Research Journal of Biological Sciences 3 (9): 1109-1114, 2008
ISSN: 1815-8846

Evaluation of *Syzygium jambolanum* Methanolic Leaf Extract for Insulin-Like Properties

S.L. Lim, J.W. Chai and U.R. Kuppusamy
Department of Molecular Medicine, Faculty of Medicine,
University of Malaya, Kuala Lumpur, Malaysia

Abstract: Traditionally, the fruits and seeds of *Syzygium jambolanum* (Lam.) DC [Family: Myrtaceae] have been used to treat diabetes mellitus. Insulin is known to inhibit lipolysis induced by epinephrine as well as promote lipogenesis and glucose uptake adipocytes. The present study evaluates the insulin-like properties of *S. jambolanum* leaf methanolic extract through lipogenic, anti-lipolytic and glucose uptake activities. Methanolic leaf extract of *S. jambolanum* induced significant lipogenesis in the dose range of 0.1-10 μg mL^{-1} with the highest activity (114%) at 10 μg mL^{-1}. This extract (1 μg mL^{-1} inhibited 29% of epinephrine (1 μM) induced lipolysis and enhanced the inhibitory effect of insulin against epinephrine induced lipolysis. This extract also inhibited (64% inhibition) of isoproterenol (1 μM) (β-adrenergic agonist) induced lipolysis. However, the extract did not enhance the antilipolytic action of propanolol (β-adrenergic antagonist). Thus, it is possible to speculate that the mode of action of this extract may involve competitive binding at β-adrenergic receptor binding sites. *S. jambolanum* (0.1-10 μg mL^{-1}) exerted a dose-dependant glucose uptake activity and further enhanced insulin mediated glucose uptake in primary rat adipocytes. These results confirm that *S. jambolanum* methanolic leaf extract has insulin-like properties and may be useful as potential therapeutic agent in the management of hyperglycemia.

Key words: *S. jambolanum* leaf extract, lipogenesis, lipolysis, glucose uptake, rat adipocytes

INTRODUCTION

Diabetes Mellitus (DM) is recognized as one of the most challenging public health problems of the 21st century and the prevalence of DM is increasing rapidly. The total number of people diagnosed with DM is estimated to increase from 171 million in 2000 to 366 million in 2030 (2.8% in 2000 to 4.4% in 2030) (Wild *et al*., 2004). DM is a life-long metabolic disorder characterized by inability of glucose transportation from bloodstream into adipose tissue due to defect in insulin action, insulin secretion or both (World Health Organization, 1999). Insulin is a pancreatic endocrine hormone that regulates carbohydrate metabolism, reduce blood glucose level and promote storage of fuel molecules in adipose tissue, liver and skeletal muscle (Voet *et al*., 1999). Insulin inhibits lipolysis elicited by epinephrine as well as to promote lipogenesis and glucose uptake in adipocytes (Okuda, 2003). The effects of insulin are counteracted by multiple hormones, such as glucagon and epinephrine which convert glycogen and triglyceride to glycerol (Voet *et al*., 1999).

Lipid metabolism in adipocytes is greatly controlled by hormonal agents such as insulin and epinephrine and deregulation of lipid metabolism has a great influence in the occurrence of Type 2 DM and obesity (Arner and Ostman, 1974). Therefore, understanding the mechanisms involved in the regulation of preadipocyte proliferation, differentiation, lipolysis as well as uptake of glucose into adipocytes are essential for the treatment of DM as well as obesity.

Syzygium jambolanum (Lam.) DC [Familiy: Myrtaceae], commonly known as 'jambol fruit', 'jamun' or 'rose apple' in English, is a large, evergreen tree found primarily in India, Pakistan, Southern Asia and Brazil (Grover *et al*., 2002). *S. jambolanum* is a well known Indian folk medicine for the treatment of DM and prickly heat (Samba-Murthy and Subrahmanyam, 1989). The antidiabetic potential of the fruit and seed extracts of *S. jambolanum* have been shown by several studies (Sridhar *et al*., 2005; Sharma *et al*., 2006). However, the potential insulin-like therapeutic value of *S. jambolanum* leaf extract in the management of hyperglycemia or DM has not been reported. Therefore, in this study, *S. jambolanum* leaf methanolic extract was tested for its insulin-like properties in enhancing lipogenesis and glucose uptake as well as inhibiting epinephrine induced lipolysis in primary rat adipocytes.

