

ORIGINAL ARTICLE

Effect of Aqueous and Ethanolic Extract of Syzygium Aromaticum on Blood Glucose in Diabetic Rats

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ABSTRACT

Objective: To compare the effect of 50% Aqueous and 50% Ethanolic extract of Syzygium aromaticum on blood glucose level in STZ induced diabetic rats in comparison with insulin.

Study Design: Randomized control trial.

Place and Duration of Study: This study was conducted at National Institute of Health Islamabad from July 2011 to December 2011.

Materials and Methods: Forty adult rats of Sprague dawaley specie were equally divided into 5 groups (I-V). Group- I control. Group (II-V) received a single intraperitoneal injection of STZ and rats having fasting blood glucose above 200mg/dl were selected. Group-II served as diabetic control, group III received 50% aqueous extract at a dose of 750 mg/kg body weight for sixty days and group IV rats received 50% ethanolic extract of Syzygium aromaticum at a dose of 750 mg/kg body weight for sixty days. Group V (standard) received the dose of 0.6 units/kg body weight of humulin insulin 70/30 subcutaneously bid for sixty days. After giving the injection of STZ fasting blood samples were taken at zero 15, 30 and 60 days and comparison is done between the glucose lowering effect of aqueous and ethanolic extract of Syzygium aromaticum.

Results: The 50% ethanolic extract of Syzygium aromaticum showed more reduction in blood glucose level than the 50% aqueous extract of Syzygium aromaticum. The levels of blood glucose markedly decreased in group-IV receiving 750 mg/kg body of ethanolic extract as compared to group III receiving the same dose of aqueous extract. Group V receiving insulin showed the level of this parameter almost closer to the blood glucose levels of group III rats.

Conclusion: The reduction in blood glucose with 750 mg/kg body weight of ethanolic extract of Syzygium aromaticum is more than with aqueous extract and insulin.

Keywords: Syzygium Aromaticum Extract, Diabetes Mellitus, Glucose Lowering Effect.

Introduction

In diabetes mellitus hyperglycemia causes cellular lesions and enhances the non-enzymatic glycosylation of proteins and advanced glycosylation end-products are formed which injure cells by structural rearrangement of proteins.¹ Diabetes mellitus is a clinical syndrome with increased blood glucose level and depending on the need of insulin it is divided into absolute deficiency of insulin (type 1) or relative deficiency of insulin (type 2).² It has become a common disorder affecting approximately 180 million people all over the globe.² Streptozotocin (STZ) is used to induce diabetes in rats and causes hyperglycaemia.³ STZ is effective after

intraperitoneal administration of single injection.³ The doses range between 40 and 60 mg/kg body weight but higher doses can also be used.³ It is a nitric oxide donor which leads to DNA damage.³ after entering the cell it brings about changes in the DNA of pancreatic beta cells leading to its fragmentation.³ Many undesirable effects of insulin and other oral hypoglycemic agents necessitated the search for more safer and effective anti diabetic agents.⁴ In the last few decades many herbal plants have shown antidiabetic potential.⁵ Syzygium aromaticum (clove) a herbal plant belongs to the family myrtaceae.⁶ genus Syzygium species aromaticum in urdu also called "laung" Scientific name of clove is Syzygium aromaticum (Linn) Merrill and Perry syn. Eugenia caryophyllata.⁶ Clove has many uses and is used as a topical antiseptic.⁷ local anaesthetic in dentistry.⁷ In the treatment of gastrointestinal symptoms.⁷ also as ant-inflammatory, insecticidal, antiplatelet, antioxidant, insulin-mimetic, and antihypertensive agent.⁷ Clove oil is used as a painkiller for dental emergencies.⁸ Clove buds are used in food products as a flavoring agent condiment.⁸ The compounds in clove are eugenol, isoeugenol, caryophyllene and triterpenes including oleanolic acid (OA).⁹ This

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compound Oleanolic acid causes attenuation of the activities of glycogenic enzymes with concomitant increases of hepatic and muscle glycogen concentrations of STZ-induced diabetic rats.¹⁰ Syzygium aromaticum has anti oxidant and antimutagenic potential.¹¹

The purpose of this experimental study is to compare glucose lowering effect of ethanolic and aqueous extract of Syzygium aromaticum on STZ induced diabetic rats in comparison with the standard drug insulin, a drug commonly used in diabetic patients.

