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Study the Effect of Iraqi Propolis Extract on Hematological Parameters in Alloxan-Induced Diabetic Rabbits

Abdul-Hadi Abbas Hadi
Department of Biology, Faculty of Science, University Of Kufa

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***Corresponding author:**
Email address:
abdulhadi.alibraheemi@yahoo.com

Abstract

Propolis, a natural product is a resinous substance that honey bees (Apis mellifera) collect from tree buds, shrubs or other botanical sources. The main chemical classes present in propolis are flavonoids, phenolics and other various aromatic compounds and has been used extensively in folk medicine due to its several pharmacological properties. This work was carried out to investigate the effect of Iraqi propolis on some hematological parameters in alloxan-induced diabetic rabbits. Diabetes was induced by a single dose of alloxan [150 mg/kg, Intravenous (IV)]. Rabbits with glycaemia were treated with alcoholic extract of propolis for 23 days. Marked significant differences ($P<0.05$) in glucose, erythrocytes, hemoglobin, packed cell volume, and leukocytes were recorded in diabetic rabbits in comparison to the control group. The findings of this study showed that oral administration of propolis can significantly ($P<0.05$) inhibit the increasing of fasting blood glucose and can improve hematological indices in diabetic animals. In conclusion, the treatment of diabetic rabbits with propolis has made a considerable hypoglycemic effect, in addition, propolis could ameliorate the disturbances in blood parameters.

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Introduction

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (ADA, 2013). Experimental animal models are one of the best strategies for the understanding the pathophysiology of any disease so treatment will be designed (Rees and Alcolado, 2005; Chatzigeorgiou *et al.*, 2009). Alloxan and streptozotocin are diabetic agents and used widely to induce experimental diabetes in animals.

Propolis is a resinous material collected by bees from buds and plant exudates, which is mixed with products of their salivary glands and wax (Sforcin, 2007). It has recently

gained popularity as a healthy food in various parts of the world because it promotes health and prevents diseases (Inokuchi *et al.,* 2006). Propolis contains approximately 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% other substances as minerals and vitamins (Cohen *et al.,* 2004). The flavonoids and polyphenolic compounds are the major constituents of propolis making 45-55% in most samples from different countries (Burdock, 1998).

The antioxidative property of propolis extract certainly is due to its chemical constituents. Phytochemical investigations of propolis have demonstrated that presence of flavonoids and polyphenolic components as main active ingredients having potent antioxidant activities (Moreno *et al.,* 2000; Hosnuter *et al.,* 2004). This study was designed to evaluate the Iraqi propolis action on diabetic rabbits and determined some hematological disturbances that accompanied alloxan-induced diabetes.

Materials and Methods

Extraction of propolis

The Iraqi propolis sample was collected from Najaf province during the year 2013 and kept in a dry place and stored at 4 °C until its processing. The propolis sample (100 g) was cut into small pieces and mixed with 70% ethanol (900 ml), in the absence of bright light (Krell, 1996). After seven days, the mixture was stirred at magnetic stirrer and filtered. The extract was evaporated to dryness in a freeze dryer (Christ - Germany). The yellow-brown powder of propolis was stored under sterile conditions.

Experimental animals

Adult female local rabbits (*Oryctolagus cuniculus*) were purchased from the local market of Najaf were used in this study. The experimental animals were kept in individual cages at faculty of veterinary medicine / animal house / University of Kufa. All animals were fed a standard laboratory pellets and water *ad libitum*. This study was approved by the ethical committee (Department of biology, Faculty of science / University of Kufa in 2013).

Induction of diabetes in rabbits

Diabetes was induced in 16 hours starved rabbits by single intravenous injection of alloxan monohydrate (150 mg/kg body weight) dissolved in physiological saline (0.9% NaCl) via the marginal ear vein. The control group was injected with the same volume of isotonic saline. To prevent hypoglycemic shock and mortalities during the hypoglycemic phase, the food was offered to animals immediately after alloxan injection. Besides, oral solution of 5% glucose in tap water was provided via water bottle for next 24 hr. After three days of injection, diabetes mellitus was confirmed by the demonstration of hyperglycemia.

