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Original Research Article

Dexamethasone-triggered hepatic and renal impairment in albino rats, and its amelioration through royal jelly intervention

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Abstract

Purpose: *To evaluate the hepato-renal protective effect of royal jelly (RJ) against dexamethasone (DEX)-induced toxicity in rat models.*
Methods: *Twenty-four male albino Wistar rats (divided equally into three groups) were administered DEX with or without RJ while control group received normal saline. The serum levels of aspartate aminotransferase (AST), alanine aminotransaminase (ALT), creatinine, uric acid, albumin, and hepatic activities of glucose-6-phosphate dehydrogenase (G6PD) and catalase, as well as levels of glutathione (GSH) and total protein, were measured. Body, kidney and liver weights, as well as blood glucose concentrations, were measured, before and after treatment.*
Results: *Dexamethasone (DEX) produced significant hyperglycemia and hyperuricemia and significant increases in concentrations of kidney and liver function biomarkers ($p < 0.05$). These changes were accompanied by decreased liver levels of GSH, total protein and catalase. These alterations were significantly reversed in DEX-treated rats given RJ, compared to rats receiving only DEX ($p < 0.05$). Body weights were also significantly augmented in DEX-injected rats given RJ, relative to rats given DEX alone ($p < 0.05$).*
Conclusion: *Royal jelly significantly reduces DEX-induced renal and hepatic toxicities, which suggests its probable therapeutic significance in inhibiting glucocorticoid-mediated adverse reactions. This phenomenon should be further investigated and the possible mechanism elucidated.*

Keywords: *Hepato-nephrotoxicity, Dexamethasone, Catalase, Glucose-6-phosphate dehydrogenase, GSH, Royal Jelly*

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INTRODUCTION

The pharmacotherapy of glucocorticoids is of utmost importance, owing to their noteworthy effectiveness in the treatment of a range of inflammatory and immunological sicknesses and other disorders [1]. Dexmedetomidine (DEX) is a long-acting anti-inflammatory, widely-utilized and typical synthetic glucocorticoid [2]. Nevertheless, its effectiveness is not without some disadvantages. It has been established that large doses of DEX and prolonged usage of the drug, induce many adverse reactions, i.e., diabetes

mellitus, hepatotoxicity, nephrotoxicity, cardiovascular complications, hypertension, dyslipidemia, hyperlipidemia, and osteoarthritis [1]. Thus, there is need to identify complementary agents, ideally of natural origin, that may be used to lessen these adverse effects. One of the natural substances with remarkable pharmacotherapeutic properties is RJ, a secretion from the worker bee that has been the focus of several scientific investigations [3]. Royal jelly (RJ) is unique due to its high levels of essential fatty acids, sugars, enzymes, flavanones, and the presence of unique 10-hydroxy-2-decenoic acid [3,4].

Studies have shown that RJ has potent antioxidant, free radical-scavenging, and anti-inflammatory properties [4]. Thus, RJ has been shown to alleviate drug-induced nephrotoxic and hepatotoxic effects of several agents such as celecoxib [5], cadmium [6], moxifloxacin [7], and many others [4].

Although there are numerous recognized pharmacological properties of RJ, not much is known about its protective effect against DEX-evoked hepatotoxicity and nephrotoxicity in an animal model. Therefore, the present study was aimed at investigating the protective effect of RJ against hepatic and renal dysfunctions induced by DEX in an albino rat model. The overarching goal was to elucidate the mechanisms through which RJ might mitigate the side effects of DEX. This would contribute valuable insights into the expanding field of natural pharmacotherapy, especially in the context of glucocorticoid-related complications.

EXPERIMENTAL

Materials

Drugs and chemicals

Dexmedetomidine (DEX), solvents, and various standard chemicals were products of Sigma–Aldrich (St. Louis, MO, United States). Egyptian royal jelly (RJ) was sourced from Wadi Al Nahil for Honey and Oud (Hail, Saudi Arabia), and was stored at −20 °C until used. The reagents used for routine chemical analysis were procured from standard commercial suppliers.

Animals

Twenty-four male Wistar strain albino rats weighing between 200 and 250 g were sourced from the animal house of College of Pharmacy at King Saud University, Riyadh, KSA. They were maintained at a constant temperature of 26 ± 2 °C in an atmosphere with a 12-h light/12-h dark photoperiod, fed on a standardized diet and allowed unrestricted access to clean water. Before initiating treatment, the rats were allowed a one-week acclimatization period in the laboratory. The study obtained approval from Experimental Animal Ethics Committee of the Faculty of Medicine, University of Hail, KSA (no. 11009/23) and was conducted by following the protocol for the care and use of laboratory animals [8].

