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Administration of Honey and Royal Jelly Ameliorate Cisplatin Induced Changes in Liver and Kidney Function in Rat

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Although cisplatin is an effective drug, its clinical use is limited because of its side effects. Honey and royal jelly are natural antioxidants that can be extracted from honey bees. The aim of this investigation is to study the ameliorative role of both honey and royal jelly against cisplatin induced changes in levels of liver and kidney function biomarkers in rat. Male wistar albino rats of almost same age and weight were divided randomly into four groups. Group I: (control group) rats were given 0.9% saline. Group II; (cisplatin group) rats were injected by cisplatin (7mg/ kg/ day) intraperitoneally for 15 days. Group III; (Honey and Royall jelly group) rats were fed orally honey (500 mg/kg/day) with royal jelly (100mg/ kg/day) for 15 days. Group IV; (cisplatin and honey with royal jelly group) rats were injected cisplatin (7mg/kg/day) intraperitoneally and fed orally honey (500mg/kg/day) with royal jelly (100mg/kg/day) daily for 15 days. At the end of experiment, blood was collected and serum was got by centrifugation at 3500 rpm. Serum obtained was analyzed for liver function test by estimating ALT, AST, ALP, total bilirubin, albumin, and total protein and kidney function test by estimating creatinine, urea, and uric acid levels. Administration of cisplatin to rats (Group, II) leads to a significant increase in serum ALT, AST, ALP enzyme activity, while the values of total bilirubin, total protein and albumin were significantly decreased as compared to control. Oral supplementation of royal jelly and honey to rats (Group, III) showed comparable enzyme activity of ALT, AST, ALP and values of total bilirubin, total protein and albumin to control. In the rat group that were administered honey and royal jelly in association of cisplatin (Group, IV) improvement was observed in liver function biomarkers. Cisplatin administrated rats (G, II) shows a significant increase in the values of kidney function biomarkers like creatinine, urea and uric acid compare to control. Oral supplementation of royal jelly and honey treated to rats (Group, III) showed comparable values of creatinine, urea and uric acid to control. In the rat group that were administered honey and royal jelly in association of cisplatin (Group, IV) improvement was observed in kidney function biomarkers. The study found that combined administration of bee honey and royal jelly attenuated the cisplatin induced alterations in liver and kidney function biomarkers, because honey and royal jelly are free radical scavengers, lipid peroxidation inhibitors and anti-inflammatory effects and hence are recommended during the cisplatin chemotherapy.

Keywords: Wister male albino rats; liver and kidney function; cisplatin; honey; royal jelly.

Cisplatin is a standout amongst the most broadly utilized anticancer medications for the treatment of different tumors [1]. However, in spite of its wide therapeutic benefits as an anticancer drug, its clinical use is limited due to its dose dependent hepatonephrotoxicity [2]. Although intensive prophylactic measures, irremediable renal and hepatic damage occurs within days in nearly

one-third of cisplatin-treated patients [3, 4]. There are many evidences that cisplatin induces oxidative stress which plays a critical role in liver and kidney diseases [5, 6]. Oxidative stress was attributed to the combination of multi-ways, such as the generation of reactive oxygen species (ROS), which could interact with the antioxidant defense system and cause oxidative damage in different tissues and reaction with glutathione and thiols in protein, which could lead to cell dysfunction in liver and kidney [7]. Reactive oxygen species (ROS) directly act on cellular units such as, proteins, lipids and DNA to devastate their structure [8]. The free radicals devastate the lipid units of the cell membrane by denaturing proteins and peroxidation, causing enzymatic deactivation and lead to mitochondrial dysfunction [9]. Although many studies have proved the role of many drugs against cisplatin-induced liver and kidney toxicity, the mechanism of hepatoprotective and neuroprotection lasts evasive [10, 11]. So, looking for procedures to prohibit cisplatin-induced nephrotoxicity and hepatotoxicity constitutes an active area of study. So that, it is rational to assume that the use of antioxidant defense of liver or kidney tissue by exogenous antioxidants having further properties such as cytoprotective and anti-inflammatory effects should be a strategy to maintain the liver and kidney from the oxidative damage [12].

