The present research aimed to evaluate the ameliorating effect of Rutin as natural antioxidant on oxidative stress induced in experimental rats exposed to chronic toxicity with Pirimiphos methyl (POM), as one of organophosphorous pesticides widely used in the Middle East, through evaluation of erythrocytes and brain biomarkers. The rats were divided into four equal groups. 1) Control Normal group (C): received no drugs. 2) POM group (P): received single oral dose of POM (50 mg/ Kg b.w) daily for 3 months. 3) Rutin group (R): received single dose of Rutin (45 mg/ Kg b.w) per os daily for 3 months. 4) Rutin + POM group (R + P): received single dose of POM (50 mg/ Kg b.w) + rutin (45 mg/ Kg b.w) orally daily for 3 months. Blood and brain samples were collected from all animal groups three times at one, two and three months from the onset of experiment and used for determination of erythrocytes and brain antioxidant enzyme activities, reduced glutathione (GSH), lipid peroxides (MDA), in addition to serum nitric oxide (NO) and ceruloplasmin. (POM) induced a significant decrease in erythrocytes and brain AChE, CAT, GSH, erythrocytes GPx, GR and GST activities, and a marked increase in erythrocytes SOD, serum GGT activities and ceruloplasmin concentration. Also a significant increase in erythrocytes and brain MDA and serum NO level were observed. Rutin administration to POM intoxicated rats was able to counter act the oxidative stress induced by POM through the significant increase in the activities of erythrocytes and brain CAT, GSH, erythrocytes GPx, GR and GST activities. In addition, a marked decrease in erythrocytes and brain MDA and serum NO level were observed. The results of the present study suggest that rutin has the potential to exert a protective effect against oxidative stress induced by organophosphorous pesticides.

Key Words: Antioxidants, brain, erythrocytes, organophosphorous pesticides, oxidative stress, Pirimiphos methyl, Rutin.

1. INTRODUCTION

Organophosphate pesticides (OP) are the class of xenobiotics that are intentionally released to the environment. Therefore, there is a growing public concern about the accumulation of these insecticides in food products and water supplies, as repeated or prolonged exposure is a likely cause of delayed toxicity [14]. Currently, the general population is primarily exposed to organophosphates by ingesting food products containing these compounds, particularly fresh fruit and vegetables. Organophosphorus (OP) pesticides form a largest and most diverse group of insecticides. The wide application of OPs in public health and agricultural programs was accompanied by potentially hazardous impact on humans, animals, plants and environment.
(water, air, soil and food) and causes severe acute and chronic poisoning. The use of OPs as insecticides has been considerably increased due to their low toxicity and low persistence in the mammalian system in comparison to organochlorine insecticides. However, the extensive use of pesticides to protect agricultural crops usually results in the transfer of these compounds into the atmosphere and their diffusion toward urban areas [33].

The cellular antioxidant status determines the susceptibility to oxidative damage and is usually altered in response to oxidative stress. The cellular antioxidant action is reinforced by the presence of dietary antioxidants. Accordingly, interest has recently grown in the role and usage of natural antioxidants as a strategy to prevent oxidative damage in various health disorders with oxidative stress as factor in their pathophysiology [13]. Natural antioxidants from fruits and vegetables are reported to provide substantial protection that slows down the process of oxidative damage caused by ROS. Hence there has been growing interest in natural antioxidants of plant origin since they also find use as nutraceuticals due to their impact on the status of human health and disease prevention [35].

Rutin (quercetin-3-rhamnosyl glucoside) is a kind of flavonoid glycoside found in buckwheat, many vegetables, fruits, and plant-derived beverages such as tea and wine. Rutin is also known as Vitamin P and has antiplatelet, antiviral, and antihypertensive properties, as well as strengthening the capillaries, which is the result of its high radical scavenging activity and antioxidant capacity [21]. In addition, hypolipidaemic, cytoprotective, antispasmodic and anticarcinogenic activities have also been reported. These properties are beneficial in preventing diseases and protecting the stability of the genetic material [50]. Flavonoids possess good potential to be protective drugs due to their iron/copper binding and ROS scavenging properties. In fact, rutin was shown to scavenge ROS, namely superoxide, hypochlorite and peroxynitrate, to bind iron and copper, and to diminish oxidative injuries catalyzed by free iron [26]. Additionally, rutin was shown to inhibit xanthine oxidase, which is a principal ROS generating enzyme. Accordingly, the aim of the present study was to evaluate the antioxidant effect of rutin in pirimiphos methyl intoxicated rats.

