THE POTENTIAL PROTECTIVE EFFECT OF NATURAL HONEY AGAINST CADMIUM-INDUCED HEPATOTOXICITY AND NEPHROTOXICITY

BY

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ABSTRACT

The therapeutic properties of natural honey once considered a form of folk or preventive medicine. It is important for the treatment of acute and chronic free radical mediated diseases and toxicity. Oxidative stress can play a key role in cadmium-induced dysfunction. The aim of this work was to study the effect of natural honey on cadmium-induced liver and kidney damage. A total of 30 adult male rats were divided into three groups. Group I animals served as control were injected daily I.P. by 1 ml saline. Group II animals were injected daily I.P. with 0.5mg/kg cadmium chloride dissolved in 1 ml saline for 4 weeks. Animals of group III were treated with 0.5 mg /kg cadmium chloride I.P. and 0.05ml of natural honey mixed with water orally concurrently for 4 weeks. Liver function (SGOT),(SGPT), (ALP) and kidney function (creatinine and urea nitrogen) tests were measured. In addition lipid peroxidation, reduced glutathione (GSH) and glutathione peroxidase (GPx) were estimated in liver and kidney tissues samples. Light and transmission electron microscopic examination were used for histological changes. The results revealed that treatment with Cd caused marked elevation in the level of free radicals (lipid peroxidation) and kidney and liver enzymes, and a decline in GPx activity and GSH level. Administration of honey with Cd induced improvement in all examined parameters. On the other hand, light microscopic examination of kidney cortex of Cd treated group revealed swelling of the cells lining the convoluted tubules and vaculation of their cytoplasm. Variable degrees of glomerular degeneration were present. The liver showed different degrees of cell degeneration, necrosis, dilatation and congestion of blood vessels. Results obtained by EM examination revealed that there were affection of mitochondria and partial loss of microvilli of some kidney tubules. Furthermore, electron dense mitochondria, depletion of glycogen granules in a rarified vaculated cytoplasm were seen in the hepatocytes. It is noticed that concurrent administration of honey with cadmium improved histological changes in both kidney and liver by light and electron microscope. It could be concluded that honey via its antioxidant activity has the ability to protect against cadmium-induced hepatotoxicity and nephrotoxicity.

INTRODUCTION

Cadmium (Cd) is a relatively rare element that occurs naturally in ores together with zinc, lead and copper or is emitted into the air through the process of volcanic
emission. It became commercial in the 20th century due to agricultural and industrial applications (WHO, 2000 and Jar- up, 2003).

Occupational exposure to cadmium, such as working with cadmium containing pigments, plastic, glass, metal alloys and electrode material in nickel - cadmium batteries, and non occupational exposure, such as food, water and cigarette smoke induces uptake of Cd from the environment into the body through pulmonary and enteral pathways (Waisberg et al., 2003). Cadmium absorbed and accumulates mainly in the kidney and liver, then it is bound to the apo-protein metallothionein (Morales et al., 2006).

The intracellular release of cadmium is responsible for the generation of reactive oxygen species, glutathione depletion, lipid peroxidation, protein cross-liking, DNA damage, culminating ultimately in oxidant-induced cell death (Brennan, 1996; Shaikh et al., 1999; Jurezuk et al., 2004 and Babu et al., 2006).

Honey has been an ingredient of traditional medicine on account of its dietary and curative properties since ancient times. Starting in the early 1970 researchers from different scientific fields have investigated the chemical and biological properties of honey, including antibacterial, bacteriostatic, anti-inflammatory, wound and sunburn healing activities. Recent views propose honey not only as a health promoting dietary supplement, but shed light on antioxidant, non-peroxide dependent properties. This makes honey more than just a nourishment of high value but a valuable dietary source of antioxidants (Beretta et al., 2005).

In recent years there has been an increased interest in the application of antioxidants to medical treatment as information is available linking the development of human diseases to oxidative stress (Al- Jadi and Kamaruddin, 2004).

Honey is a natural antioxidant which may contain flavinoids, ascorbic acid, tocopherols, catalase, and phenolic compounds all of which work together to provide a synergistic antioxidant effect, scavenging and eliminating free radicals (Johnston et al., 2005).

Little information is available on protective effect of honey against cadmium induced hepatotoxicity and nephrotoxicity. In the present study an experimental model of rats treated with Cd during four weeks as a model of Cd induced hepatotoxicity and nephrotoxicity were used. In this model the protective effect of concurrent administration of honey on Cd-induced hepatic and renal damage were assessed and if the protective effect of
honey is based on its antioxidant properties.

