Original Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

PROTECTIVE EFFECT OF *ACANTHOSPERMUM HISPIDUM* ETHYL ACETATE EXTRACT ON HYPERGLYCEMIC AND GLYCOPROTEIN COMPONENTS IN STZ INDUCED DIABETIC RATS

J.VASUNDHARAMMA[1], J. KAMAKSHAMMA[2*] AND S.VARALAKSHMI[3]

[1]Department of Botany , Rayalaseema University, Kurnool, A.P, India
[2]Department of Botany, Sri Venkateswra University,Tirupati, A.P, India*
[3]Department of Biochemistry, Sri Venkateswra University , Tirupati, A.P, India

ABSTRACT

Over the past decade, herbal medicines have been accepted universally, and they have an impact on both world health and international trade. Diabetes mellitus is a metabolic disorder in the endocrine system. This dreadful disease is found in all parts of the world and is becoming a serious threat to mankind health. There are lots of chemical agents available to control and to treat diabetic patients, but total recovery from diabetes has not been reported up to this date. Alternative to these synthetic agents, plants provide a potential source of hypoglycaemic drugs and are widely used in several traditional systems of medicine to prevent diabetes. This study was designed to evaluate the effect of *Acanthospermum hispidum* ethyl acetate extract on the levels of glycoproteins in STZ -induced diabetic Rats. Albino rats were divided equally into four groups: Group 1: control, Group 2: diabetic control , Group 3: diabetic rats treated with 300 mg/kg body weight of plant extract and group 4 diabetic rats treated with 450 mg/kg body weight of plant extract. All treatments were administered via an intragastric tube. Diabetes was induced in the rats of Group 2 by an intraperitoneal injection with 50 mg/kg body weight of STZ Oral administration of plant extract at a concentration of 300 mg/kg body weight and 450 mg/kg body weight for three weeks (21 days) days significantly reduced the levels of blood glucose, glycosylated hemoglobin, and increased in plasma insulin, body weight, as well as those of hexose, hexosamine, fucose, and sialic acid in the diabetic rats treated with the plant extract as compared to untreated diabetic rats, with no adverse effects in rats treated only with plant extract. In conclusion, *Acanthospermum hispidum* plant extract proved to have a beneficial effect on the diabetic rats in this study. In light of these advantageous results, it is advisable to broaden the scale of use of *Acanthospermum hispidum* extract in a trial to alleviate the adverse effects of diabetes.

KEYWORDS: ***Acanthospermum hispidum*, glycoproteins, blood glucose, diabetic activity, STZ**

J. KAMAKSHAMMA
Department of Botany, Sri Venkateswra University,Tirupati, A.P, India

INTRODUCTION

Since time immemorial man has used various plants in the treatment and prevention of many ailments. Historically all medicinal preparations were derived from plants whether in the simple form of plant parts or in the more complex form of crude extracts, mixtures, etc.[1] Plant derived medicines are widely used because they are relatively safer than the synthetic alternatives, they are easily available and cheaper.[2] plants continue to be an important therapeutic aid for alleviating ailments of humankind. Over the last 2500 years, there have been very strong traditional systems of medicine such as Chinese, Ayurvedic, and the Unani, born and practiced, more in the eastern continent. These traditions are still flourishing, since; approximately 80% of the people in the developing countries rely on these systems of medicine for their primary health care needs[3].Diabetes mellitus is a group of metabolic alterations charecterized by hyperglycemia resulting from defects in insulin secretion , action or both. Diabetes is a major health problem affecting major populations worldwide, it is chronic disorder in metabolism of carbohydrate, protein, and fat due to absolute or relative deficiency of insulin secretion with or without varying degree of insulin resistance. There are more than 30 million people with diabetes mellitus in India and the incidence is increasing. Also, there are many patients in the community with undiagnosed diabetes. Decreased physical activity, increasing obesity, stress and changes in food consumption have been implicated in this increasing prevalence in the past two decades. Diabetes being projected as the world's main disabler and killer in the next 25 years.[4] Effective blood glucose control is the key for preventing or reversing diabetic complications and improving quality of life in patients with diabetes . Thus sustained reduction in hyperglycemia will decrease the risk of developing microvasular complications and most likely reduce the risk of macrovascular complications[5]. Hyperglycemia, due to uncontrolled glucose regulation is considered as the causal link between diabetes and diabetic complications . A number of studies have been focused an alterations in glucose metabolism and it leads to hyperglycemia induced cell damage by four key metabolic pathways, viz., increased polyol pathway flux, increased glycation of proteins (enzymatic or nonenzymatic), increased hexosamine pathway flux and activation of protein kinase C (PKC) isoforms.[6] Among the above stated possibilities, glycosylation of proteins has been the prime subject of much interest. Glycoproteins, a carbohydrate linked protein macromolecules found in the cell surface, serves as the principal component of animal cells. Alterations in glycoprotein level leads to the pathogenesis of diabetes mellitus.[7] Many studies confirm the involvement of glycoprotein in diabetic complications[8.] With increasing severity of diabetes, there is a parallel rise in glycoprotein levels[9]. During diabetes, utilization of glucose by insulin independent pathways leads to the synthesis of glycoprotein which may be a predictor of angiopathic complications[10]. An increase in the biosynthesis and or a decrease in the metabolism of glycoproteins attributed to the deposition of these materials in the basal membrane of pancreatic cells. In recent times, many traditionally important medicinal plants have been tested for their efficacy against impaired glycoprotein levels in diabetes.[11-12]. Streptozotocin (STZ) is commonly used for experimental induction of type-I diabetes mellitus, which causes selective pancreatic islet β-cell cytotoxicity mediated through the release of nitric oxide (NO). This results in rapid reduction in pancreatic islet pyridine nucleotide concentration and subsequent β-cell necrosis. The action of STZ on mitochondria generates SOD anions, which leads to diabetic complicationsSTZ partly destroys the beta cells bringing about inadequate insulin discharge creating type 2 diabetes.