2. MATERIALS AND METHODS

2.1. Animals

Sixty adult white male albino rats weighting 150 - 200 g were used for the study. Rats were housed in separated metal cages. Rats were kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were allowed free access to standard dry rat diet and tap water ad libitum.

2.2. Experimental design:

Rats were divided into four groups:
1) Control Normal group (C): Consisted of 15 rats, received no drugs, fed on normal diet for 3 months.
2) POM group (P): Comprised 15 rats, were fed on normal diet and administrated Pirimiphos methyl orally for 3 months at a dose level of 50 mg/ Kg b.w/day (1/40 LD50).
3) Rutin group (R): Composed of 15 rats, were fed on normal diet and received single oral dose of Rutin at a dose level of 45 mg/ Kg b.w/day dissolved in propylin glycol for 3 months.
4) Rutin + POM group (R + P): Included 15 rats, were fed on normal diet and received single oral dose of Rutin at a dose level of 45 mg/ Kg b.w/day dissolved in propylin glycol for 3 months follow by oral administration of Pirimiphos methyl per os daily for 3 months at a dose of 50 mg/ Kg b.w (1/40 LD50) follow by oral administration of Rutin at a dose level of 45 mg/ Kg b.w.

2.3. Sampling:

1- Blood samples:
Blood samples were collected by ocular vein puncture from all animal groups 3 times along the duration of experiment in dry, clean and screw capped heparinized tubes and plasma were separated by centrifugation at 2500 r.p.m for 15 minutes. The clean clear plasma was separated by Pasteur pipette and kept in a deep freeze at -20°C until used for subsequent biochemical analysis. Moreover, after plasma separation, erythrocytes were washed for subsequent biochemical analysis.

2. Brain specimens:
Rats were killed by decapitation. The brain specimen quickly removed, cleaned by rinsing with cold saline and stored at -20°C. Briefly, brain tissues were minced into small pieces, homogenized in normal saline 0.9%. The homogenates were centrifuged at 10,000 for 15 minute at 4°C. The supernatant was used for subsequent biochemical analyses.

2.4. Biochemical analysis:
Biochemical analysis were determined according to the methods described previously. Erythrocytes and brain acetyl cholinesterase (AChE) [25], Reduced glutathione (GSH) [9], Lipid peroxidation (MDA) Esterbauer et al., (1982), Catalase activity (CAT) (Sinha, 1972), serum Ceruloplasmin (Scholslnsky, et al., 1974), Nitric oxide (NO) [34], Gamma glutamyle transferase (γGT) [29], erythrocytes Super oxide dismutase activity (SOD) [37], Glutathione peroxidase (GPx) [39], Glutathione reductase (GR) (Goldberg and Spooner 1983), glutathione-S-transferase (GST) [22].

2.5. Statistical analysis:
The results were expressed as mean ± SE and statistical significance was evaluated by one-way ANOVA using SPSS (version 10.0) program followed by the post hoc test, least significant difference (LSD). Values were considered statistically significant when p < 0.05.

3. RESULTS & DISCUSSION
The obtained data in table (1) revealed that, administration of POM to normal rats exhibited a significant decrease in erythrocytes and brain AChE activities as compared with C group all over the experimental period. AChE activity is known as biomarker of chronic toxicity in human following pesticide exposure. It was recorded that, in acute exposure, the main mechanism of toxicity of (OP) is irreversibly binding to the enzyme acetylcholinesterase and inhibiting its activity that results in accumulation and prolonged effect of acetylcholine and consequently followed with acute muscarinic and nicotinic effects [1]. Moreover, the recorded decrease in AChE activity might be due to the inhibition of the enzyme by the toxic metabolites of OPI. It was recorded that, phosphorothioate insecticides converted to their corresponding oxygen analogs by called mixed-function oxidases (MFO), a microsomal system of enzymes among which the enzyme cytochrome P450 (CYP450) plays a major role. The oxons are direct inhibitors of AChE [20].

