ORIGINAL ARTICLE

Comparison of Extracts of *Syzygium Aromaticum* on the Weight of STZ induced Diabetic Rats

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ABSTRACT

Objective: To observe the effect of different extracts of *Syzygium aromaticum* (50% ethanolic and 50% aqueous) on the weight of Streptozotocin (STZ) induced diabetic rats in comparison with insulin.

Study Design: Randomized control trial.

Place and Duration of Study: The 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 with weight ranging between 200-250 g were selected and were equally divided into 5 groups (I-V) with eight rats in each group. Group- I was control. Diabetes was induced to group II- V by a single intraperitoneal injection of STZ and rats with fasting blood glucose above 200mg/dl were selected. After receiving the injection of STZ, the animals were weighed on Day zero i.e 48 hours (post STZ). Group-II was diabetic control , group III received 50% aqueous extract while group IV received 50% ethanolic extract of *Syzygium aromaticum* at a dose of 750 mg/kg body weight respectively for sixty days. Group V received 70/30 humulin insulin, 0.6 units/kg body weight subcutaneously, bid for sixty days. After two months of study on Day 60 the animals were weighed again, changes in the weight which occurred with both extracts were compared with the changes in the weight which occurred with the standard drug insulin.

Results: Group-IV receiving 750 mg/kg body of 50% ethanolic extract showed marked improvement in the weight as compared to group III receiving 50% of the same dose of aqueous extract. Group V receiving insulin showed improvement in the weight which is almost closer to the increase in weight which occurred in group III diabetic rats.

Conclusion: 750 mg/kg body weight of 50% ethanolic extract of *Syzygium aromaticum* caused more improvement in the weight of diabetic rats than the increase in the weight with the 50% aqueous extract of the same dose and the drug insulin.

Key Words: *Diabetes Mellitus, Weight Improvement , Syzygium Aromaticum Extract.*

Introduction

Diabetes mellitus (DM) is a very common disease throughout the world. In Pakistan the incidence of diabetes mellitus is 22.04%.[1] In this disease there is destruction of the insulin producing beta cells of pancreas (Type1DM) and resistance to insulin action in liver and peripheral tissue leading to hyperglycemia (Type2DM).[2] It is a complex disorders that disturbs the metabolism of protein, fat and carbohydrates leading to weight loss. Several oral hypoglycemic agents lowers blood glucose and improve weight but have many side effects. Traditional antidiabetic plants might provide new oral hypoglycemic compounds, with less side effects and can counter the high cost and poor availability of antidiabetic drugs for rural populations.[3] Medicinal plant like *Syzygium aromaticum* has antidiabetic potential, its effect on STZ induced diabetic rats have been studied and its extract lowered the blood glucose level.[4] Streptozotocin (STZ) a nitrosourea compound is used to induce insulin-dependent diabetes mellitus in experimental animals.[5] It causes irreversible damage to the pancreatic beta cells resulting in degranulation and loss of capacity to secrete insulin.[5] The cytotoxic action of STZ is mediated by reactive oxygen species, which causes a rapid destruction of B cells leading to

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Funding Source: NIL ; Conflict of Interest: NIL
Received: October 09, 2015; Accepted: March 06, 2016

hyperglycemia.[6] A single intraperitoneal injection of STZ with the dosage range between 40-60 mg/kg body weight is very effective.[6] This high blood glucose causes the formation of advanced glycosylation end-products which injures the pancreatic beta cell through oxidative stress.[7] Increased serum glucose causes increase catabolism in skeletal muscles leading to increase breakdown of muscle protein and loss of adipose tissues causing a decrease in body weight.[8] A herbal plant *Syzygium aromaticum* (clove), with the scientific name (L)Merrill and Perry syn Eugenia caryophyllata, also known as "laung" belongs to the species *aromaticum*, family *myrtacea* and genus *Syzygium*.[9] It is widely cultivated in Indonesia, Sri Lanka. *Syzygium aromaticum* is commonly used as local anesthetic in dentistry and as flavoring agent in food. It treats gastrointestinal symptoms and act as ant-inflammatory, insecticidal, and antioxidant agent.[10] *Syzygium aromaticum* extract acts like insulin in hepatocytes by reducing phosphoenolpyruvate carboxykinase and glucose 6-phosphatase gene expression.[11] The active ingredient in clove is eugenol.[12] It improves gut motility by increasing the secretion of gastro-intestinal enzyme and relieves indigestion.[13] Many studies have shown that ethanolic and aqueous extract of *Syzygium aromaticum* improves and increases the weight of diabetic rats. The aim of the present study isto compare the improvement in the weight of the diabetic rats which occurs with both extracts of *Syzygium aromaticum* in comparison with the changes in the weight which occurs with the standard drug insulin.

