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Effect of aescin on β-cell physiology in fructose fed rat model

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Author`s Contribution

[1,2,5]Drafting the work or revising it critically for important intellectual content, [3] Final approval of the version to be published [4,6]Acquisition, analysis, or interpretation of data for the work

Funding Source: None
Conflict of Interest: None

Received: November 02, 2019
Accepted: March 28, 2020

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ABSTRACT

Objective: To determine the effects of Aescin on blood glucose homeostasis in fructose fed Diabetic albino Wistar rats

Methodology: This Quasi Experimental study was conducted at the Department of Physiology from July 2018 to January 2019. Sixty albino (Wistar) rats were equally divided into five groups according to diet as; Group A (normal diet) and Group B (Fructose 10 g/kg/day) and all the other experimental groups were given Fructose (10 g/kg/day) with the administration of aescin as; Group C (0.9 mg/kg/day), Group D (1.8 mg/kg/day) and Group E (3.6 mg/kg/day). After four weeks body weight was calculated and blood samples were taken from all animals to assess the blood glucose level and serum insulin level. All the data was recorded in self-made proforma.

Results: Mean of blood glucose in control group A (165.0±14.29 mg/dL) was significantly lower in contrast to experimental groups B, C and D (357.17±21.73, 246.08±21.73 and 235.67±25.73 respectively); p-0.001, while the mean difference was insignificant as compared to experimental group E (194.0±22.16 mg/dl); p=0.078. Serum insulin level was significantly lower in experimental group B (17.45± 5.17) as compared to control group A (16.88± 4.78); p-0.001, while Aescin administered groups C and D showed serum insulin level (19.05± 5.81 and 25.08± 2.42 respectively) almost equal to control group A; p-0.092. Mean serum insulin level in Aescin (3.6 mg/kg/day) administrated group E was significantly higher (29.10± 1.64 IU/L) as compared control and other experimental groups; p-0.001.

Conclusion: The Aescin showed exerts ameliorating effects on glucose homeostasis in fructose fed Diabetic albino Wistar rats. By Aescin administration, serum insulin level can be increases to control the diabetes.

Keywords: Aescin, Fructose, Glucose Homeostasis, Insulin.

Cite this article as: *Shaikh S, Malik F, Uqaili AA, Kazi N, Rahim F, Qazi S. Effect of aescin on β-cell physiology in fructose fed rat model. Ann Pak Inst Med Sci. 2020; 16(1): 15-19.*

Introduction

The use of refined sugar in the diet is on rise in this modern age. Refined sugar is a product of sugar cane that contains sucrose. Sucrose has glucose and fructose in equal ratio. Both sugars are ketohexose, and essential for cell metabolism. Fructose is being consumed in large quantities in the form of sugar cane. Fructose consumption increases the risk of metabolic and cardiovascular diseases. Fructose is an isomer of glucose that is metabolized in the liver, particularly.[1]

Hyperglycemia, hyperuricemia, hyperlipidemia and dyslipidemia, and non-alcoholic fatty liver diseases (NAFLD) have been reported in experimental rat model studies using chronic fructose rich diet.[2-4] Systemic hypertension was observed in the fructose fed experimental rat animals, and the pathogenesis was speculated as early renal damage caused by nitro-

oxidative stress.[5] A study reported that the inhibition of Nitric-oxide synthase (nNOS) induces systemic hypertension through sympathetic stimulation in metabolic syndrome.[6] Similar observations have been reported in streptozotocin (STZ)-induced diabetic rat model.[6] Currently, the Diabetes mellitus (DM) is multiplying in the World, especially in developing countries like Pakistan. Now the DM ranks 4th non-communicable disease (NCDs) that cause deaths; hence the problem needs attention for new preventive measures. DM accounts for 1.5 million global deaths each year.[7]

The estimated prevalence of DM is 9% among adults (aged ≥ 18 years) in the World, as reported in 2014.[8] The International Diabetes Federation (IDF) reported that 382 million people (aged 40-59 years) are suffering from DM across the World. It is further estimated that a 55% rise will account for an increase of 532 million diabetics by the year 2035. 80% of Diabetic subjects are living in low and middle-income countries. Approximately 175 million people are undiagnosed. In the United States, 548 million dollars were expended on the health of diabetics in 2013(9). However, Pakistan is the sixth populous country with a population of 184.35 million,[10] the true prevalence of Diabetes is lacking in Pakistan. A survey conducted by Diabetes Association of Pakistan (DAP) and WHO reported a prevalence of DM around 6.39–16.5%.[11] Contrary to this, the International Diabetes Federation reported a 6.8% prevalence of DM (population aged 20- 79 years) in 2014, in Pakistan, which means that 6.9 million cases of diabetes were present in Pakistan, in 2014.[12] However, by 2035, around 12.8 million diabetics have been estimated for Pakistan.[13]