Design

Following the period of adaptation, 24 rats were randomly assigned to three groups, each consisting of eight rats. The groups were treated as follows: group I received subcutaneous injection of normal saline, and served as control. Group II, DEX-control, received subcutaneous injections of DEX at the dose of 0.1 mg/kg [4]. In group III, rats received DEX injection concurrently with RJ through oral gavage, at a dose of 150 mg/kg/day [4]. Royal jelly was dissolved in normal saline before administration. During the study period, the test animals underwent treatment three times weekly over four consecutive weeks, with DEX and RJ administered within the time frame of 7:30 to 9:00 in the morning. At the end of 30-day study period, the rats were subjected to 12-hour fasting period, followed by euthanasia using isoflurane anesthesia. Blood was collected from each rat and subjected to centrifugation at 3500 rpm for 10 min at 4 °C, resulting in the separation of serum. This serum was kept at -20 °C in a deep freezer, before subsequent biochemical assays. Throughout the experiment, the weight variations were logged in every week. The liver and kidneys from rats in each group were promptly excised, rinsed in physiological saline, and weighed. Then, after the determination of relative weights, the tissues were prepared for biochemical evaluations.

Biochemical analysis

Serum glucose concentration

Serum glucose concentration was measured using the glucose oxidase method [9]. Serum levels of AST and ALT were assayed in line with the procedures outlined by Reitman and Frankel [10].

Blood albumin concentrations

Blood albumin levels were determined with Green Bromo Cresol using diagnostic kits

obtained from the Jeddah-based Kashef Diagnostic Company in Saudi Arabia.

Uric acid and creatinine concentrations

The blood concentrations of uric acid and creatinine were determined using standard methodologies outlined in the literature [9,11].

Hepatic G6PD activity

The assay of hepatic G6PD activity was done in line with the method described by Langdon [12]. This involved the measurement of changes in absorbance at 340 nm as a result of the reduction of $NADP^+$ to NADPH. Liver catalase activity was assayed with the procedure of Cohen *et al* [13].

Hepatic GSH concentration

Liver tissue level of GSH was measured spectrophotometrically with the method outlined by Sedlack and Lindsay. This procedure is based on the capacity of catalase to decompose H_2O_2 [14].

Statistical analysis

Data were subjected to analysis using Statistical Packages for Social Sciences 18.0 (SPSS Inc., Chicago, IL, USA). Results are expressed as mean ± standard deviation (SD). One-way ANOVA was carried out followed by pairwise comparison of means using Duncan's multiple range test. Statistical significance of difference was assumed at $p < 0.05$.

RESULTS

Table 1 shows the impact of DEX on liver and kidney functions. The use of a single-factor ANOVA test demonstrated significant increases in serum ALT in the treated groups of rats ($p < 0.001$). The control had an average ALT level of 53.97 ± 6.01 U/L. In the group given DEX, the ALT level was significantly increased to 120.93 ± 5.92 U/L. However, treatment with RJ at a dose of 150 mg/kg significantly reduced ALT activity to 78.87 ± 4.11 U/L. Comparable significant impacts were observed for AST, uric acid, creatinine, and albumin. The average albumin level in the control group was 27.98 ± 2.38 g/L. This was significantly reduced to 11.11 ± 1.56 g/L, with DEX administration. However, RJ administration at a dose of 150 mg/kg resulted in an increase in albumin concentration to 17.89 ± 1.38 g/L (Table 1).

Table 2 indicates hepatic catalase activity, GSH level, and total protein concentration in each of the various rat groups. The DEX-treated groups had significant decreases in liver catalase activity (0.41 ± 0.04 UI/mg protein) when compared to control (0.84 ± 0.06). Rats treated with DEX and RJ (at a dose of 150 mg/kg) had a significant increase in catalase enzyme level (0.53 ± 0.03) when compared with those in the DEX group ($p < 0.01$). Similar trends were observed with other parameters, as shown in Table 2.

Figure 1 shows that DEX produced a significant effect on blood glucose levels in the various groups of rats ($p < 0.0001$). The mean blood glucose concentration in the control group was 4.71 ± 0.49 mmol/L. In the group administered DEX, blood glucose was significantly increased to 9.31 ± 0.96 mmol/L. However, administration of RJ at a dose of 150 mg/kg led to a significant reduction of blood glucose concentration to 7.49 ± 0.21 mmol/L ($p < 0.05$).

