Potential Effects of Bee Honey and Propolis Against the Toxicity of Ochratoxin A in Rats

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Abstract

Background. In the recent years, extensive research work has been focused on the use of natural materials as antioxidants against the toxic oxidative materials to ameliorate their toxic and cell damaging effects.

Aim. To evaluate the antioxidant effects of bee honey and propolis against OA-induced oxidative stress in liver and kidney in rats.

Materials and Methods. 64 albino rats divided into 8 groups, group 1 as control, groups 2-4 received an oral dose of OA, honey and propolis respectively for four weeks, groups 5 and 6 were treated with a weekly dose of OA concomitant with a daily dose of bee honey in group 5 and propolis in group 6, groups 7 and 8 were treated with a daily dose of bee honey in group 7 and propolis in group 8 and single weekly dose of OA then administered starting the second week of treatment. After 4 weeks, blood samples, liver and kidney tissues were collected for the subsequent determinations.

Results. The study showed that OA exerted toxic effects on both liver and kidney tissues manifested as elevated serum alanine aminotransferase (ALT), gamma glutamyl transferase (\(\gamma\) GT), creatinine and cholesterol. OA also caused perturbation in liver and kidney antioxidant system reflected as diminished reduced glutathione (GSH), oxidized glutathione (GSSG) content and also decrease in glutathione peroxidase (GPX) and superoxide dismutase (SOD) activity. The level of malondialdehyde (MDA) which is a lipid peroxidation product was elevated. Bee honey (BH) and propolis (PR) ameliorated the toxic effects of OA on liver and kidney tissues with significant reduction of mean serum levels of ALT, \(\gamma\) GT, cholesterol and creatinine. Also BH and PR improved the reduction in the antioxidant parameters of the liver and kidney (GSH, GSSG content and GPX, SOD activity) caused by OA administration. The level of MDA was also significantly decreased.

Conclusion. Bee honey and propolis ameliorated OA-induced oxidative stress in the liver and kidney through their role in scavenging free radicals and preventing lipid peroxidation.
collected by honey bees from various plant sources. It is a popular folk medicine possessing a broad spectrum of biological activities (4). Hepatoprotective, renal protective and therapeutic effects of propolis ethanol extract were observed (5, 6). The pharmacological effects of bee propolis include reduction of the blood pressure, protection of the liver tissue against carbon tetrachloride, protection against stomach ulcer formation and maintenance of serum glucose (7).

Bee honey is a functional food which has a unique composition and antimicrobial properties (8). It is a natural product with very complex chemical composition, composed primarily of fructose and glucose but also contains 4 to 5% fructooligosaccharides, which serve as prebiotic agents (9). It contains more than 180 substances, including amino acids, vitamins, minerals and enzymes (10). Honey is used in medicine in the treatment of chronic skin ulcers, gastrointestinal diseases, chest diseases as well as in surgery (11). It is also used as chemical antiseptic, antibacterial and antifungal agent (12).

The aim of this study was to evaluate the antioxidant effects of bee honey and propolis against OA-induced oxidative stress in liver and kidney in rats.

Materials and Methods

**Experimental animals**

Male albino (Sprague Dawely) strain rats with an average weight of (100–200 g), obtained from the animal house of the National Research Center, Dokki, Egypt. They were maintained on OA-free standard laboratory diet (protein: 16.04%, fat: 3.6%, fiber: 4.1% and metabolic energy: 2887 kcal/kg) and water ad libitum. The animals were kept in ideal laboratory conditions. The experiment was carried-out in accordance with the national regulations of animal welfare and Institutional Animal Ethical Committee (IAEC), National Research Center.

**Preparation of ochratoxin (OA)**

Ochratoxin A (OA), a toxic fungal metabolite produced by Aspergillus Ochraceus was prepared according to the method of Frohlich et al., 1988 (13).

**Preparation of honey samples**

The honey was collected form Saint Katherine area in Sinai Peninsula. Samples were prepared with a modification of the method proposed by Schade et al., 1958 (14). A sample of 25 g of honey was weighed into a sterile jar and then 25 ml of sterile water was added. The honey was then dissolved rapidly by shaking at room temperature.

**Preparation of propolis samples**

The propolis was collected form Saint Katherine area. Samples were prepared with a modification of the method proposed by Hegazi, 1997 (15). A sample of 5 g of propolis was dissolved in 50 ml of distilled water, heated gently and shaken at room temperature.

