EFFECT OF SYZYGIUM AROMATICUM (CLOVE) EXTRACT ON BLOOD GLUCOSE LEVEL IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Objective: To evaluate the glucose lowering effect of 50% ethanol extract of Syzygium aromaticum in comparison with that of standard insulin in streptozotocin induced diabetic rats.

Study Design: Randomized control trial.

Place and Duration of Study: National Institute of Health Islamabad. Jul 2011- Dec 2011

Material and Methods: It was carried out on 48 adult rats of Sprague dawley specie. Rats were equally divided into 6 groups (I-VI). Group - I served as control. Diabetes was induced by giving single intraperitoneal injection of STZ in Group II to VI. Group-II served as diabetic control, while groups III, IV, V and VI served as experimental groups. Group III, IV and V rats received 50% ethanol extract of Syzygium aromaticum at a dose of 250, 500 and 750 mg/kg body weight respectively for sixty days. Group VI (standard) received humulin insulin 70/30 at dose of 0.6 units/kg body weight subcutaneously bid for sixty days. Fasting blood samples were taken at zero day, 15 day, 30 day and 60 day after giving injection STZ. Although Syzygium aromaticum with the doses of 250, 500 and 750 mg/kg body weight and insulin reduced the level of glucose in rats but on comparison Syzygium aromaticum 750 mg/kg dose reduced glucose more effectively than 250 and 500 mg/kg dose.

Results: The levels of blood glucose markedly decreased in group-V animals and reached nearly normal value. While in group III, IV subjects, blood glucose levels remained above normal level. In group VI receiving insulin the level of this parameter remained almost closer to group IV rats. On studying the weight of the animals after receiving STZ there was initial reduction in the weight of all the experimental groups but after receiving the extract of plant improvement was seen and the weight of group V getting 750 mg/kg/body weight of Syzygium aromaticum became almost closer to the weight of control group.

Conclusion: Syzygium aromaticum extract has glucose lowering effect in STZ induced diabetic rats and this effect is dose related and the dose of 750 mg/kg body weight has produced maximum effect.

Keyword: Syzygium aromaticum extract, diabetes mellitus, normoglycemic effect.

INTRODUCTION

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to absolute, (type 1) or relative deficiency of insulin (type 2). It has become a common disorder affecting approximately 180 million people all over the globe[1]. 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[2]. The acute complication of diabetes mellitus include hyperglycemia, diabetic ketoacidosis, lactic acidosis, hyperosmolar non-ketotic coma[3]. The late complications include peripheral vascular disease, coronary heart disease, retinopathy, neuropathy, nephropathy and stroke[4].

Streptozotocin (STZ) is used to induce diabetes in rats and causes hyperglycaemia[5]. It is synthesized by *Streptomycetes achromogenes* and is used to induce both type 1 and type 2 DM. The doses range between 40 and 60 mg/kg body weight but higher doses can also be used[5]. STZ is effective after intraperitoneal administration of a similar or higher dose and after entering inside the cell it brings about changes in DNA of pancreatic beta cells leading to its fragmentation[5]. It is a nitric oxide donor which leads to DNA damage[5].

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In the recent years, a number of plants have been tried on a variety of pathological conditions. Some are prescribed for the treatment of diabetes mellitus. Syzygium aromaticum (clove) in urdu also called "laung" is a herbal plant which belongs to the species *aromaticum* genus *Syzygium* and family myrtacea[6]. Scientific name of clove is Syzygium aromaticum (Linn) Merrill and Perry syn. Eugenia caryophyllata[6]. This plant is widely cultivated in Indonesia, Sri Lanka, Madagascar, Tanzania and Brazil, Zanzibar. Clove is used as a topical antiseptic and local anaesthetic in dentistry[7]. It is also used in the treatment of gastrointestinal symptoms[7]. Role of Syzygium aromaticum as ant-inflammatory, insecticidal, antiplatelet, antioxidant, insulin-mimetic, and antihypertensive have been reported[7]. Oil can be obtained from clove. Clove oil is used as a painkiller for dental emergencies as it has been relieving toothache[8]. Clove buds are used in limited amounts in food products as a fragrant, flavoring agent and condiment and also in a mixture named garam masala[8]. Clove contains eugenol, isoeugenol and caryophyllene compounds, contributing to the pharmacological effect of Syzygium aromaticum[9].

The purpose of this experimental study is to see the glucose lowering effects of ethanol 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

Animals

Adult healthy male Sprague dawley rats (n=48) of weight 200-250 g were randomly divided in 6 groups (n=8). Male rats were preferred to avoid hormonal changes which occur in females. The animals were kept for one week in the animal house in the laboratory of National Institute of Health (NIH) Islamabad to get acclimatized under standard laboratory environment, room temperature was maintained at 26^0 C, humidity at 70%, 12 hours dark and light cycle was maintained. Free access to rodent pellet and water ad labium was available throughout the study[10].

