EFFECT OF SERUM ADROPIN LEVEL AND CINNAMON WATER EXTRACT ON NORMAL AND ALLOXAN-INDUCED DIABETIC ADULT MALE ALBINO RATS

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ABSTRACT

Background: Adropin is a peptide first identified in 2008 in liver and brain tissues. It serves to modulate lipid and glucose metabolism and to maintain insulin sensitivity. It was found to be decreased in many disorders including diabetes mellitus (DM), atherosclerosis, diabetic nephropathy and many other diseases. Cinnamon tends to improve the serum glucose and lipid levels in diabetic subjects which may help in controlling DM and its disabling complications.

Objective: To study the effect of serum adropin levels and cinnamon water extract on normal and alloxan-induced diabetic adult male albino rats.

Materials and methods: Twenty-eight adult male albino rats of a local strain were used as an animal model for this study. They were divided into 4 equal groups; group 1 (control), group 2 (non-diabetic cinnamon-treated), group 3 (diabetic non treated), and group 4 (diabetic cinnamon-treated). After 4 weeks, blood samples were collected and serum was separated for the measurement of adropin level (by ELISA). Fasting blood sugar (FBS), HbA1C, cholesterol, LDL, HDL and TAGs were also measured. Collected Data were analyzed using SPSS version 25 and the difference between studied groups was considered significant when P ≤ 0.05.

Results: This study showed that there was a significant increase in serum adropin level in alloxan induced diabetic rats when compared with the normal rats. Also, the increase in serum adropin level showed a significant positive correlation with HbA1C, cholesterol, LDL and TAGs and a significant negative correlation with HDL. On the other hand, treatment with cinnamon showed a significant improvement of FBS, HbA1C and lipid profile and this was associated with reduction in serum adropin level.

Conclusion: The increase in serum adropin level in alloxan-induced diabetes is compensatory as it increases insulin sensitivity and ameliorates diabetic associated metabolic derangements and, in the future, it may be one of the members of diabetic medications.

Key words: Adropin, Diabetes Mellitus, Cinnamon, Alloxan.

INTRODUCTION

Adropin is a peptide primarily secreted by the liver and brain. It is encoded by the Energy Homeostasis Associated gene (gene symbol: Enho). It was first identified by Kumar and coworkers in the year 2008 in liver and brain tissues. This peptide was later also determined to be present in the heart, intestine, kidney, pancreas, umbilical vein, salivary glands, some peripheral tissues, and plasma (Kutlu et al., 2019). Its main function is to prevent insulin resistance, dyslipidemia,
and impaired glucose tolerance. Adropin has important roles in controlling fatty acid metabolism and protecting vascular endothelial cells. Also, it has anti-inflammatory effect. Adropin has been shown in the literature to be decreased in many diseases, such as diabetes, diabetic nephropathies, coronary atherosclerosis, hypertension, and polycystic ovary disease (Zheng et al., 2019 and Jurrissen et al., 2020). In animal studies, systemic adropin treatment improves diet-induced obesity, insulin resistance, and glucose tolerance. It also attenuates components of the metabolic distress associated with obesity independently of effects on body weight or weight loss. It could provide a promising new lead for developing therapies against the metabolic disorders associated with obesity. Significantly lower levels of adropin have been recorded in obese individuals with metabolic syndrome than obese without metabolic syndrome. In obese individuals, with increasing level of adropin, prevalence of hypertriglyceridemia, hypo-high-density lipoproteinemia, hypercholesterolemia and metabolic syndrome are significantly reduced (Yin et al., 2020). Lower levels of adropin are associated with increased risk of metabolic syndrome and DM and is a possible predisposing factor to early risk of CVD event. Future treatment of DM patients with synthetic adropin may be beneficial and worthwhile (Adetunji et al., 2020).

Diabetes mellitus is a general term for a group of metabolic disorders with the main feature of chronic hyperglycemia. It results from either impaired insulin secretion or impaired insulin efficacy or, most often, both (Petersmann et al., 2018). It is rising to an alarming epidemic, and it is among the top 10 causes of death in adults (Saeedi et al., 2019).