Few years before, the science of nutrition has been developed significantly based on the greater understanding of genetic and physiological mechanisms about effect of diet and individual food components diseases and health. Scientific proofs support the opinion that diet controls and modulate physiology of human body appropriately and share in the preservation of good health or homeostasis necessary to decrease the risk of several chronic diseases. Natural antioxidants have been studied to reduce dangerous side effects as well as enhance anticancer activities of antitumor drugs [13]. Various experimental studies indicated that diet with honey and royal jelly have profound beneficial health effects against various pathologies [14]. Royal jelly and honey have highly efficient antioxidant free radical scavenging ability against nephrotoxicity and hepatotoxicity induced by cisplatin [15]. Royal jelly and honey consist of many important compounds with biological activity [16]. Honey is affluent source of energy, and provide energy for cellular activity. This consequent effect could reduce the energy depletion and the consequent cytotoxic action of cisplatin, which has been broadly attributed to raised production of reactive oxygen species (ROS) [17], which disrupt mitochondrial membrane potential (MMP) and impair the respiratory chain [18], leading to compromised supply of energy for cellular functions.

MATERIALS AND METHODS

Animals

Healthy male Wister albino rats weighed 200-250gm (10-12 week age) were got from the animal house of R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur-India. All the experimental procedures were carried out according to the guidelines of CPCSEA and the experimental protocol approved by the Institutional Animal Ethics Committee (IAEC) of RCPIPER, Shirpur (Reg No- 651/PO/ReBi/S/02/CPCSEA).

Housing Conditions

The rats were housed in standard plastic cages. The bedding material of the cages was changed every day. Maximum of 3 rats were housed per polypropylene cage having a size of 32 X 11 cm with stainless steel grill top mesh having facility for holding food palate and a water bottle. The rats were allowed to free access to diet and water throughout the experimental period. All animals were housed in an air conditioned room at temperature range between 22 -25°C, relative humidity in between 30% - 60% and 12 hour light-dark cycle.

Acclimatization

Selected rats were divided randomly into four groups, containing 6 rats in each group and allowed to acclimatize to laboratory conditions for 7 days prior to experimentation.

Water

Water processed by reverse osmosis and UV light was supplied ad libitum to the rats.

Chemicals

Cisplatin was purchased from Cipla Ltd Company- Goa-India. Bee honey and royal jelly was collected directly from the *Apis mellifera* colonies located in the University campus. Food

pallets were purchased from Nutrivet Life Sciences, Pune, Maharashtra, India. All other chemicals used in the estimations were of analytical grade.

Preparation of Royal Jelly and Honey

500mg of honey and 100mg of royal jelly were dissolved in distilled water and administered through an intragastric tube through the mouth. The doses were weighed on digital scales where each dose relies on the relevant animal's weight, in which every single gram of the experimental rat should receive 0.5mg of honey and 0.1mg of royal jelly.

Experimental Design

For the study, 24 adult male Wister albino rats of 10-12 week age and with 200-250g weight divided randomly into 4 groups, each group contained 6 rats (n=6) and were treated for 15 days as below:

Group I (Control):0.9% (10ml/kg/day) saline solution was administered for 15 days.

Group II (Cisplatin): Cisplatin (7mg/kg/day) intraperitoneal injection for 15 days [19, 20].

Group III (Honey + Royal jelly): Honey (500mg/kg/day) +Royal jelly (100mg/kg/day) orally administered for 15 days [21, 22].

Group IV (Cisplatin+Honey+Royal jelly):7mg/kg/day of cisplatin injected intraperitoneally while honey (500mg/kg/day) and royal jelly (100mg/kg/day) were orally fed through an intragastric tube for 15 days.

Collection of Blood Sample

After 15 days of treatment, blood was collected from the retro orbital plexus under the light ether anaesthesia from experimental and control group rats. Blood collected was put in tubes without anticoagulant, and serum was got by centrifugation at 3500 rpm at 25°C for 10 minutes. The serum samples were analysed for biochemical parameters like ALT, AST, ALP, albumin, creatinine, urea, uric acid, total protein and total bilirubin by using commercially available kits obtained from Erba diagnostic Mannheim Gmbh, Germany and were determined by using spectrophotometer [23-27]. All other biochemical estimations were carried out in R. C. Patel Institute of Pharmaceutical Education and Research.

Statistical Analysis

All data was considered as mean ± S.E.M. and statistically analysed, using Graph Pad Prism 7 for Windows (Prism Inc., Chicago, IL, USA). Statistical significance of differences among different study groups was evaluated by one-way analysis of variance (ANOVA) followed by multiple comparisons test as a post hoc test. P value of 0.05 or less was taken as a criterion for a statistically significant difference.

