

Original Article

## Protective effect and potential mechanisms of propolis on streptozotocin-induced diabetic rats



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### المخلص

**أهداف البحث:** تهدف هذه الدراسة إلى معرفة التأثيرات الوقائية لمكملات العكبر ضد التأثير السام لمادة الاستربتوزوتوسين على خلايا بيتا في البنكرياس ومعرفة آلية العمل الممكنة من جراء هذا التأثير.

**طرق البحث:** تم تقسيم ٤٥ جرذا تقسيما عشوائيا إلى ٣ مجموعات، في كل مجموعة ١٥ جرذا. المجموعة الأولى: الجرذان العادية التي تتغذى على الغذاء العادي تشاؤ. والمجموعة الثانية: الجرذان المصابة بداء السكري الناجم عن الاستربتوزوتوسين. والمجموعة الثالثة: الجرذان المعالجة بالعكبر ٠.٣ غرام/كغ/ يوميا لمدة أسبوعين قبل الإصابة بالسكري الناجم عن الاستربتوزوتوسين. في نهاية الفترة التجريبية، جمعت عينات الدم الصائم لقياس مستويات الجلوكوز ونشاط بيروكسيد الدهون. وتم تجهيز أنسجة البنكرياس للجرذان المشرحة لغرض الدراسات المورفولوجية والمناعية.

**النتائج:** أظهرت نتائج الدراسة الحالية أن معالجة الجرذان بالعكبر قبل الإصابة بداء السكري، قد قلل بشكل ملحوظ من قياس الجلوكوز الصائم مقارنة مع الجرذان المصابة بداء السكري. وزادت منتجات بيروكسيد الدهون بشكل كبير في المجموعة الثانية مقارنة بالأولى. ولكن، هذه المنتجات قلت بشكل ملحوظ في المجموعة الثالثة مقارنة بالثانية. أظهر التشريح المرضي والتحليل المناعي لأنسجة البنكرياس زيادة في حجم جزر لانجرهانس وزيادة في صبغة خلايا بيتا في البنكرياس مع خلايا بيتا من الأجسام المضادة في المجموعة الثالثة مقارنة مع الثانية.

**الاستنتاجات:** أظهر العكبر تأثيرا وقائيا، وحافظ على سلامة خلايا بيتا في البنكرياس لدى الجرذان المصابة بداء السكري الناجم عن الاستربتوزوتوسين.

هذه الدراسة تحث على ضرورة إجراء مزيد من البحوث لتوضيح الآلية الدقيقة لهذا التأثير المتغير.

**الكلمات المفتاحية:** العكبر؛ الاستربتوزوتوسين داء السكري؛ بيروكسيد الدهون؛ التشريح المرضي؛ الجرذان المصابة بداء السكري.

### Abstract

**Objectives:** The objective of this study was to determine the protective effects of propolis supplementation against the toxic effect of streptozotocin (STZ) on pancreatic beta-cells and to identify the possible mechanism of actions underlying this effect.

**Methods:** Forty-five rats were randomly divided into three groups, with each group containing 15 rats. Group I consisted of normal rats fed a normal chow diet. Group II included diabetic rats induced with STZ. Group III consisted of rats treated with 0.3 g/Kg/day propolis for 2 weeks before the induction of diabetes by STZ. At the end of experimental period, blood samples were collected for the measurement of fasting blood glucose (FBG) and lipid peroxidation activity. Pancreatic tissues from the dissected rats were processed for morphological and immunohistochemical studies.

**Results:** The findings of this work showed that the treatment of rats with propolis before the induction of diabetes mellitus is associated with significantly decreased FBG levels compared to the diabetic rats. The lipid peroxidation products were significantly increased in group II compared to group I. However, these products were significantly decreased in group III compared to group II. The histopathological and

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immunohistochemical analysis of the pancreatic tissue showed an increase in the size of the islets of Langerhans and the enhanced staining of beta-cells of the pancreas with beta-cell antibodies in group III compared to group II.

**Conclusion:** Propolis showed protective and preservative effects on pancreatic beta-cell integrity in STZ-induced diabetic rats. This study suggests the need for further research to elucidate the exact mechanism of this effect.

