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Effect of Afghan *Hibiscus sabdariffa* L. and *Carum Carvi* L. Hydro-alcoholic Extracts Either Alone or in Combination on Blood Glucose Level in Diabetic Rats

Research Article

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Abstract

Combination therapy is considered as a new and effective therapeutic strategy for control of hyperglycemia. The present study aimed to determine the hypoglycemic effect of Afghan *Hibiscus sabdariffa L.* and *Carum Carvi L.* hydro-alcoholic extracts either alone or in combination on diabetic rats. Streptozotocin-induced diabetic rats were administered intraperitoneally with hydro-alcoholic extracts of *H. sabdariffa* (75, 150 and 300 mg/kg), *C. carvi* (150, 300 and 500 mg/kg) and their combinations for 21 days. The Fasting blood glucose and body weight of rats were determined on 0, 7, 14 and 21st days. The results showed a significant ($P<0.0001$) hypoglycemic effect of *H. sabdariffa* extract (75, 150 and 300 mg/kg) on 7, 14 and 21th days, *C. carvi* extract (500 mg/kg), and their effective doses combination (75:25), especially on 7th day. Only 75 mg/kg of *H. sabdariffa* had positive effect on body weight loss of diabetic rats ($P<0.05$). One can conclude that Afghan *H. sabdariffa* and *C. carvi* hydro-alcoholic extracts and their combination have a hypoglycemic effect. However, their combinations do not have any synergic effects, so the hypoglycemic effect of *H. sabdariffa* was much greater and even normalized the blood glucose level.

Key Words: *Afghanistan, Hibiscus sabdariffa, Carum carvi, Combination, Blood glucose level.*

Introduction

Diabetes mellitus is the most common endocrine disorder, which considered as one of the main causes of death and disability in many countries (1). This disorder, which is known as the silent epidemic of the present century, affects many people in the world and its prevalence is increasing. According to the International Diabetes Federation's (IDF) report, in 2017 there were 451 million people with diabetes worldwide and it is expected that this value will increase to 693 million by 2045 (2). Diabetes mellitus is a group of metabolic diseases, characterized by hyperglycemia as a result of defects in insulin secretion, insulin function, or both (3). The main cause of diabetic mortality and morbidity is diabetic complications, which resulted from hyperglycemia (4). Therefore, the major target of medicinal therapy for diabetes is balancing blood glucose level (5).

There are multiple medicinal plants which have benefits on diabetes, especially on hyperglycemia, due to their effective constituents (4). In traditional medicine, the use of herbs combinations is recommended, because it is approved that the combination of multiple herbs has more effects than individual herbs (6). Studies also have been shown that combination therapy is considered as a new and effective therapeutic strategy for control of hyperglycemia (4).

One of the medicinal herbs, which have effective constituents, is *Hibiscus sabdariffa L.* (*H. sabdariffa*) that belongs to *Malvacea* family. H. *Sabdariffa* has multiple active constituents such as organic acids, anthocyanins, polysaccharides, and flavonoids (7, 8). Its petals also contain protein, lipid, carbohydrates, different minerals, and vitamins (8, 9). Multiple studies have shown that H. sabdariffa has positive effects on different aspects of diabetes treatment, including reduction of fasting blood glucose (FBG) and postprandial glucose, improvements of glucose tolerance and insulin sensitivity (10), and inhibition of alpha-glucosidase and alpha-amylase enzymes (11).

Also, *Carum carvi* (*C. Carvi*) is another medicinal plant which belongs to *Apaiceae* family (12, 13). The main constituents of *C. carvi* are flavonoids (quercetin, kaempferol), monoterpenes (carvon, limonen and timol) and glycosides (carol and dihydrocarol) (14-16). There are multiple studies which show the positive effects of *C. carvi* extract on blood glucose level of diabetic rats (17, 18).