**MATERIAL AND METHODS**

**Chemicals:** All chemicals and reagents used were of analytical grades. Cadmium chloride (CdCl2) was purchased from ICN pharmaceutical company (USA). Reduced glutathione and Ellman’s reagent 5,5 Di-thiobis-2 nitrotenzoic acid (DTNB) were obtained from ICN Biomedica. Inc. (USA).

**Animals:** Thirty male albino wister rats weighing 150-200 gm each obtained from the Animal House, Faculty of Medicine, Assiut University were used. Rats were housed in stainless steel cages at a constant temperature 25 ± 2°C with alternating 12 hours light and dark cycles and allowed water and food (laboratory chow) ad libitum. The research was conducted in accordance with the internationally accepted guidelines for laboratory animal use and care. The experiment was approved by the Institutional Ethics Committee.

**Treatment:** Rats were divided into three groups 10 animals each.
- **Group I** (control) animals were injected intraperitoneally (I.P.) with 1 ml saline daily for four weeks.
- **Group II** animals were injected IP daily with 0.5 mg/kg CdCl2 for four weeks (Satarug and Moore, 2004).
- **Group III** (Cd ± honey) rats were concurrent by administered of 0.5 ml/kg CdCl2 I.P. and 0.05 ml natural honey (Paget and Barnes, 1964) dissolved in water orally by gastric tube daily for four weeks.

**Blood collection:**
1- Animals were anesthetized and blood samples were collected from occular vascular bed using capillary tubes.
2- Blood samples were collected into dry clean tubes on EDTA then centrifuged for 10 min to isolate plasma and stored at -70°C.

**Tissue samples:** animals were killed by cervical decapitation. The liver and kidneys were taken and each organ was divided into two parts.

**The 1st specimen** was homogenized in ice cold 100 mM phosphate buffer (pH 7.4). Homogenates were centrifuged and the resulting supernatant was storted at -70°C for biochemical analysis.

**The 2nd specimen** was preserved in 10% formaline for histological examination.

**Biochemical measurements:**
A) The plasma samples were taken for determination of :
1- **Liver function tests:** glutamyl - oxal-
oacetic acid transaminase (SGOT), glutamyl pyruvic transaminase (SGPT) and alkaline phosphatase (ALP).

2- Kidney function tests: creatinine and urea nitrogen.
The two tests were measured spectrophotometrically using standardized commercially available Diamond Kits (Modern Laboratory, Egypt).

3- Oxidative stress indices: lipid peroxidation level was estimated by the measurement of malondialdehyde (MDA), an end product of lipid peroxidation level were determined using spectrophotometre employing thiobarbituric acid reactive substances described by Thayer, (1984). Levels were expressed as nmol/ml plasma.

B) Liver and kidney homogenates: were utilized for determination of
1- Lipid peroxidation was measured as described by Thayer (1984), levels were expressed as nmol/mg protein.

2- Glutathion peroxidase (GPx): Activity was measured by the method of Tapple (1978). The method is based on oxidation of glutathione by cumen hydroperoxide via glutathione peroxidase. The reaction in the form of decrease in absorbance at 340nm was followed when oxidized glutathione converted to reduced glutathione via NADPH and glutathione reductase. The enzyme activity is expressed as U/mg protein.

3- Reduced glutathione (GSH): An equal volume of 10% metaphosphoric acid was added to a part of the homogenates and mixed by vortexing. The mixture was allowed to stand for 5 min at room temperature. After centrifugation for 5min, the supernatant was collected carefully without disturbing the precipitate. The GSH contents of the neutralized supernatant were assayed using Ellman’s reagent (5, 5’-dithiobis-2-nitrobenzoic acid (DTND solution) according to the method of Griffith (1980). A standard reference curve was prepared for each assay.

4- Protein contents were determined using the method of Lowry et al., (1951).

Preparation of histological sections:
Specimens of the liver and kidney were taken and fixed in formalin and processed for light microscope. The specimens were embedded in paraffin and sectioned at a thickness of 7 microns. The sections were stained with haematoxyline and eosine stain according to Drury and Wallington (1980). Other small pieces were fixed in glutaraldehyde and processed for electron microscope examination. Semithin sections were cut at 1/2-1 micron and stained with toluidine blue. Selected sections were
Table (3) shows the levels of lipid peroxidation in plasma, and tissues of liver and kidney. In Cd-group the level of LPO in plasma, tissues of liver and kidney was significantly higher than controls. In group received Cd + honey the level of LPO was significantly reduced to near control levels compared with the Cd - group.

