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USE OF SOME MEDICINAL HERBS TO TREAT DIABETES

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ABSTRACT

This investigation aims at using different percentages (2.5,5 and 10%) of rosemary powder for decreasing blood sugar levels. The result of its chemical composition analysis reveals that rosemary powder is a high source of crude fiber, protien, phenolic compounds and essential fatty acids. The results also show that rosemary has an effective role in lowering blood glucose sugar if used as a diet for diabetics. The infected with diabetes mellitus have been fed for 8 weeks on except for the controlling group G1 that was fed according to the basic diet. Blood samples had been taken and analysed for glucose levels at the beginning of the experiment and after 4 weeks and at the end of the study. The results are analysed statistically. The results indicate that a significant decrease in blood glucose levels for the groups given the rosemary powder diet had has been noticed. However, the difference is more significant in the group given 10 mg/100g. In conclusion, rosmary herb appears to improve hyperglycemia.
Keywords: Rosemary, diabetes, hypoglycemic, antioxidants.

Introduction

Diabetes is a serious and life-long disease, whose prevalence gradually increases, which threatens health and causes many chronic complications endangering the life when not properly managed. Diabetes is also an important public health problem that causes many burdens to the patient, his/her family in physical, emotional, and social aspects throughout life (International Diabetes Federation, 2013). According to the data of the World Health Organization; while the number of diabetic patients was 108 million in 1980, the number of diabetic patients reached 422 million in 2014. The adult population in the world has a diabetes rate of 8.5% and the number of diabetes-induced deaths in 2012 is 1.5 million (OHW, 2016). The World Health Organization has reported that diabetes is among the world's leading causes of death in the 2013-2020 global. Diabetes mellitus is achronic disease disorder of glucose intolerance. It is characterized by high blood glucose level and glycosuria from dysfunction of pancreatic cell and insulin resistance. The defective cells results in lack of total or partial synthesis of insulin. The resistance is caused by cell membrane where glucose is not transport to the cell for oxidation. As glucose is not metabolized. High amount of glucose is circulating in the blood. The kidney removes the extra sugar from the blood and excretes it in the urine. Because glucose is not utilized by the body cells, the body is under constant impression of hunger and that is why diabetes feels increased appetite and eats more frequently (Safadar *et al*., 2006). The prevalence of diabetes is increasing in every part of the world, and the impact is even greater in countries experiencing major socioeconomic development (Wild, *et al.,* 2004). And it is a risk factor for cardiovascular disease and increasing its mortality (American diabetes association 2010). Control of diabetes by herbs and other natural products is becoming popular and is more appropriate and economical for use developing countries (Safadar *et al*., 2006). Medicinal plants have been used for many centuries in the management of many diseases such as Diabetes Mellitus, CVD, hypertension, and many other diseases (El-Hilaly *et al*., 2007). Rosemary composed of dried leaves and flowers constitutes a particularly interesting source of biologically active phytochemicals as it contains a variety of phenolic compounds including carnosol, carnosic acid, rosmanol,7-methyl-epirosmanol, isorosmanol, rosmadial and caffeic acid, with substantial in vitro antioxidant activity(El Deeb, 1993). A wealth of studies demonstrated antioxidant, anti-inflammatory, anti-carcinogenic and hypoglycemic activities of rosemary (Dearlove *et al*., 2008; Nabekura *et al*., 2010). As there is a possibility for Rosemary powder to play a role as a hypoglycemic, the present study was designed to evaluate the effects of it, on profile blood sugar.

Materials and Methods

Materials

Rosemary powder is obtained from a local market, Mansoura City, Egypt.

Male albino rats (Sprague Dawley strain) weighing (130g- 150g) were obtained from the Medical Experimental Research Center, Medicine Faculty , Mansoura University, Egypt.

Analytical Methods

Gross chemical composition:

Protein, fat, crude fibres and ash contents of rosemary was determined according to AOAC (2007). While carbohydrates percentage was calculated by difference :

carbohydrates = 100 – [% protein + % fat + % ash + % crude fiber].

Chemical composition analysis was made to rosemary powder at College of food science, Al. Qasim Green University.

