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EFFECT OF WALNUT LEAF, CORIANDER AND POMEGRANATE ON BLOOD GLUCOSE AND HISTOPATHOLOGY OF PANCREAS OF ALLOXAN INDUCED DIABETIC RATS

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Abstract

Mechanism of most of herbal used for diabetes mellitus treatment has not been well defined. This study was performed to investigate hypoglycemic effect of walnut leaf (*Juglans regia L.*), coriander leaf (*Coriandrum sativum L.*) or pomegranate seed (*Punica granatum L.*), and their possible role on pancreatic tissue. Diabetes mellitus was induced in 20 adult male *Sprague Dawley* rats and the animals were divided into four groups; three of them fed a diet supplemented with about15 gram (60 g/ kg body weight /day) of mentioned plants for 15 days. The fourth diabetic untreated group (positive control) and a non-diabetic group (negative control) received standard diet. Blood glucose was measured every day and on the last day pancreases were isolated and stained with hematoxylin & eosin (H&E) and Gomeri aldehyde fuchsin (GAF). Histomorphology and following morphometric factors were studied; Volume density of β cells, volume density of islets, percent of β cells, number of islets per square centimeter and average area of islets. The results of this study indicate that only walnut leaf was able to reduce blood glucose significantly compared with diabetic untreated group (9.029vs. 14.358 mmol/l) ($P<0.05$). Hypercellularity of islets tissue, increased hyperchromic nucleus in pancreatic islets of this group was obvious. Density of islets in pancreatic tissue, percent of β cells and islets size increased significantly in this group in comparison with diabetic untreated group which may signify regeneration of islets or beta cells in group received walnut leaf ($P<0.05$).

Key words: Walnut leaf, Coriander, Pomegranate, Diabetes mellitus, Pancreas

Introduction

Many traditional plant treatments for diabetes are used throughout the world (Bailey and Day 1989; Marles and Farnsworth 1996) but most of the evidence for their beneficial effects is anecdotal. After introduction of insulin therapy the use of traditional treatment for diabetes greatly declined, although some traditional practices are continued for prophylactic purpose and adjuncts to conventional therapy (Bailey and Day 1989). In some of the societies there is strong desire to use herbs or plants for treatment, due to less side effects, easier consumption or availability. However very few of the traditional treatments for diabetes have received scientific or medical scrutiny and several have been shown to assist glycemic control in non-insulin dependent form of diabetes.

Plants may act on blood glucose through different mechanism, some of them may have insulin-like substances (Collier et al, 1987; Gray and Flatt, 1999) some may inhibit insulinase activity, others may cause increase beta cells in pancreas by activating regeneration of these cells (Shanmugasundaram et al, 1990; Abdel et al, 1997). The fiber of plants may also interfere with carbohydrate absorption; thereby affecting blood glucose (Nelson et al, 1991). Effect of walnut leaf (*Juglans regia L.*), coriander leaf (*coriandrum sativum* L.) and pomegranate seed (*Punica granatum* L.) on serum cholesterol, triglyceride, ALT, and AST of alloxan diabetic rats was reported (Jelodar and Nazifi, 1999). The aim of this study was to investigate hypoglycemic effect of mentioned plants

recommended in Persian folklore medicine as beneficial in treatment of diabetes and their possible role on pancreatic tissue.

Materials and Methods

Animal

Adult male *Sprague Dawley* rats (230-260 g and 6-7 month age) were housed at (22±2 °C) in an air condition room and supplied with standard pellet food with tap water *ad libitum*. All rats received human care according to the criteria outline in the "criteria outline in the Guide for care and use of laboratory Animals" prepared by National Academy of Science and published by The National Institutes of Health.

Preparation of alloxan-induced diabetic rats

Alloxan monohydrate (Sigma) was dissolved in sterile distilled water. Diabetes was induced in 20 rats by intraperitoneal injection of alloxan (5%) 185 mg/kg. The rats were kept fast 12h before and after alloxan injection. Animals with blood glucose above14 mmol/l, as well as with polydipsia, poly urea and polyphagia, which last for at least one week, were selected for experiment.

The range of diabetogonic dose of alloxan is quite narrow and even slight overdosing may be generally toxic causing the loss of many animals (Lenzen et al, 1996).To prevent toxic side effects, ranges of 90 to 200 mg/kg of alloxan (10 mg interval) was tested and 185mg/kg was selected as minimum and safest dose for induction of diabetes.

