Protective Role of the Royal Jelly Against Genotoxic Effect of Endoxan Drug on the Bone Marrow Chromosomes of Male Albino Mice

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Abstract: The aim of this work is to study the protective role of royal jelly on the mice after endoxan treatment. Animals were divided into seven groups. The first group served as control while the other six groups were treated with (one dose endoxan) then (endoxan and royal jelly) respectively. Each endoxan treated animal was intraperitoneally injected one day in week for 4 weeks with 50, 100, 200 mg/Kg body weight while royal jelly treated animals were orally injected daily for 4 weeks with 1g/Kg body weight. Treatment with endoxan to male mice induced chromosomal aberrations including centromeric attenuation, gap, fragment, deletion, ring and centric fusion. Chromosomal aberrations were significantly increased by time and dose. However, treatment with endoxan and royal jelly significantly reduced (P < 0.01) the frequency of chromosomal aberrations this indicates the antioxidative and scavenging ability potential of royal jelly and its protective intervention against endoxan induced cytogenetical damages.

Key words: Endoxan • Royal Jelly • Chromosomes and Mice

INTRODUCTION

The cancer is the most serious disease that causes death all over the world; it is also known as malignant tumors and neoplasms. It is defined as an abnormal growth of cells which tend to proliferate in an uncontrolled way [1].

Endoxan (EN) is a potent anti-inflammatory and immunosuppressive cytostatic and cytotoxic drug used for such diverse medical problems as neoplasia, tissue transplantation and inflammatory diseases of uncertain cause. The drug is inactive until converted to active metabolites in the liver and both metabolism and toxicity vary with body surface area [2].

Alfred et al [3] stated that cytotoxic drugs may increase the risk of infection, predispose to malignancy and increase mutations in offspring. The cytotoxic drugs interrupt nucleic acid and protein synthesis, thereby inhibiting various immune responses at different stages.

Many studies recorded that endoxan and many other chemotherapeutic agents cause gene mutations, chromosomal breaks, rearrangements and aneuploidy in somatic cells [4, 5].

Intraperitoneal administration of endoxan increased the incidences of lung adenoma and adenocarcinoma, bladder papilloma and leukaemia in mice [6-8] and mammary gland adenoma and carcinoma in rats [7].

Many natural substances are used to reduce the mutagenic effect of chemotherapeutic drugs such as cyclophosphamid (Endoxan). These substances have antitumor effect beside their protective effect, so they increase the efficiency of the therapy without any side effects on the normal cells [9]. Royal jelly, one of the honey products, stimulated cell survival, cell growth and cell differentiation and it also had a cytotoxic effect on the carcinoma cells [10]. Royal jelly has an anti-tumor effect [11] and antimetastatic effect [12].

Sabatini et al [13] reported that water content with 60-70 % is the main component of royal jelly. The dry substance is composed of carbohydrates, proteins, amino acids and fats. Smaller quantities of minerals and vitamins are also present. Proteins and peptides of RJ have many effects such as anti-oxidative [14], immunomodulating, monocyte-proliferation stimulating [12, 15], antibacterial [16, 17], anti-inflammatory [18, 19], anti-allergic [15].
Natural compounds such as RJ with antioxidant and immunomodulatory activity might be useful in the prevention of side effects of endoxan-induced bone marrow chromosomal aberrations. So, the aim of this study is to investigate the protective role of royal jelly against chromosomal aberrations induced in rat after endoxan treatment.

**MATERIALS AND METHODS**

Seventy mature male mice (CD1) of six to eight weeks old with an average body weight (26-30 g). Mice were apparently normal, healthy and were kept in animal houses under suitable conditions during the whole period of the experiment. Animals were fed on standard rodent pellet diet and supplied with water. These animals were divided into seven groups (10 animals per group). One group served as control group and other six groups served as treated group, three of those (group 2, 3, 4) treated with endoxan drug (50, 100, 200 mg/kg body weight) and the other three groups treated with endoxan drug (50, 100, 200 mg/kg body weight) plus royal jelly (1000mg/kg). These animals were injected intraperitoneally with endoxan treatment and orally with royal jelly substance. The doses were converted from human dose to mice dose by using multiplication factors for dose conversion between different species [20]. After two weeks four animals from each group were chosen to collect samples and then the experiment completed to four weeks. Bone marrow chromosome preparations according to Preston et al [21] were carried out. The statistical analysis of the obtained data was revised by SPSS 16 for windows (2007). Each treatment group was compared with the control group with independent samples T-test.

