COMPARATIVE STUDIES ON THE INFLUENCES OF *Juniperus phoenicea* AND *Hyphaene thebaica* AS HYPOGLYCEMIC FACTORS IN DIABETIC RATS

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ABSTRACT

The effects of methanolic extracts of *Juniperus phoenicea* and *Hyphaene thebaica* on carbohydrate metabolism of male streptozotocin-treated diabetic rats were studied. Blood glucose, liver glycogen and the activities of lactate dehydrogenase (LDH), hexokinase (HK) and pyruvate kinase (PK) as well as the contents of pyruvate and lactate were determined in brain, liver and kidney tissues of the experimental animals. The ingestion of both methanolic extracts significantly reduced the high level of blood glucose but elevated the liver glycogen content of the diabetic rats. PK activity reached the highest values in liver and kidneys but brain showed the minimal stimulation. HK activity had nearly the same trend as that of PK. In case of LDH, the activity was also stimulated in the examined organs of the treated animals. In addition, pyruvate contents were significantly decreased.

KEYWORDS: *Hyphaene thebaica*, *Juniperus phoenicea*, hypoglycemic factors, diabetes.

1. INTRODUCTION

Nature has been a source of medicinal agents since the beginning of time. Herbal medicine is still the most common source for primary health care of about 65-80% of the world's population, mainly in developing countries, because of better cultural acceptability, better compatibility with the human body and fewer side effects. Leaves, flowers, stems, roots, seeds, fruit and bark can all be constituents of herbal medicines [1-5]. The medicinal values of these plants lie in their phytochemical components which produce definite physiological actions on the human body. The most important of these components are alkaloids, tannins, flavonoids and phenolic compounds [6-10].

Medicinal plants are traditionally used in Egypt for the treatment of diabetes, defined as a chronic disorder of carbohydrate metabolism, characterized by hyperglycemia. It depends on a deficiency of insulin, resulting from either insufficient supply or diminished effectiveness [11, 12]. Many studies were done on the existing methods for treating diabetes, and one may conclude that non is completely satisfactory. The search for natural remedies to help in the treatment of diabetics has been as persistent as in most chronic ailments. In Egypt folk medicine, many plants are used in the treatment of diabetes mellitus e.g. *Bryonia cretica*, *Salix* species, *Lupinus termis* L., Fenugreek (*Trigonella foenum-graecum*) and *Allium* species [13].

Arar (*Juniperus phoenicea*) growing wild in the mountains of Hala, North Sinai and doum (*Hyphaene thebaica*), an African palm tree, exhibit a variety of pharmacodynamic effects, such as diuretic, carminative, antiseptic and abortive ones [14, 15], and it is reported that both have antidiabetic activities [16].

The aim of this research was planned with the following objectives: 1) to verify the hypoglycemic activity of both plants, which are traditionally used as hypoglycemic agents, 2) to evaluate the effect of administration of the chosen plants on the metabolism of carbohydrate in blood and some organ tissues (brain, liver and kidneys) of diabetic adult male albino rats.

2. MATERIALS AND METHODS

2.1. Preparation of samples

Juniperus phoenicea (Arâr*)* and *Hyphaene thebaica* (Doum) grown in Egypt were used in the present work. Whole plants of *Juniperus phoenicea* and resins of *Hyphaene thebaica* fruits were extracted successively using ether and methanol, respectively. Each fraction was concentrated under vacuum using a rotary evaporator at 40 ºC, and stored at -20 ºC until used. All the fractions were suspended at different concentrations in water or cottonseed oil before oral ingestion by the diabetic rats (daily doses of 150 mg/kg body weight (B.W.) for 6 weeks of each of the plant extracts).

2.2. Experimental animals

A total of 36 adult male albino rats of Wister strain (three months old and weight average of 150 g) were kept separately in well-aerated cages under hygienic conditions.

Rats were maintained with free access to water and a standard diet (consisting of casein 15 %, cotton seed oil 10 %, salt mixture 4 %, vitamins mixture 1 %, starch 65 % and cellulose 5%) for two weeks (adaptation period) [17]. Diet and water were supplied *ad libitum*. To induce diabetes, 30 rats were intraperitoneally (i.p.) injected with a single dose of streptozotocin (60 mg/kg B.W.) [18]. Blood samples were withdrawn from orbital venous plexuses and blood glucose was determined. The diabetic rats were then randomly assigned and divided into 5 groups, each of 6 rats, and fed on the basal diet.