Preparation of food

Walnut leaf, coriander leaf and pomegranate seed were ground, mixed and homogenized with certain amount of powdered normal food. The mixture was compressed and repelleted. Rats were first fed with prepared food and additional ordinary food was provided when they finished prepared mixed food.

Experimental procedure

One week before and one week after induction of diabetes mellitus (DM) before starting any treatment blood glucose (FBS) of all rats was checked. Following induction of diabetes in 20 rats, they were randomly divided in 4 groups; three of them were fed diet supplemented with 60 g/ kg body weight /day of mentioned plants for 15 days. While fourth diabetic group (control positive) and a non- diabetic group (control negative), received standard diet.

Blood glucose measurement

Blood glucose was tested every day. Blood was collected from tail of fasting animals. Tail was embedded in 45 °C water bath and about one millimeter of its end was cut and a drop of blood was used for blood glucose test with the help of glucometer GX (Ames, USA), further sampling need not re-cutting of tail. Accuracy of glucometer was checked with randomly sampling and testing blood glucose by O-toluidine method (Mukherjee 1988).

Histopathological study

On the last day of experiment the rats were anesthetized and tail parts (Splenic part) of pancreas were removed and were kept in 10% formaldehyde. Tissue processing was carried out by autotechnicon and the prepared 5-micron thickness sections were mounted on slide and stained with hematoxylin & eosin (H&E) or Gomeri aldehyde-fuchsin (GAF), a beta cells specific staining (Gomeri, 1950). Stained sections were quantitatively (morphometric) and qualitatively (morphological) evaluated. For quantitative analysis the following factors were evaluated.

1. Volume density of islets, in 500 microscopic field percentage of islet tissue to total tissues was determined (Findlay and Thomas 1980).
2- Volume density of β cells in islet tissue, obtained by counting approximately 1300 parts of islets and determination of density of β cells to total islet cells (O'Brien et al., 1986).
3- Percent of β cells, cells of approximately 4 islets on each tissue and 40 islets of each group were counted (Shanmugasundaram et al., 1990; Chakravarthy et al., 1980).

4-Number of islets per square centimeter
5-Average area of islets was determined by measuring diameter of 4 islets in each section and totally 40 islets in each group (Findlay and Thomas 1980).

Statistical analysis

The results are expressed as mean± SE. The significance of the differences in the value was performed by one-way ANOVA test and Duncan's multiple range test. $P<0.05$ was considered to be significant difference.

Results

The effects of plants on fasting blood glucose (FBS*)*

The effect of the plants on FBS is presented in Table1. Mean fasting blood glucose in diabetic untreated group (control positive) and non-diabetic group (control negative) was 14.358± 0.191 and 3.730±0.039 mmol/l respectively during whole period. In comparison with control positive group, the group which consumed walnut leaf showed significantly lower mean FBS (9.024 ± 0.505 mmol/l) ($P<0.05$). No significant difference of FBS observed between groups received coriander leaf or pomegranate in comparison with diabetic untreated group.

Table 1: Effect of consumed plants on mean fasting blood glucose (FBS) during 15 days treatment

Groups	Mean FBS (mmol/l)	Anti-hyperglycemic activity (%)
Control negative	3.730 ± 0.039*	-
Control positive	14.358± 0.191	-
Coriander	13.872± 0.0555	4.96
Pomegranate	12.985 ± 0.233	13.1
Walnut leaf	9.029 ±0.505*	50

Values represent mean ± SE
* $P<0.05$ compared with control positive group

Effects of consumed plants on histopathology of pancreas
Histomorphologic changes of pancreas

Pancreatic sections stained with H&E showed that alloxan caused severe necrotic changes of pancreatic islets, especially in the center of islets. Nuclear changes, karyolysis, disappearing of nucleus and in some places residue of destructed cells were visible. Relative reduction of size and number of islets especially around the large vessel and severe reduction of β cells was clearly seen (Figure 1B).

Study of pancreas of treated group showed increase size of islets and hyperchromic nucleus in section stained with H&E and also relative increase of granulated and normal β cells in group consumed walnut leaf (Figures 1C&1D). Pancreases of other treated groups showed close similarity to diabetic untreated group.

