Royal Jelly Modulates Hepatotoxic Effect of Rats Treated with Cisplatin

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ABSTRACT

Cisplatin (CP) is one of the most active cytotoxic agents in the treatment of cancer and has adverse side effects such as hepatotoxicity and nephrotoxicity. The aim of this study was focused on investigating the possible protective effect of royal jelly (RJ) against oxidative stress caused by CP injury of the liver and inflammatory changes in male albino rats. Twenty four albino rats were divided into four equal groups as follows: control group, cisplatin group: (rats injected i.p. with single dose from cisplatin (CP, 7 mg / kg body weight,)), royal jelly RJ group: rats treated for 15 consecutive days by gavage with RJ (300 mg/kg/day orally), royal jelly-cisplatin group, rats treated with royal jelly orally for 15 consecutive days by gavage (300 mg/kg/day) followed by a single injection i.p. from CP (7 mg / kg body weight). The obtained results revealed that the administration of royal jelly with cisplatin in rats significantly ameliorated the changes occurred in the biochemical parameters {GSH, GPx and CAT were significantly decreased in liver of cisplatin-treated rats and increasing ALT and AST}. The histopathological results showed distinctive pattern of hepatotoxic changes in cisplatin group, while in the royal jelly-cisplatin group the liver tissues showed reductions lesions. In conclusion, royal jelly acts in the liver as a potent scavenger of free radicals to prevent or ameliorates the toxic effects of CP as shown by biochemical and histopathological investigations and might provide substantial protection against cisplatin-induced hepatotoxicity and inflammatory damages.

Key words: Antioxidant, Cisplatin, Royal, jelly Rats.

INTRODUCTION

Cisplatin (CP) has been established as a potent chemotherapeutic agent used to treat a variety of cancers such as ovarian, bladder, testicular, head and neck, and uterine cervix carcinomas (Kuhlmann et al., 1997). The major dose-limiting side effect of cisplatin is its hepatotoxicity, and nephrotoxicity. In several studies, it had been documented that injection of cisplatin produced a marked decrease in renal blood flow and glomerular filtration rate. The alterations in the liver functions induced by cisplatin are closely associated with an increase in lipid peroxidation and reactive oxygen species (ROS) in the tissues (Silici et al., 2010). The drug can cause many toxicities (Sastry and Kellie, 2005), but the chief dose-limiting one is nephrotoxicity and hepatotoxicity (Arany and Safirstein, 2003). Cisplatin may have some mechanisms of liver injury such as functional and structural mitochondrial damage, apoptosis, and perturbation in Ca2+ homeostasis (Martins et al 2008). In addition, the interrelated mechanisms of cisplatin actions to induce the nephrotoxicity include apoptosis (Hanigan and Devarajan, 2003) and inflammatory mechanism (Ramesh and Reeves, 2004), production of tumor necrosis factor (TNF)-α by renal parenchymal cells (Zhan et al., 1999), and generation of reactive oxygen species (ROS) that include superoxide dismutase, hydrogen radical, hydrogen peroxide, singlet oxygen, and nitric oxide (Jing et al., 2007).
Reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radical, singlet oxygen, superoxide anion, and peroxyl radical are formed inside cells by exposure to several endogenous and exogenous agents, causing damage to many important biomolecules that have been implicated in several diseases (Halliwell and Gutteridge, 1999). These prooxidants are kept in check by endogenous antioxidants, but under disease conditions, the balance is shifted in favor of prooxidants, leading to oxidative stress. Excess ROS causes significant oxidative damage by attacking biomolecules such as membrane lipids, DNA, and proteins in cells (Conklin, 2000). The oxidative stress is associated with many disease states including neurological diseases such as Alzheimer’s brains and Parkinson’s disease, chronic heart disease, and kidney and liver diseases (Coyle and Puttfarcken, 1993). Endogenous antioxidants such as reduced glutathione (GSH), glutathione peroxidase (GSH-PX), superoxide dismutase (SOD) and catalase (CAT) are compounds that act as free radical scavengers. These antioxidants are electron donors and react with the free radicals to form harmless products such as water. Therefore, antioxidants protect against oxidative stress and prevent damage to cells (Valko et al., 2006).

