Research Article

The effect of citrulluscolocynthis on blood glucose profile level in diabetic rabbits

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Abstract

Introduction:
Diabetes is one of the most common diseases in this century. To cure this illness, herbal plants as well as medicine and diet therapy are used. This study is designed to determine the effect of citrullus colocynthis fruit pulp and seed on blood glucose in diabetic rabbits.
Material and Methods:
This experimental study was conducted on 36 white male New Zealand rabbits in animal laboratory of Tabriz University of Medical Sciences in 2006.To determine the effect of citrullus colocynthis fruit on blood glucose, we used the pulp and seed extracts of citrullus colocynthis. Blood glucose was measured using enzymatic kit via Elan Auto Analyzer. The results were analyzed in SPSS software.
Results:
100 mg/kg B.W of the pulp and seed extracts of citrullus colocythis significantly decreased blood glucose as compared to the diabetic control group.
Conclusion:
The results suggest that citrullus colocythis extract can probably decrease the blood glucose in diabetic rabbits, but further studies are necessary for finding the probable side-effects and also its appropriate and safe dose.

Keywords: Glucose, Blood, Diabetes Mellitus, New Zealand Rabbits

Introduction:

Diabetes mellitus includes a group of metabolic disorders with the main characteristic of chronic hyperglycemia, associated with increased morbidity and mortality rates. Hyperglycemia is caused due to defect in insulin secretion or function or both and it can lead to disturbances in carbohydrate and lipoprotein metabolism (1).

According to epidemiologic studies, the prevalence of diabetes will grow from 135 million patients in 1995 to 300 million patients in 2025 (1). Three main management approaches for diabetes include diet therapy, diet therapy with antiglycemic medications, and diet therapy with insulin therapy (2). However, in traditional medicine herbal remedies are used instead of these approaches. Reviewing published reports show that there are more than 800 herbal species with anti-diabetic effects (3). Increased use of herbs or their products have several reasons such as their lower price in comparison with synthetic drugs (4).

Administration of anti-diabetic medicinal plants has a long history in Iran. Fenugreek seed, common sage leave, pea bean pod, walnut leave, burdock root, nettle, garlic, blueberry, polygonome and colocynth are examples of these anti-diabetic plants.

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Citrullus colocynthis (Cucurbitales) commonly known as colocynth, bitter apple, vine-of-Sodom and tumba is one of the plants with a long history of anti-diabetic use in traditional medicine (5). Usable part of this plant is its fruit which is yellowish, apple-sized, and extremely bitter mainly used as a strong laxative in treatment of acute and chronic constipation. It has also been shown effective in treatment of diabetes, fever and parasitic infections (6). The fruit of this plant includes saponin glycosides such as cucurbitacin E and G, alkaloids, and caffeic acid derivatives like chlorogenic acid (7,8). In addition, Citrullus colocynthis can cause diarrhea due to increased bowel movements (9). It seems that this plant can also cause some disturbances in absorption of other nutrients with increasing glucose excretion. There are few studies regarding hypoglycemic effects of this plant (10). Considering the high prevalence of diabetes and its mortality rate and the existence of effective compounds in citrullus colocynthis, in this study we investigated the effect of using the fruit of this plant on blood glucose level of diabetic rabbits.

Material and Methods:

The study was conducted on 36 New Zealand male rabbits (1.5 to 2 months, 1683±10.5 gr) which were purchased from Pasteur Institute, Iran. Fasting blood glucose level of these rabbits was nearly 115 mg/dl. Several factors might affect blood glucose level such as fear and stress. Moreover, blood glucose level of rabbits increases with age. The most common method to induce diabetes is using phloridzin (alloxan) or streptozotocin. Considering the insensitivity of rabbits to streptozotocin, alloxan was used to induce diabetes in the study sample (11).

Alloxan causes extensive ß cell destruction in the pancreas during 18 to 24 hours after injection which leads to hyperglycemia. The reason for alloxan's selective toxicity is its structural resemblance to glucose and its mechanism of effect is producing free radicals since it can be deactivated by anti-oxidants. In addition, alloxan can cause some disorders in kidney, adrenal gland, thyroid and liver (12).