MATERIAL AND METHODS

Plant material

The plant *Acanthospermum. hispidum* was collected from Tirumala hills, AndhraPradesh, India. The taxonomical identification and authentication of the plant was done by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S. V. University, Tirupathi, Andhra Pradesh, India. The voucher specimen was deposited at the department of Botany, Sri Venkateswara University, Tirupati, for future reference.

Extraction of plant material

The aerial parts of the plant were cleaned with tap water and then left to dried at room temperature for 15 days and the dried plant material was ground into fine powder using pulverizer the powered part was sieved and kept in clean place until preparing extract. 1 kg of dried powder was extracted in soxhlet apparatus using Ethyl acetate as a solvent. The extract was concentrated on a rotary flash evaporator to semisolid consistency and then dried over a water bath. The yield of the extract obtained was 150 g. The yield plant extract was designated as *Acanthospermium hispidum* ethyl acetate extract.

Animals

Male albino Wistar rats (weighing 170- 220g, 10 weeks old) were procured from DMRT, Hyderabad and maintained in an air conditioned room [(25±1) C^0] with a 12 h light/12 h dark cycle, in Animal house, Department of Zoology, SVU college of sciences, Feed and water were provided ad libitum. The study was conducted in accordance with the Animal ethical committee, Department of zoology , Sri Venkateswara University, Tirupati. Animal ethical reference No.49/2012-2013 / (i)/a/CPCSEA/IAEC/SVU/JKS-SVR. Dt.08-0-2012.

Chemicals

Streptozotocin was obtained from Sigma-Aldrich Company (St. Louis, Missouri, USA). All other chemicals used were (St. Louis, Missouri, USA). All other chemicals used were of analytical grade obtained from E. Merck, Mumbai and HIMEDIA, Mumbai, India.

Experimental induction of diabetes

The animals were made diabetic by an intra peritoneal injection of streptozotocin (STZ, 40 mg/kg body weight, between 6:00 AM to 7:00 AM) in a freshly prepared citrate buffer (0.1M, pH 4.5) after an overnight fast. STZ

injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycaemic mortality. The animals exhibited massive glycosuria (determined by Benedict's qualitative test, Benedict 1911) and hyperglycaemia after 48 h. Diabetes was confirmed by measuring the fasting blood glucose concentration 4 days after induction. Albino rats with a blood glucose level above 250 mg/dl were considered diabetic and were used in the experiment.

Experimental design

Thirty six male albino wistar rats were used in this study. They were equally divided into four groups six animal in each. The extracts was administrated orally at two different doses such as 300, 450 mg/kg body weight.. The doses of 300, 450 mg/ kg body weight exhibited significant changes on blood glucose, plasma insulin, glycosylated haemoglobin and changes in body weight, when compared to the normal group. These two doses are more potential, so this two doses were used for further studies

Group 1- Normal control,
Group 2 - Diabetic control,
Group3 - 300 mg / kg body weight plant extract,
Group 4 -450 mg/ kg body weight plant extract treated.