Plant materials and preparation of extract

A total of 500 grams of dried Syzygium aromaticum buds were purchased from the herbal dealer in local market. The sample was submitted to the Department of Plant Sciences National University of Science and Technology Islamabad for identification (NUST / NCVI / MQH / ZRC / 001). Dried Syzygium aromaticum clove buds were crushed into small pieces and soaked into 50% ethanol. It was stirred in the flask with magnetic stirrer for 24 hours at room temperature. After 24 hours the filtrate was separated and kept in separate flask. The process was repeated thrice, and filtrate was concentrated at 40^o C under reduced pressure in a rotary evaporator. The extract was stored at -20°C till used for experimental purpose[11].

Experimental procedure

Adult healthy male Sprague dawley rats after one week of acclimatization were subjected to a 16 hour fast. Rats with fasting blood glucose level between 70-135 mg/dl were selected. Diabetes was induced by a single intraperitoneal injection of freshly dissolved streptozotocin (60 mg/ kg body weight) in 0.1m citrate buffer (pH 4.5). The experimental animals had free access to feed a solution of 5% dextrose, given over night to counter the hypoglycemic shock[12].

Blood samples were taken from the tail vein after 48 h of STZ injection. Animals having fasting blood glucose level above 200 mg/ 100 ml were considered diabetic and were selected for further experiments[12].

Grouping of animals

The animals were randomly divided into six groups (n= 8).

Group–I (control group). The animals received 10 ml/kg of 0.9% saline solution through gavage. They were fed on standard diet and tap water and received no drug therapy.

Group-II (diabetic control). Received 60 mg/kg body weight of streptozotocin as a single

intraperitoneal injection and were fed on standard diet and tap water for sixty days.

Group-III (experimental group 1). Received 60 mg/kg body weight of STZ as a single intra-peritoneal injection and the extract having strength of 250 mg/kg was administered by gavage to diabetic animals once a day for sixty days[11,12].

Group–IV (experimental group 2): Received 60 mg/kg body weight of STZ as a single intra peritoneal injection and the extract having strength of 500 mg/kg was administered by gavage to diabetic animals once a day for sixty days[11,12].

Group-V (experimental group 3): Received 60 mg / kg body weight of STZ as a single intra peritoneal injection and the extract having strength of 750 mg/kg, was administered by gavage to diabetic animals once a day for sixty days[11,12].

Group-VI (Standard): Received STZ injection at a dose of 60 mg/kg body weight and insulin (humulin 70/30) with dose of 0.6 units/kg body weight twice daily, subcutaneously for sixty days.

Estimation of body weight of rats

All the animals with weight ranging between 200-250 g were selected[12]. After receiving injection of STZ the animals were weighed on zero day i.e 48 hours (post STZ) and on 60 day (after 2 months) of the study.

Sample collection / biochemical analysis

Blood samples were taken at zero day, 15 day, 30 day and after 60 day. One drop of blood was with-drawn from the tail vein of the animals, quantitative estimation of blood glucose was done by using commercially available glucometer (Easy glucometer) and glucose oxidase based test strips[13] (Easy gluco auto coding test strips).

Twenty four hour after the last dose of the extract, the animals were weighed and 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. Quatitative estimation of blood glucose was carried out by enzymatic method using a commercially available Kit (Randox, UK) based on glucose oxidase method[14].

Table-1: Comparison of body weight of groups on zero day and after 60 days.

Groups	Body weight (grams) Mean ± SD	
	On zero day	After 60 days
Group-I (n = 8)	220.62 ± 4.56	226.50 ± 4.75¶¥€¤
Group-II (n = 8)	174.12 ± 6.73*	170.88 ± 7.08*¥€¤
Group-III (n = 8)	174.00 ± 3.82*	180.50 ± 3.89*¶€¤
Group-IV (n = 8)	175.12 ± 5.28*	190.25 ± 5.18*¶¥¤
Group-V (n = 8)	175.75 ± 6.14*	200.38 ± 6.02*¶¥€
Group-VI (n = 8)	177.38 ± 6.04*	189.25 ± 5.70*¶¥¤
p-value	< 0.001**	< 0.001**

All values have been expressed as mean ± SD
* = Significant from group-I ** = Highly significant
¶ = Significant from group-II ¥ = Significant from group-III
€ = Significant from group-IV ¤ = Significant from group-V

Statistical analysis

Data was entered into SPSS version 13. Mean and standard deviation of the parameters were calculated and results of different study groups were compared. Changes in weight and glucose level between all the groups were compared using One-Way ANOVA followed by Post-hoc Tukey test. *p*-value of < 0.05 was considered significant.

RESULTS

On estimation of the body weight of the rats 48 hrs after receiving STZ the difference between all the groups was significant ($p<0.001$). Body weight was significantly higher in group-I

as compared to all the five diabetic groups ($p < 0.001$) but the difference between all the five diabetic groups was insignificant ($p > 0.05$).

At the end of the study on 60 day it was seen that the ethanol extract of Syzygium aromaticum has caused a significant ($p < 0.001$) increase in the body weight of the rats of the group V as compared to the group III, IV and VI ($p < 0.001$), we can say a dose of 750 mg/kg body weight of Syzygium aromaticum has caused greater improvement in the body weight as compared to other two doses (Table-1).