**Keywords:** Diabetes mellitus; Diabetic rats; Histopathology; Lipid peroxidation; Propolis; Streptozotocin

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## Introduction

Diabetes mellitus is a common chronic disorder characterized by hyperglycaemia due to inadequate insulin secretion or function and under-utilization of glucose in peripheral tissues that results in the development of diabetes, a specific microvascular pathology in the peripheral nerve, retina and glomerulus. As a consequence, it is a leading cause of many debilitating neuropathies, blindness and end-stage renal disease.<sup>1</sup>

According to the International Diabetes Federation (IDF), diabetes is one of the most challenging health problems of the 21st century. Additionally, there are numerous health problems and issues associated with diabetes, which ultimately reduces the quality of life. Another tragic factor associated with diabetes is that most people are unable to afford proper treatment because it is a costly illness, particularly because it is long term. Furthermore, KSA was estimated to be among the top 10 countries with a higher prevalence of diabetes (23.9%).<sup>2</sup>

Maintaining the balance between oxidative stress and antioxidants is an important mechanism for preventing damage from oxidative stress. Therefore, supplementation with flavonoids has been used to prevent oxidative stress induced by STZ in a diabetes model.<sup>3</sup>

Propolis is a resinous hive product collected by bees from various plant sources. More than 300 components have been identified in propolis, mainly phenolic compounds (e.g., flavonoids and aromatic compounds), terpenes and essential oils.

Propolis possesses various pharmacological properties, such as antibiotic, anti-inflammatory, anti-cancer, antioxidant, and anti-hepatotoxic activities.<sup>4</sup>

The effect of propolis against the toxicity of STZ in rats was evaluated. It was reported that propolis may prevent beta-cell destruction via the free radical scavenging activity of propolis.<sup>5</sup>

Thus, we hypothesized that propolis ameliorates the toxic effects of STZ on pancreatic beta-cells in diabetic rats and glycaemic function via its antioxidant effect.

The objectives of this study were to determine whether propolis supplementation prevents the toxic effect of STZ on pancreatic beta-cells and to identify possible mechanisms of action underlying this effect.

## Materials and Methods

### Experimental designs

The study was conducted on (45) normal male Wister albino rats weighing 150–250 gm obtained from the animal house. All of the rats were housed under standard environmental conditions (temperature 25–29° C, 12 h light and 12 h darkness cycles). The animals were allowed free access to pelleted standard rat diet and water. The Scientific Research Ethics Committee of Dammam University approved this study, in accordance with the ethics standards of “Principles of Laboratory Animal Care”.

The rats were randomly divided into three groups, with 15 rats in each group (n = 15) as follows:

Group I: normal rats fed with normal chow diet;

Group II: diabetic rats induced by STZ single dose (60 mg/kg BW) IP;<sup>6</sup>

Group III: rats treated with propolis (0.3 g/Kg/day) for 2 weeks before the induction of diabetes by STZ. Propolis was obtained from Dosis Import and Export Co., Ltd., China. The best purity (70%) and freshness were guaranteed, and the propolis was supplied in powder form. Each day, freshly ground propolis was weighed and dissolved in distilled water for a final concentration of 300 mg/ml. The selected dose of propolis was administered orally using an orogastric feeding needle. This dose was selected on the basis of a previous study.<sup>7</sup>

Diabetes in experimental rats was induced by a single intraperitoneal injection of 60 mg/kg body weight of STZ (Sigma–Aldrich). Three days after drug-injections, urine strips (Medi-Test Combi 10; Macherey–Nagel GmbH & Co, Düren, Germany) were used to detect glucosuria (a dark-green colour indicates blood glucose  $\geq$  500 mg/dl). These rats were selected as diabetic rats for the experiment.<sup>8</sup>

### Blood sampling

At the end of the experimental period, food was stopped 12 h before the rats were sacrificed. Animals were weighed and then anesthetized with ketamine 50 mg/kg BW intraperitoneal. Blood was collected from the abdominal aorta through a midline incision. A plain tube was used for the separation of serum to determine the glucose level and lipid peroxidation products, malondialdehyde (MDA). Blood glucose was measured using a glucometer (Accu-Chek Go, Roche Diagnostics GmbH, Indianapolis, IN), and thiobarbituric acid reactive substance (TBARS) was used (BioAssay Systems, Hayward, CA, USA) to measure the product of the reaction