**Treatment protocol**

64 male rats were divided into 8 experimental groups each of 8 animals, (treatment duration was 4 weeks). The first group represented a control group, treated daily with 10% NaHCO₃ solution as a vehicle. The second group was given a weekly oral dose of OA in NaHCO₃ (1 mg/kg b.w equivalent to 1/5 LD₅₀), the rest of the days, the NaHCO₃ vehicle was administered daily. The third group was treated with a daily oral dose of (1 mg/kg b.w) honey in NaHCO₃. The fourth group was treated orally with a daily dose of (1 mg/kg b.w) propolis in NaHCO₃.

The fifth group each rat was given a weekly dose of OA (1/5 LD₅₀) in NaHCO₃ concomitant with a daily dose of bee honey (1 mg/kg b.w) throughout the duration of the experiment. The sixth group, each animal was administered a weekly dose of OA (1/5 LD₅₀) in NaHCO₃ concomitant with a daily dose of propolis (1 mg/kg b.w) throughout the duration of the experiment. In the seventh group, each rat was treated with a daily dose of bee honey (1 mg/kg b.w) for 4 weeks, and a single weekly dose of OA was administered, starting the second week from the initiation of the honey treatment. The eighth group, each rat was treated in a way similar to that of group seven except that honey was replaced by propolis.

**Blood and tissue sampling**

At the end of the experiment, blood samples were obtained from the retro-orbital plexus (16) using heparinized capillary tubes. Samples were left to clot, then centrifuged at 3000 r.p.m. for 15 minutes to separate blood serum. Serum was stored at -20°C until used for the determination of creatinine, total cholesterol, alanine aminotransferase and γ-glutamyltransferase. Animals were rapidly sacrificed for excision of the liver and kidney. They were removed quickly and placed in iced normal saline, perfused with the same solution to remove blood cells, blotted on filter paper and frozen at -80°C.
Homogenate preparation

The liver and kidney tissues were cut into small pieces and homogenized in 5 ml cold buffer (0.5 g of Na₂HPO₄ and 0.7 g of NaH₂PO₄ per 500 ml deionized water, pH 7.4) per gram tissue, then centrifuged at 4000 rpm for 20 minutes at 5°C, the supernatant was used for the assay of the content of (GSH) and (GSSG), the activity of (GPX) and (SOD) and the level of (MDA).

Biochemical analysis

Serum creatinine was assayed according to the method of Bartles, et al.,1972 (17) using Bio Merieux kit, France. Serum total cholesterol was performed according to the method of Richmond,1973 (18) using commercial kit supplied by Randox, U.S.A. Serum alanine aminotransferase was determined according to the method described by Reitman and Frankel, 1957 (19) using a Bio Merieux kit, France. Serum gamma-glutamyl transferase (γ-GT) was determined by a kinetic colourimetric method according to Nielsen et al., 1978 (20) using a kit supplied by Sclavo Diagnostics, Italy. Reduced glutathione (GSH) content was determined in liver and kidney according to the method described by Ellman, 1959 (21). Estimation of oxidized glutathione (GSSG) content in liver and kidney was made according to the method of Rall and Lehninger 1952 (22). Glutathione peroxidase (GPX) activity in liver and kidney was determined using the Ransel kit according to Kraus and Ganthen 1980 (23). Estimation of superoxide dismutase (SOD) activity in liver and kidney was made according to the method of Suttle, 1986 (24) using Randox Kit. Tissue malondialdehyde (MDA) was performed according to the method of Draper and Hadley, 1990 (25).

Statistical Analysis

Statistical analysis was performed using SPSS program, version 9.05 and Microsoft Excel 2003. The data was expressed as mean ± standard deviation (SD). Independent samples T-test was performed to determine the specific differences between means. The results are considered to be significant when p value is less than 0.05 and highly significant.