RESULTS

Results of Liver Function

The impact of administration of cisplatin, oral supplementation of honey and royal jelly, and administration of cisplatin with honey and royal jelly on liver function biomarkers in rats were evaluated in comparison with control and obtained results are summarized in table no.1.

The results demonstrate that cisplatin administered rats (Group, II) caused a significant increase in the activity of alanine aminotransferase (ALT), aspartate aminotransferases (AST), of alkaline phosphatase (ALP) and value of total bilirubin in experimental rats compared to control and the percent increase was 105.03%, 179.01%, 71.7% and 145.45% respectively. In contrast, the serum level of total protein and albumin were significantly decreased by 43.41%, and 21.88% respectively.

The oral supplementation of honey and royal jelly to rats, shows comparable activity of ALT, AST, ALP and the serum level of total protein, albumin and total bilirubin to control.

Administration of cisplatin with oral supplementation of honey and royal jelly to rats that leads to significant decrease in the activity of alanine aminotransferase (ALT), aspartate aminotransferases (AST), alkaline phosphatase (ALP) and values of total bilirubin compared to cisplatin administrated rats and the percent decrease was 39.05%, 37.25%, 18.65 %, and 51.85 %, respectively. While the serum level of total protein and albumin were significantly increased as compared to cisplatin administrated rats and the percent increase was 61.02% and 13. 89 %.

Results of Kidney Functions

The impact of administration of cisplatin, oral supplementation of honey and royal jelly, and administration of cisplatin with honey and royal jelly to rats on kidney function biomarkers were evaluated in comparison with control and obtained results are summarized in table no. 2.

The results demonstrate that cisplatin administered rats, caused a significant increase in the levels of serum creatinine, urea and uric acid compared to control rats and the percent increase was 137.35 %, 23.2 % and 122.2% respectively.

It was observed that oral supplementation of honey and royal jelly to rats, leads to non-significant decrease in the levels of serum creatinine, urea, and uric acid compared to control and the percentage values were 8.4, 1.14 % and 1.23 %, respectively.

Combined treatment of cisplatin along with honey and royal jelly, the level of serum creatinine, urea and uric acid in experimental rats

Table 1. Effect of Royal Jelly and Honey on Cisplatin Induced Changes in Levels of Liver Function Biomarkers in Male Wister Albino Rats

Parameter	Groups (Group I) Control	(Group II) Cisplatin	(Group III) Honey and royal jelly	(Group IV) Cisplatin with honey and royal jelly
ALT(IU/LIU/L	47.70±3.52	97.80±12.7** a# (+ 105.03%)	46.00± 3.52 NS a# (- 3.56 %)	59.50±7.79* bw (-39.05%)
AST(IU/L)	41.00±0.69	113.00±14.0*** a# (+179.01%)	40.00± 0.258NS a# (-2.43 %)	70.90±9.1** bw (-37.25 %)
ALP(IU/L)	78.00±2.6	134.00±3.8*** a# (+71.7 %)	77.00±2.3NS a# (- 1.01 %)	109.00± 7.9** bw (-18.65 %)
Total Protein (g/dl)g/dl	8.66±0.273	4.90±0.312*** a# (-43.41%)	8.80±0.193NS a# (+1.6 %)	7.89±0.344**** bw (+61.02%)
Albumin(g/dl)	5.62±0.211	4.39±0.157*** a# (- 21.88%)	5.86 ±0.122 NS a# (+ 4.27%)	5.00 ±0.191* bw (+13.89 %)
Total bilirubin(mg/dl)	1.10± 0.032	2.70±0.086*** a# (+145.45%)	1.00±0.032 NS a# (-9.09 %)	1.30± 0.067*** bw (-51.85 %)

1. ± indicate S.D. of three observations
2. # (+) or (-) indicate percent variation over respective control (G, I) rats
3. w (+) or (-) indicate percent variation over cisplatin injected (G, II) rats
4. Values are significant at *$P < 0.001$, **$P < 0.01$, ***$P < 0.05$, NS - Non-significant
5. a = $P < 0.001$, **$P < 0.01$, ***$P < 0.05$ values compared with respective control rats
6. b = $P < 0.001$, **$P < 0.01$, ***$P < 0.05$ values compared with respective cisplatin injected rats

Table 2. Effect of Royal Jelly and Honey on Cisplatin Induced Changes in Levels of Kidney Function Biomarkers in Male Wister Albino Rats