Materials and Methods

This randomized control comparative study was conducted in the department of plant and science at National Institute of Health Islamabad from July to December 2011. A total of Forty adult healthy male Sprague dawley rats were selected and randomly divided in 5 groups with eight rats in each group. Female rats and male rats with weight less than 200 gm and more than 250 gm were not included in the study. The animals were kept in the animal house NIH for one week to get acclimatized under standard laboratory environment, with the room temperature at 26°C, humidity at 70%, 12 hours dark and light cycle was maintained.¹² Free access to rodent pellet and water ad libitum was available throughout the study. A total of 250 grams of dried Syzygium aromaticum buds were purchased from the herbal dealer in the local market. The sample was submitted to the Department of Plant Sciences National University of Science and Technology Islamabad for identification of plant sample (NUST/NCVI/MQH/ZRC/001). Dried Syzygium aromaticum clove buds were divided into two groups 125g in each group and were crushed and soaked into 50% ethanol and 50% aqueous solution, each was stirred in the flask with magnetic stirrer for 24 hours at room temperature. After 24 hours the filtrate were separated and kept in a separate flask. The process was repeated thrice, and filtrate was concentrated at 40°C under reduced pressure in a rotary evaporator. The extracts were stored at temp of -20°C till used for experimental purpose.¹³

Rats with fasting blood glucose level between 70-135 mg/dl were selected after one week of acclimatization, a single intraperitoneal injection of freshly dissolved streptozotocin (60 mg/kg body weight) in 0.1M citrate buffer (pH 4.5). was used for

inducing diabetes, 5% dextrose, solution was given over night to counter the hypoglycemic shock.¹⁴ After 48 h of STZ injection blood samples were taken from the tail vein. Animals with fasting blood glucose level above 200 mg/ 100 ml were selected for further experiments. The animals were randomly divided into five groups (n= 8). All the groups received standard diet and tap water for sixty days. Group-I. (control group) received 10 ml/kg of 0.9% saline solution. Group-II till group V received STZ (60 mg/kg body weight) as a single intraperitoneal injection. Group III received an aqueous extract of strength 750mg/kg body wt by gavage, Group-IV received ethanolic extract of the strength 750mg/kg body wt by gavage.^{13,14} Group-V. (Standard) received insulin (humulin 70/30) with dose of 0.6 units/kg body wt subcutaneously twice daily, for sixty days.¹⁵ One drop of blood was with-drawn from the tail vein of the animals, at zero, 15, 30 and 60 day. quantitative estimation of blood glucose was done by using glucometer (Easy glucometer) and glucose oxidase based test strips. (Easy gluco auto coding test strips).¹⁶ Twenty four hour after the last dose of the extract, the animals were anesthetized with ether and blood was with-drawn by cardiac puncture. The blood was allowed to clot for 5 minutes. Serum was separated by centrifuge at 3000 rpm for 10 minutes and stored at -20°C. Quantitative estimation of blood glucose was carried out by enzymatic method using a commercially available Kit (Randox, UK) based on glucose oxidase method.¹⁷ Data was entered into SPSS version 20. Mean and standard deviation of the parameters were calculated and results of different study groups were compared. Changes in glucose level between all the groups were compared using One-Way ANOVA followed by Post-hoc Tukey test. A p-value of <0.05 was considered significant.

RESULTS

The readings of blood glucose showed that injection of STZ caused a significant ($p < 0.001$) increase in the serum glucose level of the rats of group II, III, IV, and V as compared to the control group. On the other hand the administration of the dose of 750mg/kg body weight of aqueous and ethanolic extract of Syzygium aromaticum caused a significant ($p < 0.001$) reduction in the blood glucose level of group III, and IV, as compared to group 11 (diabetic control). The reduction in the blood glucose level of group 1V

receiving 750 mg/kg body weight of ethanol extract of *Syzygium aromaticum* was significantly higher ($p < 0.001$) as compared to the other experimental groups. It was also seen that simultaneous administration of insulin (humulin) resulted in significant ($p < 0.001$) decrease in the serum glucose level of group V as compared to the group II (diabetic control) but the difference between group-III and group-V was insignificant ($p = 0.996$)