However, administration of rutin to POM intoxicated rats exhibited a significant increase in erythrocytes AChE activity after 2 and 3 months and in brain AChE activity after 3 months as compared with P group. The recorded increase in AChE activity may be related to the antioxidant capacity of rutin, which inhibit or decrease formation of free radicals resulted from POM administration. This suggestion was supported by the findings of [18] who mentioned that, rutin scavenge ROS, namely superoxide, hypochlorite and peroxynitrate and inhibit xanthine oxidase, which is a principal ROS generating enzyme.

The obtained data in table (1) revealed that, administration of POM to normal rats exhibited a significant increase in erythrocytes and brain (MDA) and serum NO
Table (1): Effect of Rutin administration on erythrocytes and brain AChE activity, MDA, GSH levels and serum NO, Ceruloplasmin concentration in normal and Pirimiphos Methyl (POM) intoxicated rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>Eryth. AChE (U/g Hb)</th>
<th>Brain AChE (U/g tissue)</th>
<th>Eryth. MDA (nmol/g Hg)</th>
<th>Brain MDA (nmol/g tissue)</th>
<th>Serum NO (μmol/L)</th>
<th>Eryth. GSH (mg/g Hb)</th>
<th>Brain GSH (mg/g tissue)</th>
<th>Serum Cerulo. (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>2.71± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.48± 0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.59± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>108.00± 2.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.60± 1.50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52.20± 0.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.60± 1.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.28± 0.75&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1st Month</td>
<td>P</td>
<td>2.33± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.44± 0.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.21± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>379.60± 12.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.80± 1.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.40± 0.68&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.60± 1.50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>32.06± 0.69&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>2.73± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.36± 0.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.56± 0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>109.20± 3.44&lt;sup&gt;c&lt;/sup&gt;</td>
<td>51.20± 1.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.00± 0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.20± 0.58&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R+P</td>
<td>2.33± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.64± 0.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.19± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>279.60± 5.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.00± 2.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.00± 1.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45.20± 1.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.80± 0.66&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2nd Month</td>
<td>C</td>
<td>2.71± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.48± 0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.72± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>110.40± 4.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.80± 1.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>55.00± 1.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.80± 1.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.64± 0.26&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>2.30± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>47.66± 0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.84± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>350.20± 18.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.00± 2.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.60± 1.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.40± 1.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>36.52± 1.46&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>2.78± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.54± 0.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.73± 0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>113.00± 5.58&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52.00± 0.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.40± 0.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.60± 1.94&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R+P</td>
<td>2.45± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.32± 0.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.23± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>270.00± 7.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.40± 1.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.40± 1.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.80± 1.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.40± 0.93&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3rd Month</td>
<td>C</td>
<td>2.74± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.10± 0.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.74± 0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>112.00± 4.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.80± 1.46&lt;sup&gt;c&lt;/sup&gt;</td>
<td>51.80± 3.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.60± 1.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.90± 0.42&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>2.11± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.10± 0.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.66± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>377.00± 11.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.40± 2.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.00± 1.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.80± 1.62&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.04± 1.36&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>2.79± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.12± 0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.20± 0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>119.40± 4.65&lt;sup&gt;c&lt;/sup&gt;</td>
<td>49.90± 0.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.90± 0.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>77.00± 1.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.40± 1.96&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>R+P</td>
<td>2.60± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.92± 0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.79± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>263.20± 10.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.80± 3.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.80± 3.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.20± 1.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.80± 1.77&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