Cinnamon is the inner bark of an evergreen tree that is cultivated mainly in northern east. It improves the serum glucose and lipid levels in diabetic subjects (Shen et al., 2010). The compounds found in cinnamon have insulin-potentiating properties and may be involved in the alleviation of the signs and symptoms of diabetes, and coronary vascular diseases related to insulin resistance. Cinnamon has been shown to potentiate the insulin effect in vitro through up regulation of the glucose uptake in cultured adipocytes. The polyphenolic polymers found in cinnamon, with insulin-like biological and antioxidant activities, could improve plasma fasting glucose (Hariri and Ghiasvand, 2016).

The present work aimed to study the effect of serum adropin levels and cinnamon water extract on normal and alloxan-induced diabetic adult male albino rats.

**PATIENTS AND METHODS**

Twenty-eight adult male albino rats of a local strain were used as an animal model for this study. Their ages were 8 weeks, and their weight 110 – 140 g. They were kept in suitable cages (20x32x20 cm for every 4 rats) at room temperature with the natural light-dark cycle. They were maintained on a standard diet of commercial rat chow and tap water. They were kept for 10 days for the adaptation to the new environment before starting the experiment. The animals were divided into 4 equal groups.
**Group 1:** Normal control group received 2 ml distilled water by gavaging daily.

**Group 2:** Non-diabetic cinnamon-treated group received aqueous cinnamon extract at a dose of 2 ml/rat by gavaging daily (Shalaby et al., 2016).

**Group 3:** Diabetic group received 2 ml distilled water by gavaging daily.

**Group 4:** Diabetic cinnamon-treated group received cinnamon extract at a dose of 2 ml/rat by gavaging daily.

Cinnamon bark was purchased from the local market. The bark was left to dry and finely powdered in an electrical blender. Ten grams of finely-powdered cinnamon was mixed with 100 ml of distilled water and kept in a water bath at 60°C for two hours, then filtered by chess cloth. The extract was diluted with distilled water (one-part cinnamon extract and 10 parts water) and given to rats by gavaging in a dose of 2 ml/rat daily (Shalaby et al., 2016). Rats were starved for 24 hours in specific cages with a perforated floor in order to avoid coprophagia, then alloxan was dissolved in 0.9% NaCl and injected intraperitoneally at a dose of 90 mg/kg BW (Szkudelski, 2001). Just before alloxan injection, 2ml of glucose (5%) were given orally. After 48 hours, blood samples were taken from tail vein for blood sugar estimation. Rats with blood sugar higher than 200 mg/dl were considered diabetic.

After 4 weeks from the onset of the experiment, blood samples were collected from the retro-orbital venous plexus by using a heparinized capillary tube (about 0.75 – 1.0 mm internal diameter) inserted in the medial canthus. The collected blood samples were kept in clean graduated plastic centrifuge tubes containing EDTA. About one milliliter of the blood was taken in another plastic tube and stored at 4oC till being used for estimating blood HbA1C. The remaining blood was centrifuged at 5000 rotations per minute for about 15 minutes to separate the serum. Serum was sucked out into Eppendorf tubes, and stored frozen at -20oC till used for the measurement of:

- Serum adropin level (ng/ml): ELISA kits for Human Adropin from Bioassay technology laboratory, Korain Biotech Co., Ltd. 419 Harborne Road, Edgbaston, Birmingham, England was used in the analysis.
- FBS (fasting blood sugar) (mg/dl).
- TAGs (mg/dl).
- Total cholesterol (mg/dl).
- HDL (mg/dl).
- LDL (mg/dl).

**Statistical analysis:**

Collected Data were analyzed using SPSS version 25. They were tested for normality using “Shapiro – Wilk” test, One-way Analysis of variance (ANOVA) and the post hoc “Tukey” test were used to compare means if data were normally distributed. Otherwise “Kruskal Wallis” and “Mann Whitney U” tests were used to compare means if data weren’t normally distributed. Spearman’s correlation coefficient (R) was used to evaluate the linear association between adropin and other variables. Data were expressed as means ± SD and P ≤ 0.05 was considered significant.
RESULTS

Serum level of Adropin was 0.616 ± 0.046 ng/ml in control group 1 and was 0.681 ± 0.034 ng/ml in group 2 (normal received cinnamon) whereas it was 1.02 ± 0.186 ng/ml in the diabetic non-treated group 3 and was 0.952 ± 0.205 ng/ml in the diabetic cinnamon treated group 4. Adropin level was significantly higher in diabetic non-treated group 3 when compared with group 1 (control). It was reduced in diabetic cinnamon treated group (group 4) when compared with the diabetic non treated group (group 3) however the difference was non-significant (Figure 1).

