

Effect of *Rosmarinus Officinalis* Extract on some Cardiac Enzymes of Streptozotocin-induced Diabetic Rats

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Abstract The study was undertaken to evaluate the therapeutic efficacy of water extract of *Rosmarinus officinalis* L. (rosemary) in Streptozotocin (STZ) induced diabetic rats. The effect of extract on serum cardiac enzymes in STZ induced diabetic rat models and normal control rats were determined. Extract was administered orally, to STZ-induced diabetic rats, at a dose of 200 mg / kg body weight/day for 21 days. The fasting blood glucose level of diabetic rats was decreased significantly after treatment with water extract of rosemary. Activities of aspartate transaminase (AST), creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) were recovered significantly in extract treated diabetic group in respect to untreated diabetic group. After the monitoring of CPK and LDH activities in serum, it has been noted that the extract significantly correct the activities of these enzymes in STZ-induced diabetic rat. To assess the antihyperlipidemic activities of this extract, the serum levels of total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c) and high density lipoprotein cholesterol (HDL-c) were determined. The results of this experiment demonstrated that, there was a significant recovery in the above mentioned biomarkers of lipid profile in treated STZ-induced diabetic rat.

Keywords *Rosmarinus Officinalis*, Antidiabetic, Antihyperlipidemic, Heart Enzymes

1. Introduction

Diabetes mellitus (DM) is a metabolic disorders characterized by carbohydrate disturbances and defects in insulin action. Management of diabetes is based on increased insulin secretion. Modern treatment methods can have many side effects. In addition, using medication continuously may involve economic burden on the user (1).

Some plant extracts have diagnostic markers of myocardial infarction such as creatine phosphokinase (CK), and lactate dehydrogenase (LDH) (2). These enzymes are tightly bound to the contractile apparatus of the cardiac muscle tissue and any serious insult to the heart muscle will evoke the release of these enzymes into the serum.

Rosemary, *Rosmarinus officinalis* L. (Labiatae) is an evergreen perennial shrub grown in many parts of the world. It has been reported to possess a number of therapeutic applications in folk medicines in curing or managing wide range of diseases such as diabetes mellitus, respiratory disorders, stomach problems and inflammatory diseases (3).

Rosemary contains caffeic acid and rosmarinic acid, both of which are potent antioxidants as well as anti-inflammatory agents. Rosemary is also a good source of antioxidant

vitamin E (alpha tocopherol) and other important antioxidants. In addition, rosemary contains 19 chemicals with antibacterial action and a number of volatile oils which reduce the airway constriction induced by histamine. The volatile oils in rosemary also help reduce inflammation that contributes to liver and heart disease. Herbalists think that rosemary may also help ease breast pain by acting as a natural drying agent to fluid filled cysts (4).

The present study was therefore undertaken to determine the extent to which rosemary extract would influence some cardiac enzymes such as aspartate Transaminase (AST), creatine phosphokinase (CK), and lactate dehydrogenase (LDH) in diabetic and nondiabetic rats. Our findings may provide some useful information to support the age-long practice of using rosemary as an anti-diabetic and anti-hypertensive herb.

2. Materials and Methods

2.1. Plant Material and Preparation of Extract

Leaves of Rosemary were obtained from the local herbal market of Kingdom Saudi Arabia. Voucher specimens from plant material were deposited at the Herbal Museum, Department of Pharmacology, Faculty of Science, King Abdulaziz University of Medical Sciences for identification. The fresh leaves of plant material (5 g) were soaked in 50 ml of boiled water, after 1 h stirring, at room temperature

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overnight, the supernatant was decanted and the residue was macerated two more days with dist water. The pooled supernatants were combined and filtered.

2.2. Experimental Animals

Adult male albino rats (weighing 150-200g) were obtained from Central Animal House in Jeddah, Saudi Arabia. The animals were housed in acrylic cages in standard conditions of temperature prior to the experiments for 1 week in order to adapt to the laboratory condition, fed with commercial diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Saudi Arabia, Jeddah.

2.3. Induction of Experimental Diabetes

A freshly prepared solution of Streptozotocin or STZ (45mg/kg body weight in 0.1 M citrate buffer, pH 4.5) was injected intraperitoneally to overnight fasted rats. STZ injected animals exhibited hyperglycaemia within 48-36 h (5). The rats having fasting blood glucose (FBG) values of 250 mg/dl or above were considered for the study.

