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EFFECTS OF THE AQUEOUS EXTRACT OF ROSEMARINUS OFFICINALIS L. (ROSEMARY) LEAVES ON LIPID PROFILE OF DIABETIC ADULT MALE ALBINO RATS

By

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ABSTRACT

Background: Various herbs have been used for treating several diseases. Rosemary is a thorny plant which is widely distributed in Europe and South-Eastern Asia. It is used in traditional medicine for its therapeutic properties.

Objective: Evaluation of the biological antioxidant mechanisms of Rosemary leaves extract in the reduction of blood glucose level and lipid profile.

Materials and Methods: Sixty adult male albino rats were used in this study. The rats were divided into six equal groups, i.e. Control, Rosemary control group, alloxan-treated, alloxan plus glibenclamide-treated group, alloxan plus Rosemary-treated group and finally alloxan plus both glibenclamide and Rosemary-treated group. Blood samples were obtained for determination of fasting blood glucose, total cholesterol, triglycerides, HDL- cholesterol, LDL-cholesterol, atherogenic index as well as malondialdehyde (MDA) and catalase.

Results: Treatment with rosemary leaves extract was found to be effective in ameliorating blood glucose, lipid profile and antioxidant levels.

Conclusion: Rosemary leaves extract has a beneficial effect as an anti-diabetic agent as well as improving lipid metabolism in diabetics.

Key words: Rosemary, antioxidants, diabetogenic and dyslipidemia.

INTRODUCTION

The world is facing an explosive increase in the incidence of diabetes mellitus and cost effective complementary therapies are needed (***American Diabetes Association, 2014).*** There is a continuing effort to find insulin substitutes, secretagogues, or sensitizers from synthetic or plant sources for the treatment of diabetes ***(Louise et al., 2013).***

Rosemarinus officinalis L. is an ever-green perennial aromatic shrub belonging to the family Labiatae, and commonly called Rosemary ***(Rosemary et al., 2014*)*.*** Rosemary contains some antioxidant phenolic compounds especially phenolic acids, flavonoids, caffeic acid, rosmarinic acid and vitamin E that have been shown to provide a defense against oxidative stress from oxidizing agents and free radicals ***(Matkowski, 2006).***

The present research was designed to evaluate the effects of Rosemary leaves extract on blood sugar and lipid profile as

well as the antioxidant effects of alloxan-induced diabetic rats.

MATERIAL AND METHODS

Alloxan (Sigma Pharmaceuticals Company) was dissolved in 0.9% NaCl and injected at a dose of 140 mg/kg body weight given immediately after preparation to overnight fasted animals by a single intraperitoneal injection. Blood samples was collected on the 3rd day (initial), 8th, 15th and 22nd and the level of blood glucose was determined by the first sample to ensure production of diabetes and third sample for analysis ***(Adeyi et al., 2012 and Ezazul et al., 2012).*** Rats with blood sugar higher than 200 mg/dl were considered diabetic ***(Menedez et al., 2004).***

Glibenclamide (El- Nile Company) 0.65 mg / kg body weight / rat was dissolved in aqueous solution and administered by nasogastric tube ***(Rosemary et al., 2014).***

Rosemary was obtained from the local herbal market and fifty grams of Rosemary were soaked in 150 ml hot water bath for 3 hrs, filtered with capron silic cloth, and the filtrate was stored in a refrigerator at (4 ?C). Each rat was orally administered with 0.5 ml of Rosemary extract daily by nasogastric tube ***(Abdul-Rahim and Taha, 2011).***

Animals: The experimental protocol and animal handling were approved and performed according to guidelines of animal use of the Ethical committee of Al-Azhar University. In this study, 60 healthy adult male albino rats of local strains ranging in weight from 140 -160 grams were used. The animals were housed under similar standard environmental conditions in suitable cages (35 x 32 x 35 cm for every 5 rats) with wide meshed raised floors to prevent coprophagia. They were kept on an ordinary rat chow and water at room temperature and normal light/dark cycle. They also were kept for ten days to adapt to the new conditions before any experimental interference.

Group I (Control group): Rats fed on normal standard rat chow diet.

Group II (Rosemary control group): Rats received Rosemary leaves extract 0.5 ml daily for 8 weeks by nasogastric tube.

Group III (**Alloxan-treated Group):** Rats were subjected to induction of diabetes by a single intraperitoneal injection of alloxan (140 mg/kg body weight) and fed on normal standard rat chow diet.