Corresponding Author: Assoc. Prof. Dr. Umah Rani Kuppusamy, Department of Molecular Medicine, Faculty of Medicine, 50603 Kuala Lumpur, Malaysia

MATERIALS AND METHODS

All cell culture media and culture flasks were purchased from Sigma-Aldrich (St. Louis, USA) and Nunc (Naperville, IL, USA), respectively. Penicillin-Streptomycin-Glutamine and insulin were obtained from Invitrogen (Carlsbad, California) whereas glycerol, 1-methyl-3-isobuylxanthine (IBMX), dexamethasone, adenosine-5-triphosphate (ATP) and liquid scintillation counting cocktail were purchased from ICN (Aurora, Ohio).

***S. jambolanum* methanolic leaf extract preparation:** *S. jambolanum* leaves were air dried for a few days before pulverized and refluxed with methanol at 60°C for 6 h. Excess water from the methanolic extract was removed with sodium sulphate anhydrous and filtered. Subsequently, the extract was rotary evaporated to dryness. The residue was reconstituted with DMSO and diluted with ultra pure water. The reconstituted extract was filter sterilized with syringe filters (0.2 μm) prior to the incubation with adipocytes and subsequent lipogenesis, lipolysis and glucose uptake assays.

Cell culture and differentiation: Primary preadipocytes were obtained from intra-abdominal fat depots of 6-8 week old male *Sprague dawley* rat according to an established method (Bjorntorp *et al*., 1978) with minor modifications. The rat was sacrificed by cervical dislocation and the intra-abdominal fat pads were enzymatically digested with Type-II collagenase at 37°C for 1 h. Preadipocytes obtained were cultured in 75 cm^2 culture flasks with RPMI, supplemented with 2 mM penicillin-streptomycin-glutamine, 2 mM amphotericin B and 10% Foetal Bovine Serum (FBS). The culture media was refreshed every 2 days until the preadipocytes reached confluent state. Confluent preadipocytes were seeded into 12-well plate and differentiated in Dulbecco's Modification of Eagle's Medium (DMEM) supplemented with penicillin-streptomycin-glutamine, amphotericin B, 10% FBS, 17 μM pantothenic acid, 0.5 mM 1-methyl-3-isobutylxanthine (IBMX), 1 μM dexamethasone, 10 μg mL^{-1} insulin and 33 μM biotin. After two days, DMEM differentiation media was refreshed without adding IBMX and dexamethasone. Fully differentiated adipocytes were used for subsequent studies (Madsen *et al*., 2003).

Lipogenesis assay: Various concentrations of *S. jambolanum* methanolic leaf extract were added into DMEM differentiation media either to substitute insulin (1 μM) or to supplement the differentiation media. Twelve days after initiation of preadipocyte differentiation, the cells were washed twice with PBS and fixed with 0.5% formaldehyde before stained with freshly prepared Oil Red O solution. After staining, the cells were washed twice with PBS to remove the excess dye. Oil Red O is a red dye which stains neutral lipids. Therefore the amount of Oil Red O staining is directly proportional to the amount of lipid accumulated in the cells. The differentiation efficiency of preadipocytes can be visualized microscopically or measured spectrophotometrically at 510 nm after solubilising in 100% isopropanol (Akerblad *et al*., 2002).