2.4. Experimental Design

The experiment was carried out on 5 groups of five rats in each group to study the effect of plant water extract on STZ-induced diabetes and changes in heart function as follows: Group 1: Healthy control rats. Group 2: Diabetic control rats (rats + STZ). Group 3: Normal rats administered rosemary extract (200 mg/kg body weight) (rats + rosemary extract). Group 4: Protective group received water extract of Rosemary for two weeks before STZ injection (rats + rosemary + STZ). Group 5: Diabetic treatment rats received water extract of Rosemary for 21 days after 36 hr STZ injection (rats + STZ + rosemary).

Control rats received distilled water and treated rats received Rosemary in 1 ml of distilled water. The treatment with rosemary 200 mg/kg/d, (3) was given daily for a period of 3 weeks using standard or gastric cannula (6). No detectable irritation or restlessness was observed after extract administration. No noticeable adverse effect (i.e., respiratory distress, abnormal locomotion and catalepsy) was observed in any animals after the extract administration. Throughout the experimental period, the body weight was monitored. Starting from the 1st day (3rd day of STZ-injection) of extract administration to diabetic rats, FBG level was measured in every 7th day using glucometer (7). On the 21th day of extract administration all the animals were anesthetized (Nesdonal 50 mg/kg, i.p.), blood samples were obtained from hearts of overnight fasted rats by using micro-capillary technique and allowed to clot for 20 minutes in laboratory temperature and then centrifuged at 10000 rpm for 10 minutes for serum separation.

2.5. Biochemical Assays

Haemoglobin was estimated by the method of Drabkin and Austin (8). All the enzymes were determined within 24 h of sample collection. Activities of cardiac marker enzymes

like aspartate transaminase (AST), creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) and Serum lipid profile like serum levels of triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-c), very low density lipoprotein and high density lipoprotein cholesterol (HDL-c) were measured biochemically by using the commercially available kits from Siemens Health Care Diagnostics according to their manufacturers.

Statistical Analysis: The results are expressed as mean \pm SD. Statistical significance between the groups was tested using the Student's t-independent test. Data analysis was carried out using the analysis program Statistica. P-value \leq 0.05 was considered statistically significant.

3. Results

The body weight of control and experimental groups of rats were checked up to 21 days and represented in Fig. 1. The body weight was decreased in diabetic group of rats, when compared to control rats. In the initial days of the treatment, there was no significant difference in the body weight, but in later the body weight was significant increased in extract treated rats, when compared to diabetic group of rats.

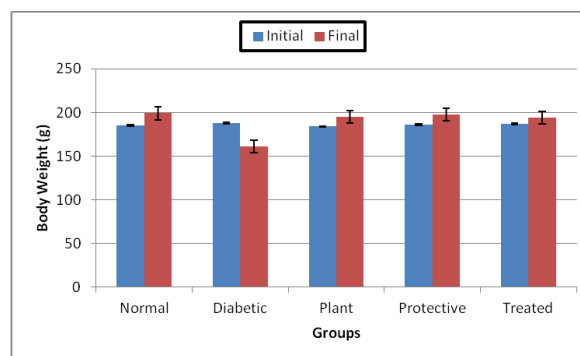


Figure 1. Effect of rosemary on body weight in control and experimental group of rats. Data were given as mean \pm standard deviation for five animals in each group. Normal (healthy control), Diabetic (rats + STZ), Plant (rats + rosemary), Protective (rats + rosemary + STZ), Treated (rats + STZ + rosemary)

Diabetes induced by STZ resulted in a significant elevation in the levels of fasting blood glucose (FBG) in comparison to the control group. After the treatment of water extract of rosemary to the diabetic animals for 21 days, a significant reduction ($p < 0.05$) in FBG level was noted in respect to untreated diabetic group. The 200 mg kg⁻¹ body weight water extract once daily for 21 days reduced the elevated blood glucose by 36.9% in respect to untreated diabetic group (Table 1).

The haemoglobin in control and experimental rats were represented in Table 2. The level of haemoglobin was significant decreased in the diabetic rats, when compared with control rats. The rosemary treated diabetic rats significant increased the level of haemoglobin.