**RESULTS**

Normally the diploid number of male mouse Mus musculus is 2n=38+XY. The chromosomes of the mouse are telocentric and their lengths forming a continuous series (Fig. 1).

**Chromosomal Aberrations:** In the present study, 500 well spread metaphases were examined in each group to show the effects of varies doses of endoxan drug and protection of royal jelly for two and four weeks on the bone marrow cells.

The results indicated that the treatment with various doses of endoxan for different periods induced certain structural and numerical aberrations in the chromosomes of the bone marrow cells of the mouse Mus musculus.

Fig. 1: Photomicrograph of metaphase chromosomes of control male albino mouse Mus musculus showing the diploid chromosome number 2n=38+XY. All chromosomes are telocentric. X: 2500

Fig. 2: Photomicrograph of metaphase chromosomes of male albino mouse Mus musculus treated with Endoxan (50 mg/kg b.wt.) for two weeks showing centromeric attenuation (Ca), deletion (D) and centric fusion (Cf). X: 2500

Structural chromosomal aberrations that induced in bone marrow cells of the mouse Mus musculus after the treatment of endoxan were centromeric attenuation (Ca) (Figs. 2-4, 6-8, 10-13), centric fusion (Cf) (Figs. 2 and 7), chromosomal gap (Chg) (Figs. 4,5,7,9 and 10), the ring chromosomes (R) (Figs. 4 and 5), end to end association (Ee) (Figs. 4-8, 10 and 11), chromatid deletion (D) (Figs. 2-13), chromatid fragment (F) (Figs. 11-13). While, numerical chromosomal aberration was polyploidy (Fig. 14).

The mean of chromosomal aberrations in bone marrow cells of the treated mice are given in (Figs. 15-20). The cells with structural and numerical chromosomal aberrations were significantly increased over the control.

The mean of cells with centromeric attenuation varies by time and significantly increased. The mean was 8, 13 and 18 after treatment for two weeks with (50, 100 and 200 mg/kg body weight) endoxan drug respectively and 5, 11 and 15.6 after treatment with (50, 100 and 200 mg/kg) in addition to RJ for the same period compared to 1 in the...
Fig. 3: Photomicrograph of metaphase chromosomes of male albino mouse *Mus musculus* treated with Endoxan (50 mg/kg b.wt.) for four weeks showing centromeric attenuation (Ca) and deletion (D). X: 2500

Fig. 4: Photomicrograph of metaphase chromosomes of male albino mouse *Mus musculus* treated with Endoxan (100 mg/kg b.wt.) for two weeks showing centromeric attenuation (Ca), deletion (D), ring form (R), chromosome gap (Chg) and end-to-end association (Ee). X: 2500

Fig. 5: Photomicrograph of metaphase chromosomes of male albino mouse *Mus musculus* treated with Endoxan (100 mg/kg b.wt.) for four weeks showing deletion (D), ring form (R), chromosome gap (Chg) and end-to-end association (Ee). X: 2500

Fig. 6: Photomicrograph of metaphase chromosomes of male albino mouse *Mus musculus* treated with Endoxan (200 mg/kg b.wt.) for two weeks showing centromeric attenuation (Ca), deletion (D) and end-to-end association (Ee). X: 2500

Fig. 7: Photomicrograph of metaphase chromosomes of male albino mouse *Mus musculus* treated with Endoxan (200 mg/kg b.wt.) for four weeks showing centromeric attenuation (Ca), deletion (D), centric fusion (Cf), chromosome gap (Chg) and end-to-end association (Ee). X: 2500

Fig. 8: Photomicrograph of metaphase chromosomes of male albino mouse *Mus musculus* treated with Endoxan (50 mg/kg b.wt.) in addition to Royal Jelly for two weeks showing centromeric attenuation (Ca), deletion (D) and end-to-end association (Ee). X: 2500
Fig. 9: Photomicrograph of metaphase chromosomes of male albino mouse *Mus musculus* treated with Endoxan (50 mg/kg b.wt.) in addition to Royal Jelly for four weeks showing deletion (D) and chromosome gap (Chg). X: 2500

Fig. 10: Photomicrograph of metaphase chromosomes of male albino mouse *Mus musculus* treated with Endoxan (100 mg/kg b.wt.) in addition to Royal Jelly for two weeks showing centromeric attenuation (Ca), deletion (D), chromosome gap (Chg) and end to end association (Ee). X: 2500

Fig. 11: Photomicrograph of metaphase chromosomes of male albino mouse *Mus musculus* treated with Endoxan (100 mg/kg b.wt.) in addition to Royal Jelly for four weeks showing centromeric attenuation (Ca), deletion (D), fragment (F) and end to end association (Ee). X: 2500