Lipolysis assay: Lipolysis or antilipolysis activities were assessed indirectly by using glycerol quantification assay according to an established method (Trinder, 1969). In lipolysis, active hormone sensitive lipase hydrolyses the triglycerides in the adipose to release glycerol and free fatty acids. Thus, glycerol level will increase if adipocytes undergo lipolysis and *vice versa* (Trinder, 1969). Twelve days after the induction of preadipocyte differentiation, the adipocytes were cultured in serum free media for one day before incubating with freshly prepared *S. jambolanum* leaf methanolic extract, epinephrine (1 μM), insulin (1 μM), IBMX (100 μM), isoproterenol (1 μM) and/or propanolol (1 μM) for five hours. After that, glycerol quantification reagent (ATP, magnesium salt, 4-aminoantipyrine, sodium N-ethyl-NO- (3-sulfoprpyl)-m-aniside, glycerol kinase, glycerol phosphate oxidase, peroxidase and TRIS buffer) was added into the cultures and incubated for 15 min at 37°C. Glycerol released correlated with the intensity of coloured complex formed which was quantitated (glycerol standard) spectrophotometrically at a wavelength of 540 nm. The increased presence of glycerol compared to control (untreated cells) indicates lipolysis, whereas a decreased level indicates antilipolysis (Trinder, 1969).

Glucose uptake assay: This assay was carried out according to an established method (Lakshmanan *et al*., 2003). Differentiated adipocytes were serum-starved for 2 h prior to treatments with various concentrations of *S. jambolanum* methanolic leaf extract in the presence or absence of insulin (100 μM) for 30 min at room temperature. Subsequently, 2-deoxy-D-[2,6-^{3}H]glucose (20 μCi mL^{-1} final concentration) was introduced and incubated for another 10 min. The cells were washed twice with ice cold PBS, lysed with 4mL of scintillant cocktail and the radioactivity was measured as counts per min (cpm) in a liquid scintillation counter (Lakshmanan *et al*., 2003).

RESULTS AND DISCUSSION

The morphology of primary preadipocytes was fibroblast-like and were cultured until confluent state.

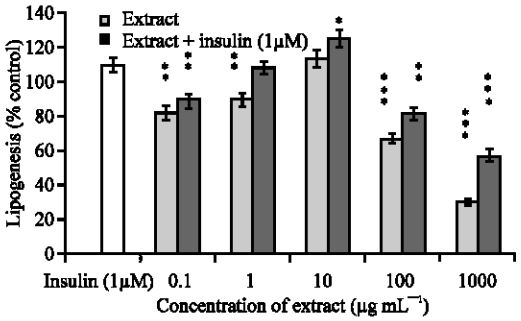

Fig. 1: Effects of Insulin and *S. jambolanum* leaf methanolic extract on lipogenesis in rat adipocytes

Confluent state is an indicator of preadipocyte growth arrest stage, which is a prerequisite for the cells to become committed to adipocyte cell lineage (Gregoire *et al*., 1998). Besides that, growth arrest stage is also induced by the addition of differentiation agents, such as insulin, dexamethasone and 1-methyl-3-isobutylxanthine (IBMX) (Gregoire *et al*., 1998). Therefore, these agents were added at the initial part of differentiation in order to enable the cells to accumulate lipids. Mature adipocytes which accumulate lipid droplets in their cytoplasm appear yellowish when viewed under the microscope. However, the lipid-filled adipocytes have higher tendency to lift from the culture plate compared to preadipocytes and thus, to avoid this, changing of media was done very gently. The rate of preadipocyte differentiation/ lipogenesis was assessed by staining the lipid droplets with Oil Red O dye before quantitated spectrophotometrically at 510 nm.

Adipose tissues are widely used as the cellular model to investigate the biochemical pathways and physiological mechanisms underlying adipose tissue development especially in finding cure for DM as well as obesity. As an insulin- responsive tissue, adipose tissue expresses many biological activities of insulin (Gregoire *et al*., 1998).

Insulin plays a vital role in preadipocyte differentiation/lipogenesis by triggering the expression of transcription factors (for example enhancer binding protein α (C/EBP-α) and PPAR-γ) which activate Insulin-Signaling Cascade (ISC). Without insulin, primary preadipocytes will not be stimulated to accumulate lipids and therefore will not differentiate into mature adipocyte (Gregoire *et al*., 1998).