Table (3) indicates the activities of cardiac enzymes of non-diabetic and diabetic rats after 21 days oral

administration of rosemary extract in 200 mg/kg body weight. STZ-induced diabetic animal resulted in a significant increase ($p < 0.05$) in AST, CPK and LDH activities with respect to the control group. After the treatment of rosemary extract to STZ-induced diabetic rat, a significant decrease ($p < 0.05$) was noted in respect to treated diabetic group. Percentage of recovery in the activities of AST, CPK and LDH were 25%, 30%, 43% respectively by rosemary extract when compared to untreated diabetic group.

Serum levels of TG and TC were increased significantly

($p < 0.05$) in STZ-induced diabetic control group when compared with control. After the treatment of water extract of plant, a significant ($p < 0.05$) recovery was noted in respect to diabetic group. Regarding the levels of TG and TC in serum, water extract treated diabetic group showed 33%, 72.9% recoveries in respect to diabetic control respectively. When comparison was made between the water extract treated diabetic group and protective diabetic group, a significant difference was noted in the levels of serum TG and TC (Table 4).

Table 1. Fasting blood glucose levels in every 7 days interval in control and different experimental groups of rats

Group	Fasting blood glucose level (mg/dl)		
	7 th day	15 th day	21 th day
Healthy control	113±3.8	115±4.8	117±5.2
Diabetic control	347±4.2 ^a	386±2.9 ^a	371±5.4 ^a
Normal+rosemary (200mg/kg)	122±5.1	82.8±4.01	86.4±3.6
Protective group	133±1.7 ^c	112±6.6 ^c	95±5.2 ^c
Diabetic treated group	305±5.2	213±4.1 ^b	137±4.6 ^b

Data were given as mean ± standard deviation for five animals in each group. Values are statistically significant * $p < 0.05$. a) Diabetic control rats vs. normal control rats. b) Rosemary treated diabetic rats vs. diabetic control rats. c) Protective diabetic rats vs. diabetic control rats

Table 2. Levels of blood glucose and hemoglobin in control and experimental groups of rats

Group	Blood glucose (mg/dl)	Hemoglobin (g/dl)
Healthy control	117±5.2	16.3±1.5
Diabetic control	371±5.4a	14±2.3a
Normal+rosemary (200mg/kg)	86.4±3.6	16.8±1.32
Protective group	95±5.2c	15±0.78
Diabetic treated group	137±4.6b	16±0.7b

Data were given as mean ± SD for five animals in each group. Values are statistically significant at * $p < 0.05$. a) Diabetic control rats were compared with normal control rats. b) Rosemary treated diabetic rats were compared with diabetic control rats. c) Protective diabetic rats were compared with diabetic control rats

Table 3. Effect of rosemary on changes in cardiac enzymes in normal and experimental rats

Group	AST (U/L)	CK (U/L)	LDH (U/L)
Healthy control	123 ±2	60±3.1	59±16.2
Diabetic control	272±44 ^a	235±4.8 ^a	77.5±32 ^a
Normal+rosemary (200mg/kg)	138±25	79.8 ±4.24	65±20
Protective group	205±20 ^c	102 ±4.14 ^c	68±20 ^c
Diabetic treated group	204±19 ^d	71.6±3.8 ^b	64±30 ^d

Data were given as mean ± standard deviation for five animals in each group. Values are statistically significant * $p < 0.05$. a) Diabetic control rats vs. normal control rats. b) Rosemary treated diabetic rats vs. diabetic control rats. c) Protective diabetic rats vs. diabetic control rats

Table 4. Effect of rosemary on changes in lipid profile in normal and experimental rats

Group	TC (mmol/L)	TG (mmol/L)	HDL-c (mmol/L)	LDL-c (mmol/L)
Healthy control	1.9 ±0.5	0.58±0.1	0.35±0.09	0.5±0.08
Diabetic control	3.7±0.4 ^a	0.94±0.8 ^a	0.2±0.05	2.4±0.12
Normal+rosemary (200mg/kg)	1.5±0.37	0.59 ±0.24	0.3±0.08	0.4±0.1
Protective group	2.3±0.2 ^c	0.59 ±0.1c	0.29±0.1	0.7±0.1
Diabetic treated group	2.7±0.9 ^d	0.63±0.8 ^b	0.28±0.11	1.9±0.15

Data were given as mean ± standard deviation for five animals in each group. Values are statistically significant * $p < 0.05$. a) Diabetic control rats vs. normal control rats. b) Rosemary treated diabetic rats vs. diabetic control rats. c) Protective diabetic rats vs. diabetic control rats