With this alarmingly rising burden of DM, there is a need of evaluating new agents and herbs for their efficacy of controlling the blood glucose levels and regulatory effects on β-cell secretory physiology. One such agent under evaluation in the present research is called as Aescin, which is a mixture of saponins derived from Aesculus hippocastanum (commonly called the horse chestnut). Medicinal properties of Aesculus hippocastanum are attributed to the aescin, which is an active compound of horse chestnut. A study[14] reported that aescin is an anti-inflammatory, veno-toner, and vasculo- protective agent. Previous studies reported that aescin is an effective therapy for chronic venous insufficiency.[14, 15] Moreover, two international studies reported that saponin-containing plants increase the production of insulin secretion from the pancreas and glucose utilization by the tissues.[16] Therefore this study has been planned to assess the effects of Aescin on blood glucose homeostasis in fructose fed Diabetic albino Wistar rats. The present research is of clinical importance so that effective newer drug molecules may be made available to tackle the diabetes epidemic.

Methodology

This Quasi Experimental study was conducted at the Department of Physiology at Isra Medical University Hospital Hyderabad, including the animal department of Zarai University Tando Jam. from July 2018 to January 2019 after taking permission from institutional ethical committee.

Inclusion criteria: sixty healthy albino (Wistar) male rats, with body weight between 150 to 250 grams

Exclusion criteria: Suffered (physically inactive) albino rats, body weight >250 grams and female rats

Animals were equally divided into five groups as;
Group A: Animals were put on a normal diet
Group B: Animals were put on Fructose (10 g/kg/day)
Group C: Animals were given Fructose (10 g/kg/day)+ Aescin (0.9 mg/kg/day)
Group D: Animals were given Fructose (10 g/kg/day)+ Aescin (1.8 mg/kg/day)
Group E: Animals were put on Fructose (10 g/kg/day)+ Aescin (3.6 mg/kg/day)
Aescin was smashed into powder, mixed in chow diet and given for 4 weeks.

After four weeks the body weight of rats was calculated by electronic weight machine. Bodyweight was noted in the proforma. Blood samples were taken from all animals to assess the blood glucose level and serum insulin level. Blood samples were drawn through a cardiac puncture after cervical dislocation of the rats and sera were isolated from the clotted blood by centrifugation. Sera were used to determine the blood glucose and serum insulin levels. Blood Glucose was estimated by glucose oxidase method on Hitachi Roche Diagnostics Chemistry Analyzer and serum insulin was detected by ELISA kit assay method. All the data was recorded in self-made proforma. Data was analyzed by SPSS version 20.

Results

Body weight of control (group A) and experimental groups B, C, D and were compared. Mean of body weight in the groups' A, B, C, D, and E was noted as 231.75±11.48, 389.75±10.87, 306.25±9.52, 285.50±8.01

and 265.83±12.34 grams respectively. Weight gain was found high in group B. While, Group E, treated with high dose aescin, showed significantly less rise in body weight. These findings show that the aescin has anti-obesity effects as shown in table I.

The mean ± SD of blood glucose in control group A was 165.0± 14.29 mg/dL. While experimental groups B, C, D, and E showed blood glucose levels of 357.17±21.73, 246.08±21.73, 235.67±25.73, and 194.0± 22.16 mg/dl respectively (Fischer`s ratio value 126.59, P=0.008). High dose Aescin treated animals (group E) showed a significant decrease in blood glucose levels as compared to control group A and experimental groups B, C, and D (P>0.05). Low dose Aescin (0.9 mg/kg) treated group C animals had high mean blood glucose levels as compared to positive control group B (not treated with aescin). Findings have been shown in table II.

Table 1: Bodyweight (grams) at the end of the experiment (n=60)

Study groups	Body weight (Mean ±SD)		p-value
A vs B	231.75±12.74	3.89±68.23	0.000
A vs C	231.75±12.74	3.06±46.61	0.000
A vs D	231.75±12.74	2.85±40.35	0.016
A vs E	231.75±12.74	2.65±42.34	0.256

A=Group A. Control Rats
B= Group B. Fructose (10 g/kg/day)
C=Group C. Fructose (10 g/kg/day)+ Aescin (0.9 mg/kg/day)
D=Group D. Fructose (10 g/kg/day)+ Aescin (1.8 mg/kg/day)
E=Group E. Fructose (10 g/kg/day)+ Aescin (3.6 mg/kg/day)

Table II: Blood Glucose (mg/dl) comparison among animal groups (n=60)

Study groups	Blood Glucose (mg/dl) (Mean ±SD)		p-value
A vs B	1.65±8.70	389.75±54.61	0.000
A vs C	1.65±8.70	2.46±30.42	0.000
A vs D	1.65±8.70	2.35±13.25	0.000
A vs E	1.65±8.70	1.93±37.61	0.134