In order to investigate the potential of *S. jambolanum* methanolic leaf extract on lipogenesis, insulin in the differentiation media was substituted with various concentrations of the extract (0.1-1000 μg mL^{-1}). At the concentration range of 0.1-10 μg mL^{-1}, the extract showed a dose-dependant increase in lipogenesis which was comparable to that of the positive control (Insulin, 1 μM) (Fig. 1). At concentrations greater than 10 μg mL^{-1}, the lipogenic effect of the extract was attenuated significantly. Co-incubation of the extract with insulin did not exert any enhancement of lipogenesis within the lower dose range (0.1-10 μg mL^{-1}) but appeared to attenuate the insulin mediated lipogenesis at higher dose-range (100-1000 μg mL^{-1}). The lipogeneic level in the presence of the extract alone was also markedly reduced. One plausible explanation for this observation is the large quantity of lipid accumulation in the adipocytes at higher concentrations of the extract could possibly enable the detachment (lifting off) of the cells from the base of the culture plates (due to increase buoyancy). The cells could have been washed away during the preparation for lipogenesis assay. The possibility of cell cytotoxicity was ruled out as it was evident from our preliminary studies that the higher concentration of extract (100-1000 μg mL^{-1}) did not affect the preadipocyte or adipocyte viability.

Lipolysis in adipocytes is highly dependent on the activity of hormone sensitive lipase which is regulated by insulin, glucagon and catecholamines such as epinephrine (Large and Arner, 1998). Epinephrine is a well known α and β-adrenergic agonist which stimulates lipolysis by promoting adenylate cyclase activity to increase cAMP level leading to the activation of hormone sensitive lipase (Burns and Langley, 1975). However, this lipase could be inactivated by insulin through the activation of phosphodiesterase. Besides that, lipolysis in adipocytes can also be inhibited by β-adrenergic antagonists, for example propanolol or by antilipolytic α-adrenergic agonist (LaFontan and Berlan, 1993).

Based on our preliminary dose-response studies of *S. jambolanum* leaf methanolic extract on lipolysis, 1 μg mL^{-1} of extract was found to be the ideal concentration at which a wide spectrum of lipolytic and/or antilipolytic activities could be observed (data not shown). *S. jambolanum* methanolic leaf extract at a concentration of 1 μg mL^{-1} exerted 31% lipolysis, whereas epinephrine (1 μM) exerted 73% lipolysis in adipocytes (Fig. 2). However, when co-incubated with epinephrine (1 μM), the extract significantly inhibited 29% of epinephrine induced lipolysis in adipocytes. In comparison, insulin inhibited 50% of epinephrine induced lipolysis. These finding clearly showed that *S. jambolanum* methanolic leaf extract has insulin-like effect in inhibiting epinephrine induced lipolysis in spite of being moderately lipolytic in itself. In addition, the extract enhanced insulin mediated anti-lipolytic activity by inhibiting 84% of epinephrine induced lipolysis (Fig. 2).

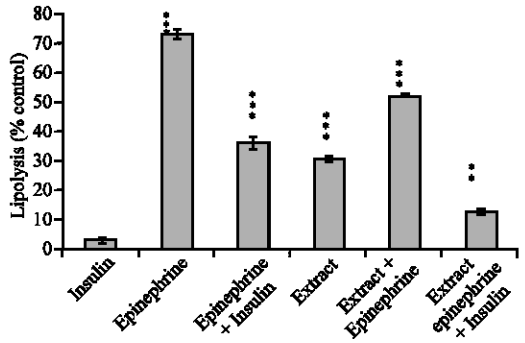

Fig. 2: Effects of *S.jambolanum* methanolic leaf extract and insulin on epinephrine induced lipolysis in rat adipocytes

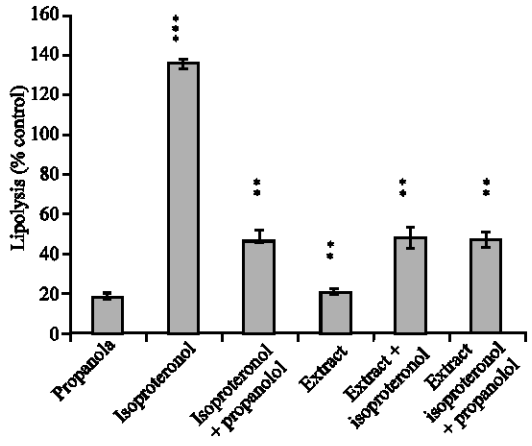

Fig. 3: Effects of *S. jambolanum* leaf methanolic extract and propanolol on isoproterenol induced lipolysis in rat adipocyte