Recently, royal jelly has received particular attention because of studies that have reported that it is a highly efficient antioxidant and has free radical scavenging capacity (Silici et al 2010). Royal jelly is a secretion produced by the hypopharyngeal and mandibular glands of worker honeybees (Apis mellifera). It contains many important compounds with biological activity such as free amino acids, proteins, sugars, fatty acids, minerals, and vitamins (Nakajima et al 2009). So far, RJ has been demonstrated to possess several physiological activities in experimental animals, including vasodilative and hypotensive activities (Takaki-Doi et al 2009), the induction of decrease in serum cholesterol levels (Guo et al 2007), antimicrobial (Chan et al 2009), antiallergic (Oka et al 2001), anti-inflammatory (Kohno et al 2004), immunomodulatory (Okamoto et al 2003) and antioxidant properties (Nakajima et al 2009). In addition, Kanbur et al (2009), studied the protective effect of RJ against paracetamol induced liver damage in mice. Apoptosis is a gene-regulated event related to special morphological changes such as shrinkage of cell, chromatin condensation, and DNA damages (Wyllie 1997). A number of factors contributed to apoptotic mechanism, that are believed to play a key role in two main families of proteins including cysteine proteases called caspase enzymes (especially caspase 3, 8, and 9) and Bcl-2 family. Caspase 3, 8, and 9 enzymes play an effective role in apoptotic process of the liver and the kidney. Caspase-3, the most important member of caspase family, is responsible for many biochemical mechanisms of apoptosis that lead to the cleavage of nuclear and cytosolic substrates, chromatin condensation, fragmentation of DNA, and apoptotic bodies (Zhan et al 1999) Whereas, Bcl-2, which acts either antiapoptotic (Bcl-xL) or proapoptotic (Bcl-xS), Bcl-xL, an important member of this family, prevents apoptotic activity by blocking cytochrome c release from mitochondria (Kong and Reynolds 2009). Thus, the aim of the present study was to investigate the protective effect of royal jelly in cisplatin-induced oxidative damages and hepatotoxicity in rat liver by evaluation of biochemical and histopathological parameters.

Materials and methods:

1-Animals:

Twenty four male Swiss Albino rats (35-50g) were obtained from the Egyptian organization for biological product and vaccines Giza, Egypt. Animal were kept under good ventilation and illumination condition and received standard diet and had free excess to water.

2-Chemicals and drugs:

Cisplatin was purchased from Sigma (Cairo) and injected intraperitoneally in a single dose of 7 mg/kg according to Jones et al. (1991). RJ was obtained from Sigma (Cairo).
3-Experimental design: The animal were divided into four groups, each consist of 6 rats.

**Group 1:** control group, animals received orally distilled water.

**Group 2:** cisplatin group, animals injection i.p. with cisplatin single dose of 7 mg/kg.

**Group 3:** royal jelly group, animals daily administrated orally by stomach tube royal jelly at a dose of 300 mg/kg/day for 15 days.

**Group 4:** royal jelly and cisplatin group, animals received royal jelly at a dose 300 mg/kg/day/orally via oral tube for 15 days then received cisplatin at a dose of 7 mg/kg, injected i.p., single dose.

4- Samples collection:

After an overnight fast, rats were anesthetized with ether and then sacrificed. Blood samples from each rat were collected by retro-orbital puncture using blood capillary tubes and urine of each rat was collected. Serum was obtained immediately by centrifugation of blood samples at 3000 rpm for 10 min. Liver was directly separated and washed in ice-cold saline then the liver samples were homogenized in bi-distilled water (10% W/V) using homogenizer then the cell debris was removed by centrifugation at 3000 rpm for 10 min. The homogenates supernatant were used for the biochemical analysis. Tissue specimens from liver were collected and fixed in 10% buffered formalin solution and fixed tissues were dehydrated, cleared and embedded in natural paraffin. Paraffin sections of 5-micron thickness were prepared and stained routinely with haematoxylin and eosin (Bancroft and Stevens 1996) and examined microscopically.

5- Estimation of biochemical parameters:

The aspartate aminotransferase (AST), alanine aminotransferase (ALT) and serum albumin were measured according to the method of the International Federation of Clinical Chemistry IFCC (Begmeyer and Herder, 1989). The GSH content was determined photometrically according to the method described by (Ellman, 1959). Glutathione peroxidase (GPx) activity was assayed according to the method of Gross et al. (1967). Catalase (CAT) activity was assayed using the method of Sinha, (1972).

6- Statistical analysis:

Data were analyzed using one way analysis of variance (ANOVA). The results obtained were expressed by mean ± standard error of the mean. Differences were considered significant at P<0.05 (George and William, 1980).

**RESULTS**

The results in table (1) clarify liver function tests; AST, ALT and albumin in different experimental groups. It showed highly significant increase in AST and ALT in the cisplatin group compared to the control group. As regard albumin level, there is a highly significant decrease in cisplatin group compared to the control group.