Figure (1): Serum adropin levels in studied groups

Serum level of FBS was 75.1 ± 9.3 mg/dl in control group 1 and was 78.8 ± 15.2 mg/dl in group 2 (normal received cinnamon) whereas it was 272.3 ± 62.8 mg/dl in the diabetic non-treated group 3 and was 186.6 ± 49.9 mg/dl in the diabetic cinnamon treated group 4. As regard HbA1C, it was 4.9 ± 0.3% in control group 1 and was 4.9 ± 0.4 % in group 2 (normal received cinnamon) whereas it was 10.1 ± 1.7% in the diabetic non-treated group 3 and was 8.3 ± 1.6% in the diabetic cinnamon treated group 4. Both FBS and HbA1C were significantly higher in the diabetic non treated group 3 when compared with control group 1. Whereas their levels were significantly lower in diabetic cinnamon treated group when compared with the diabetic non treated group (Figure 2 and 3).
Cholesterol levels were 93.8 ± 4.8, 107.6 ± 8.9, 121.2 ± 6.1 and 107.6 ± 5.5 mg/dl in groups 1, 2, 3 and 4 respectively. As regard LDL levels, they were 38.9 ± 3.4, 53.5 ± 8.8, 71.5 ± 6 and 59.4 ± 11.7 mg/dl in groups 1, 2, 3 and 4 respectively. HDL levels in these groups were 37.3 ± 1.9, 37.6 ± 1.8, 26.6 ± 1.5 and 30.4 ± 4 mg/dl respectively. Concerning TAGs levels, they were 87.9 ± 11.5, 82.6 ± 9.6, 115.5 ± 11.2 and 88.6 ± 37.6 mg/dl in groups 1, 2, 3 and 4 respectively.

Diabetic non treated group (group 3) showed significantly higher levels of cholesterol, LDL and TAGs and significant lower level of HDL when compared with control group (group 1). Cinnamon treated group (group 4) showed significant reduction in cholesterol, LDL and TAGs and significant increase in HDL level when compared with the diabetic untreated group 3 (Figure 4).
Figure (4): Lipid profile levels in studied groups

There was a significant positive correlation between adropin and HbA1C with a correlation factor (R) 0.765 (Figure 5).

Figure (5): Correlation between adropin and HbA1C “R = 0.765”

There was a significant positive correlation between adropin and cholesterol with a correlation factor 0.639. (Figure 6)
Figure (6): Correlation between adropin and cholesterol “R = 0.639”

There was a significant positive correlation between adropin and LDL with a correlation factor 0.680 (Figure 7).

Figure (7): Correlation between adropin and LDL “R=0.680”

There was a significant positive correlation between adropin and TAGs with a correlation factor 696 (Figure 8).
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Figure (8): Correlation between adropin and TAGs “R=0.696”

On the other hand, there was a significant negative correlation between adropin and HDL with a correlation factor -0.798 (Figure 9).

Figure (9): Correlation between adropin and HDL “R= -0.798”

DISCUSSION

Adropin has been shown in the literature to be reduced in many diseases of human; one of these diseases is DM. This was recorded by Zang et al. (2018), Li et al. (2019), and Jasaszwili et al. (2020). All these studies recorded significant remarkable decline in serum adropin levels in diabetes mellitus patients when compared with normal subjects. (Jasaszwili et al., 2020) reported that low levels of adropin are correlated with metabolic syndrome and hence identify it as a potentially protective agent against metabolic syndrome and DM development.
In this study serum adropin level increased significantly in alloxan induced DM in adult male albino rats (group 3) when compared with control ones (group 1). This finding is consistent with Kuo et al. (2020) who reported increase in Enho gene expression and subsequent increase in serum adropin level in streptozotocin induced diabetic rats. The increased serum adropin level was a protective mechanism to ameliorate hyperglycemia and the associated metabolic derangements including dyslipidemia. A similar finding was reported by Aydin et al. (2013) who reported that in streptozotocin induced diabetic rats, the immunoreactivity of adropin in the brain, cerebellum, kidneys, heart, liver, and pancreas tissues are increased as well as its level in the serum of diabetic rats compared to the control group which was considered a compensatory mechanism against hyperglycemia.

