210 ± 0.004 c</td>
<td>31.25</td>
<td>0.230 ± 0.007 c</td>
<td>91.67</td>
<td>3.110 ± 0.10 a</td>
<td>8.04</td>
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<tr>
<td>F-Probability</td>
<td>P &lt; 0.001</td>
<td>-</td>
<td>P &lt; 0.001</td>
<td>-</td>
<td>P &lt; 0.001</td>
<td>-</td>
<td>P &gt; 0.05</td>
<td>-</td>
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<tr>
<td>LDS at 5% level</td>
<td>0.003</td>
<td>-</td>
<td>0.042</td>
<td>-</td>
<td>0.028</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LDS at 1% level</td>
<td>0.005</td>
<td>-</td>
<td>0.035</td>
<td>-</td>
<td>0.037</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard error.
- Number of animals in each group is six.
- Mean, which have the same superscript symbol (s) , are not significantly different.
- Percentage changes (%) were calculated by comparing normal group with doxorubicin treated group and pre-treated doxorubicin groups with doxorubicin treated group.

Table 2: Effects of rutin and/or hesperidin on testes glutathione level, glutathione peroxidase and glutathione-s-transferase activities in doxorubicin-administered treated rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Glutathione (nmol/gm)</th>
<th>% change</th>
<th>Glutathione Peroxidase (U/min)</th>
<th>% change</th>
<th>Glutathione-s-transferase (U/gm)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Normal</td>
<td>486.21 ± 29.14 b</td>
<td>-</td>
<td>18.38 ± 0.51 a</td>
<td>-</td>
<td>597.25 ± 4.2 c</td>
<td>-</td>
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<tr>
<td>G2 Doxorubicin</td>
<td>371.91 ± 13.92 a</td>
<td>-23.51</td>
<td>12.06 ± 0.20 a</td>
<td>-34.39</td>
<td>494.84 ± 14.9 b</td>
<td>-17.15</td>
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<tr>
<td>G3 Rutin + Doxorubicin</td>
<td>450.11 ± 10.52 b</td>
<td>21.03</td>
<td>13.60 ± 0.41 a</td>
<td>12.77</td>
<td>517.17 ± 26.5 b</td>
<td>4.51</td>
</tr>
<tr>
<td>G4 Hesperidin + Doxorubicin</td>
<td>442.20 ± 6.91 c</td>
<td>18.90</td>
<td>12.99 ± 0.32 a</td>
<td>7.71</td>
<td>547.40 ± 2.3 a</td>
<td>10.62</td>
</tr>
<tr>
<td>G5 Rutin + Hesperidin + Doxorubicin</td>
<td>415.18 ± 2.23 c</td>
<td>11.63</td>
<td>12.44 ± 0.45 a</td>
<td>3.15</td>
<td>524.33 ± 7.6 a</td>
<td>6.00</td>
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<tr>
<td>F-Probability</td>
<td>P&lt;0.001</td>
<td>-</td>
<td>P&gt;0.05</td>
<td>-</td>
<td>P&lt;0.001</td>
<td>-</td>
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<tr>
<td>LDS at 5% level</td>
<td>45.24</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>41.30</td>
<td>-</td>
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<tr>
<td>LDS at 1% level</td>
<td>61.21</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>55.88</td>
<td>-</td>
</tr>
</tbody>
</table>

- Data are expressed as mean ± standard error.
- Number of animals in each group is six.
- Mean, which have the same superscript symbol (s), are not significantly different.
- Percentage changes (%) were calculated by comparing normal group with doxorubicin treated group and pre-treated doxorubicin groups with doxorubicin treated group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
<th>Peroxidase (u/gm)</th>
<th>% change</th>
<th>Lipid peroxidation (nmol/gm/hr)</th>
<th>% change</th>
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<tbody>
<tr>
<td></td>
<td>G1 Normal</td>
<td>59.40 ± 1.2 b</td>
<td>-</td>
<td>2.19 ± 0.16 bc</td>
<td>-</td>
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<td></td>
<td>G2 Doxorubicin</td>
<td>44.01 ± 0.93 b</td>
<td>-25.91</td>
<td>3.88 ± 0.26 a</td>
<td>77.17</td>
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<tr>
<td></td>
<td>G3 Rutin + Doxorubicin</td>
<td>56.74 ± 1.7 a</td>
<td>28.93</td>
<td>2.77 ± 0.15 c</td>
<td>-28.61</td>
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<td></td>
<td>G4 Hesperidin + Doxorubicin</td>
<td>49.37 ± 1.5 b</td>
<td>12.18</td>
<td>2.25 ± 0.15 bc</td>
<td>-42.01</td>
</tr>
<tr>
<td></td>
<td>G5 Rutin + Hesperidin + Doxorubicin</td>
<td>53.49 ± 2.0 a</td>
<td>21.54</td>
<td>2.41 ± 0.22 b</td>
<td>-37.89</td>
</tr>
<tr>
<td></td>
<td>F-Probability</td>
<td>P&lt;0.001</td>
<td>-</td>
<td>P&lt;0.001</td>
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<tr>
<td>LDS at 5% level</td>
<td></td>
<td>4.42</td>
<td>-</td>
<td>0.536</td>
<td>-</td>
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<tr>
<td>LDS at 1% level</td>
<td></td>
<td>5.98</td>
<td>-</td>
<td>0.725</td>
<td>-</td>
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</tbody>
</table>

- Data are expressed as mean ± standard error.
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- Mean, which have the same superscript symbol (s), are not significantly different.
- Percentage (%) changes were calculated by comparing normal group with doxorubicin treated group and pre-treated doxorubicin groups with doxorubicin treated group.