Biochemical estimations

The level of glucose was estimated by the method of Sasaki *et .al* ,[13] urea by the method of Natelson *et al*[14], and those of plasma protein according to the method of biuret according to the manufacturer's instructions in the Croma test kit (Cat No. 1153005; linear chemicals, S.L., Spain). Plasma insulin levels were estimated by the method of Brod and Sirota[15].

Esimation of hexose

Serum and tissue hexose content was estimated by the method of Niebes (1972)[16]. 0.2 ml of the serum or tissue homogenate is mixed with 8.5 mL of orcinol- H_2SO_4. The tubes were then heated at 80˚C for 15 min, cooled and read at 540 nm after 20 min. Standard and blank containing 0.2 mL of 0.2N H_2SO_4 were processed similarly. The hexose content of serum is expressed as mg/dL or mg/g wet tissue for tissues.

Estimation of Hexosamine

Hexosamine in the plasma and tissue was determined by the method of Elson and Morgon (1933).[17] The method involves 0.1 mL of plasma or tissue homogenate in a test tube graduated at 10 mL, 5 mL of 95% ethanol was added and mixed well, centrifuged for 15 min, decanted, and the precipitate was suspended in 3 mL of 95% ethanol. The solution was centrifuged and decanted. To the precipitated protein 2 mL of 3N HCl were added and the solution was hydrolyzed in a boiling water bath with an air condenser for 4 h. The hydro lysate was neutralized with 3N NaOH; 1 mL of the acetyl acetone was added to 1 mL of the aliquot, 1 mL of water (blank) and 1 mL of standard. The tubes were capped with marbles to prevent evaporation and were placed in a boiling water bath for 15 min. The tubes were cooled in a tap water. 5 mL of 95% ethanol was added and the solution was mixed well. 1 mL of Ehrlich reagent was added, mixed well, and diluted to 10 mL with 95% ethanol. Absorbance was measured at 530 nm after 30 min. Hexosamine content of the plasma is expressed as mg/dL or mg/g wet tissue for tissues.

Estimation of sialic acid

Sialic acid content in serum and tissues was estimated by the method of Welmer *et al*. (1952)[18]. 4.8 mL of 5% TCA was added slowly to 0.2 mL of serum or tissue homogenate, 0.2 mL of osomucoid standard in separate tubes. The test tube was placed in a boiling water bath for exactly 15 min with a glass marble to prevent evaporation, then the tubes were cooled by immersion in water and filtered. 2 mL of clear filtrate was pipetted out of each tube; 4 mL of diphenylamine (DPA) reagent were added to one of each pair of tubes and 4 mL of acid-mixture without DPA into the other tube. The reagent blank was prepared by adding 2 ml of 5% TCA and 4 mL of DPA reagent. The solutions were mixed well and capped with a glass marble and immersed in a boiling water bath for exactly 30 min.The tubes were cooled in water and the absorbance was determined at 530 nm with a reagent blank set at zero. Sialic acid content of the serum is expressed as mg/dL or mg/g wet tissue for tissues.

Estimation of fucose

Serum fucose content was estimated by the method of Dische and Shettles (1948)[19]. To 2.2 mL of serum, 4.8 mL of sulphuric acid reagent was added and heated in a boiling water bath for 3 min. The sample was cooled and 0.1 mL of cysteine hydrochloride reagent was added, 0.5 mL of 0.1 N NaOH was also treated in the same way for blank, after 25 min the optical density was measured at 393 and 430 nm. Fucose content is expressed as mg/dL for serum.

RESULT AND DISCUSSION

Table 1
Levels of Blood glucose, Plasma insulin, Glycosylated haemoglobin and changes in body weight in control, diabetic and experimental animals

Groups	Blood glucose(mg dl^{-1})	Plasma insulin (mg dl^{-1})	Glycosylated haemoglobin (mg dl^{-1})	Changes in body weight g/wet tissue
Group 1	75.65±3.57	2.78±0.39	24.47±2.36	24.09±1.25
Group 2	287.40±8.13	6.39±0.68	7.34±3.68	42.86±1.86
Group 3	105.50±13.89	3.12±0.23	16.84±1.95	28.52±1.82
Group 4	96.11±9.81	3.71±0.41	21.35±1.37	22.50±1.76

Values are given as means± SD for six animals in each group.