According to Schiller and McNamara, (1999), animals with glucose levels over 170 mg/dl but less than 400 mg/dl were classified as hyperglycemic. The fasting blood sugar of rabbits was estimated by glucometer (Accu-Chek active- Germany) using

commercially available reagent strips. The measurement of glucose level was confirmed by examining the blood taken from marginal ear vein.

Experimental design

The totally, 30 rabbits weighed 1230 to 1540 g, were used in this study. Control (negative) group received orally 1 ml distilled water per day. Diabetic (positive) group served as untreated diabetic group and did not treat with propolis. The propolis-treated animals were subdivided into three groups according to the concentration of propolis. Three oral concentrations of ethanolic extract of propolis were investigated (50, 100 and 200 mg/kg). The treatment with propolis was continued for 23 days.

Hematological analysis

After one month, blood samples were collected from all experimental animals. Five milliliters were collected from heart using disposable syringe. One milliliter of fresh blood was added to each ethylene diamine tetraacetic acid (EDTA) tube. Blood picture including the erythrocytes, hemoglobin, packed cell volume and leukocytes were made in an automatic cell counter (CYANHemato- Belgium).

Statistical analysis

Multiple comparisons between groups were done by means of one-way analysis of variance (ANOVA) followed by least significant difference (LSD). The results were expressed as (mean ± standard error). Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS-version 17). A value of $P \leq 0.05$ was considered to indicate statistical significance between experimental groups.

Results

The findings of this study showed that alloxan at concentration of 150 mg/ kg IV successfully induced diabetes in rabbits. Blood glucose level was strongly elevated on the second day after treatment (Figure.1). The results of this study showed a significant increase ($P<0.05$) in fasting blood glucose in diabetic rabbits in compare with normal control rabbits. In contrast, administration of propolis to diabetic rabbits resulted in a significant decrease ($P<0.05$) in glucose levels as compare with diabetic group throughout the experiment.

The results of hematological analysis revealed significant decreases ($P<0.05$) in the number of red blood cells (RBCs), hemoglobin (Hb) value, packed cell volume (PCV) and number of white blood cells (WBCs) of alloxan-induced diabetic rabbits in comparing with normal groups. On the other hand, treatment of the diabetic rabbits with ethanolic extract of propolis (EEP), especially at 200 mg/kg body weight showed significant increase ($P<0.05$) in blood indices levels in compare with diabetic group. Table (1).

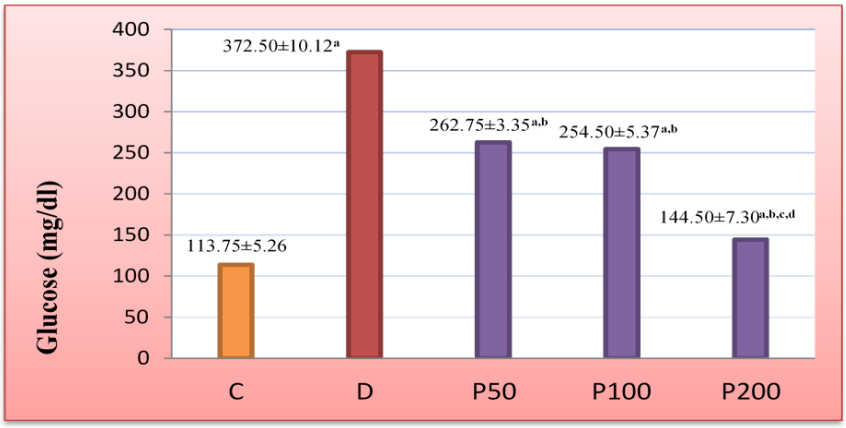

Figure (1) Effect of propolis extract on fasting blood glucose of diabetic rabbits
Experimental animal; C: control group, D: diabetic group, P: propolis-treated groups
- Data are expressed as mean ± standard error.
- Significant differences between groups are indicated with different letters.
*** Significant difference at (P<0.05).**

Table (1): Effect of propolis extract on hematological parameters of diabetic rabbits.