**Table 1:** Liver function tests in the different animal groups:

<table>
<thead>
<tr>
<th>Biochemical parameter</th>
<th>Control group</th>
<th>Cisplatin group</th>
<th>Royal jelly group</th>
<th>Royal jelly-cisplatin group</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST U/l</td>
<td>20 ± 2</td>
<td>70 ±5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20 ± 1</td>
<td>30 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALT U/l</td>
<td>19 ± 2</td>
<td>60 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18 ± 2</td>
<td>25 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Albumin gm/dl</td>
<td>3.2</td>
<td>2.3 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.3 ± 0.5</td>
<td>3 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values are expressed as mean± SE
<sup>a</sup> Significant (P < 0.05) when compared with the control group.
<sup>b</sup> Significant (P < 0.05) when compared with the irradiated group.
In Table 2, cisplatin induced significant decrease in the liver CAT, GPx activities and GSH content compared with control group. Administration of royal jelly for 15 consecutive days prior to cisplatin resulted in a significant increase in the activity of liver CAT, GPx and GSH compared to cisplatin group.

**Table 2:** Liver catalase (CAT), glutathione peroxidase (GPx) activities and reduced glutathione (GSH) level in the different animal groups.

<table>
<thead>
<tr>
<th>Biochemical parameter</th>
<th>Control group</th>
<th>Cisplatin group</th>
<th>Royal jelly group</th>
<th>Royal jelly-cisplatin group</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT (U/mg protein)</td>
<td>2.68±0.89</td>
<td>1.13±1.36 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.70±1.20</td>
<td>1.78±1.28 &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSH (mg/g wet tissue)</td>
<td>20.51±1.22</td>
<td>11.54±1.98 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.29±1.40</td>
<td>16.71±1.75 &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GPx (U/mg protein)</td>
<td>0.76±0.06</td>
<td>0.46±0.04 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.70±0.07</td>
<td>0.63±0.06 &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values are expressed as mean± SE

<sup>a</sup> Significant (P < 0.05) when compared with the control group.

<sup>b</sup> Significant (P < 0.05) when compared with the irradiated group.

**Histopathological findings:**

In control group (group 1) the hepatic lobule - between central vein and peripheral interlobular septum - consists of plates of hepatic cells. These plates branch and anastomose within the lobule. The portal venules and hepatic arterioles penetrate the connective tissue to form sinusoids. The hepatic cells are polygonal, vary in size, contain large, round nucleus and may occasionally be binucleate. The cells have a granular acidophilic cytoplasm. The sinusoids are situated between plates of hepatic cells and lined with a discontinuous type of endothelium (fig. 1). The blood in sinusoids containing erythrocytes and leukocytes and drains into the central vein. The liver of rats received cisplatin (group 2) showed an obvious widespread swelling and ballooning of hepatocytes owing to hydropic degeneration and this progresses to cell death mainly focal or zonal necrosis though the lobule (fig. 2). Moreover some sinusoids are engorged with the blood (fig. 3), beside centrolobular necrosis (fig. 4). Other cases showed periportal leukocytic infiltration mainly mononuclear, which extended out from the portal tract to involve large areas from hepatic lobules, sometimes apoptotic changes were seen (fig. 5). In royal jelly group (group 3), the hepatic tissues showed normal structure. On the other hand, most cases of royal jelly and cisplatin group (group 4) showed the histological structure of hepatic tissues relatively well preserved architecture with prominent Kuffer’s cells without degenerative or necrotic changes (fig. 6).
Fig. 1: Liver of control rat (group 1) showing normal structure (H&E × 400).

Fig. 2: Liver of cisplatin group (group 2) showing acute cell swelling and cell death around central vein (H&E × 400).

Fig. 3: Liver of cisplatin group (group 2) showing congested sinusoid and vacuolation of the hepatic cells (H&E × 400).

Fig. 4: Liver of cisplatin group (group 2) showing congested blood vessel, necrosis of hepatic tissues (H&E × 400).

Fig. 5: Liver of cisplatin group (group 2) showing necrosis with mononuclear cell infiltration around central vein (H&E × 400).

Fig. 6: Liver of royal jelly and cisplatin group (group 4) showing normal structure and prominent Kuffer's cells (H&E × 400).
DISCUSSION

Cisplatin (CP) is widely used, often in combination with radiation and other drugs, against malignant, solid, epithelial tumors (Rosa et al., 2009). The major limitation of its use is the development of resistance by tumors (Boulikas and Vougiouka, 2003) and the cumulative, dose dependent severe hepatotoxicity and nephrotoxicity that can culminate in acute hepatic and renal failure (Pabla and Dong, 2008). Despite intensive prophylactic measures, irreversible hepatic and renal damage occurs within days in approximately one-third of CP-treated patients (Santoso et al., 2003, & Jiang and Dong, 2008).

Recent studies suggest that using plant-derived chemo-preventive agents in combination with chemotherapy can enhance the efficacy of chemotherapeutic agents and lower their toxicity to normal tissues. (Kanbur et al 2009 and Gasic et al 2007). This study investigates the effects of RJ on CP-induced liver damages. Animals that received CP showed severe biochemical and histological changes. On the other hand, pretreatment with RJ caused a significant improvement in tested parameters, which were significantly altered by CP administration.