However, because of the impact of climate and geographical conditions on the quality of plants (19), as well as most effectiveness of herbs combinations on the treatment of hyperglycemia (4, 6), this study determines the hypoglycemic effects of Afghan *H. sabdariffa* and *C. carvi* hydro-alcoholic extracts effective doses, either alone or in combination on diabetic rats.

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Materials and Methods

Animals

Sixty-six adult Sprague-Dawley male rats weighing between 180 and 200 g, randomly selected from Khatam Al-Nabieen University Research and Technology Center (KNURTC), were housed in Plexy-glass cages with free access to food and water. Animals were kept under stable room temperature (23±2°C) and a 12 hours light/dark cycle (the light period started at 7 a.m.). The experimental protocol related to animal's use has complied with all the relevant national regulations and institutional policies, so approved by the ethic research board of Khatam Al-Nabieen University and were conducted following the ethical guidelines set by the 8th edition of National Institute of Health (NIH) guide for the care and use of laboratory animals. Rats were carefully handled to minimize unwanted stress during housing and experiments.

Preparation of extract

The petals of *H. sabdariffa* from Asmar, Kunar province and seeds of *C. carvi* from Behsood, Maidan Wardak province of Afghanistan, were collected freshly. Then, plants were dried in the shade, at room temperature and then powdered. 100 g of *H. sabdariffa* petals and 200 g of *C. carvi* were macerated individually with 70% ethanol (1:4) and kept for 72 hours at room temperature, with occasional shaking. After 72 hours, the mixtures were filtered and evaporated at room temperature (20).

Induction of experimental diabetes

The experimental diabetes was induced by a single intraperitoneal (i.p.) administration of streptozotocin (STZ) (Sigma-Aldrich, USA), at a dose of 55 mg/kg after 8 hours fasting. The STZ was dissolved in 0.1 M citrate buffer. 72 hours later, diabetes was confirmed by determining the FBG level. The rats with 200 mg/dl FBG level were only considered for the experiment.

Experimental groups

Rats were divided into 11 groups (n=6):

Group I (Normal), Rats of this group received Normal saline (1 ml, i.p.) for 21 days;

Group II (Diabetic), Rats of this group became diabetic and received Normal saline (1 ml, i.p.) for 21 days;

Group III-V (*H. sabdariffa* 75, 150, 300 mg/kg), Diabetic rats received *H. sabdariffa* hydro-alcoholic extract (75, 150 and 300 mg/kg, respectively, i.p.) for 21 days;

Group VI-VIII (*C. carvi* 150, 300, 500 mg/kg), Diabetic rats received *C. carvi* hydro-alcoholic extract (150, 300 and 500 mg/kg, respectively, i.p.) for 21 days;

Group IX (Combination 1), Diabetic rats received combination (75:25) of *H. sabdariffa* (56.25 mg/kg) and *C. carvi* (125 mg/kg) hydro-alcoholic extracts by i.p. administration for 21 days;

Group X (Combination 2), Diabetic rats received combination (50:50) of *H. sabdariffa* (37.5 mg/kg) and *C. carvi* (250 mg/kg) hydro-alcoholic extracts by i.p. administration for 21 days;

Group XI (Combination 3), Diabetic rats received combination (25:75) of *H. sabdariffa* (18.75 mg/kg) and *C. carvi* (375 mg/kg) hydro-alcoholic extracts by i.p. administration for 21 days.

FBG level Measurement. The FBG levels of all rats were measured after 8 hours fasting, by blood withdrawn from the tail of rats using strips and glucometer (ACCU Check Active - Roche™) on 0, 7, 14 and 21 days (considering after 3 days of STZ administration).

Body weight determination. The body weight of all rats was determined on 0, 7, 14 and 21 days.

Statistical analysis. The statistical analysis was done with Graph pad prism (6.07) software. The FBG levels were analyzed by Two-way ANOVA, followed by Dunnets test multiple comparisons. Difference between body weight of different groups was analyzed by non-parametric Kruskal Wallis test. The difference amongst means was considered statistically significant if the $P<0.05$. The results are expressed as mean ± SEM.