The effects of Cd and Cd + honey on glutathione peroxidase activity (GPx) in tissues of liver and kidney are shown in table (4). The GPx activity level was significantly lower in tissues of liver and kidney in Cd-group compared with the control group. In comparison with Cd group there was significant increase in the level of GPx activity in Cd + honey group in tissues of liver and kidney. Table (5) shows that reduced glutathione level in tissues of liver and kidney of cadmium treated group was significantly decreased in comparison to control group. In group received Cd + honey the level of reduced glutathione showed significant increase in tissues of liver and kidney in comparison to Cd group.

Histological Results:
I- Liver:
A) Light microscope results (Hx&E stain)
Group I: control group:
The control livers show normal lobular architecture with central vein and radiating cords of hepatocytes, separated by blood sinusoids. Hepatocytes are large.

Statistical analysis:
The data were presented as means ± standard errors.
For the comparison of statistical significance between two groups student Newman-keuls t-test was used.
Values were accepted as being statistically significant if a P value was less than 0.01.

RESULTS

Biochemical Results:
Table (1) displays the effects of Cd alone and Cd with honey on plasma liver enzymes. Levels of SGOT, SGPT and ALP were significantly increased in the cadmium treated group in comparison to the control group. Animals of group III received Cd + honey showed a significant reduction in SGOT, SGPT, ALP level compared with Cd group.

The effects of Cd alone and Cd + honey on plasma level of kidney function test are shown in table (2). The plasma levels of urea and creatinine were significantly higher in Cd-group than controls. Levels of urea and creatinine in plasma exhibited significant reduction in group received Cd + honey in comparison with Cd group.

contrasted and electron micrographs were taken with Jeol transmission electron microscope (TEM).

and polyhedral in shape with slightly acidophilic granular cytoplasm. They have large, rounded, vesicular nuclei with prominent nucleoli (Fig. 1).

**Group II:** animals that received CdCl2 only:

The liver cells of group II animals showed obvious histological changes, in the form of distortion in the hepatic organization, dilatation and congestion of the blood sinusoids and central vein. Some hepatocytes showed signs of degeneration in the form of hypertrophy with highly vacuolated cytoplasm and deeply stained nuclei. Other hepatocytes exhibited hyalinized cytoplasm with pale nuclei and prominent nucleoli (Fig. 2).

**Group III:** CdCl2 + honey:

The liver cells appeared more or less similar to those of the control apart from few hepatocytes appeared with vacuolated cytoplasm and pyknotic nuclei (Fig. 3).

**B) Electron microscope results:**

**Group I:** the hepatocytes of control animal exhibit a large rounded euchromatic nucleus. The cytoplasm contains numerous cisternae of rough endoplasmic reticulum, numerous well-preserved mitochondria and glycogen rosettes (Fig. 4).

**Group II:** after treatment with CdCl2 the hepatocytes showed clumping of the cellular organelles which were mainly formed of rough endoplasmic reticulum and mitochondria. These cytoplasmic organelles were separated by large areas of rarified cytoplasm. The cisternae of the rough endoplasmic reticulum showed fragmentation in the form of short segments and loss of their attached ribosomes (Fig. 5). Whorly appeared structures were demonstrated in the rarified cytoplasm. Mitochondriae were polymorphic contained electron-dense granular matrix. Some of them were swollen, lost their cristae and contained vacuoles (Fig. 6).

**Group III:** combined treatment with CdCl2 and honey led to improvement in the ultrastructure of hepatocytes which appeared nearly similar to the control. Most of the mitochondriae were intact. The cisternae of rough endoplasmic reticulum acquired their normal appearance. The nuclei of some hepatocytes appeared large, rounded and euchromatic with prominent nucleoli (Fig. 7).

**II- Kidney:**

**A) Light microscope results (Hx & E stain).**

**Group I:** the cortex of control kidney is mostly occupied by renal corpuscles and surrounding proximal and distal convoluted tubules. The renal corpuscle is formed of glomerular tuft of blood capillaries surrounded by capsular space and Bowman’s capsule.
The proximal convoluted tubules are lined with large cuboidal cells with deeply stained acidophilic cytoplasm with apical brush border and rounded vesicular nuclei. The boundaries between the adjacent cells are indistinct. The distal convoluted tubules are lined by low cuboidal cells with distinct cell boundaries and less acidophilic cytoplasm (Fig. 8).