Materials and Methods

Present study was carried out in National Institute of Health Sciences Islamabad in the department of plant and science from July to December 2011. It was a randomized comparative trial in which forty adult healthy male sprague dawley rats were selected, rats belonging to both genders with weight more than 250 gm and less than 200 gm were not included in the study. For acclimatization the rats were kept at animal house of NIH for one week at room temperature of 260C and humidity of 70% with 12 hours dark and light cycle was maintained. Rodent pellet containing proteins, fats, minerals, vitamins, fibre and water were available throughout the study.[14] Dried *Syzygium aromaticum* buds about 250gms were verified from the National University of Science and Technology Islamabad, department of Plant Sciences (NUST/NCVI/MQH/ZRC/001). About 125g of *Syzygium aromaticum* buds were soaked into 50% aqueous solution and the same amount was soaked in 50% ethanolic solution. Each was stirred with magnetic stirrer for twenty four hours in the flask at room temperature. After 24 hours the filtrates were separated and kept in a separate flask. This process was repeated thrice , the filtrates were then concentrated under reduced pressure of 40°C in a rotary evaporator. The prepared extracts was stored at a temp of -20°C to be used for further experiment.[15]

After one week of acclimatization, all the experimental groups with fasting blood glucose level between 70-135 mg/dl were induced diabetes with a single intraperitoneal injection of freshly dissolved streptozotocin (60 mg/ kg body weight) in 0.1ml citrate buffer (pH 4.5).[16] Forty eight hours after receiving the injection of STZ blood samples were taken from the tail vein of experimental animals. Quantitative estimation of blood glucose was done by using glucometer and glucose oxidase based test strips.[17](Abbott AxSYM system, USA). Rats with fasting blood glucose above 200mg/dl were selected. The animals were divided into five groups with eight rats in each group. All the rats received rodent pallets and water ad libitum for sixty days in addition 10 ml/kg of 0.9% saline solution was given to first group . Second group was (diabetic control. Third and fourth group received 750mg/kg body wt of 50% aquoeus and 50% ethanolic extract respectively by gavage. Insulin (humulin 70/30) with the dose of 0.6 units/kg body wt , subcutaneously twice daily was adminstered to fifth group.[18] The weight of the diabetic rats was estimated on Day zero i.e forty eight hours after receiving Streptozotocin and after 2 months of the study at Day60.[19] Data was analyzed using SPSS version 20. Weight of diabetic rats were expressed in Mean ± SD and the results were compared by using One Way ANOVA followed by Post-hoc Tukey test p-value of < 0.05 was considered significant.

Result

In the beginning of the study on Day zero there was significant difference in the body weight between all

the groups (p<0.001). Group I had higher body weight as compared to other groups (p < 0.001). There was insignificant difference in the body weight between all the four diabetic groups (p > 0.05). At the end of the study on Day 60 body weight was significantly higher in group-I as compared to all the four diabetic groups (p < 0.001). The weight of group II diabetic rats was significantly lower than other groups (p < 0.001). Group-III and group V showed significantly lower body weight than group-I and group-IV but higher than group II . However there was insignificant difference between group-III and group-V(p=0.941) The rats belonging to group-IV showed significantly higher body weight than other diabetic groups (p < 0.001) but significantly lower than group I.

From the above result significant increase in the weight of group IV rats, receiving 750 mg/kg body weight of 50% ethanol extract of *Syzygium aromaticum* was seen. Simultaneous administration of Insulin (humulin) also resulted in significant (p<0.001) improvement in the weight of group V as compared to the group II (diabetic control) but this increase in weight is closer to the increase in weight which occurred in group III diabetic rats .

Table I Shows the mean and standard deviation of weight taken on Day zero and Day 60.

Table I: Body weight of all study groups on Day zero and Day 60

Groups	Body Weight (grams) / Mean ± SD	
	Day zero	Day 60
Group-I (n = 8)	220.62 ± 4.56 ¶¥€ π	226.50 ± 4.75¶¥€ π
Group-II (n = 8)	174.12 ± 6.73*	170.88 ± 7.08*¥€π
Group-III (n = 8)	175.12 ± 5.28*	190.25 ± 5.18*¶€
Group-IV (n = 8)	175.75 ± 6.14*	200.38 ± 6.02*¶¥π
Group-V (n = 8)	177.38 ± 6.04*	189.25 ± 5.70*¶€
p-value	< 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 π = Significant from group-V