(C: Control Normal group, P: POM group, R: Rutin group, R + P: Rutin + POM group)
Data are presented as (Mean ± S.E). S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at (P<0.05).
### Table (2): Effect of Rutin administration on erythrocytes and brain antioxidant enzymes in normal and Pirimiphos Methyl (POM) intoxicated rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>Eryth. SOD (U/g Hb)</th>
<th>Eryth. CAT (U/g Hg)</th>
<th>Brain CAT (U/g tissue)</th>
<th>Eryth. GPx (mU/g Hb)</th>
<th>Eryth. GR (U/g Hb)</th>
<th>Eryth. GST (U/g Hb)</th>
<th>Serum GGT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Month</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>33.54±0.55 c</td>
<td>2.74±0.07 a</td>
<td>13.22±0.28 a</td>
<td>16.82±0.44 a</td>
<td>6.68±0.13 a</td>
<td>5.30±0.05 a</td>
<td>3.31±0.16 c</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>59.74±0.57 a</td>
<td>0.98±0.04 b</td>
<td>7.36±0.18 c</td>
<td>10.70±0.18 b</td>
<td>3.10±0.11 c</td>
<td>4.28±0.04 b</td>
<td>11.01±0.15 a</td>
</tr>
<tr>
<td>R</td>
<td></td>
<td>34.88±0.43 c</td>
<td>2.58±0.09 a</td>
<td>13.72±0.09 a</td>
<td>17.02±0.32 a</td>
<td>6.60±0.06 a</td>
<td>5.19±0.06 a</td>
<td>3.18±0.21 b</td>
</tr>
<tr>
<td>R+P</td>
<td></td>
<td>45.86±1.13 b</td>
<td>1.66±0.07 b</td>
<td>9.80±0.23 b</td>
<td>12.03±0.30 b</td>
<td>5.19±0.13 b</td>
<td>5.30±0.03 a</td>
<td>10.85±0.05 a</td>
</tr>
<tr>
<td>2nd Month</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>33.54±0.53 c</td>
<td>2.80±0.08 a</td>
<td>13.62±0.26 a</td>
<td>17.72±0.27 a</td>
<td>6.40±0.231 a</td>
<td>5.31±0.02 a</td>
<td>3.68±0.22 c</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>64.32±0.51 a</td>
<td>0.90±0.07 b</td>
<td>6.96±0.05 c</td>
<td>10.20±0.27 c</td>
<td>2.73±0.17 c</td>
<td>4.42±0.15 b</td>
<td>10.62±0.15 a</td>
</tr>
<tr>
<td>R</td>
<td></td>
<td>33.58±0.70 c</td>
<td>2.76±0.09 a</td>
<td>14.10±0.41 a</td>
<td>17.60±0.21 a</td>
<td>6.76±0.15 a</td>
<td>5.45±0.18 a</td>
<td>3.72±0.27 c</td>
</tr>
<tr>
<td>R+P</td>
<td></td>
<td>44.56±1.14 b</td>
<td>1.52±0.07 b</td>
<td>9.92±0.24 b</td>
<td>14.02±1.05 b</td>
<td>4.63±0.12 b</td>
<td>5.26±0.06 a</td>
<td>6.54±0.54 b</td>
</tr>
<tr>
<td>3rd Month</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>31.62±0.57 c</td>
<td>2.88±0.07 a</td>
<td>13.34±0.72 a</td>
<td>18.19±0.30 a</td>
<td>6.64±0.19 a</td>
<td>5.50±0.13 a</td>
<td>3.84±0.29 b</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>66.56±2.11 a</td>
<td>0.86±0.05 c</td>
<td>7.12±0.10 c</td>
<td>8.91±0.35 c</td>
<td>2.71±0.24 c</td>
<td>4.47±0.05 b</td>
<td>10.60±0.22 a</td>
</tr>
<tr>
<td>R</td>
<td></td>
<td>34.32±1.36 c</td>
<td>2.60±0.17 a</td>
<td>13.66±0.16 a</td>
<td>17.89±0.21 a</td>
<td>6.44±0.23 a</td>
<td>5.18±0.36 a</td>
<td>4.06±0.20 b</td>
</tr>
<tr>
<td>R+P</td>
<td></td>
<td>41.72±0.82 b</td>
<td>1.94±0.05 b</td>
<td>10.40±0.20 b</td>
<td>17.17±0.57 a</td>
<td>5.48±0.18 b</td>
<td>5.54±0.13 a</td>
<td>4.48±0.22 b</td>
</tr>
</tbody>
</table>

(C: Control Normal group, P: POM group, R: Rutin group, R + P: Rutin + POM group)

Data are presented as (Mean ± S.E). S.E = Standard error.

Mean values with different superscript letters in the same column are significantly different at (P<0.05).
levels when compared to C group allover the experimental period. LPO has been implicated in a number of deleterious effects such as increased membrane rigidity, osmotic fragility, decreased cellular deformation, reduced erythrocyte survival, and membrane fluidity. Increase in the levels of TBARS indicates enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defense mechanisms to prevent the formation of excess free radicals [12]. The recorded results might be due to induction of cytochrome 450, inhibition of AChE and disturbance in activities of GSH and GST enzymes causing lipid peroxidation as reported by [41]. Another suggestion for the recorded results stated by [46] who suggested that, dimethoate induced LPO in liver could possibly result from an enhanced microsomal oxidative capacity induced by the insecticide. Thus, elevated levels of cytochrome P450 lead to high rates of radicals production, which, in turn, increase the rate of lipid peroxidation.