**Group II:** the glomerular capillaries of some renal corpuscles of animals showed congestion and dilatation. There was swelling of some cells of the proximal convoluted tubules leading to diminution or even obliteration of the tubular lumina. Cytoplasmic vacuolation and deeply stained nuclei were observed compared to the control group. Destruction of the brush borders of the proximal convoluted tubules was also detected. The distal convoluted tubules showed degenerative changes in the form of pyknotic nuclei and vacuolated cytoplasm (Fig. 9).

**Group III:** combined treatment with honey and CdCl2 led to obvious improvement in the histological structures of the kidney compared to group II. The renal corpuscles appeared nearly similar to the control. Most of kidney tubules exhibited acidophilic cytoplasm and rounded vesicular nuclei (Fig. 10).

**B) Electron microscope results:**

**Group I:** ultrastructurally the podocytes are situated between glomerular capillaries, and their secondary foot processes were extended and rest on the basement membrane. Numerous filtration slit-membranes were observed between the processes of glomerular capillaries (Fig. 11).

Regarding the cells of the proximal convoluted tubule: The apical cell membrane is occupied by numerous microvilli. The cytoplasm contains numerous mitochondria of various sizes. Few cisternal profiles of rough endoplasmic reticulum and ribosomes were detected in the cytoplasm. The nuclei is rounded euchromatic with prominent nucleolus The basement membrane exhibited basal infoldings with elongated mitochondria between them (Fig. 13).

**Group II:** in cadmium treated animals, most of the renal corpuscles were highly affected. There was dilatation and swelling of the glomerular capillaries accompanied with narrowing of the urinary spaces. The glomerular basement membranes showed obvious thickening in most of renal glomeruli. Most of the secondary foot processes of the podocytes appeared flattened and amulgamated with each other with complete disappearance of their slit membranes. Some of these processes contained dense granules and dense irregular bodies. The lumina of the capillaries contained few exudates and degenerated...
endothelial cells. There was apparent increase in the amorphous secretion of mesangial matrix (Fig. 12).

Regarding the cells of the proximal convoluted tubules the apical brush border exhibited partial loss or erosion of their microvilli. The cytoplasm showed many vacuoles. The mitochondriae were markedly affected. They appeared small with dense matrix and destructed cristae in some of them. The tubular basement membrane displayed obvious demolishing of their basal infoldings (Fig.14).

Group III: combined treatment with Cd and honey. The glomerular capillaries and the podocytes appeared more or less similar to the control group. The cell of the proximal convoluted tubule exhibited normal cellular organelles and intact microvillous border. The apical cytoplasm of some renal tubules was rarified with some dense bodies (Fig.15).

DISCUSSION

Available literature indicates that no previous studies have been done to evaluate the antioxidant capacity of honey and its protective effect against cadmium intoxication.

The mechanisms of cadmium-induced damage include the production of free radicals that alter mitochondrial activity and genetic information (Patrick 2003 and DeBurbure et al., 2006). Therefore, some authors have postulated that antioxidants should be one of the important components of an effective treatment of cadmium poisoning (El-Demerdash et al., 2004).

The present study concentrates on the possible protective effect of honey on oxidative damage generated by cadmium induced hepatotoxicity and nephrotoxicity. Liver and kidney function tests were done for different studied groups to assess their status. Biochemical analysis was done for oxidative stress indices such as lipid peroxidase level. The activity of antioxidants was measured e.g. glutathione peroxidase (GPx) and reduced glutathione (GSH), because these antioxidants are the commonest to be affected by cadmium toxicity (Jurezuk et al., 2004). Histological changes of kidney and liver were examined by light and electron microscopes.

In this work the liver enzymes SGOT, SGPT and ALP in the Cd treated group were significantly elevated compared with the control group, denoting the presence of liver dysfunction (Shimada et al., 2004). In concurrent administration of honey and Cd, the levels of liver enzymes activity were significantly reduced as compared with the Cd-group. This finding indicates the protective effects of honey in ameliorating the hepatotoxic effect of Cd.
The plasma level of creatinine and urea was significantly increased after cadmium treatment compared to the control group indicating the impairment in the kidney function. Similar observation was obtained by Novelli et al. (1998). In fact, urea is the first acute renal marker which increases when the kidney suffers any kind of injury. Otherwise, creatinine is the most trustable renal marker and increase only when the majority of renal function is lost (Borge et al., 2005). The changes in urea and creatinine level in the present study concluded the severe injured effect of CdCl2 on kidney. Moreover, in the present study honey co-administration with cadmium significantly ameliorated the increased plasma levels of creatinine and urea.