A=Group A. Control Rats
B= Group B. Fructose (10 g/kg/day)
C=Group C. Fructose (10 g/kg/day)+ Aescin (0.9 mg/kg/day)
D=Group D. Fructose (10 g/kg/day)+ Aescin (1.8 mg/kg/day)
E=Group E. Fructose (10 g/kg/day)+ Aescin (3.6 mg/kg/day)

The mean ± SD of serum insulin in control group-A and in experimental groups (B, C, D, and E) was found as 16.88± 4.78, 17.45± 5.17, 19.05± 5.81, 25.08± 2.42 and 29.10± 1.64 IU/L respectively (Fischer`s ratio value 18.66, P=0.0001). Aescin treated animal groups C, D and E showed a significant rise in insulin secretion as compared to positive control group B. The observation may be interpreted as: aescin stimulates the β- cell functioning because insulin secretion is an exclusive function of these cells. Table III

Table III: Serum insulin (IU/L) comparison among animal groups (n=60)

Insulin level	Mean ± STD Deviation groups		p-value
A VS B	1.45±0.55	0.31±0.39	0.000
A VS C	1.45±0.55	1.74±0.56	0.490
A VS D	1.45±0.55	1.63±0.29	0.846
A VS E	1.45±0.55	2.18±0.55	0.009

A=Group A. Control Rats
B= Group B. Fructose (10 g/kg/day)
C=Group C. Fructose (10 g/kg/day)+ Aescin (0.9 mg/kg/day)
D=Group D. Fructose (10 g/kg/day)+ Aescin (1.8 mg/kg/day)
E=Group E. Fructose (10 g/kg/day)+ Aescin (3.6 mg/kg/day)

Discussion

The present study is the first research in our setup on aescin, which exerts positive effects on blood glucose homeostasis and histology of β-cell of Pancreatic Islets (Islets of Langerhans). The present study found protective effects of aescin against the fructose induced DM in rat model. The fructose is a commonly used sugar in the manufacturing of soft drinks and sweets. The use of soft drinks is on the rise throughout the World. This has resulted in metabolic disorders, which is a rising health problem. Fructose consumption is associated with metabolic disorders such as obesity, insulin resistance, and diabetes mellitus, dyslipidemia, elevated serum cholesterol, LDL-cholesterol and triglycerides. Dyslipidemia is a risk factor for cardiovascular disease.[17,18] High fructose consumption results in insulin resistance/hyperinsulinemia. Chances of liver steatosis, lipogenesis, obesity, type 2 DM, systemic hypertension, dyslipidemia, and coronary artery disease are increased by a high fructose diet. European Food Safety Authority [EFSA] stated that the use of fructose is preferred over glucose and sucrose alone in diabetics; because the fructose causes less rise in the post-prandial blood glucose levels. Moreover, fructose intake increases the risk of insulin resistance, dyslipidemia, and visceral obesity. [17,18]

In this study, the highest body weight gain was observed in group B (389.75±10.87 grams), this finding is in agreement with previous studies.[19-21] The reason is clear because fructose increases body weight and adiposity. The increase in the body was found high in positive (control group B). While the Aescin treated animals revealed less rise in body weight and thus shows anti-obesity effects of Aescin and this finding is in accordance to previous studies.[20,21] The present study suggests that

the physiological mechanism of how aescin affects body weight negatively needs further studies. Aescin administration in groups C, D and E showed amelioration of the blood glucose levels, the findings are in agreement with previous studies.[22,23]

In this study, a high dose treated Aescin animal (group E) revealed significant blood glucose lowering compared to control and experimental groups B, C, and D ($P>0.05$). Low dose Aescin (0.9 mg/kg) treated group C animals had high mean blood glucose levels as compared to control group B (not treated with aescin). The findings are in agreement with previous studies,[21,24,25] and these previous studies have reported statistically significant glucose lowering effects ($P<0.05$). However, findings are inconsistent with one study[25] that reported that aescin has glucose lowering potential, statistically insignificant ($P >0.05$). The contradictory finding of the above study is most probably because of the sub-optimal dose of aescin, as they have used low dose aescin therapy for short duration.

Gulcan Avci et al[26] conducted a study on Aescin as an anti-hyperglycemic agent and reported that blood glucose and serum insulin both showed positive effects, which is in favor of the present study, where Aescin treated animal showed a rise in insulin secretion as compared to a positive control (group B). A search of the literature shows no other studies on the effect of aescin on insulin secretion. The present study is the second study that is reporting on the effects of aescin on insulin secretion.

Conclusion

The Aescin showed exerts ameliorating effects on glucose homeostasis in fructose fed Diabetic albino Wistar rats. By Aescin administration, serum insulin level may increase and thus diabetes can be controlled. More brief large sample size experimental studies are required on this objective.

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