EFFECT OF QUERCETIN ON SOME OF ANTIOXIDANT ENZYMES IN WISTAR RATS EXPOSURE TO THE OXIDATIVE STRESS BY LEAD ACETATE

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ABSTRACT

The present study was aimed to investigate the beneficial effect of quercetin extracted from onions to improvement of some antioxidant enzymes in adult males rats exposed to the oxidative stress by lead acetate. Forty males Wistar rats about 6 months old with average weight 175±10gm were divided randomly into the four equal groups and treated for 60 days as follows: The first group C was given drinking water only as control group. The second group (T1) was given quercetin (300 mg/kg/ B.w) orally, the third group (T2) given lead acetate (10 mg/kg/ B.w) orally and the fourth group was given lead acetate (10mg/kg/B.w) orally for 30 day then given quercetin (300mg/kg/B.W) for 30 day. In the end of experiment all animals were sacrificed and samples of blood were collected from the abdominal vein by using 5 ml disposable syringe then putting in gel and clot activator tube and left at room temperature until clotted, then putting it in centrifuge at (5000 rpm) for (5 min), the serum separated from the tube and stored in -20 until using for assessment of antioxidant enzymes. The results showed there was significant increase (p≤0.05) in malondialdehyde (MDA) level in T2 group compared with other groups. Also there was a significant decrease in MDA level in T1 compared with other groups .While there were no significant difference in MDA level in T3 compared with control group. Also there was a significant increase (p≤0.05) in superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) concentration in T1 group compared...
with other groups and there was a significant decrease in T2 group compared with other groups. Also there were no significant difference in SOD and CAT in T3 group compared with control group.

**KEYWORDS:** Quercetin, antioxidant enzymes, oxidative stress, lead acetate.

**INTRODUCTION**
Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells.\(^1\) One of the most important mechanisms and the sequence of events by which free radicals interfere with the cellular functions seem to be the lipid peroxidation leading eventually the cell death.\(^2\) Heavy metal ions, such as lead, can induce generation of reactive radicals and cause cellular damage. Lead is an ubiquitous element in the environment, it is used in many industrial activities including mining, refining and producing lead–acid batteries.\(^3\) The alimentary and respiratory tracts are the major routes of lead entry into the body.\(^4\) Lead generally interferes with a number of body functions such as the central nervous system, the haematopoietic system, liver and kidneys.\(^5\) Lead is reported to cause oxidative stress by generating the release of reactive oxygen species (ROS) such as superoxide (O2−), hydrogen peroxide (H2O2) and hydroxyl (OH) radicals and lipid peroxides.\(^6\)

Antioxidants are important and essential sustenance for plants and animals’. They protect cells from the hurt caused by free radicals.\(^7\) Antioxidants react with and stabilize free radicals and can prevent the damage causes by free radicals. Moreover it causes removing intermediates of free radical and inhibit other oxidation reactions by oxidized itself.\(^8\) Generally antioxidants are reducing agents which are found both intracellular and extracellular and have the ability to react with ROS and free radicals, this action delaying or preventing the oxidative stress.\(^9\) Antioxidants can be divided into 2 categories: enzymatic antioxidants like Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GTPx), Thioredoxin (TRX), Peroxiredoxin (PRX), Glutathione transferase (GST) cited by.\(^10\) And nonenzymatic antioxidants like vitamin, minerals and phytochemicals Flavonoids are natural compounds usually found in plants as secondary metabolites have important medical properties such as antioxidant.\(^11\) Quercetin are one of the major dietary flavonoids, it is a plant’s pigment that is found in many plants especially onion, apples, tea, broccoli, and berries.\(^12\) It have importance in pharmacology as antioxidant.\(^13\) Quercetin improve the antioxidative defense system by up regulating of antioxidant enzymes.\(^14\) So the present
study was aimed to investigate the beneficial effect of quercetin in improving the antioxidant enzymes in adult males rats that exposure to oxidative stress by lead acetate.

MATERIALS AND METHODS

Laboratory animals

Used in our experiment forty adult males Wister rats with about 6 months in old, with average weight about (175±10 gm.) obtained from animal house of veterinary medicine college of Al-Qadisiyah university. The animals housed in well ventilated wire-plastic cages and reared under controlled conditions about 12 hour light and 12 hour dark at about 25°. The animals were allowed to acclimatize for 7 days before experimentation.