In the current study, lipid peroxidation level was significantly elevated in plasma, liver and kidney tissues of rats treated with cadmium compared to control group thus suggesting increased oxidative stress. These results were supported by Manca et al., (1991) who reported that LPO is an early and sensitive consequence of Cd exposure. Also, Hassoun and Stohs (1996) demonstrated that oxidative stress was induced following oral administration of cadmium chloride to rats. A similar data had been reported by Jurezuk et al., (2004). In addition, Elizabeth et al., (2003); Jahangir et al., (2005); Eybl et al., (2006) and Kawamoto et al., (2007) reported that cadmium is thought to induce lipid peroxidation and this has often been considered to be the main cause of its deleterious influence on membrane-dependent function.

In the present study the elevation in the free radicals (LPO) induced by cadmium alone was very high significantly decreased in the presence of honey. This means that honey minimized the toxic effect of cadmium via its antioxidant activity. These results are in line with the view held by Beretta et al., (2005) who confirmed the role of honey as an antioxidant agent in blood. Also Manuela et al., (2007) found that there was a direct link between the honey consumption and the level of polyphenolic antioxidants in the plasma.

In agreement with a previous study, the level of GSH was significantly decreased in the liver and kidney extracts of cadmium treated group compared to the control group. The decrease in GSH levels may be due to its consumption in the prevention of free radical-mediated lipid peroxidation (Demopoulos, 1973; Koyuturk et al., 2006). Also, GSH may be consumed in the detoxification of heavy metals (Kim et al., 1998 and Thevenod, 2003). Furthermore, it has been suggested that the decrease in GSH levels upon cadmium exposure might impair the degradation of lipid peroxides, thereby leading to its accumulation in the target organs (Sarkars et al., 1997). In controversy to the current results, Kamiyama
et al., (1995) reported an increase in GSH level in liver and kidney tissues after Cd injection which could be explained as a protective mechanism.

The co-administration of honey with cadmium increased the level of the antioxidant (GSH), and approximated to the normal values of the control group. Al-Waili, (2003) confirmed that honey increased the levels of antioxidants and this effect might be attributed to the composition of honey, which contains many nutrient elements and antioxidants as vitamin C, which is a potent antioxidant agent.

The activities of GPx was significantly decreased in the liver and kidney extracts of cadmium treated rats. The decline in the level of GPx activity resulted from Cd toxicity was previously demonstrated in liver tissues (Sidhu et al., 1993; Ossola and Tomara, 1995; Sarkars et al., 1997) and in kidney tissues (Bragadin et al., 2004). In the present study reduced glutathione peroxidase activity was recovered after co-administration of honey with cadmium in liver and kidney tissues, which in turn destructs the lipid peroxidase (Eaton et al., 1980). This confirm that honey increase antioxidant activity and able to protect from oxidative stress.

Epidemiological studies have revealed that cadmium is one of the most toxic of the heavy metals to humans; 70% of the ultrafiltered cadmium is taken up, largely by the proximal tubules of the kidney and is accumulated mainly in kidney cortex leading to proximal tubule lesions (Lars, 2002). These findings are in agreement with the current study.

The nephrotoxicity of Cd has been extensively studied in various experimental models (Mitsumori et al., 1998; Ohta et al., 2000). Recent papers show that tubular damage may appear at lower levels of Cd exposure than previously anticipated (Jar-up et al., 2000; Noonan et al., 2002).

The Cd concentrations applied in this study was in the range of previous studies (Brzoska et al., 2003; Choi and Rhee, 2003) according to average human intake data, soil Cd concentrations from contaminated sites (Blanusa et al., 2002; Satarug and Moore, 2004) and Cd levels from animals caught in polluted areas (Damek-Proprawa and Sawicka-Kapusta, 2003).

In the present study administration of Cd 0.5 mg/kg I.P. daily for four weeks induces significant damage in function and structure of kidney assessed by increased creatinine and urea concentrations in plasma and existence of atrophy of and swelling of some glomerular capillaries as well as proximal tubular necrosis and apoptosis (Damek-Proprawa and Sawicka - Kapusta, 2004). The distal convoluted tubules showed degenerative changes with
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(1996) and Shaikh et al., (1999) have reported the mechanism of Cd-induced renal damage include increased oxidative stress. Whereas increased oxygen free radicals production seems to be induced by the interaction of Cd and mitochondrial structure (Tang and Shaikh, 2001).