Results

FBG level measurement

The FBG level of all groups was measured on 0, 7, 14 and 21st days. There was a significant difference in the level of FBG among normal and diabetic groups ($P<0.0001$). The FBG level was significantly decreased in *H. sabdariffa* 75, 150 and 300 mg/kg groups as compared with diabetic group on 7, 14 and 21th days ($P<0.0001$) (Figure 1). In addition, the effect of *C. carvi* different doses was evaluated on the FBG level (Figure 2). The difference in the level of FBG only between *C. carvi* 500 mg/kg and the diabetic group was significant, on 7, 14 and 21st days, and especially on 7th day ($P<0.0001$). The FBG level was not significantly decreased in 150 and 300 mg/kg groups. As a result, the 75 mg/kg of *H. sabdariffa* and 500 mg/kg of *C. carvi* hydro-alcoholic extracts were considered as effective doses, combined in different proportions and their hypoglycemic effect was evaluated (Figure 3). The FBG level is significantly decreased only in Combination 1 group, as compared with the diabetic group on day 7 ($P<0.0001$). There was no significant difference in the FBG level between combination 2 and 3 and the diabetic group ($P>0.05$).

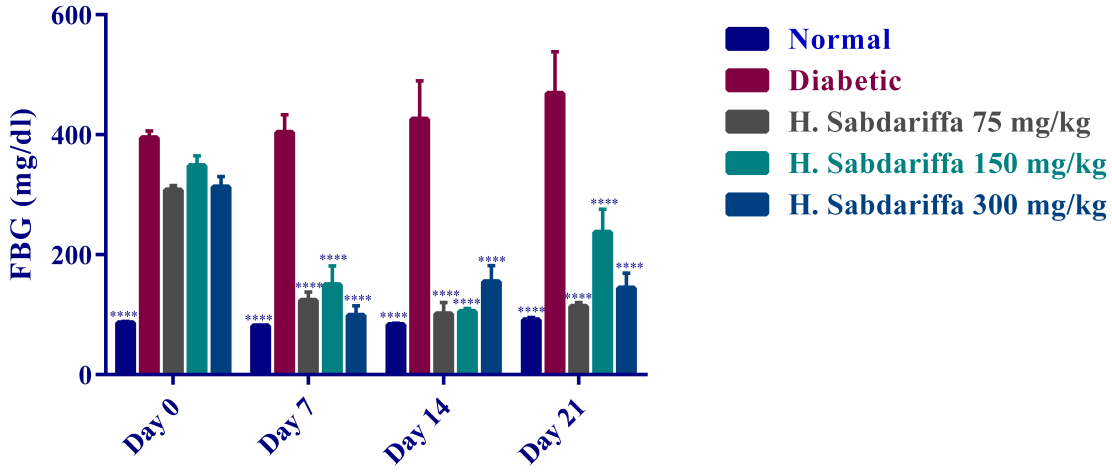

Figure 1. The effect of *H. sabdariffa* (75, 150 and 300 mg/kg) hydro-alcoholic extract on FBG level of diabetic rats. Data are shown as Mean±SEM. FBG, fasting blood glucose. ****: P<0.0001 as compared with diabetic group.