Nitrate and nitrite [a marker of endogenous nitric oxide (NO) production], possesses both antioxidant and pro-oxidant properties. An antioxidative property of NO has been reported in the many studies [6]. NO is an effective chain-breaking antioxidant in free radical-mediated LPO and reacts rapidly with peroxyl radicals as a sacrificial chain-terminating antioxidant. It is well documented that iNOS produces NO and NO-derived reactive nitrogen species such as peroxynitrite. In healthy neuronal tissue, iNOS is not commonly present, but it can be expressed by astrocyte, neurons, and endothelial cells after brain offence where it can initiate the production of high amounts of NO. Overproduction of NO may lead to neuronal damage and death. The reaction between NO and super-oxide anion generates the cytotoxic compound, peroxynitrite, that leads to neuronal toxicity [48]. Under normal physiological conditions, antioxidant enzymes are responsible to eliminate the highly reactive molecules. However, under unphysiological conditions, the excessive accumulation of reactive species induces several cellular dysfunctions [47].

However, administration of rutin to POM intoxicated rats exhibited a significant decrease in erythrocytes and brain (MDA) and serum NO levels allover the experimental period as compared with P group. The recorded results may be related to the potent free radical scavenging activity of flavonoids and polyphenolic compounds modulate the activities of various enzyme systems due to their interaction with various biomolecules as recorded by [15]. Phenolic phytochemicals due to their phenolic ring and hydroxyl substituents can function as effective antioxidants due to their ability to quench free electrons. It is therefore believed that, dietary phenolic antioxidants can scavenge harmful free radicals and thus inhibit their oxidative reactions with vital biological molecules and prevent the development of many physiological conditions, which can manifest into disease [39]. The decrease in MDA level has been also attributed to the oxidation of rutin by radicals, resulting in a more stable, less reactive radical [36]. This scavenging ability of rutin is due to its inhibitory activity on xanthine oxidase which is an important enzyme in the oxidative injury to tissue [42].

The obtained data in table (1) revealed that, administration of POM to normal rats exhibited a significant decrease in erythrocytes and brain (GSH) level and significant increase in serum Ceruloplasmin level when compared to C group allover the experimental period. The recorded results may be attributed to the utilization of GSH in the metabolism of pesticides through GST. The study of [44] revealed that, lindane, Malathion and propoxur increased the activity of GST by conjugation of GSH to pesticides in vivo. This could be understood in view of the fact that some pesticides (organochlorine and organophosphate) consume GSH through GST catalyzed
reaction as a major way of detoxification of these chemicals. In contrast, other classes of pesticides such as carbamate may utilize GSH in conjugation reaction but only in minor amounts compared to organophosphate or organochlorine [3].

However, administration of rutin to POM intoxicated rats exhibited a significant increase in erythrocytes and brain reduced glutathione (GSH) and serum Ceruloplasmin level alloover the experimental period as compared with P group. The recorded results may be related to antioxidant activity of rutin. This suggestion was supported by the findings of [49] who mentioned that, Aβ 42 may induce oxidative stress by decreasing GSH levels, but rutin can restore the decreased GSH levels. Consistently, the GSSG level was decreased with the addition of rutin. Also, GSH/GSSG redox is an ideal indicator for cellular redox status [10].

The obtained data in table (2) revealed that, administration of POM to normal rats exhibited a significant increase in erythrocytes (SOD) activity alloover the experimental period when compared to C group. The increased in SOD activity has been attributed to activation of the compensatory mechanism through the effect of OP on progenitor cells with its extent depending on the magnitude of the oxidative stress and hence, on the dose of the stressor. Supporting this idea, there is evidence that administration of malathion for 4 weeks increases the (SOD) activity in erythrocytes and liver [2].