Parameter	Groups (Group I) Control	(Group II) Cisplatin	(Group III) Honey and royal jelly	(Group IV) Cisplatin with honey and royal jelly
Creatinine(mg/ dl)	0.712 ± 0.128	1.69 ± 0.244** a# (+137.35 %)	0.65±0.11 NS a# (-8.4 %)	0.910 ± 0.122* bw (-46.1 %)
UREA(mg/ dl)	34.90 ± 1.58	43.00 ± 2.44* a# (+23.2 %)	34.50 ± 1.74NS a# (-1.14 %)	37.10 ± 0.83* bW(- 13.70)
Uric acid (mg/ dl)	0.81±0.036	1.80 ± 0.1*** a# (+122.2%)	0.80 ± 0.04NS a# (- 1.23 %)	1.30.1** bw (-27.7%)

1. ± indicate S.D. of three observations
2. # (+) or (-) indicate percent variation over respective control (G, I) rats
3. w (+) or (-) indicate percent variation over cisplatin injected (G, II) rats
4. Values are significant at *$P < 0.001$, **$P < 0.01$, ***$P < 0.05$, NS - Non-significant
5. a = $P < 0.001$, **$P < 0.01$, ***$P < 0.05$ values compared with respective control rats
6. b = $P < 0.001$, **$P < 0.01$, ***$P < 0.05$ values compared with respective cisplatin injected rats

were significantly decreased compared to rats treated with cisplatin and the percentage decrease was 46.1 %, 13.70 and 27.7%, respectively.

DISCUSSION

The obtained findings shows that the administration of cisplatin to rats, caused significant increase in alanine aminotransferase (ALT), aspartate aminotransferases (AST), and alkaline phosphatase (ALP) activity, and values of total bilirubin, while the serum level of total protein and albumin was significantly decreased compared to control. The results recorded are in harmony with the results of previous investigators [28, 29]**.** The alteration in the liver function parameters induced by cisplatin is closely associated with an increase in reactive oxygen species (ROS) and lipid peroxidation in the liver tissue [15]. The increased activity of ALT, and AST has been attributed to the destroyed structural integrity of the liver, because these are normally located in the cytoplasm and are released into the circulation after hepatic damage [30, 31]**.** These findings may refer to degenerative changes and hypo function of the liver [32] and also hepatic cell necrosis [33] which increases the release of these enzymes in the blood stream [33]. The increasing levels of these enzymes in the serum are presumptive signs of cisplatin induced necrotic lesions in the hepatocytes [34]. The boosted sensitivity of hepatocyte cell membrane to cisplatin induced peroxidative damage might have led to an increased releasing of the diagnostic sign enzymes in the systematic circulation. An increase in the AST and ALT levels refers to a reversible change of the cell membrane permeability [35].

The increase in the alkaline phosphatase (ALP) enzyme activity was attributed to the irritation of liver cells by the effects of cisplatin or due to the increasing loss of intracellular enzyme by diffusion through cell membrane, which appears to act as a stimulus to the synthesis of more enzymes [36].

The administration of cisplatin to rats leads to a noticed increase in the total bilirubin values in liver of the experimental rat. This indicates the cellular leakage of these markers from the cell membrane of the liver [32, 37, 38].

Cisplatin administrated rats shows a marked depletion in the level of total protein and albumin in liver of the experimental rat. The results recorded in the present study are matched with the results of previous investigators [32, 28, 39]. The reduction in the total protein and albumin was attributed due to oxidative stress generated by cisplatin. Cisplatin are inducers of reactive oxygen species (ROS) [40]. In the existence of reactive oxygen species (ROS), proteins can be damaged by direct oxidation of their amino acid sediments and cofactors or by secondary attack via lipid peroxidation [41]. Liver damage leads to reduce in synthetic capability of hepatocytes, causing the fall in serum total protein and albumin levels [42-43]. Also, the decrease in the serum albumin and total protein may also refer to the renal damage result in excretion in urine [44].

The royal jelly and honey significantly restore the changes of ALT, AST, ALP, bilirubin, and total protein caused by cisplatin injection towards the normal control values due to its antioxidant influence and its capacity to act as a free radical scavenger, thereby protecting membrane permeability [20]. This study indicates that the administration of honey and royal jelly prevented liver damage by preserving the integrity of the plasma membrane, suppressing the seepage of enzymes through membranes, exhibiting hepatoprotective and hepatocurative activities. This might be the main reason for the restoration in the activities of the marker enzymes during administration of honey and royal jelly oxidative damage in a cell or tissue, which occurs when the concentration of ROS generated exceeds the antioxidant capacity of free radical scavenger [45]. Many investigators reported that royal jelly and honey have a protective role against many drugs on liver function [46-47].

