Ameliorative Effect of Vanillin against Propiconazole Induced Hepatorenal and Testicular Injury in Male Albino Rats

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Abstract
Vanillin commonly used in pharmaceutical industry, food and beverage processing, and in cosmetics and perfumes preparation. To better understand the effects of vanillin in reduction of tissue injuries resulted from Fungicides misuses. Fungicides like propiconazole are used extensively in greenhouse nowadays. The hazard effects of mycotoxin occurrence affect all defense and immunocytes of animals and reduced its production. But safety of use of fungicide and develop of new generation of fungicide often take a focus from researchers and authority. Propiconazole treatments at different doses have no effect on body weight. Notably, vanillin treatment reduced toxic effects of propiconazole induced in male rats’ evidence by liver and kidney biomarkers and improvement level of antioxidants level. Also, vanillin restored tissue architecture nearly similar to control group. Taken collectively, vanillin had a protective role against propiconazole induced toxicity.

1. INTRODUCTION
Conozoles, a group of fungicides, are extensively utilized in pharmaceutical and agricultural fields. They serve as agrichemicals to preserve variety of cereal crops, vegetables, and fruits. While, in therapeutics, they are applied locally or systemically to control mycotic and yeast infections (1-3). Conozoles comprise both triazole- and imidazole-antifungal agents (4).

Despite Propiconazole valuable effect in combating fungal infections, the irrational usage of PCZ may account for potential deleterious effects in mammals (5). It elicits its effect by hindering the ergosterol biosynthesis in fungi through suppressing a cytochrome P450, lanosterol-14α-demethylase, and subsequently lead to impairment in the synthesis of cell wall (3) Prior studies have declared that PCZ caused multiple adverse effects, as hepatotoxicity in rats and mice (6), neurotoxicity (7) and reproductive toxicity via endocrine disturbing effect (8). The researcher revealed that the toxic effect of PCZ on multiple organs may be owed to enhancement production of reactive oxygen species causing oxidative damage of biological membranes (9, 10). Hence, using exogenous antioxidants may mitigate PCZ-induced toxicity in various organs.

Vanillin (4-hydroxy-3-methoxybenzaldehyde), the main component of vanilla, was extracted from several essential plant oils, particularly Vanilla pompon, Vanilla tahitensis, and Vanilla planifolia (11). Vanillin commonly used in pharmaceutical industry, food and beverage processing, and in cosmetics and perfumes preparation (12). Several pharmacological effects were recorded of vanillin as anti-inflammatory, anti-carcinogenic (13) antioxidant (14), a hepatoprotective (15) and a nephroprotective (16).

The aim this study to investigate the mitigative effect of vanillin against PCZ induced hepatorenal and testicular injury in male albino rats.
2. **MATERIALS AND METHODS**

2.1. Tested Chemical

Propiconazole was obtained from the institute for pesticide residues analysis in Doky, ministry of agriculture, Egypt. Vanillin was purchased from Sigma Cairo, Egypt.

2.2. Laboratory Animals

40 Mature males' albino rats were obtained from the Experimental Unit in the Faculty of Pharmacy, Mansoura University. Animals were weighing about 100 ±10 gm and obviously healthy then were grouping and housing in plastic cages with soft wood shavings as a bedding material that change adequately to ensure a low level of ammonia and to keep animals clean and dry.

Animals kept for 2 weeks for accommodation and maintained on a balanced ration before the start of the experiment. Also, standard laboratory pelleted diet and water were received ad libitum throughout the experiment and Light cycles of 12 hours’ light to 12 hours dark seem to be adequate in order to promote rodents' breeding.

2.3. Experimental Design for Toxicity Propiconazole and the Effects of Vanillin Curing Propiconazole Induced Injuries in Male Albino Rats

Forty males were separated into five groups with eight rats per group. The first group was received 0.5 ml distilled water and used as control; the second group was administered 150 mg/kg (1/10 LD50), of propiconazole, the third group was gavaged 75 mg/kg (1/20 LD50), of propiconazole, the fourth group was treated with 1/10 LD50 of propiconazole and was given 150 mg/kg of vanillin intraperitoneally, the fifth group was treated with 1/20 of propiconazole and was given 150 mg/kg of vanillin intraperitoneally. Both fungicide and vanillin were received twice per week for 6 weeks. The protocol was approved by Institutional Research Board of faculty of medicine, Mansoura university, Egypt (R.20.07.924) and study was done with agreement with ethical guidelines and the rest of sacrificed rats was discarded safely.