Results

The results of this study are presented in Table 1 and Figures 1 - 5. Table 1 shows the effect of Table 1: Effect of oral treatment with ochratoxin A (OA), propolis (PR), bee honey (BH) and their combinations on serum alanine aminotransferase (ALT), serum γ-glutamyl transferase (γ-GT), serum total cholestrol and serum creatinine.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cont X ± SE</th>
<th>OA X ± SE</th>
<th>BH X ± SE</th>
<th>PR X ± SE</th>
<th>OA+BH X ± SE</th>
<th>OA+PR X ± SE</th>
<th>BH+OA X ± SE</th>
<th>PR+OA X ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum alanine amino transferase - ALT (U/L)</td>
<td>12.13±0.74</td>
<td>37.25±1.91</td>
<td>10.75±0.70</td>
<td>10.50±0.56</td>
<td>23.75±1.28</td>
<td>24.50±1.09</td>
<td>18.87±1.02</td>
<td>17.25±0.81</td>
</tr>
<tr>
<td>% of change</td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>c</td>
<td>c</td>
<td>d</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>Serum γ-GT (U/L)</td>
<td>44.00±1.03</td>
<td>182.75±0.70</td>
<td>43.75±0.92</td>
<td>42.38±0.67</td>
<td>122.50±1.15</td>
<td>114.62±0.76</td>
<td>83.13±0.71</td>
<td>72.50±0.81</td>
</tr>
<tr>
<td>% of change</td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>c</td>
<td>c</td>
<td>d</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>Serum total cholesterol (mg/dl)</td>
<td>81.88±2.36</td>
<td>136.25±1.51</td>
<td>79.63±0.94</td>
<td>80.87±1.24</td>
<td>98.75±2.52</td>
<td>97.00±2.91</td>
<td>90.38±0.75</td>
<td>90.13±2.32</td>
</tr>
<tr>
<td>% of change</td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>c</td>
<td>c</td>
<td>d</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.69±0.04</td>
<td>1.78±0.15</td>
<td>0.62±0.03</td>
<td>0.64±0.02</td>
<td>1.29±0.05</td>
<td>1.27±0.07</td>
<td>1.22±0.06</td>
<td>1.10±0.08</td>
</tr>
<tr>
<td>% of change</td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>c</td>
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</table>

Values are expressed as means ±SE. Numbers between parentheses indicate the percentage of change in comparison with the corresponding control value. Means within a row with a common superscript are not significantly different (P > 0.05).

Figure 1: Effect of oral treatment with ochratoxin A (OA), bee honey (BH), propolis (PR) and their combination on liver and kidney glutathione (GSH) content (micromol/g tissue) in rats. Means with a common superscript are not significantly different (p>0.05).
There were no significant differences between the groups of rats treated with either honey or propolis and the control group regarding these serum parameters.

OA administration caused significant elevation in serum ALT, γ-GT, cholesterol and creatinine. Significant decrease in their mean serum levels was observed in the groups treated with honey or propolis either before or after OA compared to OA-treated group, but the ameliorating effect of BH or PR administration before OA was better than that after OA. Nonetheless, the mean levels of all these serum parameters did not return back to the control value.

Figures 1-3 show the effect of oral treatment with OA, BH, PR and their combination on liver and kidney reduced glutathione (GSH), oxidized glutathione (GSSG), and glutathione peroxidase (GPX). It is apparent that there was a significant decrease in the content of GSH, GSSG and activity of GPX in the rats treated with OA compared to the control group. Improvement was observed in the groups treated with BH or PR either before or after OA and the change was statistically significant, yet their levels did not reach those of the control.

Figure 4 shows similar results as regard the effect on liver and kidney superoxide dismutase enzyme (SOD) but the improvement in case of treatment with bee honey and propolis before OA was better than that after OA.
Figure 5: Effect of oral treatment with ochratoxin A (OA), bee honey (BH), propolis (PR) and their combination on liver and kidney lipid peroxidation product malondialdehyde (MDA) content (μmol/g tissue) in rats. Means with a common superscript are not significantly different (p>0.05).

Figure 5 shows the effect of OA, BH, PR and their combination on liver and kidney lipid peroxidation product, malondialdehyde (MDA). OA significantly increased its mean level above that of the control. Treatment with BH or PR either before or after OA inhibited the OA induced increase in the MDA, although its mean level is still higher than that of the control. Also figures 1-5 show that treatment with either BH or PR alone has no significant effect on either of the studied parameters as compared to the control group.

Discussion

Our results revealed that ochratoxin A has toxic effects on the liver as shown by the elevated mean serum levels of ALT and γ-GT. These results are in agreement with those of Nada et al., 1996 and Richard, 2007 (1, 2).

The liver is the most sensitive organ to preoxidative damage because it is rich in oxidizable substances. The increment of the oxidative stress on the cells of the liver and the consequent decrease in the antioxidant ability of the cells result in the occurrence of aggressive cellular damage to the liver cells with destruction of their membranes and the release of the enzymes into the blood stream (26, 27).

The more severe the liver damage the higher the release of the liver enzymes (28). In our experiment a pronounced decrease in the activities of both serum ALT and γ-GT was detected after oral treatment with bee honey or propolis either before or after OA administration. These results indicate a marked hepatoprotection induced by both agents. This protective effect may be due to the antioxidant effect of both honey and propolis which was previously confirmed (4, 29, 30).

In the current study marked increase in the mean serum cholesterol level was found in the OA supplemented group which may reflect the impairment of liver function and particularly lipid metabolism (1). Significant improvement in the serum cholesterol level in the groups of animals treated with propolis or bee honey either before or after OA supplementation was observed. Langseth, 2000 (31) obtained similar results and he explained that flavonoids in the bee honey were responsible for such effect.