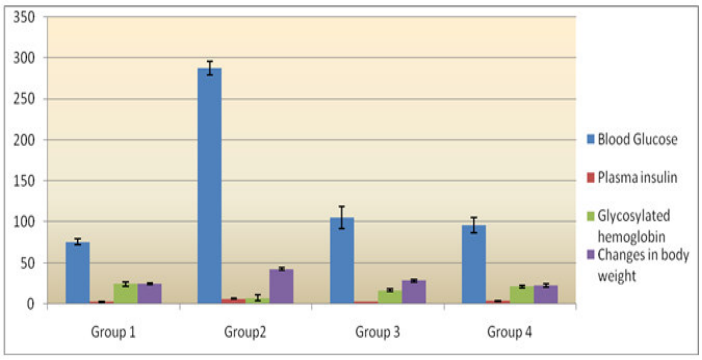

Figure 1
Values are given as means ± SD for six animals in each group.

Table 2
Levels of Glycoproteins (mg dl^{-1}) in plasma of control Diabetic and experimental animals

Groups	Hexose (mg dl^{-1})	Sexosamine (mg dl^{-1})	Sialic acid (mg dl^{-1})	Fucose (mg dl^{-1})
Group 1	91.09±3.38	72.49±2.65	57.25±3.87	32.82±2.38
Group 2	133.56±2.20	92.50±2.08	84.18±1.99	43.57±2.12
Group 3	73.09±2.57	82.48±1.84	64.39±3.32	41.41±1.24
Group 4	94.53±4.47	75.15±2.48	60.61±1.53	35.76±1.92

Values are given as means±SD for six animals in each group.

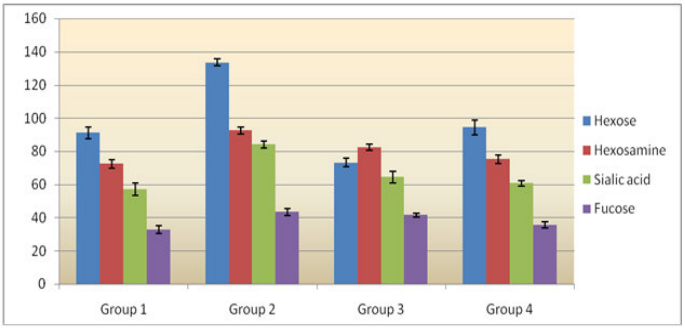

Figure 2
Values are given as means ± SD for six animals in each group.

Table 3
Levels of Glycopriteins (mg dl^{-1}) in liver of control, Diabetic and experimental animals

Groups	Hexose (mg dl^{-1})	Sexosamine (mg dl^{-1})	Sialic acid (mg dl^{-1})	Fucose (mg dl^{-1})
Group 1	20.33±1.46	5.40±0.64	7.75±0.75	9.70±1.14
Group 2	41.15±2.08	15.65±0.76	2.42±.95	26.33±1.17
Group 3	32.22±1.07	9.99±0.95	3.81±0.41	15.58±0.72
Group 4	22.22±1.07	6.59±0.54	6.01±0.56	11.88±0.75

Values are given as means±SD for six animals in each group.

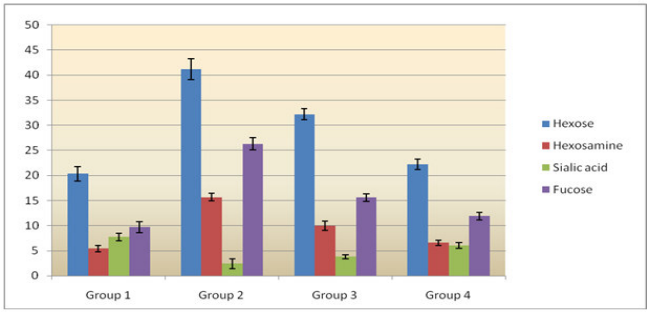

Figure 3
Values are given as means ± SD for six animals in each group.

Table 4
Levels of Glycopriteins (mg dl^{-1}) in kidney of control, diabetic and experimental animals

Groups	Hexose (mg dl^{-1})	Hexosamine (mg dl^{-1})	Sialic acid (mg dl^{-1})	Fucose (mg dl^{-1})
Group 1	20.83±1.48	7.67±0.72	9.14±0.36	12.41±1.23
Group 2	45.52±0.73	21.94±1.06	18.68±1.14	28.69±1.46
Group 3	31.07±1.03	15.64±0.84	13.71±0.85	21.26±1.09
Group 4	24.05±1.16	10.12±0.74	11.03±0.57	13.41±1.07

Values are given as means ± SD for six animals in each group.