Fig. 12: Photomicrograph of metaphase chromosomes of male albino mouse *Mus musculus* treated with Endoxan (200 mg/kg b.wt.) in addition to Royal Jelly for two weeks showing centromeric attenuation (Ca), deletion (D) and fragment (F). X: 2500

Fig. 13: Photomicrograph of metaphase chromosomes of male albino mouse *Mus musculus* treated with Endoxan (200 mg/kg b.wt.) in addition to Royal Jelly for four weeks showing centromeric attenuation (Ca), deletion (D) and fragment (F). X: 2500

Fig. 14: Photomicrograph of metaphase chromosomes of male albino mouse *Mus musculus* treated with Endoxan (200 mg/kg b.wt.) for four weeks showing polyploidy (Po). X: 2500
Fig. 15: Histogram represents the mean of chromosomal aberrations of group 2 (50 mg/kg b.wt. Endoxan) after two and four weeks and control group.

Fig. 16: Histogram represents the mean of chromosomal aberrations of group 3 (100 mg/kg b.wt. Endoxan) after two and four weeks and control group.

Fig. 17: Histogram represents the mean of chromosomal aberrations group 4 (200 mg/kg b.wt. Endoxan) after two and four weeks and control group.

Fig. 18: Histogram represents the mean of chromosomal aberrations of group 5 (50 mg/kg b.wt. Endoxan in addition to RJ- 1g/kg b.wt.) after two and four weeks and control group.
control animals. In the fourth week the mean was 11, 16 and 21 after treatment with 50, 100 and 200 mg/kg of endoxan and 9, 12.8 and 18 after treatment with (50, 100 and 200 mg/kg) in addition to RJ for the same period compared to 1 in the control animals.

The mean of centric fusion in the metaphase of control male mice chromosomes is 0. The mean of metaphases centric fusion in cells varies by the time. The mean was 1.4, 3.4 and 4 after treatment for two weeks with 50, 100 and 200 mg/kg body weight respectively and 1, 2 and 3 after treatment with (50, 100 and 200 mg/kg) in addition to RJ for the same period compared to 0 in the control animals. In the fourth week the mean was 2, 4 and 6 after treatment with 50, 100 and 200 mg/kg body weight and 1.4, 2.4 and 4 after treatment with (50, 100 and 200 mg/kg) in addition to RJ for the same period compared to 0 in the control group.

In the second week the mean of chromosome gap was 1.6, 4 and 8 after treatment with 50, 100 and 200 mg/kg body weight of endoxan respectively and in the protected group the mean was 1.2, 3 and 6 after treatment with (50, 100 and 200 mg/kg) in addition to RJ for the same period compared to 0 in the control animals. The mean was 2.4, 5 and 8 after treatment for four weeks with 50, 100 and 200 mg/kg of endoxan and 1.8, 4 and 6 after treatment with (50, 100 and 200 mg/kg) in addition to RJ for the same period compared to 0 in the control group.

The mean of metaphases ring form in cells varies by the time. The mean was 9, 10 and 12 after treatment for two weeks with 50, 100 and 200 mg/kg body weight and the mean was 5.6, 8 and 10 after treatment with (50, 100 and 200) mg/kg in addition to RJ for the same period compared to 0.4 in the control animals. In the fourth week the mean was 10, 12 and 12 after treatment with 50, 100 and 200 mg/kg body weight and 7, 9.6 and 10 after treatment with (50, 100 and 200 mg/kg) in addition to RJ for the same period compared to 0.4 in the control animals.

The cells with chromatid aberrations were significantly increased over the control by the time. The mean of deletion increases by time in treated group and decreases by time in protected group compared with treated group. The mean was 9.2, 14 and 19 after treatment for two weeks with 50, 100 and 200 mg/kg body weight of endoxan and 7, 12 and 16.2 after treatment with (50, 100 and 200 mg/kg) in addition to RJ for the same period compared to 0.8 in the control animals. In the fourth week the mean was 12, 17 and 21.4 after treatment with 50, 100 and 200 mg/kg of endoxan and 10, 13.8 and 18 after treatment with (50, 100 and 200 mg/kg) in addition to RJ.