Table I: Serum glucose levels (mg/dl) in all the study groups

Groups	Glucose level mg/dl On day zero	Glucose level mg/dl On day 15	Glucose level mg/dl On day 30	Glucose level mg/dl On day 60
Group-I (n = 8)	121.87 ± 5.86	123.50 ± 5.58 η	125.00 ± 5.73 η ¥€ π	127.75 ± 5.17 η ¥€ π
Group-II (n = 8)	232.00 ± 7.42*	234.00 ± 6.80*	236.00 ± 7.09*¥€ π	237.87 ± 6.55*¥€ π
Group-III (n = 8)	229.25 ± 5.72*	222.12 ± 5.38* η	189.87 ± 3.75* η €	167.12 ± 2.64* η €
Group-IV (n = 8)	231.62 ± 6.92*	205.75 ± 8.06* η ¥	180.37 ± 6.78* η ¥ π	154.62 ± 5.97* η ¥ π
Group-V (n = 8)	230.25 ± 7.42*	213.25 ± 7.38* η	191.25 ± 6.71* η €	166.12 ± 5.81* η €
p-value	< 0.001**	< 0.001**	< 0.001**	< 0.001**

All values have been expressed as mean ± SD

** = Highly Significant * = Significant from group-I

η = Significant from group-II ¥ = Significant from group-III

€ = Significant from group-IV π = Significant from group-V

Discussion

In our study a comparison of aqueous and ethanolic extract of *Syzygium aromaticum* on blood glucose of streptozotocin induced diabetic rats is made and the results are compared with the standard drug insulin at a dose of 0.6 units/kg body weight. We used 50% aqueous extract and 50% ethanol extract with dose of 750mg/kg body weight respectively. In our study group II showed significant elevation in the blood glucose as compared to group I (control) group. The administration of aqueous extract of *Syzygium aromaticum* to group III, ethanol extract to group IV, and humulin insulin to group V brought the level of this diagnostic parameter in all the experimental groups to almost normal as compared to group II (diabetic control group) rats. When we compare the mean values of glucose between group III, IV with group V although the reduction in the serum glucose level of all the three groups is seen but reduction is

more in group 1V as compared to the other two groups. It is seen that the group V receiving insulin also reduced the blood glucose but the level of this parameter is almost close to the reduction in blood glucose level brought by group III. It is observed that *Syzygium aromaticum* ethanol extract causes 35% reduction in the serum glucose level and the *Syzygium aromaticum* aqueous extract causes 30% reduction in blood glucose level which is close to the reduction in the blood glucose brought about by insulin. In our study we used 50% ethanol extract of plant because the constituents in the *Syzygium aromaticum* are more soluble in ethanol.¹⁸ Similar concentration of extract was used by Tajuddin A, et.al who used 50% ethanol extract of clove in rats.¹⁹ The dose 750 mg /kg body weight was selected because zunnara et al in one of her study on the diabetic rats suggested that maximum glucose lowering effect of *syzygium aromaticum* was seen with the extract having the strength of 750 mg/kg body weight.²⁰ We used aqueous extract in our study because aqueous extract of *Syzygium aromaticum* also has the potential of lowering blood glucose. Rao BK et.al conducted a study on the genus *Syzygium* and concluded that the aqueous extract of the *Syzygium* also possess the potential of lowering blood glucose.²¹ Our results indicate that group IV receiving 50% ethanolic extract at a dose of 750 mg/kg body weight causes more reduction in blood glucose as compared to group III and group V, similar results are seen with Abubakar Gidadoet et al who studied the effect of aqueous and ethanolic extract of plant on blood glucose and concluded that ethanolic extract caused more reduction in blood glucose.²² The main constituents in the *Syzygium aromaticum* are Olaenic acid and Eugenol.²³ Musabayane et al. critically reviewed the analytical chemistry of Eugenol, and Olaenic acid and found that both possess antioxidant activity and are the major scavenger of free radicals.²⁴ Segas et al, proposed that glucose lowering effect of *Syzygium aromaticum* can be through anti oxidant means.²⁵ It has been reported in many studies that extract of herbal plants when used in the treatment of diabetes mellitus resulted in the activation of pancreatic beta cells and improved granulation showing insulinogenic effect.²⁶ Khan A (2006) in one of his study said that *Syzygium aromaticum* has the

potential to cause regeneration of pancreatic beta cells and stimulate the functioning cells of islet of

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