between malondialdehyde, a product of lipid peroxidation, and TBARS.<sup>9</sup>

### Immunohistochemistry study

The pancreases of the dissected rats were removed, fixed in 4% paraformaldehyde and in buffered formalin 10% for 24 h and then embedded in paraffin. The pancreases were processed for routine haematoxylin and eosin and the slides were visualized using a digital microscope.<sup>10</sup> Immunohistochemistry was performed to visualize the antigen–antibody complex. Sections were incubated with a polyclonal antibody against insulin (Santa Cruz Biotechnology) at a dilution of 1:500 for 18 h at 4 °C. The samples were incubated for 1 h with horseradish peroxidase-conjugated secondary antibody (1:500 dilutions) at room temperature. Images were taken at a magnification of X400. Controls were processed by omitting the primary antibody in the immune-labelling procedure. The different regional distribution of insulin-secreting cells and their relative frequency in the pancreatic islets were studied.<sup>11</sup>

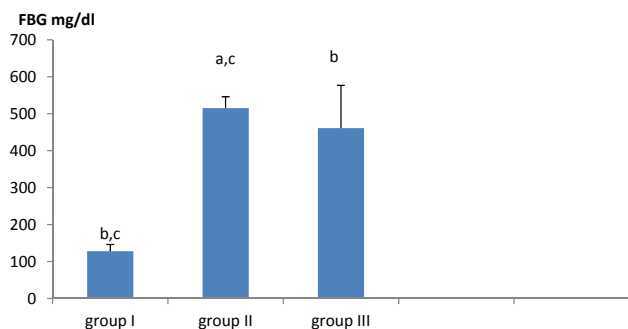
Islet morphology and the presence or absence of inflammatory infiltrate was recorded using a descriptive method. The number of pancreatic islets and the islet size were measured and expressed as the ratio of islet tissue to total pancreatic tissue, and the area of beta-cells was measured and expressed as the ratio of beta-cells to islet tissue.

### Statistical analysis

All reported values are expressed as the mean  $\pm$  standard deviation (SD). Differences among means were analysed for significance by analysis of variance using SPSS software version 19. Groups were compared by ANOVA, and a P-value  $< 0.05$  was considered statistically significant.

## Results

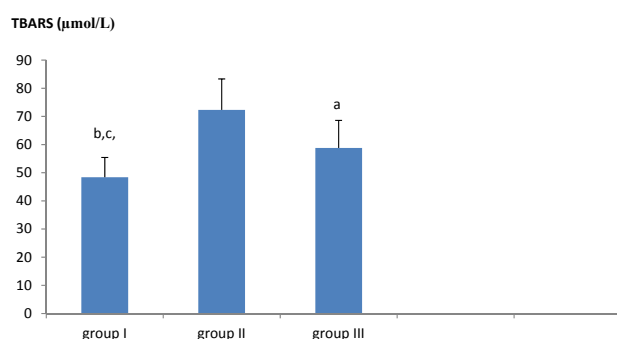
The results of the present study showed that the FBG was  $128.2 \pm 18$  in group I,  $514.9 \pm 30.5$  in group II and  $460.9 \pm 116.3$  in group III. The increase in FBG in group II is highly significant compared to both group I (with a P-value  $\leq 0.000$ ) and group III (P-value 0.021). See Figure 1.



**Figure 1:** Fasting blood glucose (FBG) levels in group I (control), group II (diabetic) and group III (propolis treated, 0.3 g/Kg). a Significantly different compared to group I. b Significantly different compared to group II. c Significantly different compared to group III.

The mean serum TBARS levels, an indicator of lipid peroxidation, were significantly higher in group II ( $72.3 \pm 11.4$ ) than in group I ( $48.4 \pm 7$ ), with a P-value  $\leq 0.000$  (see Figure 2). Furthermore, the mean serum TBARS level of the group that received propolis (III) was significantly lower ( $58.8 \pm 9.8$ ) than group II (P-value = 0.003).

The semi-quantitative analysis of the immunohistochemical staining of pancreatic cells is shown in Tables 1 and 2. The area of insulin beta-cells in the Langerhans islets was measured. The histopathology studies of group I (Figure 3) showed normal healthy histological characteristics. Numerous pancreatic islets represented approximately 15% of the pancreatic tissues. The staining of beta-cells with insulin antibody in the immunohistochemistry study was strong and showed a dark brown colour. The majority of the



**Figure 2:** Thiobarbituric acid reactive substances (TBARS) levels in group I (control), group II (diabetic) and group III (propolis treated, 0.3 g/Kg). a Significantly different compared to group I. b Significantly different compared to group II. c Significantly different compared to group III.

