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Effect of feeding some herbs on body weight and blood glucose and insulin levels in normal and alloxan - diabetic rats

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Abstract

The present work was undertaken to study the effect of feeding 3 herbs viz.: *Ambrosia maritima* (Damsissa), *Aloe vera* (Sabbar) and *Lactuca virosa* (Lebbien), alone and in combination, on body weight , blood glucose and insulin levels in normal and alloxan - diabetic rats. The obtained results revealed that feeding normal and diabetic rats on diets supplemented with *Ambrosia maritima* or *Aloe vera* or *Lactuca virosa* or a mixture of them did not affect body weight at end of the study period. Diets containing *Ambrosia maritima* or *Aloe vera* or *Lactuca virosa* at 10 % concentration significantly decreased blood glucose levels in normal and diabetic rats. Feeding diets supplemented with *Ambrosia maritima* or *Aloe vera* at 10 % concentration caused significant increases in serum insulin levels in diabetic rats, while the diet containing *Lactuca virosa* at 10 % concentration did not. Diet mixed with the 3 tested herbs at 5 % concentration when fed for 3 weeks to both normal and diabetic rats caused significant decreases in blood glucose and increases in serum insulin levels at end of the study period. In conclusion, feeding normal and diabetic rats on diets supplemented with a mixture consisting of *Ambrosia* maritima, *Aloe* vera and Lactuca virosa at 5 % concentration for 3 weeks produced significant hypoglycemic and hyperinsulinemic effects, but did not affect body weight. Therefore, daily intake of mixture of these herbs for 3 weeks may be useful in the management of type 2 diabetes mellitus.

Key words: Herbs - Ambrosia maritima - Aloe vera - Lactuca virosa – Body weight gain Blood glucose - Insulin - Alloxan diabetic rat.

Introduction

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia due to insulin deficiency or insulin resistance. Hyperglycemia occurs because the liver and skeletal muscles can not store glycogen and the tissues are unable to utilize glucose. This disease is one of the common causes of deaths in Egypt. The prevalent treatment of diabetes mellitus besides controlling food

intake and proper exercise includes insulin injection and administration of oral hypoglycemic drugs ***(Rang and Dale, 1991).***

From time immemorial, it has been accustomed to treat diabetes mellitus through folk medicine in Egyptian villages. Egyptian folk medicine has described 25 kinds of herbs, belonging to 13 families, to be concerned with the treatment of diabetes mellitus. These herbs were examined for their hypoglycemic and antidiabetic activities in rats, rabbits and mice and some of them have been selected to develop new drug resources to be safely used for treating diabetes mellitus ***(Eskander and Jun, 1995).*** Some of these herbs and plants such as lupinus termis ***(Shani et.al., 1974);*** Trigonella foenum greacum ***(Sharma, 1986) ;*** Balanites aegyptiaca ***(Eskander, 1994) ;*** Zizyphus spina christi ***(Glombitza et.al., 1994)*** ; Urtica dioica ***(Bijan Farzamie et.al., 2003)*** and Ginseng panax ***(Xie et.al., 2005)*** decreased blood glucose level, while others such as Ginseng radix ***(Waki et.al.,1982) ;*** Trigonella foenum greacum ***(Sharma, 1986) ;*** Vinca rosea ***(Chattopadhyay et.al.,1991); Rhazya stricta (Ali, 1997) and Urtica dioica (Bijan Farzamie et.al., 2003)*** increased insulin levels in diabetic rats and mice. It was found that feeding rats a ration containing lupinus termis powder decreased the fasting blood glucose level due to its content of alkaloids ***(Youness, et.al., 1985). Sharma, (1986)*** reported that feeding ***Fenugreek (Hulba)*** seeds caused hypoglycemic and antidiabetic effects in human subjects. ***Eskander et.al. (1994)*** reported that a formulation containing 3 herbs caused hypoglycemic effect in alloxan - diabetic rats. Moreover, a high dietary fiber in herbs and plants stabilized glucose and insulin levels and might be useful in treating type 2 *diabetes mellitus* ***(Leeuw et.al., 2004).***

The present study was designed to investigate the effect of feeding three herbs viz.: *Ambrosia* ***maritima (Damsissa), Aloe vera (Sabbar), Lactuca virosa (Lebbien),*** alone and combined, on body weight, blood glucose and insulin levels in normal and diabetic rats.