However, administration of rutin to POM intoxicated rats exhibited a significant decrease in erythrocytes and brain CAT, erythrocytes GPx, GR and GST activities alloover the experimental period as compared with C group. The decrease in antioxidant enzymes has been interpreted as an indirect inhibition of the enzymes resulting from the binding of oxidative molecules produced during pesticide metabolism. Three enzyme systems (GST, esterases and monoxygenases) are involved in the detoxification of organophosphate insecticide class. These enzymes act by rapidly metabolizing the insecticide to non-toxic products or by rapidly binding and very slowly turning over the insecticide [28]. The present results confirm the previous reports of [16], who showed that repeated administration of dimethoate induced disturbances in the activities of the enzyme regulating GSH metabolism. Glutathione S-transferases are detoxifying enzymes that catalyze the conjugation of a variety of electrophilic substrates to the thiol group of GSH, producing less toxic forms. As demonstrated in other studies, the activities of antioxidant enzymes can be altered in a variety of animal tissues poisoned with OPI [24]. Generally, oxidative stress results in reduction in tissue antioxidants because these agents are utilized in terminating the lipid peroxidation chain reactions.

However, administration of rutin to POM intoxicated rats exhibited a significant increase in erythrocytes and CAT, erythrocytes GPx, GR and GST activities alloover the experimental period when compared to P group. The disorders provoked due to POM administration can be prevented using antioxidant substances capable of settling on the membrane and counteracting lipid peroxide formation [23]. Numerous papers show that among flavonoids there are strong scavengers of lipid radicals. Rutin has been found to be an important antioxidant agent and also has well-established properties against lipid peroxidation [32]. The recorded results may be related to the antioxidant
capacity of rutin. This suggestion was supported by the findings of [4] who mentioned that, rats given rutin showed significant improvement regarding the activity of GSH, GPx, GR and catalase thus suggesting its role in scavenging the free radicals generated by cisplatin.

However, administration of rutin to POM intoxicated rats exhibited a significant decrease after 2 and 3 months as compared with P group. It has been hypothesized that one of the principal causes of leakage of cellular enzyme into plasma is hepatic injury as reported by [27]. When the liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into blood stream. The recorded results may be related to antioxidant activity of rutin and its radical scavenging capacity to free radicals produced due to metabolism of organophosphorous pesticides by cytochrome P450 [46]. These radicals increase the rate of lipid peroxidation, increased membrane rigidity, osmotic fragility, decreased cellular deformation, reduced cellular survival, and membrane fluidity [12]. Administration of antioxidants restores the imbalance in antioxidant defense mechanism and preserves the structural integrity of the hepatocellular membrane against free radicals [31]. This suggestion was supported by the findings of [12] who reported that, pretreatment of the STZ-diabetic group with rutin, successfully restored the activities of liver enzymes to their normal levels.

4. CONCLUSION

In conclusion, the findings of the present study demonstrated that rutin administration provided an effective protection against oxidative damage in erythrocytes and brain induced by POM in rats, since rutin was able to ameliorate enzymatic and non-enzymatic antioxidant defense system and to prevent the lipid peroxidation in these tissues.

5. REFERENCES

of the aldehyde products of ADP/Fe+2 C stimulated lipid peroxidation in rat liver microsomes. Biochem. J, 208: 129-140.
Antioxidant Effect of Rutin


التأثير الواقي للروتن على نظام الدفاع المضاد للأكسدة والإجهاد التأكسدي في دم ومخ الفئران المسممة بالبيريميفوس ميثيل

سامي علي حسين - أمينة أحمد رجب - حسين عبد المجيد علي - عفاف دسوقي عبد المجيد- الشيماء محمد سعيد
قسم الكيمياء الحيوية – كلية الطب البيطري – جامعة بنها

الفقرة العربية


هذا وقد أظهرت النتائج وجود نقص واضح في نشاط الإنزيمات مضادة للأكسدة في المجموعة الثانية وعلى العكس ظهر تحسن واضح في النتائج في المجموعة الرابعة. كذلك مستوى مضادات الأكسدة غير إنزيمية في دم ومخ الفئران أظهرت النتائج وجود نقص واضح في المجموعة الثانية وعلى العكس ظهر تحسن واضح في نتائج المجموعة الرابعة.

ما سبق ينتج أن الأروتين له تأثير وقائي واضح في حماية الفئران من التأثير الضار لمادة البيريميفوس ميثيل ولذلك ننصح بضرورة استخدام مبيدات عاملة في العناصر المستخدمة للوقاية من الإجهاد التأكسدي الناتج عن التعرض للعديد من ملوثات البيئة ومن ضمنها المبيدات الحشرية.

(مجلة بنها للعلوم الطبية البيطريه: عدد 24 (1)، يونيو 2013: 172-183)