The animals were binary housed in aluminum caging with free access to food and water in Animal Lab Centre of Tabriz University of Medical Sciences for a total of seven weeks including two weeks of adaptation period and five weeks of testing period (13,14). They were kept in 18-22 °C temperature on a 12 : 12 hour light : dark cycle (15). During the adaptation period, rabbits were fed by standard basic laboratory rabbit diet (Niru-Sahand Co, Tabriz).

At the end of the adaptation period, 5 cc fasting blood samples (12-13 hours fasting) were collected from rabbits' ear vein with careful considerations to avoid hemolysis during sampling and transfer. Blood samples were immediately transported to the Nutrition Department Laboratory and the clot was separated by applicator. In order to separate the plasma serum, blood specimens were centrifuged at 1500 rpm for 15 minutes. The serum of the blood samples was then transferred to test tubes by eppendorf sampler and blood glucose level was determined using enzymatic kit (Parsazmun, Iran) and spectrophotometer. After the adaptation period, diabetes was induced in 24 rabbits by injecting 100 mg/kg of 10% alloxan monohydrate solution to their marginal auricular veins. Alloxan induces diabetes through destructing Langerhans islands of the pancreas. Therefore, a large amount of insulin is released from the pancreas cells after the injection. In order to prevent hypoglycemic shock during the first 24 hours after the alloxan injection, the rabbits received 10% dextrose instead of water (14,15). After seven days, 12-hour fasting blood samples were collected from rabbits' marginal auricular veins. A fasting

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blood glucose level higher than 300 mg/dl was considered diabetic (16).

Diabetic rabbits were randomly assigned into four groups of six as the intervention groups and one remaining group as the diabetic control group. The rabbits in the intervention groups received oral 100 and 200 kg/kg bw of pulp and seed extract of Citrullus colocynthis, respectively. Fasting blood glucose was measured by collecting blood samples from all rabbits at days of 7, 14, 21 and 28.

Results:

According to the results of the present study, alloxan injection caused a significant increase in mean fasting blood glucose level in all intervention groups in comparison with healthy controls (table 1). All rabbits receiving 200 mg/kg bw of Citrullus colocynthis seed extract were expired by the sixth day due to severe diarrhea.

Table 1: Mean fasting serum glucose levels of the study groups before and seven days after alloxan injection

groups	Biochemical variable	Number	Fasting serum glucose level		P value*
			Before alloxan injection	7 days after alloxan injection	
Healthy control		N=6	93.3±1.8**	99±1.8	NS**
Diabetic control		N=6	97.8±1.4	311.7±6.1	P<0.0001
Diabetic	100 mg/kg bw of citrullus pulp extract	N=6	99±1.1	309±1.8	P<0.0001
Diabetic	200 mg/kg bw of citrullus pulp extract	N=6	98.9±1.5	311±0.9	P<0.0001
Diabetic	100 mg/kg bw of citrullus seed extract	N=6	98.2±4.2	310.2±6.2	P<0.0001

*P value <0.05 indicates statistically significant difference between mean fasting serum glucose of the study groups before and 7 days after alloxan injection
**NS indicates no statistically significant difference between the means

During a week, mean blood glucose level decreased in all three intervention groups in comparison with control group. Using Dunnett's test showed that the highest decrease in serum glucose level belonged to the group receiving 200 mg/kg bw of citrullus pulp extract and that compared to the control group, the drop in mean blood glucose level in the group receiving 100 mg/dl bw of citrullus seed extract was not statistically significant (table 2).

Table 2: Comparison of mean difference of fasting blood glucose levels between intervention and control groups during the first week

Groups (I)	Groups (J)	Mean Difference (I-J) (±SE)	P value	95% confidence interval	
				Lower limit*	upper limit*
100 mg/kg bw pulp extract	Diabetic control	-9.1 ± 4.7	0.157	-20.9	2.8
200 mg/kg bw pulp extract	Diabetic control	-17.8 ± 4.7	0.003	-29.7	-6
100 mg/kg bw seed extract	Diabetic control	-12.1 ± 4.7	0.044	-23.9	-3

*values are reported as milligrams per deciliter (mg/dl).
(All rabbits receiving 200mg/kg bw of citrollus seed extract expired between days 10 to 12 due to diarrhea)

Dunnett's test showed that during the four week period of intervention, mean blood glucose level in the rabbits receiving 100 mg/kg citrullus seed extract decreased significantly in comparison with the 26 trol group. The blood glucose level also decreased in the 100 mg/kg citrullus pulp extract group but the difference wasn't statistically significant (table 3).