Materials and Methods

Materials:

Herbs:

Ambrosia maritima (Damsissa) Family Compositae, Aloe vera (Sabbar) Family Liliceae and Lactuca virosa (Lebbien) Family Compositae were purchased as dried materials from a local Company for Medicinal Plants and Herbs, Egypt.

Rats:

A total of 70 male albino rats weighing 140 -155 gm body weight and 10-12 week age of Sprague Dawley strain were obtained from the Laboratory Animal Colony, Helwan, Egypt. Rats

were kept under hygienic conditions in plastic cages, fed on basal diet only and water was provided ad libitum. Animals were left for one week before the start of the experiment for **Acclimatization.**

Alloxan:

It was purchased from El-Gomhoryia Company for Chemicals; Cairo, Egypt in the form of a white power packed in dark brown bottles each containing 25gm alloxan monohydrate.

Biochemical kits:

Glucose enzymatic kits of BioMeriuex were obtained from Alkan Company for Chemicals and Biodiagnostics, Dokki, Egypt for the determination of serum glucose level. Radioimmunoassay kits were obtained from Diagnostic Products Corporation, 5700 West 96th street, Los Angeles, USA for the estimation of serum insulin level.

Methods:

Preparation of basal diet:

Basal diet was prepared according to Reeves *et.al.* (1993). It consisted of: 20 % protein (casein), 10% sucrose, 4.7% corn oil, 2% choline chloride, 1% vitamin mixture, 3.5 % salt mixture and 5% fibers (cellulose). The remainder is corn starch.

Preparation of experimental diets:

Four diets supplemented with the tested herbs were prepared. The studied herbs were grinded in an electrical mixer into a fine powder. Each 100 gm of *Ambrosia maritima* or *Aloe vera* or *Lactuca virosa* herb was properly mixed with 900 gm of the basal diet to form three different experimental diets, each at 10% concentration (w/w of the diet). The 4th experimental diet contained a mixture of the 3 tested herbs and was prepared by proper mixing of 50 gm from each herb with 850 gm of the basal diet (5% concentration of herb mixture in the diet).

Grouping and feeding of rats:

All rats were fed for one week on the basal diet for acclimatization. The rats were then divided into two main groups (n=35 rats). The 1st main group was subdivided into 5 subgroups (n=7 rats) and rats was fed for 3 weeks as following :-

Subgroup (1): received the basal diet only and served as a normal control.

Subgroup (2): received Ambrosia maritima - supplemented diet (10% Concentration) for 3 weeks.

Subgroup (3): received *Aloe* vera - supplemented diet at the same concentration and for the same period.

Subgroup (4): received Lactuca virosa - supplemented diet at the same concentration and for the same period.

Subgroup (5): received basal diet supplemented with a mixture of the 3 tested herbs, at 5 % from each herb, for the same period.

All rats of the 2nd main group were rendered diabetic by S/C injection of alloxan in a single daily dose of 150 mg/kg body weight for 3 days to induce acute early diabetes according to the method described by Katsumata and Katsumata (2006). On the 4th day, the diabetic rats were subdivided and fed in the same manner and for the same period such as in the 1st main group.

Effect of dietary supplement on body weight:

Rats were weighed at beginning and end of the study period. The effect of feeding different experimental diets on body weight gain (%) of rats was calculated at end of the feeding period based on the differences between initial and final weights using the following equation:

BWG =((FBW – IBW) / IBW) × 100

Where: BWG = Body weight gain

FBW = Final body weight at end of the study period

IBW = Initial body weight before the beginning of the study period

Blood sampling and biochemical analysis:

Before sampling, all rats were fasted for 18-20 hours and allowed to excess of free water. Blood samples were collected from all rats in each group randomly (normal and diabetic groups) before start of the study period to estimate the normal and diabetic blood glucose and insulin pre feeding levels. Blood samples were then collected at weeks 1, 2, and 3 post feeding. Blood was withdrawn from the orbital sinus by puncture and left to clot for separating serum by centrifugation at 1500 rpm for 15 minutes. Serum samples were directly frozen at –10°C till biochemical analysis. Estimation of glucose in the serum was carried out using enzymatic glucose kits according to the method described by ***Siest et.al., (1981).*** Serum insulin was determined using radioimmunoassay kits according to the method of Yallow and ***Bauman, (1983).***The latter assay was performed in Hormone unit, Medical Division, National Research Center, Dokki, Egypt.

