Ameliorative Effect of Honey and Bee Venom against Renal Injury Induced by Lipopolysaccharide (Lps) And Carbon Tetrachloride (Ccl₄) in Male Albino Rats

Nagy S. Tawfik, Suzan Alaa Ismail and Noha M. Meligi

Zoology Department, Faculty of Science, Minia University 61519, Minia, Egypt

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ABSTRACT
The objective of the study was to assess the potential effect of honey and bee venom (BV) on nephrotoxicity caused by LPS (Lipopolysaccharide) and CCl₄ (Carbon Tetrachloride) in rats. Sixty-four male albino rats of an average weight of 120–150 g were included in this study. Rats were divided into 8 equal groups of 8. The results showed that treatment with LPS/CCl₄ caused a significant increase in serum urea, creatinine as well as uric acid. The use of honey (25 mg/kg b.wt.) and BV (1 mg/kg b.wt.) attenuates to LPS and CCl₄ induced renal dysfunction was demonstrated. Honey and/or BV may therefore be a potential therapeutic method to prevent LPS/CCl₄-induced renal dysfunction.

Keywords: Carbon Tetrachloride CCl₄, Lipopolysaccharid (LPS), honey, bee venom, Kidney functions, Albino Rats.

1. Introduction

Honey is one such naturally occurring food substance produced by bees. It is recognized worldwide for its high nutrient components that are beneficial to humans. Honey constituents include major sugars and some minor phytochemicals, such as phenolic acids, vitamins and certain minerals. It has traditionally been used by Egyptians, Greeks, Romans, and Chinese to cure wounds and intestinal diseases, including gastric ulcers. It has also been used as a cure for cough, sore throat, and earache (Subramanian et al., 2016; Pasupulet et al., 2017). Honey has recently also been used for its antimicrobial, anti-inflammatory and antioxidant activities, as well as for boosting the immune system (Abeshu and Geleta, 2016). Evidence of the biological action of honey can be attributed to its polyphenolic content, which in turn is usually associated with its antioxidant and anti-inflammatory action, as well as its cardiovascular, anti-proliferative and anti-microbial benefit (Alvarez-Suarez et al., 2013).

Bee venom (BV) has also been recognized for its therapeutic properties by Chinese, Egyptians, Ancient Mexicans and Greeks for thousands of years (Abel, 2015). BV therapy is the use of live bee stings (or injectable venoms) to treat various diseases. BV is a complex mixture of active peptides, enzymes and amines. There are at least 18 active components in the venom that have some pharmaceutical properties (Ali, 2012). Among these compounds, melittin, a small linear peptide consisting of 26 amino acids, is the major potent toxin of BV, which contains about 50 % of BV (Kim et al., 2015). BV has been used as a traditional medicine for the treatment of various diseases such as arthritis, rheumatism, back pain and skin diseases (Son et al., 2007).

LPS is an endotoxin that is produced by gram-negative bacteria. Endotoxin can trigger cytokine synthesis, secretion, and consequent chronic inflammation. Earlier studies have shown that LPS is one of the most important causes of sepsis and is associated with sepsis-associated acute kidney injury pathogenesis, which resulting in oxidative stress induction, low blood pressure, renal hypoperfusion, and, eventually, a slow reduction in renal function.(Peerapornratana et al., 2019). It is well recognized that an animal model of LPS-induced acute kidney injury has been widely used to investigate the
mechanisms by which endotoxemia causes acute kidney injury and to assess possible new preventive or therapeutic agents for the disease. (Jung-Yeon et al., 2020).

CCl₄ is an active, lipid-soluble hepatotoxin that enhances the peroxidative process, has been shown to produce reactive oxygen species (ROS) in a variety of tissues other than the liver, including the kidney, heart and blood. In acute and chronic renal injuries, free radicals that cause lipid peroxidation damage cell membranes, resulting in a variety of pathological changes. The main enzyme involved in CCl₄-induced nephrotoxicity is cytochrome P450, which is found in cortical tubule cells and causes an increase in lipid peroxidation in the renal brush border. Renal mitochondrial activity, including calcium flux through mitochondrial membranes, is also affected by CCl₄ (Manal et al., 2011). Renal fibrosis is the principal process involved in the progression of chronic kidney disease (Manal et al., 2011).

The progression of kidney damage is marked by the rise in two important chemical substances in the blood - creatinine and urea whose evaluation in serum helps to assess Glomerular Filtration Rate (GFR) followed by renal function. However, neither creatinine nor urea is directly toxic and they are only a measure of kidney function. Creatinine is produced from muscles and is excreted through the kidneys along with other waste products (Nisha et al., 2017). Urea & creatinine are the parameters to diagnose functioning of the kidney. Changes in serum creatinine concentration more reliably reflect changes in GFR than do changes in serum urea concentrations. Creatinine is formed spontaneously at a constant rate from creatinine, and blood concentrations depend almost solely upon GFR. Urea formation is influenced by a number of factors such as liver function, protein intake and rate of protein catabolism (Griffin et al., 2008).