Discussion:

The findings of the present study showed that using citrullus extract in the rabbits with the forms of 100 and 200 mg/kg citrullus pulp extract and 100 mg/kg citrullus seed extract can decrease blood glucose level in comparison with the controls. This decrease was found statistically significant in the groups receiving 200 mg/kg citrullus pulp extract and 100 mg/kg citrullus seed extract. Rabbits receiving 200 mg/kg citrullus pulp extract expired by the days 7-10 of the study and those receiving 200 mg/kg citrullus seed extract expired by the day 6 of the study, all due to severe diarrhea. On the other hand, in the groups receiving 100 mg/kg citrullus pulp and seed extract, mean blood glucose level remained lower than the control group until the end of the intervention period; however, the decline was only significant in the 100 mg/kg citrullus seed extract. Abdel-hasan *et al* also found that oral administration of 200 mg/kg bw citrullus extract in healthy rabbits can cause significant drop off in the blood glucose level 1, 2 and 6 hours after the administration (17). They also administered 50 mg/kg bw of alkaloid-glycoside and saponin extracts derived from citrullus orally that resulted in significant decrease in blood glucose level in comparison with controls. This significant decrease was more dominant for saponin derivatives compared with glycoside derivatives. In the next stage of their investigation, they studied the effect of citrullus saponin derivatives with doses of 10, 15 and 20 mg/kg bw on alloxan-induced diabetic rabbits and found a significant decrease in blood glucose with 15 and 20 mg/kg doses. The difference was not statistically significant for 10 mg/kg dose.

Nmila *et al* in their study investigated the effect of Citrullus colocynthis fruits on insulin secretion stimulation in rats and showed that raw, hydro-alcoholic and refined extracts of the seeds of this fruit with concentration of 0.1 mg/kg can cause a significant increase in insulin secretion after 20 minutes of administration at the presence of 8.3 mmol glucose (18). Jorge *et al* reported that glycoside flavnoid in the leaves of Hedyotis verticillata named Kaempferitrin has a severe hypoglycemic effect on blood glucose level of alloxan-induced diabetic rats and can stimulate the uptake of glucose by the muscles of healthy rats (19). According to their report, this glycoside didn't show any effect on glycosuria or protein synthesis in healthy or diabetic animals. In addition, radioactive glucose uptake by muscle cells at the presence of this glycoside was similar to glucose uptake mediated by insulin. Therefore this compound is capable of reducing the glucose level of the diabetic plasma and its mechanism of effect is not through insulin secretion stimulation or reducing glucose intestinal absorption, but it directly increases glucose uptake by muscular tissue which leads to plasma glucose level reduction in diabetic rabbits. Diatewa *et al* showed a 40% drop in blood glucose concentration of the healthy rats, 3 hours after oral administration of 100 mg/kg ethyl extract of leaves of a type of cogniauia containing glycoside and saponin flavnoids ($P<0.001$) (20). This reduction was similar to the effect of tolbutamide-the antiglycemic agent- in the control group. Moreover, 50 mg/kg and 100 mg/kg doses of this extract was found to be capable of reducing blood glucose level in alloxan-induced diabetic rats to 29.4% and 44.5% and 44.4% and 70.4%, 3 and 4 hours after administration, respectively. This reduction was found statistically significant in comparison to the control group and the effect was similar to tolbutamide. Anoja *et al* reported that using 50 and 150 mg/kg ginseng seed extract containing saponin can cause significant decrease in blood glucose level in ob/ob mice (21). They found that on the twelfth day of intervention with 15 mg/kg bw of this extract, blood glucose became normal in these mice and no significant difference was found between their blood glucose level with healthy lean mice in the control group.

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Conclusion: Considering that according to our findings, 200 mg/kg bw of citrullus pulp extract and 100 mg/kg of citrullus seed extract showed significant reduction in blood glucose level but 100 mg/kg citrullus pulp extract was not found effective, it can be concluded that probably citrullus pulp contains higher amounts of saponin glycosides than its seeds. In addition, given the fact that administration of citrullus pulp and seed extract with the dose of 200 mg/kg led to expiration of all the studied rabbits, further studies are recommended on finding effective doses between 100 and 200 mg/kg of citrullus pulp and seed extracts.

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