Original Article

Antihyperglycaemic and Antihyperlipidemic Effects of Ethanolic Extract of Syzygium Aromaticum(Clove) / In Streptozotocin induced Diabetic Rats

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ABSTRACT

Objective: The purpose of the present study was to assess the effect of ethanolic extract of Syzygium aromaticum buds in streptozotocin(STZ) induced diabetes in rats.

Study Design: Randomized controlled trial

Place and Duration of Study: This study was conducted at the Department of Pharmacology, Islamic International Medical College, Rawalpindi from 1st Jan 2011 to 30st June 2011.

Materials and Methods: Single injection of STZ was given intraperitoneally to rats and rats showed fasting glucose level over 280mg/dl were included in the study. After induction of diabetes all rats were divided into, normal control group (A), diabetes positive control group (B), and the two groups (C and D) served as experimental groups while group E served as standard as it received glibenclamide. Group C and D diabetic experimental rats received ethanolic extract of Syzygium aromaticum at 250 mg/kg and 500mg/kg of body weight orally for eight weeks on daily basis. On the other hand group E rats received glibenclamide at 0.5 mg/kg body weight orally for eight weeks. Blood samples were collected after eight weeks.

Results: The diabetic positive group rats showed variable increase in serum levels of glucose, triglycerides, low density lipoprotein (LDL) and total cholesterol levels. Serum high density lipoprotein (HDL) levels decreased in diabetic positive group. Syzygium aromaticum 250mg/kg and 500mg/kg dose and glibenclamide significantly decreased the levels of these parameters in rats. On comparison Syzygium aromaticum 500mg/kg dose reduced glucose and lipid levels more, effectively than the 250mg/kg dose of Syzygium aromaticum and glibenclamide. Syzygium aromaticum constituents, especially polyphenols and flavonoids have strong anti-oxidant activity which might be involved in glucose and lipid lowering effect.

Conclusion: Syzygium aromaticum ethanolic extract decrease glucose and lipid levels in experimentally induced diabetic rats.

Key Words: Syzygium aromaticum, diabetes, oxidative stress, streptozotocin, total cholesterol, serum triglycerides, LDL (low density lipoproteins), HDL (High density lipoproteins)

INTRODUCTION

Diabetes mellitus (DM) is a syndrome characterized by chronic hyperglycaemia and relative insulin deficiency, resistance or both.[1] Diabetes mellitus is not a single disease but basically a group of disorder which is characterized by hyperglycemia, hyperlipidemia, glycosuria, ketonemia and if prolong leads to diabetic complications such as nephropathy, neuropathy and retinopathy.[2] Worldwide more than 140 million people suffer from DM, making it one of the most common non-communicable diseases. Diabetes and hyperlipidemia are also the major cause of conditions associated with atherosclerosis like coronary artery disease, cerebrovascular disease and peripheral vascular disease.[3] Different Studies indicate that reactive oxygen species play a key intermediate role in the pathophysiology of diabetes and its complications.[4-5] Hyperglycaemia also attenuates anti-oxidative mechanisms through non enzymatic glycosylation of anti-oxidant enzymes.[6-7] Dietary modifications and drug therapy have shown promising results to regulate glucose, HDL and LDL cholesterol levels and to reduce subsequent risk of coronary artery disease associated pathological conditions. But due to high cost and adverse effects of glucose and lipid lowering drugs, peoples are now diverting to certain natural substances. The use of such substances are grown faster over the past few years which is undoubtedly driven by the belief that they are relatively safe, easily available and affordable.[8] STZ induced hyperglycaemic rats have been used mainly as a model of diabetes. STZ is synthesized by streptomycetes achromogenes and is used to induce both type 1 and type 2 DM.[9]

Syzygium aromaticum are the aromatic dried flower buds of a tree in the family Myrtaceae. Syzygium

aromaticum is widely cultivated in Indonesia, Sri-Lanka, Brazil, Pakistan and India. It is used in limited amounts in food products as a fragrant, flavoring agent and condiment. Syzygium aromaticum, usually called as clove, is used as a topical antiseptic and local anaesthetic in dentistry.[10-11]. It is also used as antibacterial, antifungal, antimicrobial, antiinflammatory and insecticidal.[12-13] Clove oil mainly constitutes eugenol, isoeugenol and caryophyllene contributing pharmacological role to Syzygium aromaticum. Eugenol comprises 72-90% of the essential oil extracted from cloves. Other important constituents of clove include polyphenols, beta-caryophyllene , tannins, flavonoids, kaempferol, rhamnetin, terpenoids like oleanolic acid, stigmasterol and campesterol.[14]
The purpose of the present experimental model was to observe the effect of Syzygium aromaticum in STZ induced diabetes in wistar rats.