Yosae et al. (2017) stated that adropin is a potentially protective agent against metabolic syndrome development and this may explain its elevation in serum of rats after induction of diabetes. They reported that variation in adropin levels may partly explain the “healthy obese” phenomenon where some obese persons have no remarkable metabolic derangements because they have higher serum adropin levels in comparison with obese persons with metabolic syndrome.

Butler et al. (2019) stated that Low adropin expression is associated with metabolic dysregulation and liability to have DM in nonhuman primate model. Experimental studies showed that adropin deficiency in adropin knockout mice was associated with insulin resistance, obesity, dyslipidemia and endothelial dysfunction and development of DM whereas treatment with adropin was associated with improvement of insulin resistance and diabetic related complications whether microvascular or macrovascular (Ganesh-Kumar et al., 2012).

The limited efficacy of current treatment methods and the continued upward trend in the diagnosis of diabetes mellitus are an incentive for investigating how metabolic homeostasis is maintained to improve treatment efficacy and identify novel treatment methods. Several peptide hormones secreted by the endocrine pancreas, gut, adipocytes, and liver modulate insulin activity to maintain glucose homeostasis; these hormones – and adropin is one of them- are considered promising leads in the development of therapies against diabetes mellitus (Shelest and Buriakowska, 2019).

This study showed a significant increase of cholesterol, TAGs and LDL and significant reduction in HDL in diabetic group (group 3) when compared with the control group (group 1). Insulin is a potent anti-lipolytic hormone and it restrains lipolysis from the adipocyte by inhibiting the enzyme "hormone sensitive lipase". So, in DM and as a result of insulin deficiency lipolysis is enhanced. This, in turn, results in increase of free fatty acids flux to the liver and drives cholesterol, and TAGs and LDL synthesis and secretion (Dahlman et al., 2018).

The diabetic cinnamon-treated group (group 4) showed significant improvement in blood glucose, HbA1C levels and lipid profile when compared with the diabetic untreated group. Hafizur et al. (2015) stated that cinnamic acid, a pure
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A compound from cinnamon, decreased blood glucose levels in diabetic rats in a time- and dose-dependent manner. The improvement was comparable to that of standard drug glibenclamide. Cinnamic acid significantly enhanced glucose-stimulated insulin secretion from pancreatic islets. The beneficial effects of cinnamon in animals include attenuation of diabetes associated weight loss, reduction of fasting blood glucose, HbA1C, improving lipid profile and increasing circulating insulin levels with no significant toxic effects on liver and kidney and a significantly high therapeutic window (Ranasinghe et al., 2012).

The lipid lowering effect of cinnamon was also reported by Cao et al. (2010), Ping et al. (2010), Vafa et al. (2012) and Shalaby et al. (2016) whose studies concluded that cinnamon extract exerts a blood glucose-suppressing and lipid lowering effect by increasing insulin secretion, improving insulin sensitivity and slowing absorption of carbohydrates in the small intestine. Serum adropin level in cinnamon treated group (group 4) was lower than diabetic untreated group (group 3) however the difference was statistically insignificant.

CONCLUSION

Serum adropin level was significantly higher in alloxan induced diabetic rats when compared with normal ones. There was a significant correlation between adropin level and HbA1C and lipid profile. The increase in serum adropin levels was compensatory as it increased insulin sensitivity and ameliorated diabetic associated metabolic derangements and, in the future, it may be one of the members of diabetic medications.