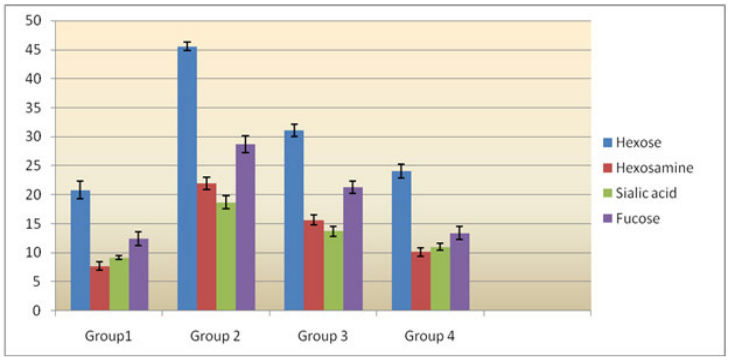

Figure 4
Values are given as means ± SD for six animals in each group.

DISCUSSION

Table 1 shows the levels of blood glucose, plasma insulin glycated haemoglobin, and changes in body weight. Diabetic control rats showed significant increase in the levels of blood glucose and decrease of plasma insulin glycated haemoglobin, and changes in body weight. Oral administration of plant extract significantly decreased in the levels of blood glucose increase in plasma insulin, glycated haemoglobin and change in body weight in diabetic rats. Table 2, 3, 4 shows the changes in the levels of plasma glycoprotein, liver and kidney glycoproteins in the experimental rats. The levels of glycoprotein containing hexose, Hexosamine Sialic acid and fucose were significantly increased in diabetic rats oral administration of plant extract significantly decreased these changes in the glycoprotein levels in the plasma insulin, liver and kidney of diabetic rats Streptozotocin selectively destroys the pancreatic insulin secreting β (beta) cells, leaving less active cells and resulting in a diabetic state[20,21]. The fundamental mechanism underlying hyperglycaemia in diabetes mellitus involves the overproduction (excessive hepatic glycogenolysis andgluconeogenesis) and decreased utilization of glucose by the tissues[22], and studies have shown that the level of blood glucose was elevated in STZ-induced diabetic rats. Hence, in the present study, we observed an increased level of blood glucose Glycoproteins are carbohydrate linked protein macromolecules found in the cell surface, which form the principle components of animal cells. They play an important role in membrane transport, cell differentiation and recognition, the adhesion of macromolecules to cell surface and the excretion and absorption of macromolecules[23]. Prolonged elevation of blood glucose in diabetes may result in structural and functional alterations of both circulating and membrane bound proteins[24]. Alterations in the diabetic state of the composition of the carbohydrate components of glycoprotein's especially serum glyloproteins and glycoproteins of the capillary basement membrane have been reported[25]. Protein bound hexoses in the cell membrane provides hydrophobic areas, whereas protein bound Hexosamine provides cationic charges on the cell membrane surface and make the membrane more polar. The elevated level of hexoses in diabetic rats may be associated with disturbances with carbohydrate metabolism. Treatment with *Acanthospermum* in diabetic rats showed significantly decreased hexoses due to improved glycemic control. Hexosamines function as physiologic glucose sensors that serve as an adaptor in diverting excess calories toward storage as fat[26]. One pathway through which glucose is sensed subacutely is hexosamine synthesis. The amination of fructose-6-phosphate to glucosamine-6-phosphate is rate limiting and is catalysed by glutamine fructose- 6-phosphate amino transferase (GFA) [27]. In accordance with previous report diabetic rats had elevated level of hexosamines, which could be due to, increased expression of GFA and increased plasma glucose. In our report, diabetic rats had elevated level of hexosamine in plasma and tissues when compared with normal rats. Diabetic rats treated with Acanthospermum showed significantly decreased hexosamines in the plasma and tissues when compared to diabetic rats, which could be due to improved glycaemic control. Sialic acid is the terminal residue of the oligosaccharide side chain of glycoproteins and widely occurs in the exposed positions of molecules like hormones, enzymes and also on tissues. Elevated levels of serum sialic acid are considered to be a good predictor of cardiovascular disease[28]. Diabetic rats had increased level of sialic acid in the plasma and tissues[29]. In our study, the level of sialic acid in plasma and tissues of diabetic control rats significantly elevated when compared to normal rats. The elevated level of Sialic acid in diabetic rats might be due to either enhanced Sialic acid synthesis or