Groups / Parameters	C	D	P50	P100	P200
RBC (10^6 µl)	6.50 ±0.13	5.48 [a] ±0.14	5.54 [a] ±0.23	6.26 [a,c] ±0.15	6.44 [b,c] ±0.11
Hb (g/dl)	14.6 ±0.51	12.4 [a] ±0.43	12.9 [a] ±0.38	13.2 [a] ±0.47	14.6 [b,c,d] ±0.51
PCV (%)	36.88 ±0.82	32.04 [a] ±1.13	34.19 ±1.28	34.66 ±1.02	40.09 [b,c,d] ±1.46
WBC (10^3 µl)	8.76 ±0.11	4.82 [a] ±0.14	4.95 [a] ±0.77	7.20 [b,c] ±0.77	8.35 [b,c] ±0.39

Experimental animal; C: control group, D: diabetic group, P: propolis-treated groups.
- Data are expressed as mean ± standard error.
- Significant differences between groups are indicated with different letters.
*** Significant difference at (P<0.05).**

Discussion

The alloxan is one of the chemical agents, which are used to induce diabetes mellitus experimentally. Its mechanism in inducing diabetes is the partial destruction of the β-cells of islets of Langerhans (Szkudelski, 2001). The alloxan is selectively taken up into the β-cells by a glucose transporter (GLUT2) (Gorus *et al.*, 1982). In addition, hyperglycemia occurs due to the defects in the liver and skeletal muscle glycogen storage and inability of the tissues to take up and utilize glucose (Lamba *et al.*, 2000).

In this study, Iraqi propolis acted to suppress the high blood glucose levels in diabetic rabbits. The significant antihyperglycemic effect of propolis extract is probably due to its antioxidant chemical contents. Previous clinical trials approved that the improvement of oxidative stress may prevent the progression of both type 1 and type 2 diabetes (Varvarovska *et al.*, 2004; Franzini *et al.*, 2008). The results of this study are in agreement

with data reported by Matsushige *et al.*, (1996); Wang and Li, (2004). These studies suggested that the propolis extract has a beneficial effect on reduction of blood sugar levels in alloxan-induced diabetes mice.

The findings of this study also showed that alloxan has significant effects ($P < 0.05$) on hemoglobin concentration, packed cell volume, and white blood cell number. The alloxan treated group showed decrease in RBCs number, which might be resulted from the increase in free radical generation, decreased antioxidant defenses, and oxidative modifications of the membrane increase fragility of RBCs. These mechanisms led to anemia and consequent depletion of endogenous antioxidant reserves (Wagner *et al.*, 1988; Umar *et al.*, 2007).

In particular, reactive O_2 species generated during alloxan metabolism is implicated in red cell damage (Rao *et al.*, 2003). According to Schwartz *et al.*, (1991) glucose derived oxidative stress may be responsible for osmotic fragility and altered deformability of RBCs due to membrane lipid peroxidation. This suggested an important role of oxidative damage in the impaired functions of erythrocyte.

Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce RBCs (Ohlsson and Aher, 2006). From human data, early reports demonstrated diminished erythropoietin production as a predictor of anemia in patients with diabetes and without overt nephropathy (Winkler *et al.*, 1999; Cotroneo *et al.*, 2000; Bosman *et al.*, 2001). This anemia could be attributed to destruction of RBCs and reduced rate of its release from the bone marrow to blood. Several studies attributed this anemia to increase in lipid peroxidation of the erythrocyte cell membrane (Kang-Xin *et al.*, 1990; Helal, 2000).