In the current study treatment with CdCl2 caused liver damage demonstrated functionally by increasing the activity of SGOT, SGPT and ALP, and histological alterations. These alterations were in the form of dilatation in the hepatic organization, dilatation and congestion of central veins and blood sinusoids. Hypertrophy or degeneration of some hepatocytes with either hyalinized and vacuolated cytoplasm with pale nuclei and prominent nucleoli. These results coincide with results of Borges et al., (2005). These alterations were observed with electron microscopy. Most of the hepatocytes showed clumping of the cellular organelles. The cisternae of the rough endoplasmic reticulum showed fragmentation in the form of short segments and loss of their attached ribosomes. Mitochondriae were polymorphic contained electron-dense granular matrix. Some of them were swollen, lost their cristae and contained vacuoles. These results are in agreement with study of Mousa, (2004). Co-administration of honey with cadmium markedly ameliorated these histopathological changes induced by Cd. Most of mitochondrie were intact. The

These results reveal that honey has a marked protective effect on renal tubular toxicity and showed decreased lipid peroxidation and increased tissue levels of GSH and GPx activity a potent endogenous antioxidant (Meister and Anderson, 1983). This protective effect of honey was confirmed when renal tissues were observed by electron microscopy. Most of kidney tubules exhibited acidophilic cytoplasm and rounded vesicular nuclei. Moreover, the observation that Cd+honey treated animals did not have mitochondrial damage might be seen as supporting the anti-oxidant properties of honey. In agreement with this suggestion, Brennan, (1996) and Shaikh et al., (1999) have reported the mechanism of Cd-induced renal damage include increased oxidative stress. Whereas increased oxygen free radicals production seems to be induced by the interaction of Cd and mitochondrial structure (Tang and Shaikh, 2001).

In the current study treatment with CdCl2 caused liver damage demonstrated functionally by increasing the activity of SGOT, SGPT and ALP, and histological alterations. These alterations were in the form of dilatation in the hepatic organization, dilatation and congestion of central veins and blood sinusoids. Hypertrophy or degeneration of some hepatocytes with either hyalinized and vacuolated cytoplasm with pale nuclei and prominent nucleoli. These results coincide with results of Borges et al., (2005). These alterations were observed with electron microscopy. Most of the hepatocytes showed clumping of the cellular organelles. The cisternae of the rough endoplasmic reticulum showed fragmentation in the form of short segments and loss of their attached ribosomes. Mitochondriae were polymorphic contained electron-dense granular matrix. Some of them were swollen, lost their cristae and contained vacuoles. These results are in agreement with study of Mousa, (2004). Co-administration of honey with cadmium markedly ameliorated these histopathological changes induced by Cd. Most of mitochondrie were intact. The
cisternae of rough endoplasmic reticulum acquired their normal appearance.

In the present study CdCl2 exposure increased LPO levels in plasma and tissues with alterations in antioxidant defenses (GPx and GSH). Thus it may be possible that oxidative stress and disturbance in antioxidant defenses were the causes for liver and kidney damage induced by CdCl2 in this experimental model.

On hypothesis to explain the beneficial effects of honey in ameliorating biochemical parameters and histological changes is that honey may contain flavonoids, ascorbic acid, tocopherols, catalase and phenolic compounds. All of which work together to provide a synergistic antioxidant effect, scavenging and eliminating free radicals (Johnston et al., 2005).

**In conclusion:** The present study demonstrated that honey administered in combination with cadmium minimized its hazards. Honey can protect against oxidative stress induced by cadmium, by lowering the free radicals and increased the levels of antioxidants. Further studies are required to recommend the use of honey and its therapeutic potential in human. Several antioxidant assays were utilized in order to evaluate the biological and chemical properties of honey. In addition, the exposure to cadmium should be reduced and attention paid to sources of cadmium in food, water and personal-care products. Furthermore using diets rich in honey could be beneficial in alleviating cadmium toxicity.
Table (1): The effect of cadmium alone and with honey on the liver enzymes.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>12.08 ± 0.74</td>
<td>10.12 ± 0.56</td>
<td>142.21 ± 9.63</td>
</tr>
<tr>
<td>Group II (Cd)</td>
<td>58.20 ± 4.98**</td>
<td>65.21 ± 3.42**</td>
<td>1278.40±4.92**</td>
</tr>
<tr>
<td>Group III (Cd+Honey)</td>
<td>15.11 ± 1.1***</td>
<td>12.34 ± 1.20***</td>
<td>150.28± 6.63***</td>
</tr>
</tbody>
</table>