The mean of fragments was 9.2, 13 and 17 after treatment for two weeks with endoxan 50, 100 and 200 mg/kg body weight of endoxan and in the protected group the mean was 7.6, 12 and 14 after treatment with both
endoxan (50, 100 and 200 mg/kg) and royal jelly for the same period compared to 0.8 in the control animals. In the fourth week the mean was 12, 15 and 21 after treatment with 50, 100 and 200 mg/kg of endoxan and 2, 12 and 18 after treatment with (50, 100 and 200 mg/kg) of endoxan and royal jelly for the same period compared to 0.8 in the control group.

Also the results indicated that there was a change in the number of chromosomes as a result of effect of endoxan. Polyploidy is the form of numerical change, which was observed significantly increased over the control. The present study shows that there was a non-significant chromosomal aberration such as end to end association.

Results indicated that chromosomal aberrations were significantly increased by time (Fig. 21). The mean of chromosomal aberrations of treated groups and protected groups compared to control group during two and four weeks (2W and 4W). The mean of total aberrant cells in animals treated with 50, 100 and 200 mg/kg for two weeks was 38.4, 61 and 84 compared to 27.4, 50 and 68.8 in animals treated with both endoxan (50, 100 and 200 mg/kg) and RJ. While the mean of total aberrant cells in animals treated for four weeks with endoxan 50, 100 and 200 mg/kg was 52.2, 73.4 and 97.4 compared to 31.2, 58.2 and 78 in animals treated with both endoxan (50, 100 and 200 mg/kg) and RJ.

Our result indicated that treatment with royal jelly reduce the mean of chromosomal aberrations in the bone marrow chromosomes of male albino mice Mus musculus treated with various doses of endoxan for different periods (Fig. 22). The mean of total aberrations for control was 3 compared to 45.3, 67.2 and 90.7 in animals treated with endoxan 50, 100 and 200 mg/kg and 29.3, 54.1 and 73.4 in animals treated with endoxan (50, 100 and 200 mg/kg) in addition to RJ.

DISCUSSION

The present results show that the chromosomes and chromatid aberrations were significantly increased in mice bone marrow cells after intraperitoneal injection of the different doses of endoxan. And the results indicate that RJ treatment significantly reduced the mean of structural chromosomal aberrations induced by endoxan in bone marrow cells. However, treatment with RJ did not return
the mean of structural chromosomal aberrations to the normal level. Similarly, Valadares et al [22], Barekati et al [23], Tripathi and Jena [24] and Tohamy et al [25] recorded that endoxan induced highly significant number of structural chromosomal aberrations (p ≤ 0.001) in male mice.

The results of the present study show that the maximum chromosome aberrations were attained in the fourth week after treatment with different doses of endoxan. The observed aberrations which were induced by endoxan in bone marrow cells were structural and numerical aberrations. Structural aberrations such as centromeric attenuation, centric fusion, chromosomal gap, ring formation, deletion and fragment. While, numerical aberration was polyploidy. These results agreed with reports [26-28].

The results also demonstrated that deletion was most common type of aberration that occurred after treatment with endoxan. The deletion was very high in cells obtained from mice treated for four weeks with endoxan in various doses. This result is in agreement with Moore et al [29] and Murata et al [30] who elucidated that endoxan and its metabolites can bind DNA, causing damage that may result in chromosome breaks, micronucleus formation and cell death.

Povirk and Shuker [4] mentioned that endoxan damages chromosomes through generate of free-radicals and alkylating DNA thereby producing mutation. The hydroxyl radical is highly reactive and is responsible for DNA damage [31].

Newman et al [32] reported that several metabolites of endoxan have a functional OH group. And the induction of DNA strand breaks could be attributed to its ability to generate free-radicals [33].

Royal jelly (RJ) is a honeybee product secreted from the hypopharyngeal and mandibular glands of the worker honeybees mainly between the sixth and twelfth days of their life primarily for developing and maintaining the queen bee. RJ consists mainly of proteins, sugars, lipids, vitamins and free amino acids [34]. RJ has been shown to possess several pharmacologic activities, including antitumor activity, anti-inflammatory, antioxidative and scavenging ability, hypoglycemic and wound healing activity, immunomodulatory and estrogenic activity [35, 36].

The protective effect of royal jelly may be due to its component vitamins, antioxidant vitamins A, E, C, vitamin D and vitamin B complex [37]. These vitamins themselves had anticancer effect [38-42] and protective effects against the genotoxicity of chemotherapy and radiotherapy [43-51]. Royal jelly also contained protein fractions [10] that they had also anticancer effects.

In conclusion royal jelly plays a very important role in protecting the gene pool from the severe effects of endoxan. Hence, its judicious application can minimize and mitigate the dreaded side-effects of endoxan on the normal cells and tissues of the body.

REFERENCES