2.4. Clinical Signs

The animals were noticed daily throughout the experimental period for any abnormal behavior, findings, or alteration.

2.5. Samples Collection

At end of treatment, rats were euthanized with overdose of thiopental Na (40 mg/Kg i.p.), whole blood was collected in gel tube (not contain anticoagulant) then kept for overnight in refrigerator followed by serum separation in centrifuge at 3000 rpm for 15 minutes then was store at -20°C in Eppendorf tubes. Liver, kidney, and testicle tissues were removed and wash with saline solution and preserve in 10% buffered formalin and another one gram of each homogenized tissue was preserve in falcon tube with 1 ml ice cold phosphate puffer (PBS). PH7.4 at –20 deep freezing.

2.6. Biochemical Analysis

*a. biochemical assays*

Sera of all rats of all groups was test for AST activity, ALT activity, urea and creatinine levels.

*b. Antioxidant and oxidative stress biochemical analysis*

Liver, kidney and testicular tissue homogenates were analyzed for GSH, GST, CAT, SOD1 and MDA levels by using specific kits.

2.7. Histopathologic Examinations

Specimens from liver, kidney and testicular tissue were fix in 10% formalin and 5μ thickness sections of specimens prepared then stained with hematoxylin and eosin (H&E) and examined microscopically.

2.8. Statistical Analysis

Data was analyzed statically for variance by one-way ANOVA and least standard difference LSD, homogeneity and sample size by using SPSS program version 20. P value ≤ 0.05 was consider significant (17).

3. **RESULTS**

PLZ is used as a fungicide in agriculture especially in greenhouse. PLZ reported to induce different organ toxicity in animal models and also in human intoxication. In the current study PLZ not resulted in change in body weight or organ to body weight ratio.

In table 1, PLZ increased significantly liver enzymes, blood urea nitrogen and creatinine. While vanillin retained liver and kidney biomarkers when compared to control group. Additionally, vanillin hadn’t induced any alteration in activity of liver markers or kidney parameters levels.
leucocytic infiltration in different tissue in figure

Table 1. Biochemical effects of treated groups by PLZ and or vanillin

<table>
<thead>
<tr>
<th></th>
<th>ALT</th>
<th>GGT</th>
<th>Urea</th>
<th>creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>35.2 ± 1.2</td>
<td>24.3 ± 0.9</td>
<td>47.3 ± 1.6</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>G2</td>
<td>35.4 ± 1.02</td>
<td>24.6 ± 0.91</td>
<td>47.8 ± 1.06</td>
<td>0.66 ± 0.02</td>
</tr>
<tr>
<td>G3</td>
<td>60.74 ± 2.03</td>
<td>53.97 ± 2.02</td>
<td>77.62 ± 2.1</td>
<td>1.82 ± 0.06</td>
</tr>
<tr>
<td>G4</td>
<td>44.43 ± 2.07</td>
<td>33.34 ± 1.14</td>
<td>59.02 ± 1.55</td>
<td>0.86 ± 0.44</td>
</tr>
<tr>
<td>G5</td>
<td>36.2 ± 1.22</td>
<td>25.3 ± 0.9</td>
<td>48.3 ± 1.76</td>
<td>0.66 ± 0.02</td>
</tr>
<tr>
<td>G6</td>
<td>35.12 ± 1.02</td>
<td>24.43 ± 0.93</td>
<td>47.53 ± 1.66</td>
<td>0.67 ± 0.02</td>
</tr>
</tbody>
</table>

*a, b significant at p value ≤ 0.05

In table 2, 3, and 4 PLZ reduced level of antioxidant like GSH, GST, SOD1 and catalase in liver, kidney and testicular tissue. Also, PLZ increased lipid peroxidation evident by level of MDA when compared to control group. Notably, vanillin restored level of antioxidant levels of PLZ-treated groups with vanillin quite similar to control group. While group treated with vanillin only showed increased level of antioxidant and maintained level of MDA similar to control group.