REFERENCES


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تأثر مستوي أدروبين المصل والمستخلص المائي للقرفة علي ذكور الجرذان البيضاء البالغة الطبيعية والمصابة بالسكر المحدث بالألوكسان

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خلفية البحث: الأدروبين هو ببتيد متعدد أكتشفي لأول مرة بانسجة الكبد والمخ
في العام 1998 وثامنية. الوظيفة الرئيسية للأدروبين هو تنظيم عمليات التمثيل
الغذائي للجلوكوز والدهون والحفاظ على حساسية الجسم للإنسولين. لوحظ أن
الأدروبين يقل في كثير من الأمراض مثل داء السكري وتصلب الشرايين
والإعتلاك الكلوي السكري وغير ذلك من الأمراض. تساعد القرفة على تحسين
حساسية الجسم للإنسولين وتنظيم مستويات السكر والدهون في الدم مما يساعد
علي التحكم في داء السكري والوقاية من مضاعفته.

الهدف من البحث: دراسة تأثير مستوي الأدروبين في المصل والمستخلص المائي
للقرفة على ذكور الجرذان البيضاء البالغة الطبيعية والمصابة بالسكر المحدث
بالألوكسان.

مواد وطرق البحث: تم استخدام ثمانية وعشرون جرذًا أبيضًا ذكورًا من سلالة
محلية كنماذج لهذه الدراسة. تم تقسيمهم إلى أربع مجموعات متساوية؛ المجموعة
الأولى (ضابطة)، المجموعة الثانية (طبيعة، معالجة بالمستخلص الماني للقرفة)،
mجموعة الثالثة (مصابة بالسكر)، المجموعة الرابعة (مصابة بالسكر ومعالجة
بالمستخلص المائي للقرفة). بعد أربعة أسابيع تم أخذ عينات الدم وفصل المصل
لقياس مستوي الأدروبين والسكر الصائم والسكر التراكمي والكوليسترول
والأيبروتينات الدهنية مخفض الكثافة والأيبروتينات الدهنية عالي الكثافة والدهون
التانثية. تم تحليل البيانات المجمعة باستخدام الإصدار الخمس والعشرون من
برنامج "الحزمة الإحصائية للعلوم الإجتماعية" واعتبار الفرق بين مجموعات
الدراسة ذا دلالة إحصائية حينما كانت قيمة "P" أقل من أو يساوي 0.05.
نتائج البحث: أظهرت هذه الدراسة إرتفاع مستويات الأدروبين في مصل الجرذان المصابية بالسكر ارتفاعًا ذا دلالة إحصائية عند مقارنته بمستويات الأدروبين في مصل الجرذان الطبيعي. كما كان هناك ارتباطًا موجباً بين دلالة إحصائية بين التغير في مستوي الأدروبين والتغير في مستوي كلاً من السكر التراكمي والكوليسترول والدهون الثلاثية والبروتين الدهني منخفض الكثافة، في حين كان هناك ارتباطًا سلبيًا ذا دلالة إحصائية بين التغير في مستوي الأدروبين والتغير في مستوي البروتين الدهني عالي الكثافة. كما أظهرت الجرذان التي تم علاجها بالمستخلص المائي للقرفة إنخفاضًا ذا دلالة إحصائية في مستويات السكر التراكمي والكوليسترول والدهون الثلاثية والبروتين الدهني منخفض الكثافة وارتفاعًا ذا دلالة إحصائية في مستوي البروتين الدهني عالي الكثافة وكان ذلك مصحوباً بانخفاض مستوي الأدروبين.

الاستنتاج: يمثل الارتفاع في مستوي الأدروبين في الجرذان المصابية بالسكر المحدث بالألوكسان ميكانيكية حماية تقوم بها الجسم لمقاومة اضطراب عمليات التمثيل الغذائي لكل من الجلوكوز والدهون بعد إحداث داء السكري. وبناءً عليه فقد يمثل النقص في هذا الهرمون عاملًا مريسًا في الإصابة بمرض السكر في البشر كما أن الاستخدام الدوائي للأدروبين ربما يكون مثاباً مستقبلاً لعلاج داء السكري.

الكلمات الدالة: الأدروبين، داء السكري، القرفة، الألوكسان.