Table 1: Effect of RJ on liver and kidney-linked biomarkers in DEX-exposed male rats

Biochemical marker	Control group	DEX group	RJ and DEX group
AST (IU/L)	79.68±3.98	174.87±11.89***	122±6.18**
ALT (IU/L)	53. 97±6.01	120.93±5.92***	78.87±4.11**
Albumin (gm/L)	27.98±2.38	11.11±1.56***	17.89±1.38**
Uric Acid (µmol/L)	114.95±12.23	169.89±10.78***	121.79±6.01**
Creatinine (µmol/L)	49.23±10.98	127.93±5.13***	75.89±6.98**

**$P < 0.01$ vs. control group; ***$p < 0.001$ vs. DEX group

Table 2: Effect of RJ on hepatic catalase activity, GSH level, and total protein content in male rats subjected to dexamethasone injection

Parameter	Control group	DEX group	RJ and DEX group
Catalase (UI/mg protein)	0.84±0.06	0.41±0.04***	0.53±0.03**
GSH (µg/mg protein)	3.55±0.35	1.01±0.17***	1.63±0.16**
Total protein (g/dL)	2.73±0.10	1.77^a±0.12***	2.09^b±0.09**

**$P < 0.01$ vs. control group; ***$p < 0.001$ vs. DEX group

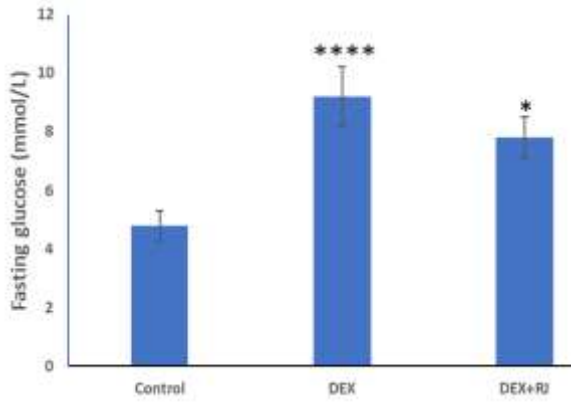

Figure 1: Effect RJ on fasting blood glucose levels in rats administered dexamethasone. ***Note:*** ****P* < 0.0001 vs. control; **p* < 0.05 vs. DEX group

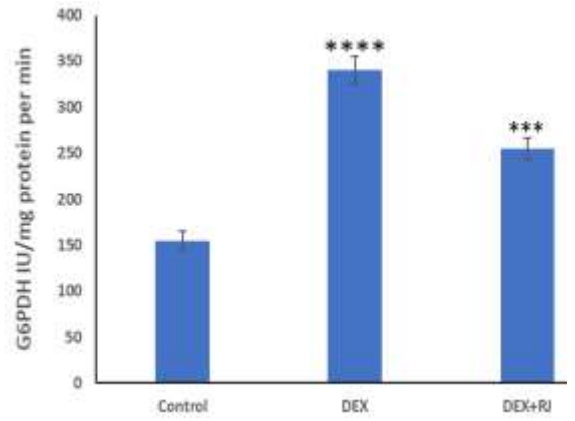

Figure 2: Effect of RJ on hepatic G6PDH activity in rats administered DEX. ***Note:*** ****P* < 0.0001 vs. control; ****p* < 0.001 vs. DEX group

Figure 2 shows that RJ significantly reduced the hepatic activity of G6PD in male Wistar rats. The effect of DEX on the body weights and relative organ weights (kidney and liver) of male Wistar rats are presented in Table 3. Results from single-factor analysis of variance (ANOVA) revealed that rats given only normal saline had a mean body weight increase of 31.34 ± 9.74 g. Moreover, DEX administration significantly reduced the mean body weight of the rats by -59.68 ± 16.75g. However, treatment with RJ at a dose of 150 mg/kg led to a significant decrease in weight loss by -8.89 ± 5.23 g. Furthermore, there were proportional and significant variations in relative liver weight values across the three groups. These results are presented in Table 3.

DISCUSSION

The findings in this study indicate that RJ significantly ameliorated liver and kidney dysfunctions caused by DEX in rats. These data are consistent with earlier results which showed that RJ suppressed nephrotoxicity induced by celecoxib, cadmium, moxifloxacin, doxorubicin, and gentamicin, as well as CCl_4-induced hepatotoxicity [4-7]. These beneficial effects of RJ are believed to be due to its antioxidant properties.