The increase in AST and ALT activities in the CP group was found to be related to damage in the liver and change in hepatic functions. The rise in levels of serum AST and ALT has been attributed to the damaged structural integrity of the liver, because these are normally located in the cytoplasm and are released into the circulation after hepatic damage (Sallie et al 1991). Greggi et al. (2001) and Mora et al. (2003) have also described the increasing effects of CP on changes in serum AST and ALT activities and decrease of albumin level. On the other hand, pretreatment with the RJ remarkably inhibited CP-induced liver damage as evidenced by decreased serum activities of AST and ALT and increase of albumin level, as showed in present study (table1). As regard albumin level, there is a highly significant decrease in cisplatin group compared to the control group. Additionally, histopathological study of liver sections indicated centrilobular necrosis, congested sinusoids, hydropic degeneration and mononuclear cells infiltration in rats treated with CP alone. On the other hand, liver of royal jelly and cisplatin group (group 4) showed relatively well preserved architecture.

Cisplatin acts on cancer cells by releasing free radicals such as superoxide radicals, hydroxy radicals, peroxyl radicals, and singlet oxygen, which at the same time damage liver and kidney cells. Free radicals are known to attack the highly unsaturated fatty acids of the cell membrane to induce lipid peroxidation, which is considered a key process in many pathological events and is one of the reactions induced by oxidative stress (Schinella et al 2002). Cisplatin treatment causes an increase in lipid peroxide levels and a decrease in the activities of antioxidant enzymes that protect against lipid peroxidation in the tissues such as liver and kidney (Golstein and Mayor 1983). Many cellular pathways have been suggested to contribute to induction of a state of oxidative stress and lipid peroxides. For example, it is possible that cisplatin-induced oxidative stress and cytochrome P450 2E1-(CYP2E1-) which mediated oxidative stress synergize to produce hepatotoxicity.

A number of studies reported that CP has been found to have an apoptotic effect on kidney and liver (Kang and Reynolds 2009). Apoptosis is characterized by phosphatidylserine externalization, membrane budding, cell shrinkage, and chromatin condensation. The apoptotic caspase enzymes are named as initiator caspases (Valko et al 2006) and effector caspases (Halliwell and Gutteridge 1999). The effector caspases act via the activation of initiator caspases which trigger the apoptotic process (Zhan et al 1999), whereas Bcl-2 family proteins, which are generally antiapoptotic, are inhibited by cytochrome c release from mitochondria and programmed cell death inducing enzymes (Kang and Reynolds 2009). The cisplatin-induced apoptosis is believed to be the result of DNA damage (Cummings and Schnellmann 2002).
Royal jelly contains biologically active amino acids such as aspartic acid, cysteine, cystine, tyrosine, glycine, lysine, leucine, valine, and isoleucine. As indicated by previous researchers, the antioxidant effect of RJ may be related to its free amino acid content (Tamura et al. 2009). Reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anions, and hydroxyl radicals are generated under normal cellular conditions and are immediately detoxified by major scavenger enzymes (glutathione based enzymes such as GSH-Px, GST, and CAT). However, excessive ROS production by CP causes antioxidant imbalance and leads to lipid peroxidation and antioxidant depletion. In our study (Table 2), the corroborate (GSH, GPx and CAT) were significantly decreased in liver of cisplatin-treated rats. This result may be connected with the CP-induced increase in free radical generation or a decrease in amounts of protecting enzymes against lipid peroxidation. In previous studies, CP has been found to have a peroxidative effect on the liver tissues (Silici et al. 2010). However, the treatment with RJ ameliorated the cisplatin-induced liver damages due to free radical production. Meanwhile, the elevated GSH level and activities of GSH-Px and CAT enzymes in the CP plus RJ group implied a decrease in the number of free radicals after cisplatin administration and reflected that these enzymes played important roles in scavenging free radical. Our findings are similar to results of other investigators such as Al-Majed et al. (2006) and Arafa (2008) for liver tissues in which cisplatin injections caused low GSH, GSH-Px, and nitric oxide levels. Furthermore, in this study, it was observed that levels of GSH, GPx and CAT in CP plus RJ-treated group were higher than in the CP group. These results suggested that RJ has a supporting effect on the antioxidant system because of increases in GSH, GPx, and CAT activities. Our results corroborate previous findings that RJ prevents carbon tetrachloride- [Cemek et al. 2010], cadmium- (Avus glu et al. 2009) and paracetamol- (Kanbur et al. 2009) induced liver toxicity genotoxicity and nephrotoxicity, respectively.

In conclusion, RJ acts in the liver as a potent scavenger of free radicals to prevent or ameliorates the toxic effects of CP as shown by the biochemical and histopathological investigate and might provide substantial protection against cisplatin-induced inflammatory damages.

REFERENCES