The first group (6 normal rats without injection of streptozotocin) was used as normal control. The 2nd group (6 of diabetic rats) was used as diabetic control. The 3rd group (6 of diabetic rats) was ingested with 150 mg/kg B.W. of *Juniperus phoenicea* ether extract, and the 4th one (6 of diabetic rats) with 150 mg/kg B.W. of *Juniperus phoenicea* methanolic extract. For the 5th and 6th groups each of 6 diabetic rats, ether and methanol extracts of doum fruits were applied analogously. All of the 6 animal groups were fed on the basal diet for 6 weeks (experimental period). Then, all rats were killed by decapitation. Blood, brain, liver and kidneys of the experimental animals were removed and chilled up for analysis.

2.3. Blood biochemical analysis

Determination of blood glucose and liver glycogen were adopted using the methods of Trinder [19] and Rerup and Lundguist [20], respectively. Determination of pyruvate kinase (PK), lactate dehydrogenase (LDH) and hexokinase (HK) activities in brain, liver and kidney tissues were carried out according to Czok and Lamprecht [21] as well as Bergmeyer [22], respectively.

All analyses were performed in triplicate (n=3). Statistical analysis was done using SPSS (version 10) program. Mean and standard error were descriptive measures of quantitative data using the analysis of variance (ANOVA) test for independent samples (P <0.05 considered to be significant).

3. RESULTS AND DISCUSSION

The hyperglycemia produced in animals is the main aberration of carbohydrate metabolism, observed in detail during certain phases. The first one is an inhibition in glucose utilization in the animal body tissues (muscle and adipose, or others) [23].

The results in Table 1 show the effects of ingestion of ether and methanolic extracts on blood glucose and liver glycogen levels of diabetic rats. However, diabetes caused highly significant elevation of blood glucose (309 mg/ 100 ml). Diabetes may elevate hepatic glycogenolysis and gluconeogensis as well as reduce the removal of glucose from blood into body tissues. Ingestion of ether extracts did not affect blood glucose levels of diabetic rats but that of methanolic extracts significantly reduced these levels, observed with both experimental plants. These reduced levels, however, were still higher than those of the normal control. It can be revealed that the methanolic extracts of both plants had potent hypoglycemic effects, possibly be due to the occurrence of active principles (like insulin action on entry of glucose into cells and achieving better utilization of glucose).

Table 1 shows the effects of both plant extracts on hepatic glycogen content of diabetic rats. Diabetes generally elevated blood glucose, accompanied by reduction in liver glycogen. As shown, methanolic extracts of both plants increased the contents of liver glycogen similarly and significantly whereas the increase with ether extracts was lower and not significantly.

Methanolic extracts` ingestion reduced the blood glucose levels in diabetic animals through conversion of glucose to glycogen in the liver, and, therefore, this ingestion might stimulate hepatic glycogenosis by enhancing of hepatic glycogen synthesis activity. But this stimulation by methanolic extracts probably was not the sole factor for reduction of blood glucose in diabetic rats. These above agents might also stimulate the utilization of glucose by peripheral tissues [24, 25].

LDH, PK and HK activities in the three organ tissues (liver, brain and kidneys) of normal and diabetic rats ingesting ether and methanolic extracts of the experimental plants were determined and results are shown in Table 2. Diabetes was characterized by alterations of HK and PK activities in all studied organs. HK activity reached a maximal value in liver tissues, whereas kidneys showed minimal values relative to control. In case of PK activity, brain tissues showed the highest values, followed by kidneys and liver tissues. In addition, LDH activity of brain

TABLE 1 - Plant extract influences on blood glucose and liver glycogen levels of diabetic rats.

Treatments	Blood glucose		Liver glycogen	
	mg/100 ml	%	mg/100g	%
Normal control	93±7 [c]	100	5.20±0.4 [a]	100
Diabetic control	309±20 [a]	332	0.51±0.04 [c]	9.80
Ether extract of *Juniperus phoenicea*	300±25 [a]	323	0.48±0.03 [c]	9.23
Methanolic extract of *Juniperus phoenicea*	191±17 [b]	205	1.00±0.07 [b]	19.2
Ether extract of *Hyphaene thebaica*	302±26 [a]	325	0.50±0.04 [c]	9.61
Methanolic extract of *Hyphaene thebaica*	187±19 [b]	201	1.01±0.10 [b]	19.4

%: relative to normal control; each value represents the mean ± SD; the mean values with different letters within a column indicate significant differences (P< 0.05).

TABLE 2 - Plant extract influences on LDH, HK and PK activities of diabetic rats organs.