Isoproterenol showed a higher potency in inducing lipolysis in the adipocytes compared to epinephrine (Fig. 2 and 3). When β-adrenergic antagonist, propanolol (1 μM) was co-incubated with isoproterenol, the lipolytic action elicited by isoproterenol was inhibited. These data are consistent with other findings which showed that the stimulation of lipolysis in adipocytes is mediated through β receptors and isoproterenol is a stronger β-adrenergic agonist compared to epinephrine (Schimmel, 1976).

When propanolol was substituted with *S. jambolanum* methanolic leaf extract, a significant inhibition (64%) of isoproterenol induced lipolysis was observed (Fig. 3). This result was comparable to propanolol's antilipolytic action. However, this extract was unable to enhance propanolol induced anti-lipolytic action (Fig. 3). Thus, it is possible to speculate that *S. jambolanum* methanolic leaf extract could be inhibiting lipolysis through the similar pathway to propanolol and

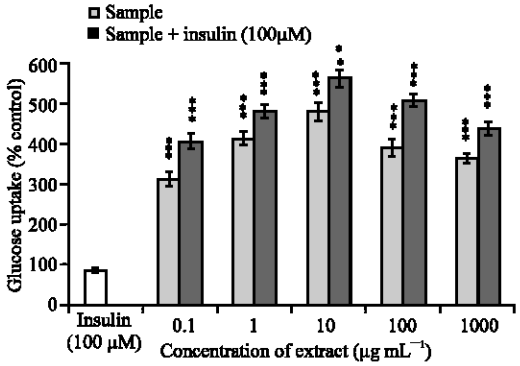

Fig. 4: Effects of *S. jambolanum* methanolic leaf extract and insulin on glucose uptake in rat adipocytes

possibly could interfere with the agonist binding to β-adrenergic receptor binding sites.

Besides lipogenesis and lipolysis, insulin is also known to stimulate glucose uptake in adipocytes (White, 1997). Insulin activates several insulin-signalling proteins, namely Insulin Receptor Substrates (IRS), phosphatidylinositol 3-kinase (PI3-K) and protein kinase B (PKB), which will eventually lead to the translocation of insulin-sensitive glucose transporter 4 (GLUT4) vesicles to the plasma membrane to facilitate glucose uptake into the cells (White, 1997).

A preliminary dose-response study of insulin on glucose uptake showed that 10,000 μM of insulin exerted the highest glucose uptake activity in rat adipocytes (data not shown). Thus, the sub-maximum concentration of insulin (100 μM) was chosen to be the positive control so that a wide spectrum of glucose uptake activity could be observed. *S. jambolanum* leaf methanolic extract strongly promoted glucose uptake in rat adipocytes even in the absence of insulin (Fig. 4). The degree of glucose uptake exerted by *S. jambolanum* leaf methanolic extract was at least 2 fold higher than the positive control used At the concentration range of 0.1-10 μg mL^{-1}, *S. jambolanum* methanolic leaf extract significantly stimulated glucose uptake in a dose-dependant manner (2-3 fold greater than insulin) (Fig. 4). When co-incubated with insulin (100 μM), *S. jambolanum* methanolic leaf extract enhanced almost 20% of insulin-stimulated glucose uptake in rat adipocytes (Fig. 4).