** P< 0.01 group II vs control.  *** P< 0.01 group III vs group II.

Table (2): The effect of cadmium alone and with honey on kidney function tests (creatinine and urea).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>32.24 ± 2.13</td>
<td>0.92 ± 0.04</td>
</tr>
<tr>
<td>Group II</td>
<td>86.5 ± 6.42**</td>
<td>3.01 ± 0.3**</td>
</tr>
<tr>
<td>Group III</td>
<td>35.47 ± 2.26***</td>
<td>0.95 ± 0.06***</td>
</tr>
</tbody>
</table>

** P< 0.01 group II vs control.  *** P< 0.01 group III vs group II.

Table (3): The effect of cadmium alone and with honey on lipid peroxidation level.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Groups</th>
<th>GroupI Control</th>
<th>GroupII Cd</th>
<th>GroupIII Cd + honey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma nmol/ml</td>
<td>3.56 ± 0.12</td>
<td>9.10 ± 0.53**</td>
<td>4.82 ± 0.11***</td>
<td></td>
</tr>
<tr>
<td>Liver nmol/mg</td>
<td>0.79 ± 0.08</td>
<td>2.76 ± 0.38**</td>
<td>0.8 ± 0.04***</td>
<td></td>
</tr>
<tr>
<td>Kidney nmol/mg</td>
<td>1.28 ± 0.09</td>
<td>2.67 ± 0.10**</td>
<td>1.58 ± 0.03***</td>
<td></td>
</tr>
</tbody>
</table>

** P< 0.01 group II vs control.  *** P< 0.01 group III vs group II.
Table (4): The effect of cadmium alone and with honey on glutathione peroxidase activity (GPx).

<table>
<thead>
<tr>
<th>Organs</th>
<th>Groups</th>
<th>Group I Control</th>
<th>Group II Cd</th>
<th>Group III Cd + honey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver U/mg protein</td>
<td></td>
<td>328.50 ± 5.18</td>
<td>231.28 ± 15.38**</td>
<td>331.92 ± 10.36***</td>
</tr>
<tr>
<td>Kidney U/mg protein</td>
<td></td>
<td>333.06 ± 4.80</td>
<td>224.20 ± 16.63**</td>
<td>331.20 ± 10.18***</td>
</tr>
</tbody>
</table>

** P< 0.01 group II vs control. *** P< 0.01 group III vs group II.

Table (5): The effect of cadmium alone and with honey on reduced glutathione (GSH) level.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Groups</th>
<th>Group I Control</th>
<th>Group II Cd</th>
<th>Group III Cd + honey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver nmol/mg</td>
<td></td>
<td>6.64 ± 0.35</td>
<td>2.65 ± 0.16**</td>
<td>6.22 ± 0.43***</td>
</tr>
<tr>
<td>Kidney nmol/mg</td>
<td></td>
<td>2.96 ± 0.21</td>
<td>1.28 ± 0.09**</td>
<td>2.66 ± 0.29***</td>
</tr>
</tbody>
</table>

** P< 0.01 group II vs control. *** P< 0.01 group III vs group II.
**Fig. (1):** A photomicrograph of a section in the liver of control adult male albino rat (group I) showing; central vein (C) and hepatocytes with vesicular nuclei (↑) separated by blood sinusoids (S). *(H&E X200).*

**Fig. (2):** A photomicrograph of a section in the liver of rat of (group II) showing; disorganization of the hepatocytes with vacuolated cytoplasm (V) and deeply stained nuclei. Some of them are hypertrophied with deep acidophilic cytoplasm (↑). Note: dilated and congested central vein (C). *(H&E X400).*

**Fig. (3):** A photomicrograph of a section in the liver of rat of (group III) showing; hepatocytes appeared more or less similar to control apart from few cells with vacuolated cytoplasm (↑). *(H&E X400).*

**Fig. (4):** An electron micrograph of control liver cells showing; large nucleus with euchromatin (N). The cytoplasm contain numerous mitochondriae (M), strands of rER and fine glycogen granules (g) *(X5000).*
**Fig.(5):** An electron micrograph of liver cell of (group II) animals showing: clumping of cellular organelles, whorly appeared structures (↑) in a rarified cytoplasm (X4000).

**Fig.(6):** A magnified part of a hepatocyte obtained from the previous group. Showing: polymorphic mitochondria with electron dense matrix. Note: vacuole (v) and dense bodies (D) inside the degenerated mitochondria (X8000).

**Fig.(7):** An electron-micrograph of the hepatocyte of (group III) showing: more or less normal ultrastructure with large vesicular nucleus (N), strands of rER and numerous mitochondria (M).