Treatment	PK activity (µmol glucose phpsphate/min/mg protein)						HK activity (µmol glucose phpsphate/min/mg protein)						LDH activity (µmol NADH.H$^+$/min/mg protein)					
	Brain		Liver		Kidneys		Brain		Liver		kidneys		Brain		Liver		kidneys	
	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%
Normal control	7.29 ^{a} ±0.66	100	2.72 ^{b} ±0.17	100	3.88 ^{b} ±0.40	100	0.062 ^{a} ±0.007	100	0.043 ^{a} ±0.003	100	0.052 ^{a} ±0.004	100	0.111 ^{a} ±0.012	100	0.051 ^{a} ±0.004	100	0.041 ^{a} ±0.003	100
Diabetic control	7.20 ^{a} ±0.71	98.8	2.59 ^{b} ±0.21	95.2	3.60 ^{b} ±0.29	92.8	0.056 ^{b} ±0.004	90.3	0.040 ^{a} ±0.004	93.0	0.045 ^{b} ±0.003	86.5	0.082 ^{b} ±0.007	73.9	0.045 ^{b} ±0.004	88.2	0.038 ^{a} ±0.004	92.7
Ether extract of *Juniperus phoenicea*	7.22 ^{a} ±0.69	99.0	2.60 ^{b} ±.19	95.6	3.58 ^{b} ±0.31	92.3	0.055 ^{b} ±0.005	88.7	0.041 ^{a} ±0.004	95.3	0.044 ^{b} ±0.004	84.6	0.081 ^{b} ±0.008	73.0	0.045 ^{b} ±0.005	88.2	0.037 ^{a} ±0.003	90.2
Methanolic extract of *Juniperus phoenicea*	7.90 ^{a} ±0.80	108	3.40 ^{a} ±0.30	125	4.56 ^{a} ±0.42	117	0.065 ^{a} ±0.001	105	0.044 ^{a} ±0.003	102	0.055 ^{a} ±0.004	106	0.109 ^{a} ±0.009	98.2	0.051 ^{a} ±0.004	100	0.040 ^{a} ±0.002	97.6
Ether extract of *Hyphaene thebaica*	7.23 ^{a} ±0.64	99.2	2.61 ^{b} ±0.16	96.0	3.61 ^{b} ±0.29	93.0	0.056 ^{b} ±0.004	90.3	0.040 ^{a} ±0.004	93.0	0.045 ^{b} ±0.005	86.5	0.082 ^{b} ±0.007	73.9	0.043 ^{b} ±0.005	84.3	0.039 ^{a} ±0.002	95.1
Methanolic extract of *Hyphaene thebaica*	7.60 ^{a} ±0.70	104	3.11 ^{a} ±0.29	114	4.30 ^{b} ±0.41	111	0.060 ^{a} ±0.002	96.8	0.048 ^{a} ±0.005	112	0.053 ^{a} ±0.004	102	0.099 ^{a} ±0.009	89.2	0.048 ^{ab} ±0.004	94.1	0.038 ^{a} ±0.003	92.7

% relative to normal control; each value represents the mean ± SD; mean values with different letters within a column indicate significant differences (P< 0.05)

was highest, followed by that of liver and kidney tissues. Ingestion of both methanolic plant extracts stimulated PK, HK and LDH activities in all three organ tissues (Table 2) but was inhibited by injection of streptozotocin (diabetic rats). Effects of *Juniperus phoenicea* methanolic extract were more distinctive than that of *Hyphaene thebaica*.

HK, the enzyme controlling (key) or rate limiting reaction governing glucose utilization in different tissues followed nearly the same trend as PK. After ingestion of methanolic *Juniperus phoenicea* extracts, all enzyme activities reached a maximum value relative to control. The observed stimulations in LDH, HK and PK activities by methanolic extracts of both plants in the three organ tissues were paralleled by stimulation of glycolysis/utilization of pyruvate and glucose [26].

The observed alterations in PK and HK activities in the brain, liver and kidneys of diabetic rats were combined with the inhibition of glycolysis confirmed by the obtained results in Table 3 (effects of plant extract ingestion on lactate and pyruvate contents of brain, liver and kidneys of diabetic animals). There was a highly significant decrease in pyruvate content in all three organs (maximum and minimum decrease noticed in liver and kidneys, respectively). On the other hand, lactate content in diabetic rat liver was significantly increased, and maximum and minimum values were noticed in liver and kidneys, respectively. The high increase in lactate content was mainly due to inhibition of muscular pyruvate dehydrogenase, or degradation of muscular proteins [27].

Thus, the lactate formed by skeletal muscles and erythrocytes was recycled to the liver reforming glucose again by stimulatory gluconeogenic processes [28].