Results of histomorphometric study

The results of this section are summarized in Figures 2 to 6. In diabetic untreated group all 5 mentioned measured factors were significantly low in comparison with normal health group ($P<0.05$). The group, which consumed walnut leaf, showed significantly higher value of volume density of islets in pancreas, percent of β cells and islets size in comparison with diabetic untreated group (Figures 2 to 4) ($P<0.05$). No significant difference was observed in other measured parameters between treated and untreated diabetic groups (Figures 5 and 6).

Discussion

In this study the pancreatic beta cells were destroyed with the help of alloxan. Alloxan and streptozotocin are the most usual applicable substance for induction of diabetes mellitus (Szkudelski, 2001). Rats were fasted 12h before and after injection of alloxan, unfed animals are more susceptible for alloxan induction diabetes (Szkudelski et al,

1998; Katsumata et al, 1992). Following injection of alloxan, beta cells were selectively destroyed and it was confirmed by GAF staining. Of consumed plant only walnut leaf was able to reduce blood glucose, but coriander

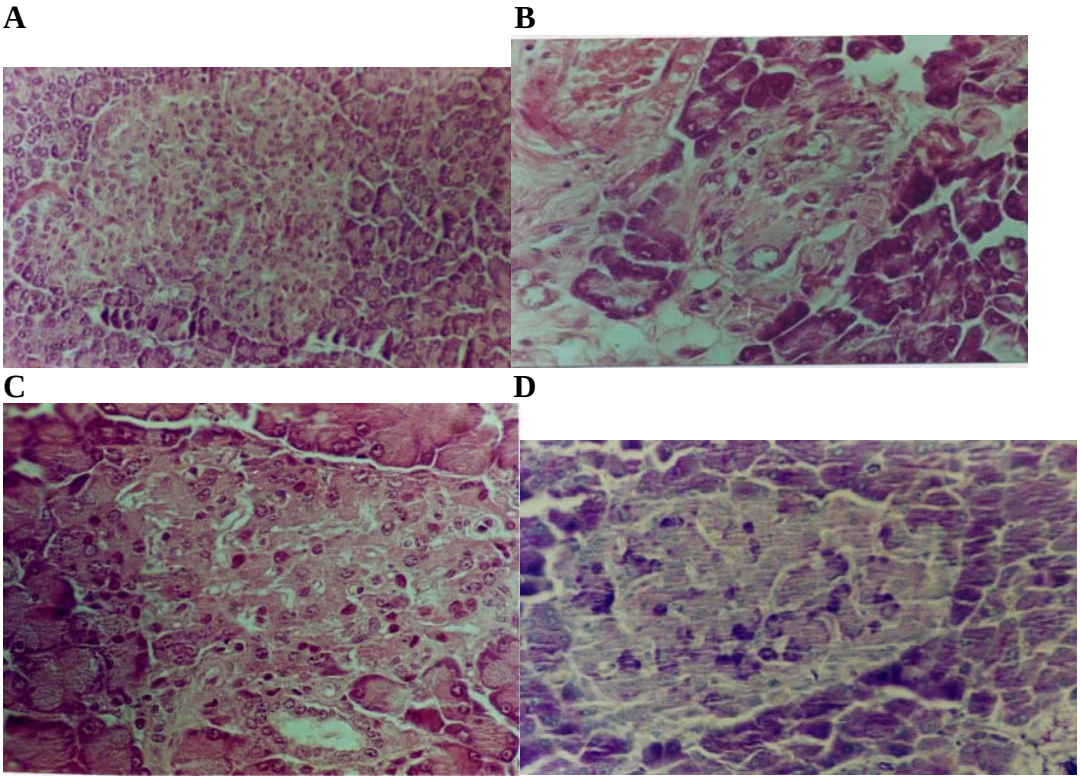
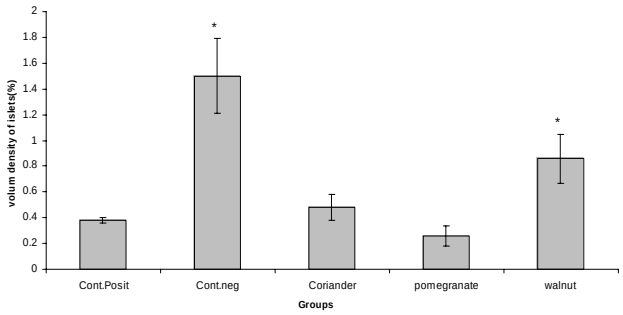