MATERIALS AND METHODS

Animals: Sixty adult male Wistar rats weighing 200-250g were procured for this study. They were kept in the experimental research laboratory of Islamic International Medical College, Rawalpindi, under day and night conditions. Prior to the commencement of the experiments, all animals were kept for one week under the same laboratory conditions, at a temperature of 22 ± 2 ^{0}C, relative humidity of 70 ± 4% and 12 hour light/day cycle. They received nutritionally standard diet and tap water. The care and handling of rats were in accordance with the internationally accepted standard guidelines for use of experimental animals.

Chemicals/Instruments: Commercially available kits (Randox) for biochemical analysis of glucose, cholesterol, triglycerides, LDL and HDL, 95% ethanol, pre-coated TLC(Thin Layer Chromatography) plate silica gel GF254 and toluene. The standard compounds used are ellagic acid, gallic acid and protocatechuic acid. The instruments used were soxhlet and rotary evaporator and centrifuge (Germany), TLC scanner III (Camag, Switzerland) with win CATS software.

Plant materials and preparation of the extract: Clove buds were collected from local market of Rawalpindi and were authenticated from a botanist. Clove buds were coarsely powdered using a grinder. 500 g powdered form of clove was dissolved in 2L of 95% ethanol. The extraction was carried out by mixing the powdered clove in ethanol by Soxhlet apparatus for 72 hr. The extract was filtered and the solvent i.e., ethanol was allowed to evaporate using rotary evaporator at temperature 40-45°C. Thus the highly concentrated ethanolic extract was obtained .The yield of extract was 8.6% w/w in terms of dried starting material. The extracts obtained were stored at -20°C till used for experimental purposes.[15] The clove buds (voucher no. 0525) and extract (voucher no. 0526) were deposited in Pharmacology laboratory, Islamic International Medical College, Rawalpindi. This extract got standardized from Riphah institute of Pharmaceutical Sciences, Islamabad.

Experimental Procedure: After acclimatization, 10 rats were labeled as control. All other rats were starved for 16 hours and diabetes was induced by using a single intraperitoneal injection of freshly dissolved streptozotocin (60 mg/kg) in 0.01M citrate buffer (pH 4.5).One week after the streptozotocin injection, rats were assessed for diabetes and those with fasting blood glucose over 280 mg/dl were included in this study.[16] Thereafter, all rats were divided into five groups each having 10 animals. The control rats (Group A) were fed on standard diet with tap water and received no drug. Group B i.e. diabetic control rats received 60 mg/kg of STZ as a single intraperitoneal injection and were fed on standard diet and tap water. Group C i.e. experimental group rats received 60 mg/kg of STZ as a single intraperitoneal injection and ethanolic extract of *Syzygium aromaticum* buds in a daily oral dose of 250 mg/kg for a period of sixty days. Group D i.e. experimental group rats received 60 mg/kg of STZ as a single intraperitoneal injection and and ethanolic extract of *Syzygium aromaticum* buds in a dose of 500 mg/kg body weight daily (orally) for a period of sixty days .Group E standard group rats received 60 mg/kg of STZ as a single intraperitoneal injection and glibenclamide orally at a dose of 0.5mg/kg for sixty days.

Sample collection: Blood sampling through tail vein was performed at 2 intervals (0, 8 weeks) following same protocol every time. Twenty four hour after administration of the last dose of extract i.e. on 60th day and after overnight fasting, the animals were weighed and anaesthetized under ether vapours. A sample of 2ml blood was drawn from tail vein from all animals. Blood was transferred to the sterile vacuotainers with gel and allowed to clot at room temperature for one hour. It was then centrifuged for ten minutes at a speed of 3000 rpm. Serum was separated and stored in sterile eppendorf tubes at -20°C for analysis of biochemical parameters.[17]

Biochemical Analysis: Glucose levels were estimated using commercially available kit (Randox, UK) based on glucose oxidase method.[18] Total cholesterol levels were estimated using commercially available kit (Randox, UK) based on enzymatic endpoint method.[19] Serum triglycerides were estimated by commercially available kits (Randox, UK), based on GPO-PAP method[20] while serum HDL by precipitant method.[21] Serum LDL was estimated using commercially available kit (Randox, UK) based on an established method.[22]

Statistical Analysis: The data was entered and analysed using SPSS 17.0 (Statistical Package for Social Sciences). All data are shown as mean ± S.E.M

(standard error of mean). One way ANOVA (analysis of variance) was applied to observe group mean differences. Post Hoc Tukey test was applied to observe mean differences among the groups. A p-value of <0.05 was considered as statistically significant.

RESULTS

The biochemical parameters showed that the injection of STZ caused a significantly (p<0.01) increased serum glucose levels in the rats of group B, C , D and E as compared to control group. On the other hand, simultaneous administration of ethanolic extract of *Syzygium aromaticum* resulted in a significant (p<0.01) decrease in the serum glucose levels of rats in groups C and D when compared with that of group B. Also simultaneous administration of glibenclamide resulted in a significant (p<0.01) decrease in the serum glucose levels of the rats in groups E when compared with that of group B.