Ingestion of methanolic plant extracts was characterized by a significant increase in pyruvate but decrease in lactate, respectively, in the organ tissues. Therefore, these treatments might stimulate the activity of muscular pyruvate dehydrogenase to a normal level, or might prevent the muscular protein degradation [29].

Activity of hepatic lactate dehydrogenase might be stimulated by the present methanolic extracts. This conclusion was confirmed when hepatic LDH was measured as indicated in Tables 2 and 3. The decreasing rate of hepatic lactate content did not correspond to the increasing rate of hepatic pyruvate.

TABLE 3 - Plant extract influences on lactate and pyruvate contents of diabetic rat organs.

Treatment	Lactate (µmol/g tissue)						Pyruvate (µmol/g tissue)					
	Brain		Liver		Kidneys		Brain		Liver		Kidneys	
	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%
Normal control	2.84 ^{b} ± 0.29	100	1.31 ^{b} ± 0.11	100	1.62 ^{b} ± 0.12	100	0.72 ^{a} ± 0.06	100	0.81 ^{a} ± 0.07	100	0.21 ^{a} ± 0.011	100
Diabetic control	3.65 ^{a} ± 0.31	128	1.75 ^{a} ± 0.12	134	1.98 ^{a} ± 0.19	122	0.33 ^{d} ± 0.03	45.8	0.31 ^{d} ± 0.02	38.3	0.11 ^{b} ± 0.010	52.4
Ether extract of *Juniperus phoenicea*	3.66 ^{a} ± 0.24	129	1.78 ^{a} ± .17	136	2.00 ^{a} ± 0.14	123	0.32 ^{d} ± 0.02	44.4	0.30 ^{d} ± 0.03	37.0	0.12 ^{b} ± 0.010	57.1
Methanolic extract of *Juniperus phoenicea*	2.90 ^{b} ± 0.28	102	1.33 ^{b} ± 0.11	101	1.64 ^{b} ± 0.15	101	0.55 ^{b} ± 0.06	76.4	0.58 ^{b} ± 0.06	71.6	0.13 ^{b} ± 0.011	61.9
Ether extract of *Hyphaene thebaica*	3.59 ^{a} ± 0.36	126	1.80 ^{a} ± 0.17	137	1.99 ^{a} ± 0.20	123	0.34 ^{d} ± 0.02	47.2	0.29 ^{d} ± 0.03	35.8	0.11 ^{b} ± 0.010	52.4
Methanolic extract of *Hyphaene thebaica*	3.11 ^{b} ± 0.30	109	1.47 ^{b} ± 0.14	112	1.84 ^{b} ± 0.17	114	0.43 ^{c} ± 0.03	59.7	0.49 ^{c} ± 0.04	60.5	0.12 ^{b} ± 0.009	57.1

%: relative to normal control; each value represents the mean ± SD; mean values with different letters within a column indicate significant differences (P< 0.05).

Probably, pyruvate was rapidly consumed by several enzymes, such as pyruvate dehydrogenase, pyruvate carboxylase and pyruvate transaminase, in many reactions [30].

In addition, the ingestion of methanolic extracts of both plants showed their hypoglycemic influences on blood glucose content in diabetic animals relative to normal control. The hypoglycemic effect of *Prunus davidiana* methanolic extract was due to the presence of the main flavanone glycoside and pruning components [31]. Also, the presence of glycosidic components in aqueous extracts of *Citrullus colocynthis* fruits induced hypoglycemic action [32]. In addition, the same behavior of *Anastatica hierochuntica* extract was due to the presence of glycosidic flavones, which were responsible for reducing the blood glucose levels in streptozotocin-diabetic rats [33].

The results herein confirmed above suggestions because the phytochemical screening of successive extracts of *Juniperus* plants revealed the presence of alkaloids, saponins, resins, flavonoids, tannins and phenols in the aqueous extracts but not in ether extracts which had sterols, lipid fractions and lipid-soluble vitamins [25, 34]. In addition, *Juniperus phoenicea* (Arar) and *Hyphaene thebaica* (Doum) showed an antioxidant activity; substantially due to high amounts of phenols, flavonoids, glycosides, β-carotene and lycopene components (soluble in water and methanol) [1, 16, 35].

4. CONCLUSION

The present results of the ingestion of methanolic extracts of *Juniperus phoenicea* and *Hyphaene thebaica* into diabetic animals improved the carbohydrate metabolism of diabetic rats. In contrast, the administration of ether extracts of both plants did not improve carbohydrate metabolism.

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