Determination and Identification of phenolic compounds

Phenolic compounds fractionated and identified, by HPLC according to the method of Goupy *et al*. (1999) as follow: 5 g of sample were mixed with methanol and centrifuged at 10000 rpm for 10 min and the supermant was filtered through a 0.2 pm Millipore membrane filter, then 1-3 ml was collected in avail for injection in to HPLC (Agilent 1200 series) auto sampling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm and quaternary HP pump (series 1100). The column temperature was maintained at 35°c. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. Phenolic acid standard from Sigma Co were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used for calculation of phenolic compounds concentration by the data analysis of HEWLLET packaged software.

Biological Experiments

Experimental design

Twenty five adult albino rats (130 - 150 g) were kept under normal healthy conditions, all animals were housed in bottomed cages, fresh and clean drinking water was supplied through specific nipple. Rats were kept at a constant environmental and nutritional conditions throughout the period of the experiment (Temp 24 ± 2 ºC) and 12 hr light-dark cycle). Rats were fed on basal diet for acclimatization, for 10 days. The composition of basal diet (gm/100 gm) as described by **AOAC(1990)**, salt and vitamin mixtures were described by Abo-El Naga (2002). After adaptation, the animals were randomly divided into five groups of equal number and weight (five animals each), which were fed with rosemary powder 2.5,5 and 7.5, respectively.

The body weight was measured on days 0, 28, 60 days of the experiment.

Design of hypoglycemic experiment:

After feeding on basal diet for ten days (adaptation period). Five rats were kept as control which fed basal diet during the experiment period which called group one G1(normal control). The other rats (20) were injected with alloxan solution (120mg/kg of body weight) after 72 hours fasting, to induce hyperglycemia (Eskander and jun 1995). After 72 hours of injection with alloxan. Animals were having diabetes as reported by (Esmerino, 1998). the second main group(20) rats were divided into four subgroups (5 rats each). One group of rats control positive continued on hypoglycemic diet without any addition. the three remaining groups received hypoglycemic diet with level 2.5, 5 and 7.5 % of rosemary powder After (1 week) of injection with alloxan, blood glucose concentration was determined.

Hyperglycemia experiment of medicine plant:

G1**:** feeding on the basal diet (negative control)

G2 : feeding on hyperglycemia diet (positive control)

G3 : feeding on (HD) + 2.5% rosemary powder

G4 : feeding on (HD) + 5% rosemary powder.

G5 : feeding on (HD) + 7.5% rosemary powder.

Table 1 : Composition of different tested diabetes from rosemary powder.

Ingredients / Groups	G1	HD	HD+2.5% Rosemary powder	HD+5% Rosemary powder	HD+7.5% Rosemary powder
		G2	G3	G4	G5
Corn starch	600	600	575	550	500
Corn oil	100	100	100	100	100
casein	200	200	200	200	200
cellulose	50	50	50	50	50
vitamin	10	10	10	10	10
mineral	40	40	40	40	40
Rosemary powder	0	0	25	50	100

G1: the basal diet (negative control). G2(HD): hyperglycemic diet (positive control).

Biochemical analysis of blood

Blood glucose concentrations were measured three times during the study. First time, at the beginning. Secondly, at the fourth week. Finally, at the end of experimental period (8 weeks). Blood samples were collected by tail vein of the rats after being fasted for 12 hr. the blood glucose levels determination was carried out using a single touch glucometer (Accucheck, bayer). The results were calibrated and verified by colorimetric measurement as described by trinder (1969). Using glucose oxidase (GOD) *spinreact* enzymatic kits.

Statistical analysis

Data were statistically analyzed according to the technique of analysis variance (ANOVA), the least significant difference (L.S.D) and Duncan's methods was used to compare the deference between the means of treatment values to the methods described by Gomez and Gomez (1984). All statistical analyses were performed using analysis of variance technique by means of Co STATE Computer Software.

Results and Discussion

Gross chemical composition of rosemary powder

Gross chemical compositions of rosemary powder was determined and the results are presented in Table (1).

It is clear that protein content was 6 % for rosemary powder. the results show that the herb contain considerable amounts of fiber 35%, also show that the fat content was higher in rosemary powder 20% while the ash quantity was 7.95% .These results are in accordance with those of (Lahlou *et al*., 2012).

Table 2 : Chemical composition of rosemary powder (on dry weight)

Samples	Fat %	Protein %	Fiber%	Ash%	Total carbohydrate%
Rosemary	20	6	35	7.95	31.05

Phenolic compounds of rosemary powder

Phenolic compounds are known as antioxidants which have long been recognized to have protective function against oxidative damage in diet they may provide health benefits associaticl with reduced risk of chronic disease (Karppinen *et al.,* 2003).