Figure 1: Histopathological sections of pancreas **A:** pancreas of normal health rat, H&E staining(X512) **B:** pancreas of diabetic rat, H&E staining(X512) **C:** pancreas of diabetic rat, treated with walnut leaf, H&E staining (X512) **D:** pancreas of diabetic rat, treated with walnut leaf, GAF staining (X512)

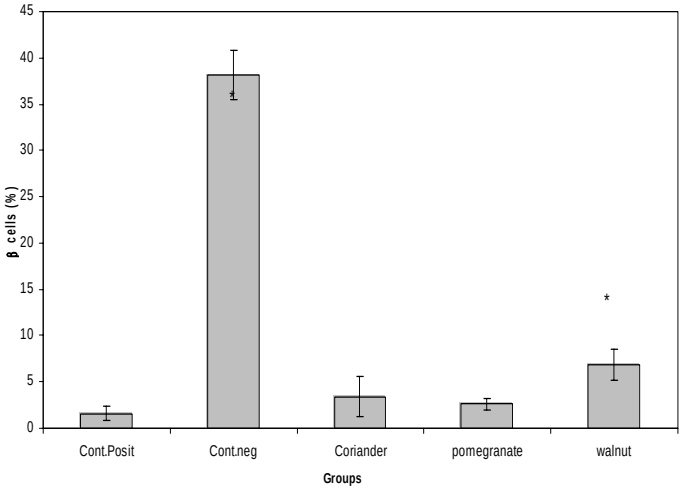


Values represent mean ± SE *P<0.05 compared with control positive

Figure 2: Effect of consumed plants on volume density of islets

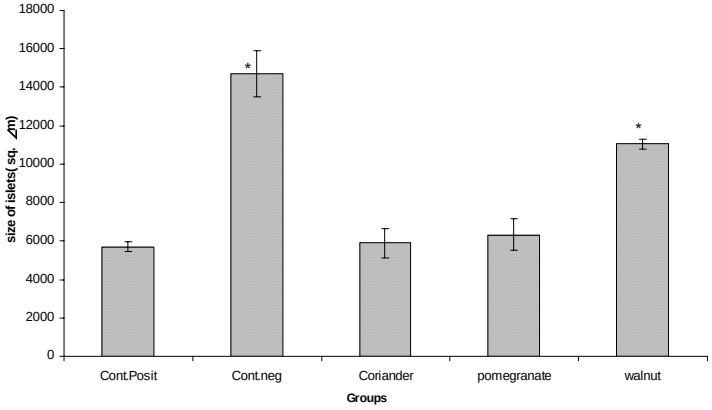
and pomegranate didn't have significant effect on blood glucose. It appears to us that there is no documented report about hypoglycemic effect of walnut leaf, however black walnut was reported to have hypoglycemic effect (Broadhurst, 1997). Treatment with pomegranate seed had minor effect on FBS with no significant difference in comparison with control group; however, extract of pomegranate seed was reported to have hypoglycemic effect

(Das et al, 2001). These findings may indicate the presence of some hypoglycemic agents in this fruit, which have been concentrated in extract and were insufficient in our prescription. Consumption of coriander leaf did not lower



Values represent mean ± SE *P<0.05 compared with control positive

Figure 3: Effect of consumed plants on percent of β cells in each islet



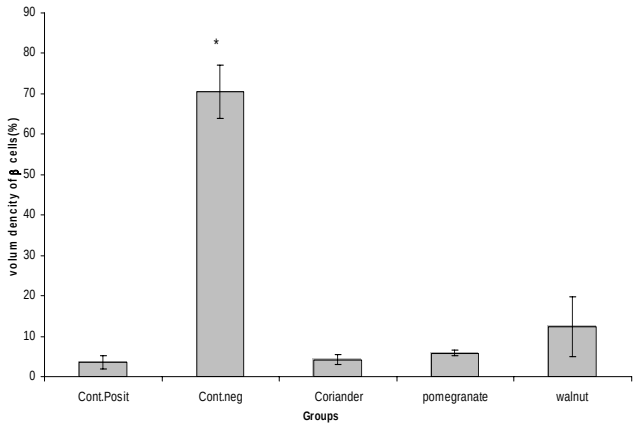
Values represent mean ± SE *P<0.05 compared with control positive

Figure 4: Effect of consumed plants on average size of islets (μm^2)

FBS significantly; since most of reports about hypoglycemic activity of coriander are focused on coriander seed (Swanston et al, 1990; Gray and Flatt, 1999) they may not be comparable with our results.