The occurrence of anemia in diabetes mellitus has been reported due to the increased non-enzymatic glycosylation of erythrocyte membrane proteins (Oyedemi *et al.*, 2011). Thus, oxidation of these proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to hemolysis of RBCs (Arun and Ramesh, 2002).

The results of this study demonstrated that EEP, especially at concentration (200 mg/kg), resulted in marked increased in RBCs levels in compare with diabetic group. These results are in agreement with Oršolić *et al.*, (2012), who showed that administration of propolis to diabetic mice significantly elevated hematological parameters such as the total number of red blood cells, hemoglobin and hematocrit level in compare to untreated diabetic mice.

Available data suggest that propolis induced extensive proliferation of hematopoietic cells in the spleen and bone marrow (Oršolić and Bašić, 2005). In addition, the high content of flavonoids in propolis improves the expression level of erythropoietin hormone and accelerates the generation of erythrocyte and hemoglobin (Dong *et al.*, 2005). It is well known that propolis is a potent antioxidant contains high phenolic compounds such as flavonoids, which are able to scavenge free radicals (Banskota *et al.*, 2001). Other study revealed that supplementation of rabbit does with bee propolis has a beneficial effect on the hematobiological parameters and economic values. These effects may be due to the bee propolis content of substantial levels of antioxidant nutrients, including vitamins, minerals, phenolic constituents and enzymes (Kamel *et al.*, 2007).

The results of this study demonstrated that alloxan resulted in significant decreased in Hb concentration. The decrease in Hb content in diabetic rabbits suggests the direct effect

of the excess glucose present in blood. Previous studies demonstrated that during diabetes the excess glucose present in blood reacts with hemoglobin to form glycosylated hemoglobin. So, the total hemoglobin level is decreased in alloxan diabetic animals (Sheela and Augusti, 1992; Luo *et al.,* 2004).

In addition to the previously mentioned, the improvement in Hb value may be due to that bee propolis is rich in most microelements including iron and selenium (El-Ansarey , 2004). Moreover, propolis improves the digestive utilization of iron and increases the regeneration efficiency of hemoglobin especially during recovery from an anemic syndrome (Haro *et al.,* 2000).

PCV represents the percentage of erythrocytes to blood plasma. Therefore, the decrease in the number of erythrocytes leads to a decline in the value of PCV. The results of this study showed that these rabbits suffered from anemia which might be resulted from the toxic effect of alloxan used to induce diabetes in these animals. Similar result recorded in rats (Helal *et al.,* 2005). This study found that high-dose propolis (200mg/kg) causes an improvement in the level of PCV in diabetic rabbit's due to the increase in the number of erythrocytes. This indicates that the propolis could reduce the destructive effect of alloxan.

In the diabetic group, total leukocytes were decreased in number. This decrease in total WBCs number might be due to the decreased hemopoietic activity. In previous study, the intraperitoneal injection of streptozotocin into rats significantly reduced the WBCs number and its differentials such as basophils, monocytes, eosinophils, lymphocytes and neutrophils in diabetic rats. The reduction of these parameters could be linked to suppression of leucocytosis from the bone marrow which may account for poor defensive mechanisms against infection (Oyedemi *et al.,* 2010).

In the present study, the numbers of WBCs were significantly improved after propolis administration at both concentrations (100 and 200mg/kg). According to Oršolić and Basic, (2005), propolis increased the proliferation of leukocyte precursors from pluripotent stem cells. Furthermore, prolonged administration of propolis elevated the myeloid and megakaryocytic type of colony forming units.

In conclusion, this study revealed that ethanolic extract of Iraqi propolis reduced hyperglycemia as well as improved blood indices in alloxan-diabetic rabbits. The highest treatment dose (200 mg/kg) of propolis extract showed treatment effects on diabetes rabbits and revealed greater protection against oxidative stress. The author recommends future studies on propolis that explore the chemical compounds responsible for the antihyperglycemic effect.

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