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Original Article

Anti-hyperglycemic effects of Aescin at different doses: An experimental animal study

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Abstract:

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

Methodology: Quasi Experimental study was conducted at the Department of Pharmacology from September 2020 to March 2021. Fifty albino Wistar rats were equally divided into five groups according to dose as; Group A (normal diet), Group B (Fructose 10 g/kg/day), Group C (Fructose 10 g/kg/day plus aescin0.9 mg/kg/day), Group D (Fructose 10 g/kg/day plus aescin1.8 mg/kg/day) and Group E (Fructose 10 g/kg/day plus aescin3.6 mg/kg/day). Post-experimental body weight was measured after completion of four weeks. While blood samples were also collected from all animals to assess the blood glucose level and serum insulin level at the end of experiment duration. Data was analyzed using SPSS version 20.

Results: Mean blood glucose in control group A (165.0±14.29 mg/dL) was significantly ($p<0.05$) lower in contrast to experimental groups B, C and D (357.17±21.73, 246.08±21.73 and 235.67±25.73 respectively) while no significant difference observed between group A and group E (194.0±22.16 mg/dl). Serum insulin level was significantly lower ($p<0.05$) in experimental group B (17.45± 5.17) as compared to control group A (16.88± 4.78). Mean serum insulin level in Aescin administrated (group E, 3.6 mg/kg/day) was significantly higher ($p<0.05$) (29.10± 1.64 IU/L) as compared control and other experimental groups.

Conclusion: The Aescin exerts the ameliorating effects on glucose homeostasis; by its administration serum insulin level also increases that may help to control diabetes.

Keywords: Aescin, Fructose, Glucose Homeostasis, Insulin.

Introduction:

The use of refined sugar in the diet is on rise. 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 particularly metabolized in the liver.[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 prevalence of Diabetes mellitus (DM) in the world is rocket high; particularly developing countries like Pakistan. Now the DM ranks 4th non-communicable disease (NCDs) that cause deaths; hence the problem needs search for more effective 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. An rise of 55% is estimated; if happens than there will be 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 United States, management of diabetes costs 548 million dollars in 2013.[9] However, Pakistan is the sixth populous country with a population of 184.35 million, the true prevalence of Diabetes in Pakistan is not avialable.[10] A survey conducted by Diabetes Association of Pakistan (DAP) and WHO reported prevalence of DM around 6.39–16.5%.[11] Contrary to this, in 2014, International Diabetes Federation reported 6.8% prevalence of DM in Pakistan (population aged 20-79 years). It means that 6.9 million cases of diabetes were present in Pakistan, in 2014.[12] By 2035, it is estimated that Pakistan will have 12.8 million diabetics.[13] With this alarmingly rising burden of DM, there is a need to find newer agents and herbs having efficacy of controlling the blood glucose levels and regulatory effects on β-cell secretory physiology.

An herb called Aesculus hippocastanum (commonly called the horse chestnut) is presently focused of research. Medicinal properties of Aesculus hippocastanum are attributed to the Aescin, which is an active compound of horse chestnut. A study reported that aescin is an anti-inflammatory, veno-toner, and vasculo-protective agent.[14] Previous studies reported that aescin is an effective therapy for chronic venousinsufficiency.[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]

Objective:

To assess the effects of Aescin at different doses on blood glucose homeostasis in fructose fed Diabetic albino Wistar rats.

Methodology:

This Quasi-Experimental study was conducted at the Department of Pharmacology at Isra Medical University Hospital Hyderabad from September 2020 to March 2021 after getting approval from the institutional ethical committee. Fifty healthy albino (Wistar) male rats, with body weight between 150 to 250 grams were included in the study. All the procured animals were equally divided into five groups as;

Group A: *Animals were put on a normal diet (normal rat chow with clean water)*

Group B: *Animals were put on Fructose (10 g/kg/day)*

Group C: *Animals were given Fructose (10 g/kg/day) +Aescin (0.9mg/kg/day)*

Group D: *Animals were given Fructose (10 g/kg/day) +Aescin (1.8mg/kg/day)*

Group E: *Animals were put on Fructose (10 g/kg/day) +Aescin (3.6mg/kg/day)*

Aescin was smashed into powder, mixed in chow diet and given for duration of 4 weeks. After four weeks the bodyweight of rats was calculated by electronic weight machine. Bodyweight was noted in the proforma. 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 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 predesign proforma. Data was analyzed by SPSS version20.

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).

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

High dose Aescin treated animals (group E) showed a significant decrease of 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 II: Blood Glucose (mg/dl) comparison among all 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

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 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: Insulin level (g/dl) comparison among animal groups (n=60)

Insulin level groups	Mean ±SD		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

Discussion:

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 cardiovasculardisease.[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, 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.

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