Biological material

Quercetin 95% from onion provided by brightol company/ China.

Experiment design

Forty adult male Wister rats divided randomly to the four equal groups and treated for 60 days as following:-:Control group(C) given drinking water only. Second group (T1): given quercetin orally in dose (300mg/kg/B.w).[15] The third group (T2): given lead acetate orally in dose (10mg/kg/B.w).[16] The four group (T3): given lead acetate orally in dose(10mg/kg/B.w) for 30 days then treated by quercetin orally in dose (300mg/kg/B.w) for 30 day.

Samples Collection

Blood was collected from abdominal vein by using 5 ml disposable syringe, then putting in gel and clot activator tube and left at room temperature until clotted, then putting it in centrifuge at (5000 rpm) for (5 min) , the serum separated from the tube and stored in -20 until using for assessment of antioxidant enzymes.

Measurement of antioxidant enzymes

Antioxidant enzymes are determinate by spectrophotometer kits to SOD, GSH and CAT concentration that provided from US bio USA.

Measurement of malondialdehyde (MDA)

MDA is determinate by spectrophotometer kit that provided from US bio USA.
Statistical analysis
For analysis the results of study used Anova 1 (one way analysis of variance) with smallest significant difference LSD, was detected to compare between groups by using statistically analyzed by (SPSS) program version software.\textsuperscript{[17]}

RESULTS
Table 1 show there was a significant difference (p$\leq 0.05$) represented by increase in MDA level in T2 group (2.8±0.02) compared with other groups. And there was a significant difference represented by decrease in T1 group (1.2±0.01) compared with other groups. Also there were no significant difference between T3 group (1.50±0.03) and control group (1.58±0.01).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control group</th>
<th>T1 group</th>
<th>T2 group</th>
<th>T3 group</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µmole/ml)</td>
<td>b 1.58±0.01</td>
<td>c 1.2±0.01</td>
<td>a 2.8±0.02</td>
<td>b 1.50±0.03</td>
</tr>
</tbody>
</table>

Number= mean± S.E.
Different litters= Significant differences (p<0.05).
C group = control group.
T1 group = Orally gavage quercetin (300mg /kg/B.W once daily, dissolved in 1 ml drinking water) for 60 days.
T2 group = Orally gavage lead acetate (10mg/kg/ B.W once daily ,dissolved in 1 ml drinking water) for 60 days.
T3 group = Orally gavage lead acetate (10mg/kg/B.W once daily ,dissolved in 1 ml drinking water) for 30 days then given quercetin (300mg/kg/b. w) for 30 days.

SOD concentration
Table 2 show there was a significant difference (p$\leq 0.05$) represented by increase in SOD level in T1 group (2.8±0. 01) compared with other groups. And there was a significant difference represented by decrease in T2 group (1.2±0.04) compared with other groups. Also there were no significant difference between T3 group (1.91±0.03) and control group(1.94±0.01).
GSH concentration
Table 2 show there was a significant difference (p≤0.05) represented by increase in GSH level T1 group (2.8±0.01) compared with other groups. And there was a significant difference represented by increase in T3 group (2.4±0.03) compared with T2 group and control group. Also there was a significant difference represented by decrease in T2 group (1.1±0.05) compared with other groups.

CAT concentration
Table 2 show there was a significant difference (p≤0.05) represented by increase in CAT level in T1 group (0.60±0.09) compared with other groups. And there was a significant difference represented by decrease in T2 group (0.33±0.08) compared with other groups. Also there were no significant difference between T3 group (0.49±0.02) and control group(0.53±0.01).