**Table 1:** Ratio of islet tissues to pancreatic tissue among fasting blood glucose (FBG) in group I (control), group II (diabetic) and group III (propolis treated, 0.3 g/Kg). (The values are expressed as the mean  $\pm$  SD).

Group	Ratio of islets tissue to pancreatic tissue.
I	14.13 $\pm$ 1.35
II	4.00 $\pm$ 1.29 <sup>a,b</sup>
III	9.07 $\pm$ 1.32

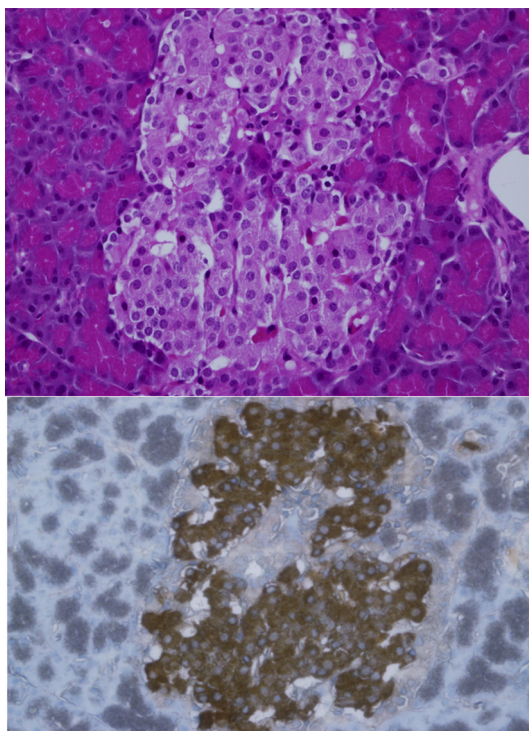
a Significantly different compared to group I. b Significantly different compared to group III.

**Table 2:** Ratio of beta-cells to islet tissue among fasting blood glucose (FBG) in group I (control), group II (diabetic) and group III (propolis treated, 0.3 g/Kg). (The values are expressed as the mean  $\pm$  SD).

Group	Ratio of islets tissue to pancreatic tissue.
I	59.33 $\pm$ 2.58
II	20.15 $\pm$ 4.94 <sup>a,b</sup>
III	48.78 $\pm$ 5.12

a Significantly different compared to group I. b Significantly different compared to group III.





**Figure 3:** Control group showing normal beta-cells in the islet of Langerhans and showing strong staining with the anti-insulin antibody. Haematoxylin counterstain  $\times 420$ .

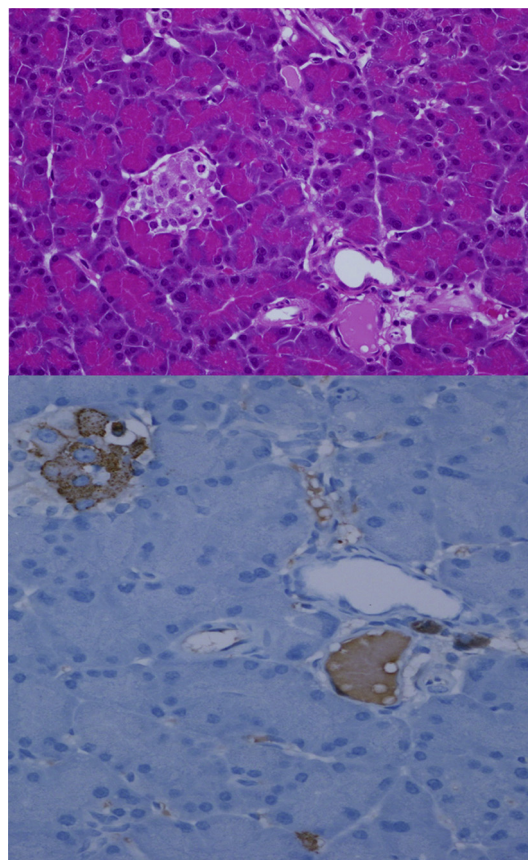
beta-cells contained brownish granules. STZ-induced diabetic rats from group II (Figure 4) showed clear injury to the islets of Langerhans, with few small pancreatic islets of approximately less than 5% of the pancreatic tissues. The most consistent findings in the histological sections of beta-cells were necrotic and degenerative changes of the beta-cells. In the group that received propolis (group III) (Figure 5), there were many prominent pancreatic islets representing approximately 10% of the pancreatic tissues. In addition, based on the immunohistochemical staining of the pancreatic tissues, propolis significantly increased the area of insulin immunoreactive beta-cells.