Statistical analysis:

Data were statistically analyzed using one way analysis of variance (ANOVA) test Student "t" test was used for the significance between groups according to Snedecor and ***Cochran (1980).***

Results and Discussion

The obtained results revealed that feeding of *Ambrosia* maritima (Damsissa) or *Aloe* vera (Sabbar) or Lactuca virosa (Lebbien) or a mixture of them to normal and diabetic rats for 3 weeks did not significantly affect body weight gain. While diabetic rats fed the basal or experimental diets significantly ($p \leq 0.05$) gained less body weight as compared to the normal rats. For diabetic control rats, the body weight gain was 8.50 ± 1.22 gm versus 13.90 ± 1.20 gm for normal control rats at

end of the study period, as recorded in Table (1).The decrease in body weight of diabetic rats may be attributed to effect of the disease as it is well known that diabetes mellitus is characterized by body weight loss ***(Xie et.al., 2005).***

As show in Tables (2) and (3) fasting blood glucose level in normal rats before start of the study period was 82.55 mg/dL versus 345.55 mg/dL in diabetic rats. This finding indicated that S/C injection of alloxan in a dose of 150 mg/kg b.wt. for 3 days induced an early acute diabetes mellitus. A similar effect was previously reported by ***Atta et.al, (1983), Eskander and Jun, (1995) and Katsumata and Katsumata (2006).***

Feeding normal and diabetic rats diets supplemented with *Ambrosia maritima* or *Aloe vera* or *Lactuca virosa* at 10 % or a mixture of them at 5 % concentration significantly ($P \leq 0.01$ or $P \leq 0.001$) decreased blood glucose levels . At end of the study period (3 weeks), the percentages of decrease in blood glucose levels in normal control rats were 31.5, 25.1, 23.9 or 39.1% for diets mixed with *Ambrosia maritima, Aloe vera, Lactuca virosa* or their combined mixture, respectively. In diabetic rats, the corresponding percentages of decrease in blood glucose levels were 18.7, 16.8, 11.9 or 24.2%, respectively (Tables 2 and 3 respectively).

The reported hypoglycemic and antihyperglycemic (antidiabetic) effects of *Ambrosia maritima* in the present study were nearly similar to those reported by ***Eskander and Jun, (1995).*** These effects were attributed to the presence of two active sesquiterpenes (ambrosin and damsin) isolated from *Ambrosia maritima* as mentioned by ***Ammar et.al., (1993).*** Feeding *Aloe vera* to normal and diabetic rats produced hypoglycemic effects as reported in this study. These effects were previously reported by ***Ajabnoor, (1991) and Tanaka et.al., (2006)*** in mice and by ***Eskander and Jun, (1995) in rats. Tanaka et.al., (2006)*** mentioned that hypoglycemic and antihyperglycemic effects of *Aloe vera* were due to presence of five phytosteroles isolated from *Aloe* leaves. The hypoglycemic and antihyperglycemic effects of *Lactuca virosa*, reported in the present study, were in accordance with those reported by ***Eskander and Jun, (1995).***These effects were attributed to the presence of a bitter compound called lactcopicrin as mentioned by ***Eskander and Jun, (1995) and Jia et.al., (2003).***

Concerning the effect of the studied herbs on serum insulin levels in normal and diabetic rats, the results in Tables (4) and (5) showed that feeding the experimental diets contained *Ambrosia maritima* or *Aloe vera* herb significantly($P \leq 0.05$) increased serum insulin levels; while that contained *Lactuca virosa* did not. The experimental diet contained a mixture of the 3 tested herbs produced a significant ($P \leq 0.01$) increase in serum insulin levels in normal and diabetic rats. The hyperinsulinemic effect produced by diet mixed with the tested herbs could be attributed to the presence of *Ambrosia maritima* and *Aloe vera* because both herbs significantly increased serum insulin levels; while Lactuca *virosa* was devoid of this activity. The increase in serum insulin levels in normal and diabetic rats fed experimental diets contained *Ambrosia maritima* or *Aloe vera* or a

mixture of both was similar to that previously reported by ***Eskander and Jun, (1995).*** However, other herbs and medicinal plants were reported to increase insulin secretion by stimulating the β cells of islets of Langerhans or to contain active components which exerted insulin like effects ***(Chattopadhyay, 1993; Eskander et.al., 1994; Al- Habori and Rhaman, 1998; Gray and Flatt, 1999; and Aritajat et.al., 2004).***