The total cholesterol, serum triglycerides and serum LDL levels showed significantly (p<0.01) increased levels in the rats of group B, C, D and E as compared to control group. On the other hand, simultaneous administration of ethanolic extract of *Syzygium aromaticum* resulted in a significant (p<0.01) decrease in the cholesterol ,serum triglycerides and serum LDL levels of rats in groups C and D when compared with that of group B. Also simultaneous administration of glibenclamide resulted in a significant (p<0.01) decrease in the cholesterol and serum triglycerides levels of the rats in groups E when compared with that of group B. However the simultaneous administration of glibenclamide resulted in an insignificant decrease in LDL levels of the rats in groups E (p=0.25) animals when compared with that of group B (diabetic group) rats.

Serum HDL showed significantly (p<0.01) decreased levels in the rats of group B, C, D and E as compared to control group. On the other hand, administration of ethanolic extract of *Syzygium aromaticum* to group C and D and glibenclamide to group E for eight weeks resulted in a significant (p<0.01) increase in the serum HDL levels of the rats in groups C ,D and E when compared with that of group B.

DISCUSSION

Diabetes is a chronic and systemic disease that triggers life-changing complications in virtually every system of the body.[23] Hyperglycemia, abnormal lipid and antioxidant profiles are the most usual complications in diabetes mellitus. It has been established that hyperglycemia is the principal cause of diabetic complications. People with diabetes exhibit a pattern of dyslipidemia characterized by elevated triglycerides, LDL and low levels of HDL.[24] Many in vivo and in vitro studies indicated that oxidative stress is one of the major pathophysiological mechanisms involved in the development of diabetes.[25,26] Effective control of blood glucose level is a key step in reversing diabetic complications and improving the quality of life in diabetic patients.[27] A number of plants are being assessed for their therapeutic potential as there is a growing trend towards the use of natural remedies as adjuncts to conventional therapy. It is well documented that modulations of oxidative stress through treatment with antioxidants can effectively reduce glucose and lipid levels.[28,29]

Table No. 1: Mean± SEM values of different biochemical parameters in all groups (A, B, C , D and E)

Parameter	Group A	Group B	Group C	Group D	Group E
Serum Glucose mg/dl	130.54±5.18	298.49±1.95*	200.23±6.41**	148.80±4.72**	209.70±4.87**
Total Cholesterol (mg/dl)	119.43±8.07	208.95±7.57*	180.59±3.63**	160.72±4.18**	184.29±3.67**
Serum Triglycerides(mg/dl)	65.45±4.61	177.47±4.68*	152.83±4.54**	117.53±4.85**	158.05±4.43**
Serum LDL (mg/dl)	39.02±2.42	151.48±6.07*	130.30±4.59**	103.30±4.11**	138.41±4.47
Serum HDL (mg/dl)	31.51±1.56	14.74±1.12*	23.85±1.48**	34.13±1.25**	23.68±0.90**

* p<0.05 when compared with group A (control)

** p<0.05 when compared with group B (diabetic)

The present study showed a significant elevation in the levels of serum glucose, triglycerides, LDL and total cholesterol of group B diabetic rats as compared to group A normal rats. Serum HDL levels was reduced in group B rats as compared to group A rats. Administration of syzygium aromaticum ethanolic extract to group C and D and Glibenclamide to group E brought the levels of these diagnostic parameters in the serum of group C , D and E animals towards normal as compared to group B rats (Table 1). When we compare mean values of group C and D with group B, although both decrease lipid levels, but group D reduced the levels more as compared to group C. When we compare mean values of group C and D with group E, although Glibenclamide decrease glucose and lipid levels, but syzygium aromaticum ethanolic extract reduced the levels more as compared to glibenclamide. Syzygium aromaticum also increased the level of HDL more as compared to glibenclamide (Table 1), showing better effectiveness of syzygium aromaticum ethanolic extract over glibenclamide. Our results are in accordance with the reports by others who used chemical antioxidants and diet of natural antioxidant plants.[30-31]

The main constituents in syzygium aromaticum are eugenol, polyphenols and flavonoids. The proposed mechanism of syzygium aromaticum in reducing the glucose and lipid levels could be due to the antioxidant mechanism. Atawodi et al. in 2011 showed that polyphenols in syzygium aromaticum have antioxidant activity.[32] Robards and Antolovich in 1997 have critically reviewed the analytical chemistry of bioflavonoid and it was found that flavonoids possess antioxidant activity, they are potent free radical scavengers and metal chelators and they also inhibit lipid oxidation which is a key step in the formation of atherosclerotic plaque.[33]Therefore, in our study polyphenols and flavonoids in syzygium aromaticum might have a role in decreasing glucose and lipid levels in rats. Further experiments are needed to determine the actual mechanism of action of the active constituents of the syzygium aromaticum plant fractions.

CONCLUSION

The results of the present study indicate that the treatment with *syzygium aromaticum* ethanolic extract decreased glucose and lipid levels in diabetic rats. The *syzygium aromaticum* ethanolic extract, showed better results as compared to glibenclamide.

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