The present study was therefore designed to evaluate the beneficial effects of honey and BV either alone or combined on nephropathy induced by both LPS and CCl₄. The assessment was carried out by measuring renal function values such as urea, creatinine and uric acid.

2. Materials and Methods

2.1. Chemicals

LPS were extracted from (Escherichia coli serotype O127:B8), purchased as a lyophilized powder from Sigma-Aldrich chemical, and purified by phenol extraction. Carbon tetrachloride (CCl₄, >99.5%) was purchased from Sigma Aldrich. Honey (moisture 60%) lyophilized whole BV, and olive oil were purchased from GHADA Company, Borgalarb, Alexandria, Egypt. All other chemicals and reagents used were of analytical grade.

Urea was determined according to (Kaplan et al., 1984) using reagent kits purchased from Analyticon Biotechnologies chemical company, Egypt. Serum creatinine level was determined spectrophotometrically according to the method described by (Young, 2001). Serum uric acid level was determined spectrophotometrically according to the method described by (Young, 2001).

2.2. Collection of blood samples

By the end of the experimental period, all animals were scarified under diethyl ether anesthesia at fasting state, after which blood samples were taken. Blood samples were centrifuged at 4000 rpm for 10 minutes to isolate serum samples. For biochemical analysis, serum samples were maintained frozen at -80°C.

2.3. Animals and Experimental Design

Sixty-four adult males albino rats (Sprague Dawely) with average weight 120 - 150 g were used. The rats were obtained from the Animal House of the Faculty of Agriculture, Minia University, Minia, Egypt. All animals were housed in well aerated and isolated polypropylene cages under good hygienic laboratory conditions. Rats were provided commercial rodent diet containing all the necessary nutritive elements. Food and water were available throughout the experiment. Animals were housed at 25 ± 2 °C under a 12-h light/dark cycle.

Rats were divided into eight groups (eight rats each) as follows: the first group is control group which rats were fed on a balanced diet during the experimental period without any treatment, the second group (honey group) rats were administrated with honey (25 mg/kg b. wt.) every day for 2 months, rats of the third group (BV group) were injected intraperitoneally with BV (1 mg/kg b. wt.) every day for 2
months. The fourth group (honey + BV group) were received with honey bee and BV as described in groups 2 and 3. Primarily, the fifth group were injected intraperitoneally with a single dose of LPS (1 mg/kg b.wt.) followed by an intraperitoneal injection of CCl4 (0.5 mg/kg b.wt.) two times a week for 4 weeks (to assure liver injury models in animals). The sixth group (LPS + CCl4 + honey treated group) were received the same treatment as described above in group 5 then honey was co-administered orally (25 mg/kg b. wt. daily) for 2 months. Rats of the seventh group (LPS + CCl4 + BV treated group) were administered with LPS + CCl4 as previously cited and BV was co-administered intraperitoneally injection (1 mg/kg b.wt. daily) for 2 months. Finally, the eighth group (LPS + CCl4 + honey + BV treated group) were received the same treatment as described above in Group 5 then honey + BV were co-administered for 2 months under the same procedure and dosage, as described above.

2.4. Statistical analysis

The results of the present study were analyzed using SPSS version 22 for Windows. The significance was designed via one-way analysis of variance (ANOVA), followed by Tukey’s multiple comparison procedure. The results were expressed as the mean ± SE, and P < 0.05 was assayed as the level of significance.

3. Results

The results of the present study observed that the significant alternation in blood urea level in group treated with honey (group 2) and observed significant in group treated with BV (group 3) and their combination (group 4) when compared with control group (group 1), whereas there was a significant increase at (P < 0.05) in group Lps+CCl4 (group 5) when compared with control group. There were a significant decrease in the groups (6,7,8) when compared with the group of Lps+CCl4 as shown in (figure 1). The changes in serum creatinine level, demonstrated that no significant alternation showed in the groups treated with honey in group 2 and the group treated with bee venom in (group 3) or their combination in (group 4) when compared with control group (group 1). But in group 5 (Lps+CCl4), there were a significant elevated in the level of serum creatinine when compared with the control group (group 1) (figure 2). The changes in the serum uric acid, showed the significant increase in the group Lps+CCl4 (group 5) when compared with the control group (group 1) at (P < 0.05), however, no significant demonstrated in the groups 2,3 and 4 when compared with control group (group 1) shown in (figure 3). we can also showed the significance In (table 1).