Group IV (Alloxan plus glibenclamide-treated group): Diabetic rats received glibenclamide 0.65 mg /kg body weight in aqueous solution for 8 weeks by nasogastric tube.

Group V (Alloxan plus Rosemary-treated group): Diabetic rats received Rosemary leaves extract 0.5 ml daily for 8 weeks by nasogastric tube.

Group VI (Alloxan plus Rosemary- and glibenclamide-treated group): Diabetic rats received both Rosemary leaves extract 0.5 ml and glibenclamide (0.65 mg /kg body weight) daily for 8 weeks by nasogastric tube.

Collection of Blood Samples: At the end of the 8th week, all rats except group III (22nd day sample) were fasted overnight. Blood was collected (4 ml of blood for each) from the retro-orbital plexus using heparinized capillary tube (0.75 - 1.0 mm

internal diameter) inserted in the medial canthus. To obtain serum, the blood was collected into a dry clean graduated glass centrifuge tube under sterile conditions. It was rapidly set to centrifuge at 5000 r.p.m. for 10 minutes. About half of the supernatant serum was sucked out into Eppendorf tubes and stored frozen at -20?C for subsequent biochemical analysis ***(Ezazul, et al., 2012).***

Statistical Analysis: Data input and analysis were done using SPSS computer program. All results were expressed as the mean ± standard deviation (SD). Mean values of the different groups were compared using a one way analysis of variance (ANOVA). Least significant difference (LSD) post hoc analysis was used to identify significantly different mean values. *P* value < 0.05 was accepted to denote a significant difference**.**

RESULTS

Diabetes induced by alloxan resulted in a significant elevation in the levels of fasting blood glucose (FBG) in group III (diabetic group). The mean ± standard deviation was 459 ± 68.8 mg/dl in comparison with control group, while the treatment with Rosemary leaves extract reduced the elevated fasting blood glucose by 283.4± 55.5, 204.5±53.7 and 101.1 ± 11.5 mg/dl in groups, IV, V and VI respectively in respect to untreated alloxan-induced diabetic group (Tables 1 and 2).

Induction of diabetes significantly increased the levels (Mean ±SD) of total serum cholesterol was 121.9 ± 16.09 mg/dl, triglycerides (TG) was 138.4 ±12.08 mg/dl and LDL was 63.8 ± 13.12 mg/dl levels associated with significant decrease in the HDL level was 24.1 ± 3.69 mg/dl in comparison with control group (Table 1).

Rosemary leaves extract have no significant influence on plasma glucose level and lipid profile of normal rats (Table 1).

In treatment with glibenclamide, there was a significant decrease in the level of total serum cholesterol was 97.7 ± 7.79 mg/dl, triglycerides was 95.9 ± 12.56 mg/dl, and LDL was 32.5 ± 4.98 mg/dl levels associated with significant increase in the HDL level (39.1 ± 2.92 mg/dl in comparison with control group. After treatment with Rosemary leaves extract, a significant decrease was in the level of total serum cholesterol (98.2 ± 8.8 mg/dl), triglycerides (TG) was 96.3 ± 8.84 mg/dl, and LDL (33.54 ± 11.82 mg/dl) levels associated with significant increase in the HDL level was (40.7 ± 3.47 mg/dl) in comparison with control group (Table 1). These results were more significant in treatment with glibenclamide than treatment with rosemary leaves extract. There was a synergistic effect to the combination with both glibenclamide and Rosemary (Tables 1 and 2). On the other hand, there was a significant increase in atherogenic index (AI) in untreated alloxan-induced diabetic group in respect to control was (0.64 ± 0.06 and 0.54 ± 0.019 respectively). After treatment of alloxan-induced diabetic rats with glibenclamide, was 0.37 ± 0.43. The level of this biomarker significantly decreased in respect to untreated alloxan-induced diabetic group (Table 2). Treatment with Rosemary leaves extract caused insignificant decrease in that parameter (0.49 ± 0.06) and combination of both

treatment caused a significant decrease (0.41 ± 0.07).

In diabetic group, there was a significant decrease in the level of catalase as an antioxidant (21.7 ± 2.83 U/ml)) and significantly increased the level of MDA as an oxidant (23.73 ± 3.29 µmol/l) in this group in comparison to control group (Table 1). On the other hand, after treatment of alloxan-induced diabetic rats with Rosemary leaves extract, the level of catalase was significantly increased (39.5± 2.27 U/ml), and MDA level significantly decreased (16.37 ± 2.77 µmol/l) in respect to untreated alloxan-induced diabetic group (Table 2).