**Fig.(8):** A photomicrograph of a section of control renal cortex (group I) of adult male albino rat showing: renal corpuscle (↑), Proximal (P) and distal (D) convoluted tubules. (H&E X200).
**Fig.(9):** A photomicrograph of a section of renal cortex of (group II) showing: congestion of the glomerular capillaries (G), cytoplasmic vacuolation (v) and pyknotic nuclei (p) in most of the tubules. Note: some tubular cells with highly acidophilic cytoplasm (↑)(H&E X200).

**Fig.(10):** A photomicrograph of a section of renal cortex of (group III) showing: more or less normal structure (H&E X200).

**Fig.(11):** An electron micrograph of a renal corpuscle of (group I) showing: podocytes (P) with their minor or secondary foot processes (P2) that rest on normal basement membrane (↑) of capillary endothelium. (X 6700).

**Fig.(12):** An electron micrograph of a renal corpuscle of (group II) showing: parts of podocytes with fused minor processes (↑) rest on a thick basement membrane (B). (X6700).
**Fig.(13):** An electron-micrograph of proximal convoluted tubule of (group I) showing: long microvillus border (MV), many elongated mitochondria (M), basal spherical nucleus with euchromatin (N). Note: normal basal infoldings.

**Fig.(14):** An electron-micrograph of proximal convoluted tubule of (group II) showing: loss of the microvilli (↑) small dense mitochondria (M) and cytoplasmic vacuoles (V). Note: loss of basal infolding (X5000).

**Fig.(15):** An electron-micrograph of proximal convoluted tubules of (group III) showing: intact microvillous border (MV), intact mitochondria. Note: rarification of apical cytoplasm with some dense particles (X5000).
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التأثير الواقع المحتمل للعسل الطبيعي ضد التسمم الكبدى والكلوي الناتج من الكادميوم

المشتركون في البحث

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تم إجراء هذا البحث لدراسة التأثير الواقع للعسل الطبيعي على التسمم الكبدى والكلوي بالكادميوم في الفئران البضاء. أجريت هذه الدراسة على عدد 60 فأر أُبيض بالعسل متوسط وزنهم من 150-200 جم تم تقسيم الفئران إلى ثلاث مجموعات حيث استخدمت المجموعة الأولى كمجموعة ضابطة وحقت داخل التجريب البروتيني بحلول الملح الفسيولوجي. المجموعة الثانية. حقن بكادميوم الكادميوم (5 مجم/كجم) + عمل نحل 5 ر. مليلتر عن طريق الفم يومياً.

تم حقن عينات من عينات الفئران بعوضة يومياً لمدة أربعة أسابيع. تم تشريح الفئران في نهاية الدراسة. تم أخذ عينات دم وكبد وكليك لإجراء التحاليل الكيمياوية ودراسة التغييرات الهستولوجية في الخلايا بالمنزليكروسب وصورة الإلكترونية. أجريت التحاليل الكيميائية لدراسة وظائف الكبد والكلي. كما تم قياس مستويات مضادات الأكسدة مثل الجلوتاتيون المختزلا والقولونوسرن فوق المؤكد في نتائج الكبد والكليك. وتم قياس مستوى دالات حدد الضغوط الأكسيدية مثل مستويات الدهن فوق المؤكد. في البالاما وفي أنسجة الكبد والكليك.

وقد دلت النتائج على وجود قصور في وظائف الكبد والكلي في المجموعة الثانية بالمحتوى بالكادميوم فقط مع وجود ارتفاع الضغوط الأكسيدية (ارتفاع نسبة الدهن فوق المؤكد) في كل من مصل الدم وأنسجة الكبد والكلي وانخفاض في مستوى مضادات الأكسدة. وقد أظهرت الدراسة الهستولوجية للمجموعة الثانية من الخلايا الكلية في الخلايا المبطنة للأنابيب المثلثية ونجات في سينتولازم بعض الخلايا وبعض ظواهر التحلل في أوعية الكريات الكلوية. أما بالنسبة لخلايا الكبد فقد ظهر بعض التخلل والتحلل، كما ظهر إنسداد واحتقان في الأوردة المركزية.

وقد دلت النتائج أن استخدام العسل الطبيعي أدى إلى زيادة في مستويات مضادات الأكسدة في الخلايا المردولية ونقص في مستوى الشفافية الحرة (الضغط الأكسيدية) وفي نفس الوقت استعادت خلايا الكبد والكلي تدفقًا بشكل طبيعي ومن ذلك نستنتج أن استخدام العسل له تأثير واقع على حدوث تأثيرات سامة بالكادميوم في خلايا الكبد والكلي من الناحية الكيميائية والهستولوجية.