In this study nephrotoxicity was manifested by inhibition of kidney function as indicated by increased mean serum creatinine level. OA acts essentially in the proximal renal tubules, inhibiting the enzyme phosphoenol-pyruvate carboxylase, which is a lipid peroxidant, and it alters the structural and functional renal ability to metabolize calcium (32). Morphologically, the kidney shows atrophy and sclerosis of the proximal tubules. Functionally, tubular functions are reduced (33). Our results coincide with others by Kuiper-Goodman and Scott 1989 (34) and Marquardt et al., 1990 (35).

Effective reduction of serum creatinine level was noticed after administration of bee honey or propolis. These results are probably due to the antioxidant protective effect of propolis and honey which could have accumulated in the cells of the proximal convoluted tubule of the kidney where propolis was reported to be collected and secreted (36).

Reduced glutathione (GSH) is an important naturally occurring antioxidant and its level in a tissue is considered a critical determinant of the threshold for tissue injury. Our results proved OA toxic effects similar to those of cadmium resulting in a significant depletion of GSH in both liver and kidney cells which might have led to their damage due to enhancing of lipid peroxidation (37).

OA combines with iron, facilitating its reduction. The iron–OA complex produces the
extremely damaging hydroxyl radical in the presence of NADPH cytochrome p-450 reductase system, this radical species may be partly responsible for OA toxicity (38, 39). Similar results were obtained for liver and kidney oxidized glutathione (GSSG) with OA treatment. Since GSH represents the substrate of glutathione peroxidase which catalyzes the formation of GSSG, so if the reduced glutathione decreases, this is likely to be followed by a decrease of oxidized glutathione (40).

Propolis or honey treatment ameliorated this effect in both kidney and liver but to a some extent as the values were still lower than that of the control group. This protective effect is probably a result of their antioxidant and free- radical scavenging properties which in turn help to maintain the intracellular level of both reduced glutathione and oxidized glutathione (41).

The present results proved significant increase of lipid peroxidation secondary to OA administration which is manifested by high malondialdehyde level. MDA is an end product of lipid peroxidation and it is considered a late biomarker of oxidative stress and cellular damage (42). Pervious studies suggested that the toxicity of OA may be the result of three major effects: inhibition of ATP synthesis, inhibition of protein synthesis and enhanced lipid peroxidation (35). Similar results were obtained by others (39, 43) who demonstrated that OA enhanced lipid peroxidation when added to liver and kidney microsomes in vitro or when administered to rats in vivo. They ascribed this effect to an OA- stimulated NADPH-dependent and ascorbate -dependent lipid peroxidation with iron being an essential cofactor.

The efficiency of several ochratoxins (ochratoxins A, B, C, alpha and O- methyl ochratoxin C) to enhance lipid peroxidation was also related to the presence of a reactive phenolic hydroxyl group in these compounds capable of complexing with transitional metals. Furthermore, substantial lipid peroxidation occurs only when cellular defense mechanisms have been weakened or overcomed by prolonged oxidative stress (43, 44). Lipid peroxidation was greatly reduced in the groups of rats treated with propolis or bee honey before or after OA treatment, as manifested by decreased MDA level.

The antioxidant effects of bee honey was attributed to its constituents of the most important antioxidant trace elements and to the antioxidant activity of its flavonoid compounds. Therefore bee honey has been suggested to be able to decrease the nitric oxide and lipid peroxidation. Similarly propolis was reported to decrease lipid peroxidation (45-48).

Concerning the effect of OA on the activity of liver and kidney glutathione peroxidase (GPX) and superoxide dismutase (SOD), significant decrease in their activity was observed in the OA treated group compared to the control group. The reduction in their activity is probably due to the increase in malondialdehyde level caused by OA (49).

The study showed significant improvement in GPX and SOD activity after treatment with bee honey or propolis. This improvement plays an important role in the cellular defense against the oxidative stress of OA (50). In case of SOD the improvement was better when they were administered before OA. Machlin and Bendich 1987 (51) explained that bee honey constituents include the antioxidant trace elements iron, zinc and selenium which are essential cofactors for the enzymatic antioxidant defense system represented by catalase, superoxide dismutase and glutathione peroxidase.

**Conclusion**

OA is a concern of public health by the consumption of contaminated food. The kidney and liver are its main target tissues. This study provides an easy and relatively cheap natural products (bee honey and propolis) as natural antioxidants for protecting against oxidative toxic effects of OA. The results confirmed counteracting most of OA-induced hepato- and nephrotoxicity by these bee products and showed that their administration improved the tissue specific antioxidant enzymes, reduced lipid peroxides thus enhance tissue antioxidant defense capacity.

**References**


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