Discussion

In the present study the results have proved that *Syzygium aromaticum* ethanolic extract improves the weight of diabetic rats. In this study the effect of differents extracts of *Syzygium aromaticum* on the weight of streptozotocin induced diabetic rats is seen and the results are compared with the standard drug insulin at a dose of 0.6 units/kg body weight. We used 50% aqueous and 50% ethanol extract with the dose of 750mg/kg body weight respectively. There is significant reduction in the weight of group II diabetic rats as compared to control group I. On comparing the improvement in the weight of the group III, group IV and group V with the diabetic group II it is observed that *Syzygium aromaticum* ethanol extract causes 8% improvement and the *Syzygium aromaticum* aqueous extract causes 5.6 % increase in the weight which is close to the increase in the weight which occurred with insulin receiving group 5.5% . Tajuddin et al., (2003) used 50% ethanol extract of clove in rats.[20] in our study Similar concentration of ethanolic extract showed more impovement in the weight as compared to aquoeous extract and insulin. Namasivayam et al., (2008) conducted a study on the genus *Syzygium* and concluded that the aqueous extract of the *Syzygium* also possess the potential of improving the weight of diabetic rats.[21] Singh et al., (2007) studied the effect of ethanolic extract of *Syzygium* on diabetic rats and concluded that ethanolic extract lowered the blood glucose and improved the weight of diabetic rats.[22] In diabetes mellitus free radicles and reactive oxygen specie are produced which damages nucleic acid, carbohydrates , proteins and lipids, this may lead to weight loss.[23] Musabayane et. al.,(2010) on doing the analytical chemistry of constituents of *syzygium aromaticum* mainly eugenol and olaenic acid suggested that both act as antioxidant and are the major scavenger of free radicals, thus preventing the weight loss.[24] Many studies have reported that aqueous and ethanolic extracts of plants causes glucose lowering effect by activation of pancreatic beta cells , improving its granulation, increasing insulin production and have insulin mimetic effect.[25] Khan et al.,(2006) in one of his study on the *Syzygium aromaticum* suggested that this herbal plant has the potential to stimulate the functioning cells of islet of langerhans and causes regeneration of pancreatic beta cells thus increasing insulin release.[26] Laizuman et al., (2010) suggested that improvement in the weight of diabetic rats by *Syzygium* can be by either due to release of insulin from pancreatic beta cell or from the bound form and insulin also inhibit gluconeogenesis from proteins thus preventing loss of proteins and maintan body weight.[27] Insulin causes increase food intake and retains energy leading to

the improvement in the weight of diabetic rats. Willing et al.,(1990) suggested that insulin stimulates daily food intake and body weight gain in diabetic rats.[28] So we can say that improvement in the weight of diabetic rats by *Syzygium aromaticum* can be due to the stimulation of functioning pancreatic beta cells, to increase the release of insulin or this may be due to regeneration of beta cells. Admin, (2013) suggested that *Syzygium aromaticum* activates digestive enzymes , aid in digestion , smoothens the lining of digestive tract and has myorelaxant action. He further reported that high amount of carbohydrates, protein , dietary fibers, multivitamins and minerals are present in clove which maintain the weight of body.[29] Lester et al., (2004) suggested that eugenol the main constituent of *Syzygium* causes stimulation of gastric secretion and improves digestion.[30] In our study the ethanolic extract caused more improvement in the weight than the aqueous extract we can say that this effect is probably because the constituents of the *Syzygium aromaticum* are more soluble in the ethanol solution than the aqueous solution . San et al., (1995) suggested that *Syzygium aromaticum* compounds have more solubility in the ethanolic solution .[31] We can say in our study that the possible mechanism by which ethanol extract improves the weight of diabetic rats is that the free radical scavenging property of ethanolic extract is more than aqueous or it may be due the presence of antioxidant active principles in the ethanol extract in excess amount than aqueous extract. This improvement in weight can also be due to the induction of insulin secreting pancreatic β cells of islets of langerhan or it may be due to enhanced transport of blood glucose to the peripheral tissue and proper glucose utilization by diabetic rats.[32] The antispasmodic and myorelaxant property of the compounds of *Syzygium aromaticum* can also lead to the improvement in the body weight.[33]

Conclusion

Our results from this study indicate that 50% ethanolic extract of *Syzygium aromaticum* at a dose of 750 mg/kg body weight has more potential to improve the weight of diabetic rats than the 50% aqueous extract of the same dose and the drug insulin because of its insulin mimetic action , free radical scavenging property and the increased solubility and presence of excess amount of antioxidant active principles in it.

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