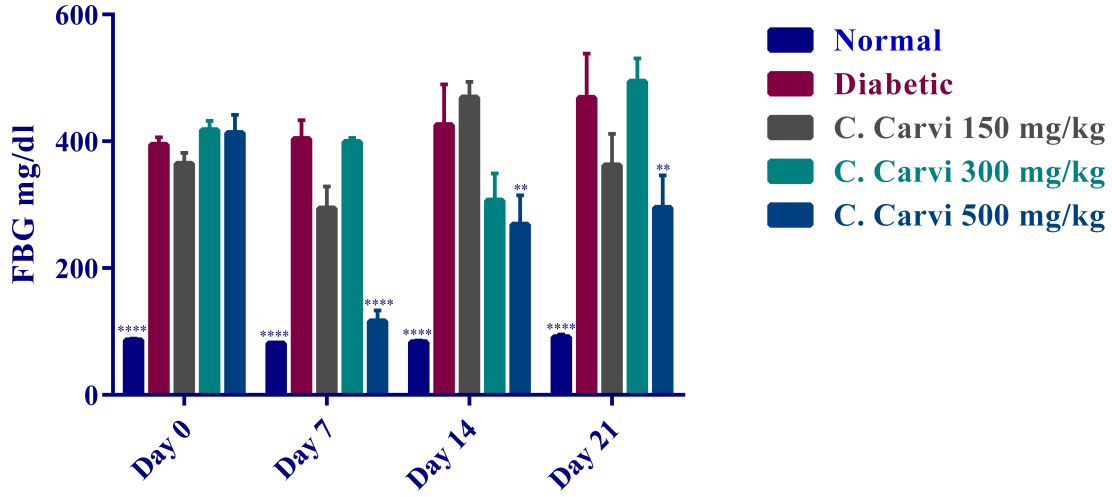

Figure 2. The effect of *C. carvi* (150, 300 and 500 mg/kg) hydro-alcoholic extract on FBG level of diabetic rats. Data are shown as Mean±SEM. FBG, fasting blood glucose. **: P<0.01, ****: P<0.0001 as compared with diabetic group.

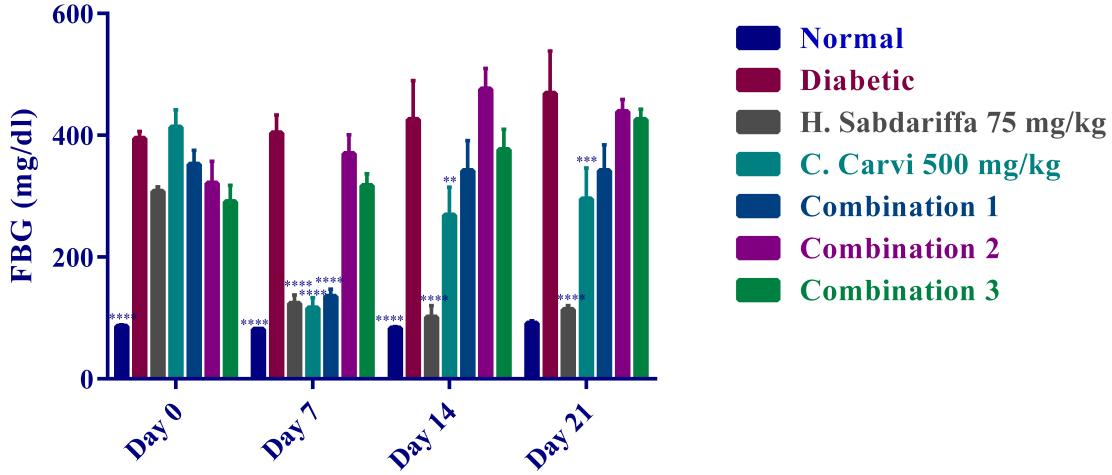

Figure 3. The effect of *H. sabdariffa* and *C. carvi* hydro-alcoholic extracts combinations on FBG level of diabetic rats. Data are shown as Mean±SEM. FBG, fasting blood glucose. **: P<0.01, ***: P<0.001, ****: P<0.0001 as compared with diabetic group.

Body weight determination

The body weight of all groups was determined on 0, 7, 14 and 21th days. There was a significant body weight loss in a diabetic group as compared with the normal group (P<0.001). The body weight was significantly increased only in *H. sabdariffa* 75 mg/kg group as compared with the diabetic group (P<0.01). However, there was not a significant difference in the body weight among *H. sabdariffa* 150, 300 mg/kg, *C. carvi* 150, 300, 500 mg/kg, combination 1, 2 and 3 groups and diabetic group (P>0.05) (Table 1).