Table 1: Biochemical parameters of control and experimental groups.

| Group | Parameters | (G1) Control | (G2) Honey bee | (G3) Bee venom | (G4) Honey bee + Bee venom | (G5) Lps+CCl4 | (G6) Lps+CCl4 + Honey bee | (G7) Lps+CCl4 + BV | (G8) Lps+CCl4 + Honey +BV |
|-------|------------|-------------|---------------|----------------|--------------------------|----------------|------------------------|----------------|--------------------------|
|       |            | M ± SE      | M ±SE         | M±SE          | M±SE                     | M±SE          | M±SE                   | M±SE          | M±SE                     |
| Blood Urea |          | 21.58±     | 41.35±       | 70.06±        | 52.82±                   | 84.64±        | 62.75±                 | 54.31±        | 45.19±                   |
|        |            | 1.147       | 0.836         | 0.379         | ±1.421                   | 1.560         | 0.620                  | 0.982         | 1.351                    |
| S.creatinine |       | 0.617±     | 0.481±       | 1.533±        | 1.062±                   | 6.065±        | 4.231±                 | 3.475±        | 1.125±                   |
|        |            | 0.025       | 0.018         | 0.093±        | ±0.052                   | 0.277         | 0.122                  | 0.190         | 0.045                    |
| S.uric acid |        | 3.278±     | 3.151±       | 5.921±        | 3.838±                   | 10.241±       | 8.428±                 | 7.351±        | 3.878±                   |
|        |            | 0.207       | 0.187         | 0.146±        | ±0.234                   | 0.223         | 0.113                  | 0.141         | 0.225                    |

Values are means ± SE for eight rats in each group. Significance at P < 0.05. (a) Comparison of control with other groups. (b) Comparison of LPs+ CCl4 group with LPs+ CCl4+honey and LPs+ CCl4 +BV and LPs+ CCl4 +honey + BV groups.
Fig. 1: Serum urea activities of control and experimental rats, data are presented as (mean ± S.E, n = 8), significance at $P < 0.05$, (a) significantly different from control group, (b) significantly different from LPS + CCl$_4$ group.

Fig. 2: Serum creatinine level of control and experimental rats, data are presented as (mean ± S.E, n = 8), significance at $P < 0.05$, (a) significantly different from control group, (b) significantly different from LPS + CCl$_4$ group.

Fig. 3: Serum uric acid level of control and experimental rats, data are presented as (mean ± S.E, n = 8), significance at $P < 0.05$, (a) significantly different from control group, (b) significantly different from LPS + CCl$_4$ group.
4. Discussion
The administration of CCl\textsubscript{4} and LPS to rats resulted in a significant increase in the levels of creatinine, urea and uric acid in serum group 5 compared to control. These findings are in agreement with earlier studies (Hanaa et al., 2018) Increased levels of creatinine, urea and uric acid were attributed to oxidative stress induced by CCl\textsubscript{4} and LPS due to elevation of ROS, lipid peroxidation and reduction of the antioxidant defense system (Borges, et al., 2005) reported that the urea, creatinine, and uric acid levels are the most reliable renal markers only after the majority of kidney function has been disturbed.
From the present study, the administration of CCl\textsubscript{4} and LPS with oral supplementation of honey to rats, leads to a significant decrease in the level of creatinine, urea and uric acid in rats compared to group 5. Honey has a protective role against many drugs on kidney function (Chilwant, et al., 2004). Honey's high antioxidant capacity allowed it to scavenge free radicals, lowering nitric oxide levels and, as a result, lowering lipid peroxidation levels. (De camargo, 1999; Oliveira et al., 2007). The antioxidant effects of honey was attributed to its constituents like flavonoids, phenolic compounds, enzymes (glucose oxidase, catalase and peroxidase) and L-ascorbic acid (Heba et al., 2009) have mostly shown how natural honey has a beneficial effect on kidney injury due to oxidative damage caused by lead in rats (Abeer, et al., 2012)
Renal dysfunction was observed in LPS and CCl\textsubscript{4}-treated rats, as evidenced by elevated serum levels of creatinine, urea and uric acid, which were substantially revesed by administration of BV (Kim, et al., 2020). demonstrated that bee venom and its component apamin have protective effects against LPS-induced acute renal failure and structural damage. Moreover [24] reported that melittin, the main component of BV, was found to ameliorate an acute decline in renal function, as illustrated by decreased plasma creatinine, tubular dilatation, tubular epithelial cell swelling, and brush border loss. They suggested that melittin protect against endotoxin-induced renal injury and mortality by suppressing inflammatory reactions, oxidative stress, and apoptotic and necrototic cell death (Jung-Yeon et al., 2021).

5. Conclusion
It is concluded that the combined oral supplementation of honey and/or BV protected and improved changes in some renal parameters induced by CCl\textsubscript{4} and LPS in rats. This protective effect can be attributed to the presence of antioxidant and lipid peroxidation inhibitors and anti-inflammatory effects.

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