Serum levels of LDL-c was increased significantly ($p < 0.05$) in untreated diabetic group in respect to control. But after treatment of water extract of rosemary to diabetic rats the level of this biomarker was corrected significantly ($p < 0.05$) in respect to diabetic group. Percentage of recovery in the serum level of LDL-c was 79% by water extract in respect to diabetic control. HDL-c level was decreased significantly ($p < 0.05$) in diabetic control group in respect to the control. But after treatment of the plant extract, a significant recovery ($p < 0.05$) was noted in respect to diabetic control. The recovery in serum level of HDL-c was 40% in water extract treated diabetic group (Table 4)

4. Discussion

The STZ is a broad-spectrum antibiotic extracted from *Streptomyces acromogenes*. The STZ induced diabetes causes the destruction of β -cells of the islets, which leads to a reduction in insulin release (9). An insufficient release of insulin leads to high blood glucose namely hyperglycaemia. The body weights of STZ-induced diabetic rats were reduced and also recovered after hypoglycaemic treatment. In our study also the body weight was gain in rosemary treated diabetic rats. The enhancement of body weight in STZ-induced diabetic treated rats may be due to the increase of glucose metabolism. The treatment with medicinal plant extract to the STZ-induced diabetic rats, activated the β cells and granulation returned to normal, like insulinogenic effect (10).

The decreased level of blood glucose was observed in our present study, which indicates that rosemary stimulates insulin secretion from the remnant β cells or regenerated β cells. Some plants have antidiabetic activity through insulin releasing stimulatory effects (11). The excess glucose presents in the blood during diabetes, reacts with haemoglobin and forms glycosylated haemoglobin. The various proteins including haemoglobin, albumin, collagen, and low density lipoprotein (LDL)/crystalline proteins undergo nonenzymatic glycation in diabetes (7). The haemoglobin level was decreased in diabetic rats that may increase the formation of glycosylated haemoglobin.

It is well known that diagnosis of cardiac enzymes is important. Serum CK activity is a more sensitive indicator in early stage of myocardial ischemia, while peak rises in LDH is roughly proportional to the extent of the myocardial tissue (2). Also, the integrity of the cardiac apparatus in drug biotransformation and metabolism could be assessed by evaluating the levels of AST, CK, and LDH in serum. The results in diabetic animals in this experiment showed a protective effect of rosemary water extract on the heart of experimental animals at a dose level of 200 mg/kg body weight. Moreover, the significantly lowered activities of AST, CK, and LDH at 200 mg/kg body weight scientifically suggest that the extract of rosemary may have the potential of reducing the factors that produce myocardial infarction. This is so because the metabolism of STZ -induced infarcted

myocardium may be studied by assessing the level of marker enzyme proteins in the serum. It is interesting to know that as myocardial diseases are rich sources of CKMB, so are skeletal muscular diseases are good sources of creatine kinase isoenzyme (12). Pathological value has been estimated in injured skeletal muscle. Therefore the significant reduction in CK enzyme at the dose of 200 mg/kg body weight of rosemary extract may be due to some physiological effects on muscular activity. This fact may be associated with the efficacy of rosemary extract in the treatment of muscular pains, arthritis and inflammation (13).

Lipid profile, which is altered in the serum of STZ-induced diabetic rats, appears to be a vital factor in the development of atherosclerosis which is noted in diabetes (14). Elevated levels in serum TG and TC in diabetes are in consistent with our previous observations (15) and by others (16). In this study, water extract significantly recovered the levels of serum lipid profile in treated diabetic rats when compared to untreated diabetic rats. From these results, it may be stated that the water extract leads to regeneration of the β -cells of the pancreas and potentiating of insulin secretion from surviving β cells.

The increase in insulin secretion and consequent decrease in blood glucose level may lead to inhibition of lipid peroxidation and control of lipolytic hormones. In this context, a number of other plants have also been reported to have insulin stimulatory along with antihyperlipidemic effects (17).

So, on the basis of the results in this experiment it may be stated that the extract of rosemary has a beneficial effect in preventing diabetes and its complications as well as improving lipid metabolism in diabetics. Further studies will be conducted to purify the bioactive compound (s), and use the purified compound (s) for bioassay guided experiments in near future.

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