Table 2. Antioxidant/ Oxidant level in liver tissue homogenates in treated groups versus control one

<table>
<thead>
<tr>
<th></th>
<th>GSH mg/g tissue</th>
<th>GST U/g tissue</th>
<th>SOD U/g tissue</th>
<th>CAT U/g tissue</th>
<th>MDA nmol/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>g1</td>
<td>28.69 ± 0.19</td>
<td>10.78 ± 0.18</td>
<td>27.42 ± 1.04</td>
<td>17.96 ± 0.19</td>
<td>30.97 ± 0.19</td>
</tr>
<tr>
<td>g2</td>
<td>28.69 ± 0.29</td>
<td>10.78 ± 0.28</td>
<td>27.42 ± 1.24</td>
<td>17.96 ± 0.24</td>
<td>30.97 ± 0.23</td>
</tr>
<tr>
<td>g3</td>
<td>15.91 ± 1.34</td>
<td>6.05 ± 0.37</td>
<td>16.08 ± 1.33</td>
<td>10.94 ± 0.31</td>
<td>73.96 ± 1.39</td>
</tr>
<tr>
<td>g4</td>
<td>19.17 ± 1.98</td>
<td>7.69 ± 0.54</td>
<td>24.82 ± 1.49</td>
<td>13.54 ± 0.36</td>
<td>61.14 ± 1.28</td>
</tr>
<tr>
<td>g5</td>
<td>28.16 ± 0.92</td>
<td>10.78 ± 0.28</td>
<td>27.42 ± 1.24</td>
<td>17.96 ± 0.39</td>
<td>30.17 ± 0.39</td>
</tr>
<tr>
<td>g6</td>
<td>28.69 ± 0.15</td>
<td>10.78 ± 0.21</td>
<td>27.42 ± 1.4</td>
<td>17.96 ± 0.24</td>
<td>30.17 ± 0.43</td>
</tr>
</tbody>
</table>

*a, b, c significant at p value ≤ 0.05

In histopathological figure 1, 2, 3, A showed normal histological structure of liver, kidney and testicular tissue (normal seminiferous tubules with complete spermatic series) in control group 1 and also in group received vanillin. PLZ treated group showed necrosis, degeneration and leucocytic infiltration in different tissue in figure 1, 2, 3 B. Notably, vanillin treatment restored injury induced by PLZ quite similar to normal tissue marketecture of normal rats. Taken collectively, from the biochemical and histological biomarkers, vanillin had a ameliorative effects against injury induced PLZ.
4. DISCUSSION

Propiconazole (PCZ) is a triazole fungicide had extensively used in agriculture (7). The PLZ had no effects on the body weight or relative organ weights as previously mentioned before (18). PLZ had a hepatotoxic effects (5, 19, 20), nephrotoxic (21), induced testicular damage (18, 22, 23), induction of oxidative stress (7, 9) and tumorin rats (24). Previously we found that vanillin had hepatorenal protections against gentamycin induced toxicity in male albino rats (25). In current study, vanillin improved the liver enzymes, creatinine and blood urea nitrogen of rats treated with different dose of propiconazole. Similarly, vanillin had a hepatorenal protections (16, 25) through increased the antioxidant levels (14, 15). Also, Vanillin increased regeneration in injured tissue as a common modulator (26, 27) and vanillin could enhanced the expression of recombinational repair genes in tissues of rats treated by azoxymethane (28). Notably, vanillin had a protective role, restoring of testicular and renal tissues (29) and had a vital role in mitigation of oxidative stress and toxicity induced by toxic agents (30). Only noticed that vanillin reported to increase the antioxidant level and maintained oxidant at basal level (31), similarly, other plant extract could increase the antioxidant than basal level. On the conclusions, vanillin could have a role in protection against injuries induced by propiconazole fungicide.

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