Table 1. The body weight of different groups

Groups	Body weight (g)				Weight gain/lost
	Day 0	Day 7	Day 14	Day 21	Day 0-21
Normal	181.50±0.62	185.83±0.48	201.50±1.78	208.50±1.18	27.00±1.37**
Diabetic	165.67±0.88	170.50±4.14	158.00±02.25	155.83±2.24	-9.83±2.45
H. sabdariffa 75 mg/kg	177.67±0.80	179.50±4.10	188.83±0.75	198.33±2.82	20.67±3.08*
H. sabdariffa 150 mg/kg	171.83±1.99	174.33±3.40	173.17±4.01	179.00±5.44	7.17±6.08
H. sabdariffa 300 mg/kg	176.83±1.85	170.0±2.57	170.83±4.40	175.83±6.31	-1.00±4.97
C. carvi 150 mg/kg	165.8±2.06	157.0±2.28	150.2±2.87	137.2±2.60	-28.67±1.43
C. carvi 300 mg/kg	164.8±1.85	160.70±3.95	155.30±2.72	148.70±3.15	-16.67±2.51
C. carvi 500 mg/kg	176.20±2.81	161.3±2.36	160.70±2.78	163.0±3.25	-11.67±2.50
Combination 1	175.0±5.12	170.50±4.82	171.50±3.57	181.50±4.31	6.50±5.40
Combination 2	181.20±2.53	185.0±1.51	179.80±2.47	182.8±2.86	-0.67±1.75
Combination 3	192.30±2.75	192.0±3.26	185.30±2.33	185.70±2.50	-6.67±3.0

Data are shown as Mean±SEM. *: P<0.05, **: P<0.01 as compared with the diabetic group.

Discussion

In this study, the effect of *H. sabdariffa* and *C. carvi* hydro-alcoholic extracts either alone or in combination on the FBG level of STZ-induced diabetic rats was evaluated. STZ is a toxic, diabetogenic compound, which inhibits insulin secretion and induces insulin-dependent diabetes (21). This compound causes pancreatic beta cells necrosis through the oxidative stress pathway (21-23). Administration of STZ in animals leads to an increase in blood glucose level and a decrease in body weight (22, 24).

Similarly, this study showed that a single i.p. administration of STZ increased the FBG level and decreased body weight in a diabetic group as compared with the normal group. So, we can conclude that STZ in this study could develop experimental diabetes model.

Besides, i.p. administration of *H. sabdariffa* hydro-alcoholic extract (75, 150 and 300 mg/kg) for 21 days could decrease the FBG level significantly. However, only 75 mg/kg of *H. sabdariffa* extract could significantly increase the body weight of rats. This shown that Afghan *H. sabdariffa* hydro-alcoholic extract has a hypoglycemic effect and its effective dose for reducing the FBG level and compensating the body weight loss of diabetic rats is 75 mg/kg.

Similarly, previous studies showed that *H. sabdariffa* can decrease the FBG level in diabetic rats and confirm the results of this study. For instance, in one study, the effect of oral administration of *H. sabdariffa* ethanolic extract in 1.0 and 0.1 g/kg doses for 6 weeks on blood glucose level and insulin secretion rate in normal and diabetic rats was evaluated. The results have shown that only 1.0 g/kg dose of the extract could decrease the blood glucose level in diabetic rats, not in normal rats. The extract increased the number of pancreatic islets. They concluded that the antidiabetic activity of *H. sabdariffa* extract may be partially mediated by its stimulating effect on insulin secretion (10). Also, another study was evaluated the effect of *H. sabdariffa* polyphenolic extract on hyperglycemia, hyperlipidemia and glycation-oxidative stress and insulin resistance in type 2 diabetic rats. They showed that oral treatment with *H. sabdariffa* hydro-alcoholic extract reduced hyperglycemia, hyperlipidemia and glycation-oxidative stress and improved insulin resistance, especially at the dose of 200 mg/kg (25). In summary, different studies have been shown that the possible mechanism of the effect of *H. sabdariffa* extract on blood glucose level may be the increase of basal insulin level or induction of insulin secretion by pancreatic beta cells, as a result of an increase in the number of beta cells, as well as the increase in blood glucose transport into peripheral tissues. Also, these studies are emphasizing on the potent anti-oxidant constituents of *H. sabdariffa* which are protecting the pancreatic beta cells from destruction by STZ and actions of free radicals (7, 10, 26, 27).