Insulin and several common oral antidiabetic drugs, such as rosiglitazone are known to have similar activities in promoting lipogenesis (Schoonjans *et al*., 1996), inhibit epinephrine induced lipolysis (Festuccia *et al*., 2006) as well as stimulate glucose uptake (Young *et al*., 1995) in primary rat adipocytes. Various plants with lipogenic, glucose uptake and/or antilipolytic effects were claimed to have insulin-like activity and have been utilized as

antidiabetic agents. For example *Amomi semen*, the dried seed of *Amomum xanthioides* (Zingiberaceae) was reported to posses antidiabetic potential through the stimulation of glucose uptake as well as potentiate insulin action in 3T3-L1 adipocytes (Kang and Kim, 2004). Besides that, Korean red ginseng (*Panax ginseng*) which was traditionally used to improve pathological conditions of DM, was found to promote lipogenesis as well as inhibit epinephrine induced lipolysis both in the presence and absence of insulin (Okuda, 2003). In this study, *S. jambolanum* methanolic leaf extract significantly promoted lipogenesis and glucose uptake as well as inhibited epinephrine induced lipolysis in adipocytes both in the presence and absence of insulin. These findings correlated with insulin action on adipocytes and clearly showed that *S. jambolanum* methanolic leaf extract has insulin-like properties. Based on an ethnopharmacological survey in Brazil, dry leaf decocotion of *S. jambolanum* leaves exerted hypoglycemic effect (Teixeira *et al*., 1992). However, Pepato *et al.* (2001) had reported that decoction of *S. jambolanum* leaves (15% w/v) showed no statistically significant difference in biochemical and physiological parameter when given to streptozotocin induced diabetic rats (Pepato *et al*., 2001). These contradictory results may have been due to several factors, such as humidity, soil, climatic conditions and the geographical location of the specific *S. jambolanum* leaves used which could contribute to variability in the chemical constituents (Evans, 1996).

CONCLUSION

Based on our findings, we conclude that *S. jambolanum* leaf methanolic extract possess insulin-mimicking potential and the active compound(s) present in *S. jambolanum* leaf methanolic extract might potentially serve as a adjuvant or substitute to insulin in treating DM since it mimics insulin's action in adipose tissues. However, further investigations are needed to identify the active compound(s) and the exact mode of action(s) involved in the insulin-mimicking effect of *S. jambolanum* leaves.

ACKNOWLEDGEMENT

This study was supported by the Intensification of Research in Priority Areas (IRPA) fund by the Ministry of Science, Technology and Environment (MOSTE) (Grant number: IRPA 06-02-03-1017). The authors wish to thank Prof. Rais Mustafa for his advice on glucose uptake assay and Mr. Sivarajah A. Palanisamy for providing the *Syzygium jambolanum* leaves.

REFERENCES

Akerblad, P., U. Lind, D. Liberg, K. Bamberg and M. Sigvardsson, 2002. Early B-Cell Factor (O/E-1) is a promoter of adipogenesis and involved in control of genes important for terminal adipocyte differentiation. Mol. Cell Biol., 22: 8015-8025.

Arner, P. and J. Ostman, 1974. Mono-acid diacylglycerols in human adipose tissue. Biochem. Biophys. Acta, 369: 209-221.

Bjorntorp, P., M. Karisson, H. Pertoft, P. Pettersson, L. Sjostrom and U. Smith, 1978. Isolation and characterization of cells from rat adipose tissue developing into adipocytes. J. Lipid. Res., 19: 316-324.

Burns, T.W. and P.E. Langley, 1975. The effect of alpha and beta adrenergic receptor stimulation on the adenylate cyclase activity of human adipocytes. J. Cyclic Nucleotide Res., 1: 321-328.

Evans,W.C., 1996. Commerce and Production: Principles Related to the Commercial Production, Quality and Standardization of Natural Products. In: Pharmacognosy. 14th Edn. Saunders, London.

Festuccia, W., M. Laplante, M. Berthiaume, Y. Gélinas and Y. Deshaies, 2006. PPAR agonism increases rat adipose tissue lipolysis, expression of glyceride lipases and the response of lipolysis to hormonal control. Diabetologia, 49: 2427-2436.

Gregoire, F.M., C.M. Smas and S.H. Sul, 1998. Understanding adipocyte differentiation. Physiol. Rev., 78: 783-809.

Grover, J.K., S. Yadav and V. Vats, 2002. Medicinal plants of India with anti-diabetic potential. J. Ethnopharmacol., 81: 81-100.