The mechanism(s) of hypoglycemic and antihyperglycemic (antidiabetic) actions of *Ambrosia maritima* and *Aloe vera* reported in the present study can be possibly explained by: (a) enhancing glucose metabolism ***(Eskander and Jun, 1995)*** or (b) decreasing the intestinal absorption of glucose ***(Leeuw et.al., 2004)*** and / or (c) increasing insulin secretion from β cells of pancreas ***(Ajabnoor, 1991 and Ammar et.al., 1993).*** *Lactuca virosa* herb may exert its hypoglycemic effect by decreasing glucose absorption or enhancing glucose metabolism, but not by acting on pancreatic β cells of Langerhans as it did not affect insulin level.

In conclusion, feeding normal and diabetic rats diets supplemented with a mixture of herbs containing *Ambrosia* maritima, Aloe vera and Lactuca virosa at 5 % concentration for 3 weeks produced significant hypoglycemic and hyperinsulinemic effects, but did not affect body weight. Therefore, daily intake of this herb mixture for 3 weeks may be useful in the management of type 2 diabetes mellitus.

Table (1): Effect of feeding of some herbs mixed with the basal diet on body weight gain (%) of normal and diabetic rats. (n= 7 rats)

Subgroups	Body weight gain (%) at the end of dietary period (3 weeks)	
	Normal rats	Diabetic rats
Control (basal diet only)	13.90 ± 1.20 [a]	8.50 ± 1.22 [b]
Ambrosia maritima (10%)	13.85 ± 1.50 [a]	8.45 ± 1.16 [b]
Aloe vera (10%)	13.70 ± 1.40 [a]	8.30 ± 1.14 [b]
Lactuca virosa (10%)	13.85 ± 1.30 [a]	8.20± 1.12 [b]
Mixture of the 3 herbs (5%)	13.60 ± 1.20 [a]	8.25 ± 1.30 [b]

Values with different superscript letter differ significantly at $P \leq 0.05$ (ANOVA test)

Table (2): Effect of feeding of herbs on blood glucose level in normal rats.

Subgroups (n = 7 rats)	Mean ± SEM of serum glucose level (mg/dL)				
	After feeding for				
	PSL	1 week	2 weeks	3 weeks	% decrease
Control (basal diet only)	82.55± 2.40	82.60 ± 2.35	82.60 ± 2.40	82.55 ± 2.25	—
Ambrosia maritima (10%)		61.35 ± 3.40 *	60.50 ± 2.54 *	56.50 ± 3.36 *	31.5
Aloe vera (10%)		63.50 ± 2.30 *	62.50 ± 2.60 *	61.80 ± 2.45 *	25.1
Lactuca virosa (10%)		65.45 ± 2.25 *	64.80 ± 2.40 *	62.80 ± 2.10 *	23.9
Mixture of the 3 herbs (5%)		53.35 ± 2.33 **	52.50 ± 2.43 **	50.20 ± 2.39 **	39.1

PSL =Prestudy level of serum glucose for 7 rats of the 1st main group.

* Significant at P ≤ 0.01 ** Significant at P ≤ 0.001

Table (3): Effect of feeding of herbs on blood glucose level diabetic rats.

Subgroups (n = 7 rats)	Mean ± SEM of serum glucose level (mg/dL)				
	After feeding for				
	PSL	1 week	2 weeks	3 weeks	% decrease
Control (basal diet only)	345.55 ± 12.65	344.55 ± 12.20	343.55 ± 18.25	343.55 ± 13.35	—
Ambrosia maritima (10%)		292.65 ± 12.20 *	290.35 ± 19.20 *	279.25 ± 13.20 *	18.7
Aloe vera (10%)		298.65 ± 15.30 *	294.65 ± 17.24 *	285.65 ± 13.65 *	16.8
Lactuca virosa (10%)		312.55 ± 19.24 *	305.55 ± 17.33 *	302.55 ± 18.22 *	11.9
Mixture of the 3 herbs (5%)		265.30 ± 18.60 **	264.20 ± 16.80 **	260.25 ± 14.20 **	24.2

PSL =Prestudy level of serum glucose for 7 rats from the 2nd main group.

* Significant at P ≤ 0.01 ** Significant at P ≤ 0.001

Table (4): Effect of feeding of herbs on serum insulin level in normal rats.