Table: 2 Effect of quercetin on antioxidant enzymes in adult males wistar rats exposed to oxidative stress by lead acetate

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control group</th>
<th>T1 group</th>
<th>T2 group</th>
<th>T3 group</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/ml)</td>
<td>b 1.94±0.01</td>
<td>a 2.8±0.01</td>
<td>c 1.2±0.04</td>
<td>b 1.91±0.03</td>
</tr>
<tr>
<td>GSH (µmole/ml)</td>
<td>c 2.22±0.01</td>
<td>a 3.1±0.05</td>
<td>d 1.1±0.05</td>
<td>b 2.4±0.03</td>
</tr>
<tr>
<td>CAT (U/ml)</td>
<td>b 0.53±0.01</td>
<td>a 0.60±0.09</td>
<td>c 0.33±0.08</td>
<td>b 0.49±0.02</td>
</tr>
</tbody>
</table>

Number= mean± S.E.
Different litters= Significant differences (p<0.05).
C group = control group.
T1 group = Orally gavage quercetin (300mg /kg/B.W once daily, dissolved in 1 ml drinking water)for 60 days.
T2 group = Orally gavage lead acetate (10mg/kg/ B.W once daily, dissolved in 1 ml drinking water) for 60 days.
T3 group = Orally gavage lead acetate ( 10mg/kg/B.W once daily, dissolved in 1 ml drinking water) for 30 days then given quercetin (300mg/kg/b. w) for 30 d

DISCUSSION
The present study was aimed to investigate the role of quercetin to improvement antioxidant enzymes by using males rats as a model of mammalian through study SOD, GSH and CAT concentration, also study MDA that give indicator for lipid peroxidation.
Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components. They act by reducing the energy of the free radicals or by giving up some of their electrons for its use, thereby causing it to become stable. In addition, they may also interrupt with the oxidizing chain reaction to minimize the damage caused by free radicals.\[18\] Animal CAT are heme-containing enzymes that convert hydrogen peroxide (H2O2) to water and O2, and they are largely localized in subcellular organelles such as peroxisomes. Mitochondria and the endoplasmic reticulum contain little CAT, GSH removes H2O2 by coupling its reduction with the oxidation of GSH. Also reduce other peroxides, such as fatty acid hydro peroxides. These enzymes are present in the cytoplasm at millimolar concentrations and also present in the mitochondrial matrix. Most animal tissues contain both CAT and GSH activity. SODs are metal-containing proteins that catalyze the removal of superoxide, generating water peroxide as a final product of the dismutation. Three isoforms have been identified, and they all are present in all eukaryotic cells. The copper-zinc SOD isoform is present in the cytoplasm, nucleus, and plasma. On the other hand, the manganese SOD isoform is primarily located in mitochondria.\[19\]

Antioxidant substances like quercetin trigger reactive oxygen species-sensitive intracellular pathways that regulate the induction of specific gene.\[20\]

InT1 that given quercetin (300 mg/kg) there is a significant decrease in MDA due to quercetin act as strong antioxidant which causes scavenging to the free radicals and restore to the antioxidant enzymes, this leads to decreasing in free radicals which causes lipid peroxidation, subsequently decreasing in MDA level which give indicator to the lipid peroxidation and this agreed with.\[21\] Quercetin and its derivatives have been found to prevent oxidative cell damage by either increasing glutathione or reducing the activity of glutathione peroxidase.\[22\] Flavonoids have also been reported to protect cells from glutathione depletion with the cooperation of ascorbic acids.\[23\]

In T2 group which is exposed to oxidative stress by lead acetate there is increase MDA level due to lead acetate increases NO and lipid peroxidation in body tissues and serum, associate with depletion of antioxidant enzymes as SOD, GSH and CAT are one of the primary factors which permits lipid peroxidation, suggested to be closely related to lead acetate tissue damage.\[24\]
In T3 group that received lead acetate then treated by quercetin, there is a significant increase in SOD, GSH and CAT and significant decrease in MDA due to quercetin treatment reduced the harmful effect of lead acetate through scavenging free radicals and restore antioxidant enzymes. The present results indicate that the preventive effects of quercetin may be due to inhibition of lipid peroxidation by its antioxidant nature. In this study, exogenous application of quercetin has resulted in the influence on synthesis of antioxidant enzymes. Effect of these flavonoids was concentration dependent and tissue specific. Results have first time documented the effect of flavonoids on the antioxidant system of the body. This study would help in further understanding the cross talk between flavonoid and antioxidant pathways.

CONCLUSION
1. Quercetin have excellent therapeutic effects on oxidative stress accrue by lead acetate toxicity.
2. Quercetin have role in improvement the antioxidant enzymes and decreasing lipid peroxidation.
3. That use of the quercetin at a dose of (300 mg/kg) did not causes any side effects along period of experiment.

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REFERENCES