Table (1): **Effect of blood glucose, lipid profile and antioxidants levels in different groups (Mean± SD).**

Groups / Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
Fasting blood glucose (mg/dl)	98.8± 8.8	101.7 ± 10.2	459 ± 68.6	283.9 ± 41	204 ± 53.5	101±11.8
		P > 0.05	P < 0.05	P < 0.05	P < 0.05	P > 0.05
Triglycerides (mg/dl)	116 ± 5.11	102.2 ± 13.18	138.4 ±12.08	95.9 ± 12.56	96.3 ± 8.84	96.1±9.46
		P > 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05
Total cholesterol (mg/dl)	108.7 ± 36.8	91.9 ± 10.67	121.9 ± 16.09	97.7 ± 7.79	98.2 ± 8.8	84.8±14.3
		P > 0.05	P < 0.05	P < 0.05	P > 0.05	P > 0.05
HDL (mg/dl)	30 ± 6.58	33.7 ± 5.42	24.1 ± 3.69	39.1 ± 2.92	40.7 ± 3.47	36.3 ±4.78
		P > 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05
LDL (mg/dl)	55.5 ± 25.86	37.76 ± 9.69	63.8 ± 13.12	32.5 ± 4.98	33.54±11.82	29.28 ±37.24
		P > 0.05	P > 0.05	P < 0.05	P < 0.05	P < 0.05
Atherogenic index	0.54 ± 0.019	0.47 ± 0.09	0.64 ± 0.06	0.37 ± 0.43	0.49 ± 0.06	0.41 ± 0.07
		P > 0.05	P < 0.05	P < 0.05	P > 0.05	P < 0.05
Catalase (U/ml)	29.9 ± 3.03	33.1 ± 3.0	21.7 ± 2.83	37.8 ± 2.1	39.5± 2.27	38.20 ± 3.08
		P > 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05
MDA (µmol/l)	16.1 ± 3.62	15.05 ± 2.68	23.73 ± 3.29	20.49 ± 2.93	16.37 ± 2.77	14.1 ± 2.54
		P > 0.05	P < 0.05	P < 0.05	P > 0.05	P > 0.05

Number of rats in each group = 10.
Group I: Control group.
Group II: Rosemary-treated group.
Group III: Alloxan treated group
Group IV: Alloxan-and glibenclamide-treated group.
Group IV: Alloxan-and Rosemary-treated group.
Group V: Alloxan-, Rosemary-and glibenclamide-treated group.

Table (2): **Effects of Rosemary leaves extract and glibenclamide treatment in groups IV, V and VI (Mean ± SD).**

Groups / Parameters	Group III	Group IV	Group V	Group VI
Fasting blood glucose (mg/dl)	459 ± 68.6	283.9± 4	204 ± 53.5	101±11.8
		P < 0.05	P < 0.05	P < 0.05
Triglyceride (mg/dl)	138.4 ±12.08	95.9± 12.56	96.3 ± 8.84	96.1±9.46
		P < 0.05	P < 0.05	P < 0.05
Total cholesterol (mg/dl)	114.9 ± 16.09	111.7 ± 7.79	98.2 ± 8.8	84.8±14.3
		P < 0.05	P < 0.05	P < 0.05
HDL (mg/dl)	28.1 ± 3.69	34.1 ± 2.92	40.7 ± 3.47	36.3 ±4.78
		P > 0.05	P < 0.05	P < 0.05
LDL (mg/dl)	63.8 ± 13.12	54.5 ± 4.98	33.54±11.82	39.28 ±37.24
		P > 0.05	P < 0.05	P < 0.05
Atherogenic index	0.64±0.06	0.37± 0.43	0.49±0.06	0.41±0.07
		P < 0.05	P < 0.05	P < 0.05
Catalase(U/ml)	24.7 ± 2.83	37.8 ± 2.1	39.5± 2.27	38.20± 3.08
		P < 0.05	P < 0.05	P < 0.05
MDA(µmol/l)	23.73 ± 3.29	20.49 ± 2.93	20.4 ± 53.5	14.1± 2.54
		P < 0.05	P < 0.05	P < 0.05

Number of rats in each group = 10.
Group III: Alloxan-treated group
Group IV: Alloxan-and glibenclamide-treated group.
Group IV: Alloxan-and Rosemary-treated group.
Group V: Alloxan-, Rosemary-and glibenclamide-treated group.