Blood glucose level (mg/dl)

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, V and VI as compared to the control group. On the other hand the simultaneous administration of different doses of ethanolic extract of Syzygium aromaticum caused a significant ($p<0.001$) reduction in the blood glucose level of group III, IV, V as compared to group 11 (diabetic control). The reduction in the blood glucose level of group V 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 VI as compared to the group II (diabetic control) but the difference between group-IV and group-VI was insignificant ($p=1.000$)(Table-2).

Table-2: 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)	121.88 ± 5.87	123.50 ± 5.58¶	125.00 ± 5.73¶¥€¤	127.75 ± 5.18¶¥€¤
Group-I1 (n = 8)	231.25 ± 6.78*	233.38 ± 6.28*	235.12 ± 6.36*¥€¤	237.25 ± 5.99*¥€¤
Group-I11(n = 8)	229.75 ± 7.88*	218.75 ± 8.70*¶	205.62 ± 8.48*¶€¤	193.00 ± 6.09*¶€¤
Group-IV (n = 8)	228.88 ± 7.92*	212.75 ± 8.12*¶	190.62 ± 6.50*¶¥¤	168.38 ± 5.93*¶¥¤
Group-V (n = 8)	231.62 ± 6.93*	2205.75 ± 8.07*¶¥	180.38 ± 6.78*¶¥€	154.62 ± 5.97*¶¥€
Group-V (n = 8)	230.25 ± 7.42*	213.25 ± 7.38*¶	191.25 ± 6.71*¶¥¤	166.12 ± 5.82*¶¥¤
p-value	< 0.001**	< 0.001**	< 0.001**	< 0.001**

All values have been expressed as mean±SD

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

¥ = Significant from group-III € = Significant from group-IV ¤ = Significant from group-V

Herbal medicine has long been used for the treatment of diabetic patients and continues to be currently accepted as an alternate therapy. In our study the glucose lowering effect of 50% ethanolic extract of Syzygium aromaticum in STZ induced diabetic rats was observed. We used 50% ethanol extract of Szygium aromaticum because its constituents are more soluble in ethanol[18]. In the present study significant elevation in the blood glucose level of group II was seen as compared to group I (control) group. However administration of Syzygium aromaticum ethanol

DISCUSSION

During diabetes mellitus persistent hyperglycemias may liberate oxidizing radicals causing damage to the tissue responsible for lowering blood glucose. Tight control of blood glucose can reduce clinical complications and oxidative stress in diabetic patient through treatment with antioxidants[15]. Insulin and other hypoglycemic agents are used to control blood glucose but they also produce a number of undesirable effects[16]. Therefore the use of oral hypoglycemic agents and insulin is restricted due to their management and treatment failure having several adverse effects[17].

extract to group III, IV, V and humulin insulin to group VI 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 group III, IV with group V although the reduction in the serum glucose level of all the three groups was seen but reduction was more in group V as compared to the other two groups. When we compare the mean values of this parameter in all the three experimental groups receiving Syzygium aromaticum with group VI receiving humulin, although insulin reduced the blood glucose level but Syzygium aromaticum ethanol extract brought greater reduction in the serum glucose level as compared to insulin especially in group V. The main constituents in the syzygium aromaticum are Olaenic acid and Eugenol[19]. Segas et al, proposed that glucose lowering effect of Syzygium aromaticum could be through anti oxidant means[20]. Musabayane et al. critically reviewed the analytical chemistry of Eugenol, and Olaenic acid and found that both posses antioxidant activity and are the major scavenger of free radicals[21]. 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[8]. Khan et al, in one of his study reported that Syzygium aromaticum has insulin mimetic action and improves insulin efficiency[22]. So we can say that glucose lowering effect of Syzygium aromaticum could be due to stimulation of functioning pancreatic beta cells, to increase the release of insulin[22]. Or this may be due to regeneration of beta cells[22]. Toda et al in his study reported that Syzygium aromaticum posses alpha-glucosidase inhibiting properties, it is presumed that anti hyperglycemic effect of clove may be due to its inhibitory action on alpha–glucosidase[23]. On comparing the weight of diabetic rats it was seen that high dose of Syzygium aromaticum extract (750 mg/kg) improved the weight of group V animals almost closer to normal than other groups. This increase in weight may be due to the insulin like action of extract on muscle, adipose tissue and hepatocytes. Agbaje et al in their study showed improved body weight by Syzygium aromaticum in rats[24]. Further laboratory investigations are needed to evaluate the actual mechanism of action of the compounds present in Syzygium aromaticum. However this study will pave the way for certain plant based treatment of diabetes avoiding the complications of artificial drug substances.

CONCLUSION

It was concluded that glucose lowering effect was dose dependent with ethanol extract of Syzygium aromaticum and at higher doses (750 mg/kg) the reduction was more as compared to the reduction in blood glucose brought about by standard insulin.

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