## Discussion

The present study was conducted to investigate the possible prophylactic effect of propolis supplementation against STZ-induced diabetes in rats and to investigate the effect on FBG and oxidative stress of beta-cells of the pancreas.

Experimental diabetes induced by STZ in this study was considered type 1 diabetes (insulin-dependent) because STZ causes destruction of the beta-cells of the islets of Langerhans.<sup>12</sup> The results of the present study showed a highly significant elevation of FBG in the untreated diabetic group II compared to the other groups (I and III). Interestingly, prophylactic administration of propolis (group III) caused a significant decrease in FBG compared with group II, indicating a prophylactic effect.

For lipid peroxidation, our results showed a significant increase in the TBARS levels in the untreated diabetic group

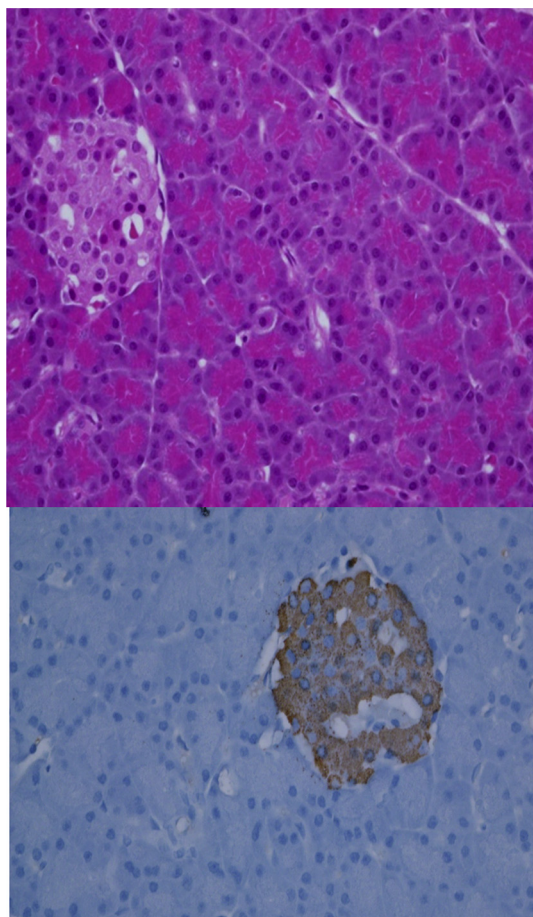


**Figure 4:** Untreated diabetic group showing shrunken islets of Langerhans, displaying necrotic changes and that have weak insulin-immunoreactivity in a few beta-cells in the islet of Langerhans. Haematoxylin counterstain  $\times 420$ .

II compared with the other studied groups, whereas after supplementation with propolis (group III), the TBARS levels were significantly decreased. This finding provides an important mechanism explaining the protective effect of propolis on the beta-cells of the pancreas. Lipid peroxidation is the most potent oxidative defect affecting the cells during type 1 diabetes.<sup>13</sup>

Maintaining the balance between the production of reactive oxygen species and their catabolism by antioxidants is a critical mechanism for preventing damage due to oxidative stress. Therefore, supplementation with antioxidants such as propolis that contain flavonoids has been used to prevent the STZ-induced diabetic complications.<sup>14</sup>

Lipid peroxidation may cause protein damage and inactivation of membrane-bound enzymes through direct attack by free radicals. In our study, TBARS levels were significantly increased in the diabetic rats (group II). Propolis supplementation before induction (group III) decreased the elevated lipid peroxidation activity. This finding was also consistent with previous studies that reported that the protective effect of propolis against the toxicity of STZ on pancreatic beta-cells may occur via the free radical scavenging activity of propolis together with its inhibitory activities on IL-1 beta-cells synthase and nitric oxide synthase.<sup>5</sup> This effect is supported by the



**Figure 5:** Propolis treated group (0.3 g/Kg) has protected beta-cells in the islet of Langerhans and moderate staining with the anti-insulin antibody. Haematoxylin counterstain  $\times 420$ .

histopathological and immunohistochemical slides of the pancreatic beta-cells. The current immunohistochemical finding showed that propolis supplementation protected the majority of pancreatic beta-cells that were destroyed by STZ, whereas propolis partially prevents the degeneration of beta-cells. Moreover, propolis significantly increased the area of insulin immunoreactive cells, possibly due to the inhibition of lipid peroxidation because of its antioxidant nature.