In addition, the present study was shown that the i.p. administration of *C. carvi* hydro-alcoholic extract for 21 days could decrease the FBG level significantly. However, 500 mg/kg was the only effective dose for reducing the FBG level. Its effect was much better on 7th day. So, we can conclude that the Afghan *H. sabdariffa* hydro-alcoholic extract can decrease the FBG level effectively, especially at high-dose and short-term period of administration.

Previous studies have also confirmed the results of this study. A study evaluated the antidiabetic effect of *C. carvi* ethanolic extract on blood glucose level of normal and diabetic rats. They administrated *C. carvi* ethanolic extract at 0.1, 0.2, 0.4 and 0.6 g/kg, i.p., and then measured the blood glucose level of rats 1, 3 and 5 hours after extract administration. Their results showed

that 0.2, 0.4 and 0.6 doses of extracts could significantly decrease the blood glucose level and the insulin level in diabetic rats in 3 and 5 hours, but not in normal rats (17). Similarly, another study was evaluated the effect of *C. carvi* aqueous extract on body weight, serum blood glucose, and blood lipids in diabetic rats after feeding 1 g/kg dose of extract for 21 days. They have been shown that aqueous extract of *C. carvi* could decrease the blood glucose level, total cholesterol and LDL, and increase the body weight (18). Also, another study showed that after a single dose or 14 daily doses of the *C. carvi* aqueous extract (20 mg/kg) oral administration, a significant decrease in blood glucose levels of diabetic rats was produced. Also, there was no significant effect on basal plasma insulin concentrations in either normal or diabetic rats, so they concluded that the underlying mechanism of this pharmacological activity seems to be independent of insulin secretion (16). Based on these results, it assumed that *C. carvi* inhibits hepatic glucose production, and stimulates glucose utilization by peripheral tissues, and its hypoglycemic effects are independent of insulin secretion. Also, it can act as an inhibitor of renal glucose reabsorption and intestinal glucose absorption. These all beneficial effects of *C. carvi* may be related to its main anti-oxidant constituents (16-18).

After determining the effective doses of Afghan *H. sabdariffa* and *C. carvi* hydro-alcoholic extracts on FBG level, the effect of the combination of their effective doses in different proportions on FBG level of diabetic rats, was evaluated. The results showed that only 75:25 combination of *H. sabdariffa* and *C. carvi* effective doses were significantly decreased the FBG level in diabetic rats, especially on 7th day, and even normalized. However, as we compare the effect of these individual extracts with combinations of them, one can simply conclude that the effect of individual extracts on the FBG level is much greater than their combinations. Especially important, all doses of *H. sabdariffa* hydro-alcoholic extract which used in this study, could normalize the FBG level in diabetic rats. As a result, Afghan *H. sabdariffa* hydro-alcoholic extract has higher hypoglycemic activity, as compared with *C. carvi* hydro-alcoholic extract and also with different proportions of their combinations. Also, despite the multiple different constituents and different hypoglycemic action mechanisms of *H. sabdariffa* (insulin-dependent) and *C. carvi* (non-insulin dependent) extracts, they don't have any synergic effects on the reduction of FBG level in diabetic rats.

Conclusion

In summary, one can conclude that Afghan *H. sabdariffa* and *C. carvi* hydro-alcoholic extracts and their combination (75:50) have a hypoglycemic effect. However, despite the multiple different constituents and different hypoglycemic action mechanisms, their combinations do not have any synergic effects, so the hypoglycemic effect of *H. sabdariffa* was much greater and even normalized.

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