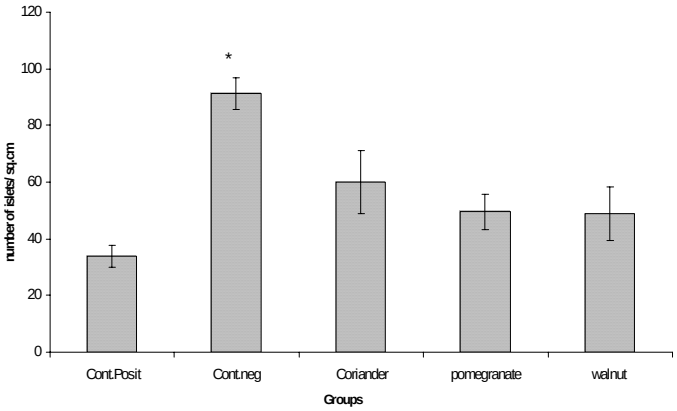
Hypoglycemic effect of plants may be due to presence of insulin-like substances in plants (Collier et al, 1987; Gray and Flatt, 1999), stimulation of β cells to produce more insulin(Chang and Johnson., 1980; Khan et al, 1990), improving insulin action and binding(Khan et al, 1990), increasing glucose metabolism (Broadhurst, 1997), high level of fiber which interfere with carbohydrate absorption (Nelson et al, 1991) or regenerative effect of plants on pancreatic tissue (Shanmugasundaram et al, 1990). Specific staining along with H&E staining has been used to clarify the effect of applied plants on pancreatic β cells. Histopathological study of diabetic untreated rats showed almost complete destruction of β cells, which was due to proper dose of alloxan used in this study. Decrease number of islets in pancreatic tissue observed in diabetic rats could be due to conversion of large islets to small. Reduction

of numbers and volume density of islets by 70% was also reported in diabetic dogs (Sudha Rastogi et al, 1990).



Values represent mean ± SE *P<0.05 compared with control positive

Figure 5: Effect of consumed plants on volume density of β cells in islets



Values represent mean ± SE *P<0.05 compared with control positive

Figure 6: Effect of consumed plants on number of islets (per cm^2)

The histopathological study of diabetic treated groups indicates; increase volume density of islets, percent of β cells and size of islet in the group received walnut leaf, which may be a sign of regeneration. Increase percent of β cells without changes of volume density of β cells in islets may be due to increase number or size of other types of pancreatic cells specially α cells. Severe reduction of β cells in diabetic dogs with no significant change in size of islets was suggested to be due to increase of other pancreatic cells specially α cells (Sudha Rastogi et al, 1990). Sign of regeneration of β cells has been reported following consumption of some other plants. A flavonoid fraction extracted from *pterocarpus marsupium* caused decrease of blood glucose and increase of beta cells (Chakravarthy et al, 1980). *Nigella sativa* and gliclazide also was reported to increase beta cells(Abdel et al, 1997). Two components extracted from *Gymnema sylvestera* (GS4 and GS3) was reported to decrease blood glucose and increase number of beta cells and islets in diabetic rats (Shanmugasundaram et al, 1990). Walnut leaf may also have some chemical components that exert regenerative effect on β cells, stimulate these cells to produce more insulin or it may have some insulin-like substances, moreover walnut leaf is rich of fiber, paraffin and minerals such as calcium and manganese that along with other factors may cause hypoglycemic activity. Manganese chloride was reported to exert hypoglycemic action (Rubenstein et al, 1965).

Conclusion: The findings of this study indicate that consumption of pomegranate seed or coriander leaf did not exert significant hypoglycemic effect in diabetic rats, however walnut leaf reduced FBS significantly and histomorphometric study of pancreas of treated group showed evidence which can be sign of regeneration of beta cells in group that received walnut leaf. This finding supports traditional use of walnut leaf for controlling hyperglycemia in diabetics, hence further investigation with longer period or higher dose may show clearer feature of this finding.

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