Administration of cisplatin to rats caused a significant increase in the level of creatinine, urea and uric acid in serum of experimental rats compared to control. These results are in agreement with the previous investigators [48-49]. The increasing in the level of creatinine, urea, and uric acid was attributed to cisplatin induced oxidative stress through elevation of ROS, lipid peroxidation and reduction of the antioxidant defence system [4, 50]. Moreover, the kidney provides the final common pathway for the excretion of many drugs and their metabolites. That's why it is frequently subjected to high concentrations of potentially

toxic materials. Drugs and their metabolites are taken up selectively and concentrated by the renal tubular cells before excretion into the urine. Therefore, high intracellular concentrations are attained, especially in the renal medulla, which has relatively little vasculature in comparison with the cortex [51]. In fact, the level of urea is the first severe renal marker of the kidney sufferers at any type of injury. In addition, creatinine and uric acid are the most trusted renal marker, and those parameters increase only when the majority of renal function is lost [52].

Administration of cisplatin with oral supplementation of honey and royal jelly to rats, leads to a significant decrease in the level of creatinine, urea and uric acid in rats compared to cisplatin administrated rats. Thus the results indicated that there are ameliorative effects of honey and royal jelly on the kidney function parameters of rats. Royal jelly and honey are functional food, having naturally high antioxidant potential. Royal jelly contains water, free amino acids, proteins, sugars, fatty acids, mineral salts, vitamins and antioxidants [16]. Royal jelly possesses antioxidant, antitumor, antibacterial, hypoglycemic, anti-inflammatory antihypercholesterolemic, and immunomodulatory activities [53, 54]. Furthermore, honey and royal jelly had a protective effect against cisplatin induced kidney damage in rats [15]. [55-56] showed that royal jelly has a protective role against many drugs on kidney damage. Honey consists of at least 200 components, including phenolics (caffeic acid, chrysin, quercetin, kaempferol), flavonoids, ascorbic acid, carotenoid-like substances, organic acids, amino acids, proteins and certain enzymes such as glucose oxidase, catalase...etc [57]. Many studies reported that honey has a protective role against many drugs on kidney function [58-60]**.** The ability of honey to prevent cadmium-induced hepato-nephrotoxicity in rats was also reported by some authors**.**

The oral supplementation of honey and royal jelly has a protective and curative role on alteration which caused by cisplatin in kidney and liver function parameters. However, these observations may be attributed to the antioxidant properties of honey and royal jelly which contain zinc and selenium [61], in addition to many forms of flavonoid compounds [62]. These compounds were known for their hydrogen donating antioxidant activities as well as their ability to form complexes with divalent transition metal cations [63]. Thus, this highly antioxidant capacity of honey and royal jelly made it able to scavenge the free radicals, reducing the level of nitric oxide and consequently decrease the level of lipid peroxidation as well as to prevent protein oxidation as reflected by the observed reduction liver and kidney function parameters. Also, royal jelly and honey plays an important role in the development of normal cellular immunity [64].

These obtained results support the hypothesis that cisplatin hepatotoxicity and nephrotoxicity are related to the free radical generation, while the nephroprotection and hepatoprotection are caused by oral supplementations of honey and royal jelly. In agreement with our hypothesis, [65-69] confirmed that honey and royal jelly could effectively remove a variety of ROS.

Royal jelly and honey, the most important antioxidants, spares the other antioxidants by forming the first line defence against free radicals and peroxides that are generated during cellular metabolism as it demonstrated by the significantly restored changes of liver and kidney function parameters due to theirs antioxidant effect and theirs capacity to act as a free radical scavenger in case the cisplatin nephrotoxicity and hepatotoxicity, thereby protecting membrane permeability.

CONCLUSION

It is concluded that the combined oral supplementation of honey and royal jelly protected and ameliorated the alterations in liver and kidney function induced by cisplatin in rats. Therefore, it is suggested that at the time of chemotherapy treatment honey and royal jelly should be given to the patient as therapeutic agents to prevent nephrotoxicity and hepatotoxicity caused by chemotherapy drug, cisplatin because honey and royal jelly contain antioxidant, lipid peroxidation inhibitors and anti-inflammatory effects.

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