Kang, Y. and H.Y. Kim, 2004 Glucose uptake-stimulatory activity of Amomi Semen in 3T3-L1 adipocytes. J. Ethnopharmacol., 92: 103-105.

LaFontan M. and M. Berlan, 1993. Fat cell adrenergic receptors and the control of white and brown fat cell function. J. Lipid. Res., 34: 1057-1091.

Lakshmanan J., J.S. Elmendorf and S. Ozcan, 2003. Analysis of insulin-stimulated glucose uptake in differentiated 3T3-L1 adipocytes. Methods Mol. Med., 83: 97-103.

Large, V. and P. Arner, 1998. Regulation of lipolysis in humans. Pathophysiological modulation in obesity, diabetes and hyperlipidaemia. Diabetes Metab., 24: 409-418.

Madsen, L., R.K. Petersen, M.B. Sorensen, C. Jorgensen, P. Hallenborg and L. Pridals *et al*., 2003. Adipocyte differentiation of 3T3-L1 preadipocytes is dependent on lipoxygenase activity during the initial stages of the differentiation process. Biochem. J., 375: 539-549.

Okuda, H., 2003. Obesity and diabetes: Patho-physiological mechanisms and therapeutic approaches. Exp. Biol. Med., 228: 1250-1255.

Pepato, M.T., V.B.B. Folgado, I.C. Kettelhut and I.L. Brunetti, 2001. Lack of antidiabetic effect of a Eugenia jambolana leaf decoction on rat streptozotocin diabetes. Braz. J. Med. Biol. Res., 34: 389-395.

Samba-Murthy, A.V.S.S. and N.S. Subrahmanyam, 1989. Fruits. In: A text book of economic botany, Wiley, New Delhi, India, pp: 629.

Schimmel, R.J., 1976. Roles of alpha and beta adrenergic receptors in control of glucose oxidation in hamster epididymal adipocytes. Biochem. Biophys. Acta, 428: 379-387.

Schoonjans, K., B. Staels and J. Auwerx, 1996. The peroxisome proliferator activated receptors (PPARs) and their effects on lipid metabolism and adipocyte differentiation. Biochem. Biophys. Acta, 1302: 93-109.

Sharma, S.B., A. Nasir, K.M. Prabhu and P.S. Murthy, 2006. Antihyperglycemic effect of the fruit-pulp of Eugenia jambolana in experimental diabetes mellitus. J. Ethnopharmacol., 104: 367-373.

Sridhar, S.B., U.D. Sheetal, M.R.S.M. Pai and M.S. Shastri, 2005. Preclinical evaluation of the antidiabetic effect of Eugenia jambolana seed powder in streptozotocin-diabetic rats. Braz. J. Med. Biol. Res., 38: 463-468.

Teixeira, C.C., R.M. Blotta, A.P. Costa, D.G. Mussnich, G.G. Ranquetat and F.D. Fuchs, 1992. Plants employed in the treatment of diabetes mellitus results of an ethnopharmacological survey in Porto Alegre, Brazil. Fitoterapia, 4: 320-322.

Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem., 6: 24.

Voet, D., J.G. Voet and C.W. Pratt, 1999. Mamalian Fuel Metabolism: Integration and Regulation. In: Fundamentals of Biochemistry. 4th Edn. John Wiley and Sons Inc., New York, pp: 663-692.

White, M.F., 1997. The insulin signalling system and the IRS proteins. Diabetologia, 40: 2-17.

Wild, S., G. Roglic, A. Green, R. Sicree and H. King, 2004. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care, 27: 1047-1053.

World Health Organization 1999. Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO Consultation, WHO, Geneva.

Young, P.W., M.A. Cawthorne, P.J. Coyle, J.C. Holder, G.D. Holman, Kozka I.J., D.M. Kirkham, C.A. Lister and S.A. Smith, 1995. Repeat treatment of obese mice with BRL 49653, a new potent insulin sensitizer, enhances insulin action in white adipocytes: Association with increased insulin binding and cell-surface GLUT4 as measured by photoaffinity labeling. Diabetes, 44: 1087-1092.