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DISCUSSION

In the present study, there was a significant increase in blood glucose level in diabetic group (III) when compared with the control group (I). In treated groups with glibenclamide and Rosemary leaves extract (IV and V), there were significant decrease in blood glucose levels when compared with diabetic group (IV). The mechanisms by which alloxan brought about its diabetic state included selective destruction of pancreatic insulin secreting ?-cells, which make cells less active and lead to poor glucose utilization by tissues ***(Lenzen, 2008). Bakirel et al. (2008)*** attributed the anti-diabetic effect of many Labiatae species including Rosemary, to their essential oil which is composed of mono sesquiterpenes; phenolic compounds and flavonoides (caffeic acid and rosmarinic acid phenolic acid content) which have hypoglycemic effects.

The result of this study revealed that treatment of alloxan-induced diabetic rats with Rosemary leaves extract significantly reduced blood glucose level, and this triggered the liver to revert to its normal homeostasis during experimental diabetes (***International Journal of Pharma Tech Research, 2014)***. The anti-hyperglycemic activity of Rosemary leaves extract may be through a stimulatory effect on insulin secretion or through improvement of insulin action ***(Alnahdi, 2012)***. Also, Rosemary may have extra pancreatic mechanism of action or through rosmarinic acid which improves pancreatic ?-cell function, and thus enhance insulin secretion ***(Tavafi et al., 2011).***

In addition, the remarkable antidiabe-togenic effects of Rosemary leaves extract could be due to its potent antioxidant properties. It also might be producing its hypoglycemic activity by a mechanism independent from insulin secretion such as inhibition of protein glycation and the inhibition of endogenous glucose production ***(Bakirel et al., 2008).***

Glibenclamide appears to lower the blood glucose acutely in healthy individuals and patients with type II diabetes by stimulating the release of insulin from the pancreas, an effect dependent upon functioning beta cells. It acts in concert with glucose (improved sensitivity of beta cells to physiological glucose stimulus), and leads to an insulin secretion in the rhythm of meals. Other mechanisms of the hypoglycemic action associated with short term therapy appear to include reduction of basal hepatic glucose production and enhancement of peripheral insulin action at post-receptor (probably intracellular) sites, and increase in insulin binding and/or the number of insulin receptors ***(Qaseem, 2012).*** Glibenclamide also exerts a direct inhibitory effect on glucagon-producing alpha cells of the pancreas and increases the release of somatostatin. However, these two pancreatic extra-beta cell actions may play only a minor clinical role ***(Vaidyanathan et al., 2012).***

Administration of both glibenclamide and dried Rosemary leaves powder leads to more reduction of blood glucose level than glibenclamide or Rosemary alone, thus reducing the dose of oral hypoglycaemic drug (adjuvant effect).

The present study revealed a significant change in plasma lipid profile of alloxan- induced diabetic rats, which appeared to be a vital factor in the development of atherosclerosis which is noted in diabetes ***(Chattopadhyay and Bandyopadhyay, 2005).*** Elevated levels of plasma TG and TC in alloxan-induced diabetes were in agreement with ***Yadav et al. (2008)***.

Oral administration of Rosemary leaves extract caused significant decline in the blood levels of total cholesterol, LDL-C, TG and an increase in HDL-C. Rosemary leaves extract had a hypolipidemic potential due to progressive metabolic control of Rosemary leaves extract on mechanisms involved in the elimination of the lipids from the body. Such decrease may be due to antioxidant effect of constituents of Rosemary as rosmarinic acid, which changed the rate of fatty acids oxidation in the liver and reduced the rate of triglycerides biosynthesis in rats ***(Iweala and Oludare, 2011)***. *Relative* regeneration of the ?-cells

of the pancreas and potentiation of insulin secretion from surviving ?-cells may lead to inhibition of lipid peroxidation ***(Alnahdi, 2012).***

The reduction of TC and LDL-C by Rosemary leave extract may be due to the inhibition of pancreatic lipase and hormone sensitive lipase by a variety of constituents in the extract especially rosmarinic acid and other phenolics ***(Fawzy et al ., 2012)***. ***Alaa and Brahamachair (2010)*** showed an increase in HDL-C in experimental rats after Rosemary leaves extract. This could be due to glucose metabolism improvement as this directs protein metabolism into anabolic instead of catabolic process, which results in synthesis of proteins such as apolipoprotein that constitute 70% of HDL-C structure which in turn results in increase of HDL-C concentration ***(Abdul-Rahim and Taha, 2011)***