Phenolic compounds were determined in studied rosemary and the results are presented in Table (3). From these results, it is evident that these herbs have considerable amounts of phenolic compounds, flavonoids and diterpenes have been found in rosemary pwoder, example vanillic acid, caffeic acid, rosmarinic acid, naringin, hispiduling, cirsimaritin, carnosol, and carnosic acid were present in sufficient amount to be identified and quantified in this study. It was observed that carnosic acid had the highest percentage of (12.59 mg/100g), followed by rosmarinic acid (2.16 mg/100g), while the total of compounds that have antioxidant activity reached (15.59 mg/100g). These data are in agreement with those reported by Saber and Hawazen, (2012) ; Matkowski, (2006).

Table 3 : Phenolic compounds of rosemary powder (mg/100g).

Phenolic compound	Concentration
Caffeic acid	0.013 ± 0.0005
Carnosic acid	12.19 ± 0.608
Carnosol	0.54 ± 0.0218
Cirsimaritin	0.081 ± 0.0039
Carnosol	0.54 ± 0.0218
Hispulin	0.021 ± 0.009
Vanillic acid	0.005 ± 0.0001
Rosmarinic acid	2.16 ± 0.103
Naringin	0.58 ± 0.027
Apigenin	ND
Total phenolics	15.59 ± 0.773

Effect of feeding on rosemary powder on blood glucose in rats:

Table (4) illustrated the mean blood glucose level of negative control G1 and hypoglycemic group G2 through the experimental period. Blood glucose levels of hypoglycemic groups2 were markedly higher than the normal control G1.It should be notated that, treating group with 2.5 mg/100g of rosmary powder for 8 weeks caused no significant decreasing in fasting blood glucose. While that data dealing with this case that given in the same table clarified that, diets containing rosemary powder at level 5% and 10% led to cause a significant decreased in blood glucose level of the hypercholesterolemia groups G4 and G5, comparing with the hypercholesterolemia group fed on control diets G2. Such reduction was observed after 4 week of feeding till the end of experiment period, also, the reduction was increased with increasing the feeding period. Apparent also from the table (4) and that rosemary powder at level 10% had more effect on blood glucose level than rosemary powder at level 5%. The results were in agreement with many researchers in this field.It should be notated that, treating group with 2.5mg/100g of rosmary powder for 8 weeks caused no significant decreasing in fasting blood glucose. On contrast, treating groups with 5 and 10%mg/100g of rosmary powder for 8 weeks caused significant decreasing in fasting blood glucose.

Table 4 : Effect of feeding on rosemary powder on blood glucose level in rats.

Groups	Diet	Glucose mg/dl (M±SD)		
		After adaptatin	After 4 weeks	After 8 weeks
G1	Negative control (BD)	103.34^{b}±2.07	105.00^{a}±2.00	105.01^{b}±2.07
G2	Positive control (HD)	103.34^{b}±1.51	117.68^{c}±0.56	128.68^{d}±1.51
G3	HD+rosemary 2.5%	103.00^{b}±1.00	112.34^{b}±1.51	114.00^{c}±1.00
G4	HD+rosemary 5%	102.66^{ab}±0.57	110.68^{b}±0.56	111.48^{c}±1.51
G5	HD+rosemary 10%	100.68^{a}±1.51	106.68^{a}±2.07	106.00^{b}±1.00

A potential component of the hypoglycemic activity of rosemary was recommended to be through expanding the insulin level (Vanithadevi and Anuradha, 2008). In addition, ongoing investigation revealed that rosemary prompts recovery of the cells of the pancreas and potentiating of insulin discharge from enduring cells, which shows that rosemary decline blood glucose level by invigorating insulin emit from the remainder cells or recovered cells (Alnahdi, 2012). Additionally the decrease of fasting blood glucose might be because of rosemary may hinder the intestinal assimilation of glucose by hindrance of intestinal an amylase compound (McCue and Shetty, 2004) or a-glycosidase enzyme (Koga *et al*., 2006). Furthermore, the striking antidiabetogenic effects of rosemary herb could be due to its potent antioxidant properties. It also might be producing its hypoglycemic activity by a mechanism independent from insulin secretion. For example restraint of protein glycation, and the hindrance of endogenous glucose creation (Bakirel *et al*., 2008).

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