Subgroups (n = 7 rats)	Mean ± SEM of serum insulin level (µU/ml)			
	After feeding for			
	PSL	1 week	2 weeks	3 weeks
Control (basal diet only)	4.55 ± 0.88	4.50 ± 0.58	4.52 ± 0.38	4.55 ± 0.48
Ambrosia maritima (10%)		6.55 ± 0.38 *	6.35 ± 0.26 *	6.27 ± 0.25 *
Aloe vera (10%)		7.35 ± 0.18 *	7.15 ± 0.32 *	7.30 ± 0.28 *
Lactuca virosa (10%)		4.35 ± 0.55	4.45 ± 0.38	4.44 ± 0.78
Mixture of the 3 herbs (5%)		9.45 ± 0.38 **	9.35 ± 0.26 **	9.27 ± 0.25 **

PSL =Prestudy level of serum insulin for 7 rats from the 1st main group.

* Significant at P ≤ 0.05 ** Significant at P ≤ 0.01

Table (5): Effect of feeding of herbs on serum insulin level in alloxan - diabetic rats.

Subgroups (n = 7 rats)	Mean ± SEM of serum insulin level (µU/ml)			
	After feeding for			
	PSL	1 week	2 weeks	3 weeks
Control (basal diet only)	2.65 ± 0.38	2.55 ± 0.28	2.45 ± 0.36	2.38 ± 0.25
Ambrosia maritima (10%)		4.35 ± 0.37 *	4.55 ± 0.38 *	4.65 ± 0.26 *
Aloe vera (10%)		5.75 ± 0.35 *	5.73 ± 0.30 *	5.75 ± 0.28 *
Lactuca virosa (10%)		2.45 ± 0.28	2.35 ± 0.36	2.55 ± 0.32
Mixture of the 3 herbs (5%)		7.45 ± 0.14 **	7.25 ± 0.13 **	7.65 ± 0.18 **

PSL =Prestudy level of serum insulin for 7 rats from the 2nd main group.

* Significant at p ≤ 0.05 ** Significant at p ≤ 0.01

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تأثير التغذية ببعض الأعشاب على وزن الجسم ومستوى سكر الدم وهرمون الانسولين فى الفئران السليمة والمصابة بداء السكرى

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الملخص العربي

استهدف هذا البحث دراسة تأثير التغذية ببعض الأعشاب وهى الدمسيسة ، الصبار و حصى لبان - منفردة و متحدة معا - على وزن الجسم ومستوى سكر الدم وهرمون الأنسولين فى الفئران السليمة والمصابة بداء السكرى.

وأظهرت النتائج أن تغذية الفئران السليمة والمصابة بداء السكرى على عليقة مدعمة بهذه الأعشاب سواء كانت منفردة أومتحدة معا لفترة ثلاثة اسابيع ليس لها تأثير على وزن الجسم فى نهاية فترة التغذية.
و أدت التغذية على العليقة المدعمة باعشاب الدمسيسة أو الصبار أو حصى لبان بتركيز ١٠% إلى نقص معنوى فى مستوى سكرالدم فى الفئران السليمة والمصابة بداء السكرى ، وزيادة معنوية فى مستوى هرمون الأنسولين فى مصل هذه الفئران ، بينما لم تسبب العليقة المدعمة بحصى لبان بتركيز ١٠% أى تغييرات معنوية فى مستوى هرمون الأنسولين . كما أدت التغذية على العليقة المدعمة بالأعشاب الثلاثة متحدة معا لفترة ثلاثة اسابيع بتركيز ٥% إلى نقص معنوى فى مستوى سكرالدم وزيادة معنوية فى مستوى هرمون الأنسولين فى مصل الفئران السليمة والمصابة بداء السكرى .

ويتضح من هذة الدراسة أن تغذية الفئران السليمة والمصابة بداء السكرى على عليقة مدعمة بالدمسيسة والصبار و حصىلبان متحدة معا بتركيز ٥ % لفترة ثلاثة اسابيع تؤدى إلى نقص معنوى فى مستوى سكرالدم ، وزيادة معنوية فى مستوى هرمون الأنسولين فى مصل هذه الفئران ، ولا تؤثر على وزن الجسم فى نهاية فترة التغذية. و توصى الدراسة بأن تناول تركيبة مكونة من أعشاب الدمسيسة والصبار و حصىلبان متحدة معا لفترة ثلاثة اسابيع قد يكون مفيدا فى علاج المرضى بداء السكرى .