Rosemary is able to stabilize free radicals through donation of electrons to them. High scavenging capacity, mostly for free radicals, of Rosemary is considered as one of antioxidant action mechanism ***(Moreno et al., 2006).***

On evaluation of lipid ratios in the current study, we observed that atherogenic index of plasma (AIP) was significantly higher in alloxan-treated group as compared to controls groups. AIP is a significant predictor of atherosclerosis. The Canadian working group had chosen TC/HDL-C ratio as a secondary goal of therapy considering it to be a more sensitive ***(Nwagha et al., 2010).***

In alloxan-treated group, free radicals are formed disproportionately in diabetes by glucose auto-oxidation, thus resulting in consumption of antioxidant defenses (catalase level) which lead to disruption of cellular function and oxidative damage to membranes and enhance susceptibility to lipid peroxidation ***(Akomas et al., 2014)***. ?-cell is particularly sensitive to damage by free radicals because of their low level of free radical scavenging enzymes that leads to hyperglycemic condition ***(Sanders et al., 2001).***

Intake of Rosemary leaves extract and glibenclamide reduced MDA levels significantly in respect to diabetic non-treated group. Such decrease in the MDA level by Rosemary leaves extract may be attributed to the antioxidant properties that inhibited lipid peroxidation. This in turn stabilizes the reactive radicals preserving the cellular functions (***El Kader et al., 2012)***. These results agreed with the previous study which reported that Rosemary extract significantly decreases malondialdehyde (MDA) contents. The crude extract also acted as a cyto-protective agent when reflected a free radical–scavenging activity that elicited widespread damage to cell constituents such as membrane lipids, and significantly increased the normal cells viability and the antioxidant enzymes activity as catalase enzyme ***(Labban, 2014). Zhang et al. (2012)*** showed that Rosemary extract significantly decreased MDA content and increase the activities of catalase.

CONCLUSION

Rosemary leaves extract is one of the dietary components that is known as safe and used every day in our food products. Its extract exerts a hypoglycemic effect and improves the lipid profile in alloxan-induced type II diabetic rats. These effects should be studied further in human volunteers and diabetic patients.

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خلفية البحث: العلاج بالأعشاب الطبيعية يستخدم في علاج أمراض عديدة هذه الأيام. إن الروزمارى (حصا البان) هو عشب واسع الانتشار فى أوروبا وشمال شرق آسيا ويستخدم في الطب التقليدي لخواصه العلاجية

الهدف من البحث: تقييم تأثير خلاصة منقوع حصا البان على ذكور الجرذان البيضاء المصابة بمرض السكر من حيث نسب كل من السكر والدهون بالدم وعوامل الاكسدة ومضاداتها.

طرق ومواد البحث: إستخدم في هذا البحث ستون جرذا ذكراً أبيضاً من سلالة محلية كنموذج للدراسة. وقد قسمت الفئران إلي ست مجموعات متساوية كالآتي: مجموعة ضابطة ومجموعة ضابطة تم إعطاؤها خلاصة منقوع حصا البان ومجموعة مصابة بالسكر ومجموعة مصابة بالسكر تم إعطاؤها فقط عقار الجليبنكلاميد ومجموعة مصابة بالسكر تم إعطاؤها خلاصة حصا البان المنقوع ومجموعة مصابة بالسكر تم إعطاؤها خلاصة حصا البان بالإضافة إلى عقار الجليبنكلاميد.

وتم سحب عينات الدم في آخر التجربة وذلك لقياس:

- نسبة السكر بالدم (صائم).
- الكوليستيرول.
- الدهون الثلاثية.
- البروتين الدهني عالي الكثافة.
- البروتين الدهني منخفض الكثافة
- معامل التصلب في الدم
- انزيم المالون داى الدهيد في الدم
- انزيم الكاتاليز في الدم

نتائج البحث: خلاصة منقوع حصا البان تسببت في خفض نسبة السكر والدهون في دم الجرذان التي تم إحداث السكر بها تجريبيا. كما تسبب أيضا في تحسين نسبة مضادات الأكسدة وخفض عوامل التأكسد في هذه الجرذان.

الإستنتاج: إستخدام حصا البان يمكن أن يكون ذا فائدة تطبيقية وعاملا مساعداً في علاج مرض السكر والوقاية من مضاعفاته حتى عن العقارات التى تستخدم فى علاجه. وقد يرجع ذلك إلى قدرته علي زيادة إفراز هرمون الإنسولين من البنكرياس وزيادة حساسية الجسم له وقلة إمتصاص الدهون وزيادة مضادات الاكسدة.