## Conclusion

Based on these results, propolis administration can protect and preserve pancreatic beta-cell integrity against the toxic effects of STZ. Propolis administration as a natural agent can be used as a prophylactic agent to prevent the occurrence of diabetes by any harmful agent or strong oxidants via its strong antioxidant effect, which protects beta-cells. This makes it a promising medication for complementary medicine.

## Recommendation

Further studies and investigation are required to evaluate the potential molecular mechanisms by which propolis exerts its effects.

## Conflict of interest

The authors have no conflicts of interest to declare.

## Authors' contributions

All authors have made substantial contributions to the conception, design, acquisition of data and interpretation. They participated in drafting the article, revising it critically for important intellectual content, and approved the final version submitted.

## Acknowledgements

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## References

1. Bergman RN. Lilly lecture: toward physiological understanding of glucose 1989 tolerance. Minimal model approach. *Diabetes* **1989**; *38*: 1512–1527.
2. International Diabetes Federation. *Diabetes atlas sixth edition*. p. 1. Available from: <http://www.idf.org/diabetesatlas>; 2013.
3. Xu Y, et al. Prophylactic protective effects and its potential mechanisms of icaridell on streptozotocin induced spermatogenic dysfunction. *Int J Mol Sci* **2014**; *15*(9): 16100–16113.
4. Khalil ML. Biological activity of bee propolis in health and disease. *Asian Pac J Cancer Prev* **2006**; *7*: 22–31.
5. Matsushige K, Basnet P, Hase K, Kadota S, Tanaka K, Namba T. Propolis protects pancreatic-cells against toxicity of streptozotocin (STZ). *J Phytomedicine* **1996**; *3*: 203–209.
6. Al-Hariri MT. Comparison the rate of diabetes mellitus induction using STZ dissolved in different solvents in male rats. *J Comp Clin Path Res* **2012**; *2252–0422*: 96–99.
7. Al-Hariri M, Gamal Eldin T, Abu-Hozaifa B, Elnour A. Glycemic control and anti-osteopathic effect of propolis in diabetic rats. *Diabetes, Metab Syndr Obes Targets Ther* **2011**; *4*: 377–384.
8. Wang NZ, Li D. Effect of combined propolis-ethanol-extract and Shaoyao- Gancao-tang on blood sugar levels in rabbits with alloxan induced experimental diabetes. *Asia Pac J Clin Nutr* **2004**; *13*: S66.
9. Afshari AT, Shirpoor A, Farshid A, et al. The effect of ginger on diabetic nephropathy, plasma antioxidant capacity and lipid peroxidation in rats. *Food Chem* **2007**; *101*: 148–153.
10. Eliakim-Ikechukwu CF, Obri AI. Histological changes in the pancreas following administration of ethanolic extract of alchornea cordifolia leaf in alloxan- induced diabetic wistar rats. *Niger J Physiol Sci* **2009**; *24*(2): 153–155.
11. Hsu SM, Raine L, Fanger H. Use of avidin–biotin–peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* **1981**; *29*: 577–580.

12. Weiss RB. Streptozotocin. A review of its pharmacology, efficacy and toxicity. **Cancer Treat Rep** 1982; 3: 427–438.
13. Okutan H, Ozcelik N, Yilmaz RH, Efsan Uz. Effects of caffeic acid phenethyl ester on lipid peroxidation and antioxidant enzymes in diabetic rat heart. **Clin Bioch** 2005; 38: 191–196.
14. Khaki A, Fathiazad F, Nouri M, Khaki A, Maleki N, Khamnei HJ, Ahmadi P. Beneficial effects of quercetin on sperm parameters in streptozotocin-induced diabetic male rats. **Phytother Res** 2010; 24: 1285–1291.