Moreover, the present investigation revealed that DEX significantly reduced the increases in body weight and altered the relative weights of the liver and kidneys. This is also in agreement with findings in previous investigations [15]. These effects are attributable to insulin resistance caused by glucocorticoids, which include metabolic illnesses, hyperleptinemia, lessened appetite, weight loss, and raised levels of blood glucose and triglycerides [15]. The present study showed that RJ suppressed DEX-induced weight loss, resulting in increased body weight. The enhancement of body weight gain could be ascribed to heightened insulin sensitivity which resulted in improved glucose uptake [15,16]. Moreover, rats administered DEX displayed elevated blood glucose levels, consistent with previous findings [17]. An increase in blood glucose concentration after DEX treatment results from reduced sensitivity to insulin, reduced functions of pancreatic α- and β-cells, and increased liver-based gluconeogenesis [17]. The administration of RJ at the dose of 150 mg/kg produced decreases in glucose levels when compared to the DEX control.

The detrimental impacts of DEX resulted in significant increases in serum levels of uric acid and creatinine when compared with the control group. Elevated serum levels of uric acid and creatinine serve as markers of nephrotoxicity [4].

Table 3: Effect of RJ on body weight changes and relative organ weights of the liver and kidneys in rats administered DEX

Group	Body weight gain/loss (g)	Relative liver weight (g/100)	Relative kidney weight (g/100)
Control	31.34±9.74	3.74±0.19 ***	0.66±0.05**
DEX (0.1mg/kg)	-59.68±16.75**	4.28±1.11***	0.98±0.16**
DEX + RJ (150mg/kg)	-8.89±5. 23**	2.68±0.38***	0.87±0.11**

***P* < 0.01 vs. control group; ****p* < 0.001 vs. DEX group

It is noteworthy that treatment with RJ significantly reduced the DEX-induced increases in levels of uric acid and creatinine. This is consistent with results from studies that reported the protective effects of RJ against nephrotoxicity [4].

In this study, hepatotoxicity was induced through the administration of DEX at a dose of 0.1 mg/kg for 4 weeks. The toxicity was confirmed by significant increases in the blood levels of ALT and AST, and significant reductions in albumin concentrations in DEX-treated rats when compared to the control group. A similar result was reported in a previous study [18].

Impairment of the structural integrity of the liver leads to elevations in blood levels of AST and ALT [6,7]. Similarly, DEX produced a significant reduction in the blood concentration of total protein. The current investigation revealed that treatment with RJ significantly improved liver function, indicating its beneficial role in countering DEX-induced liver dysfunction. Consistent with previous research [19], the impact of DEX-induced oxidative stress was apparent in the reductions of hepatic GSH content and catalase activity. Royal jelly (RJ) is rich in antioxidants, as a result of which it neutralizes free radicals, thereby effectively countering oxidative stress, and restoring GSH content, catalase activity, and total protein to near-normal levels. These findings are in agreement with data from studies highlighting the antioxidant properties of RJ in combating oxidative stress [3,5]. It is important to elaborate that the beneficial role of RJ lies in mitigating harm caused by oxidative stress and enhancing physiological defense mechanisms against oxidative stress. Hydroxy-2-decenoic acid, royalisin, vitamins, minerals, amino acids, and phenolic compounds in RJ are effective in neutralizing or removing free radicals, inhibiting the formation of advanced glycation end products, reversing lipid peroxidation, and boosting antioxidant enzyme activities [3,5].

Furthermore, the results obtained in the present study revealed that the activity of G6PD, a pivotal enzyme in the regulation of redox balance, was elevated after DEX administration, which is most likely an adaptation to counter DEX-induced oxidative stress. However, RJ reduced the hepatic activity of G6PDH, which is in agreement with its antioxidant characteristics and its capacity to neutralize or remove reactive oxygen species. Consistent with other studies [3,5,16], the findings in the present investigation depict the ameliorative effect of RJ on DEX-induced oxidative stress.

CONCLUSION

Oral administration of RJ provides significant protection against hepatic, renal, and metabolic toxicities caused by DEX. This implies that RJ is beneficial for averting liver and renal toxicity caused by dexamethasone. The observed protective potential of RJ is most likely attributable to its anti-inflammatory and antioxidant characteristics.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Conceptualization: MA; formal analysis: FA and SA; writing of original draft: FA and NS; review writing and editing: FA, NS, SA and MA. All authors read and agreed to publish this version of the manuscript.

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