decreased sialidase activity. Sialic acid contributes to the negative charges on this membrane, thus possibly playing a role in the selective glomerular permeability to negative charged proteins[30]. It has been postulated that an increased activity of sialidase, an enzyme which catalyses the removal of sialic acid residues from sialoconjugates which might be responsible for the depletion of glomerular sialic acid[31]. Treatment with *Acanthospermum. hispidum* ethyl acetae extract had significantly decreased sialic acid in the plasma and tissues of diabetic rats, which could be due to the regulation of sialidase activity by insulin, since insulin is a more likely mediator of sialic acid changes than any other alterations in plasma glucose levels[29]. Fucose (6-deoxy-L-galactose) is a characteristic constituent of many glycoproteins, and is a mobile component of plasma glycoproteins of particular physiological and pathological significance. In our study, diabetic rats had elevated level of fucose, which could be due to elevated blood glucose level, which is in line with previous report[29]. Treatment with *Acanthospermum. hispidum* ethyl acetate extract in diabetic rats had significantly decreased fucose levels, which could be due to improved glycaemic control. The biosynthesis of the carbohydrate moieties of glycoprotein forms the insulin independent pathways for the use of glucose 6-phosphate. But the deficiency of insulin during diabetes produces derangement of glycoprotein metabolism, resulting in the thickening of the basal membrane of pancreatic beta cells. In hyperglycemic state, the excess availability of glucose accelerates the synthesis of glucose basement membrane components i.e., glycoproteins[31]. Agents with antioxidant or free radical scavenging property may inhibit oxidative reactions associated with glycation. In this context, previous studies have shown that decrease in hyperglycemia could lead to a decrease in glycoprotein levels[29]. Administration of *Acanthospermum. hispidum* ethyl acetae extract to diabetic rats resulted in a significant reversal of all these changes to near normal. In conclusion, the decreased hyperglycemic state in *Acanthospermum. hispidum* ethyl acetate extract treated diabetic rats might have been responsible for the decrease of glycoproteins in plasma, liver and kidney. The observed effect of *Acanthospermum. hispidum* ethyl acetate extract on reversing the adverse effects of hyperglycemia provides an insight into the pathogenesis of diabetic complications, and may be used to advantage in therapeutic approaches.

CONCLUSION

In conclusion, the administration of plant extract to diabetic rats has a beneficial effect on the on the glycoproteins in addition to its anti diabetic effect. Present results revealed the potential therapeutic value of *Acanthosprmum hispidum* plant extract as an alternative medicine for the better control, management and prevention of diabetes mellitus.

AKNOWLEDGEMENT

Authors are thankful to department of Biochemistry , Sri Venkateswara University, Tirupati, A.P., India. For providing the lab facility

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Ayyanar, M., Ignacimuthu, S. Herbal medicines for wound healing among tribal people in Southern India: Ethnobotanical and Scientific evidences. *Int. J. Appl. Res. Nat. Prod.* 2009; 2(3): 29-42.
2. Iwu, M. M., Duncan, A. R. and Okunji, C. O. “New antimicrobials of plant origin”. In J. nick (ed). *Prospective on new crops and new uses,* ASHS press, Alexandria, V.A.1999; pp.457-62.
3. Tsay HS, Agrawal DC. Tissue Culture Technology of Chinese Medicinal Plant Resources in Taiwan and their Sustainable Utilization. Int J App Sci Eng 2005; 3:215- 23.
4. Jarald, E., Joshi, S. B. and Jain, D. C. Diabetes and Herbal Medicines. *Iran. J. Pharmaco. &Therapeut.,2008;* 7(1): 97-106.
5. Latha, M. *Scoparia dulcis,* a traditional antidiabetic plant, protects against streptozotocin induced oxidative stress and apoptosis in vitro and in vivo. *J. Biochem. Mol. Toxicol*.2004; 18(5): 261-72.
6. Rolo AP, Palmeira CM. Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. Toxicol Appl Pharmacol 2006; 212: 167-78.
7. Michael J, Fowler MD. Microvascular and macrovascular complications of diabetes. Clin Diabetes 2008; 26:
8. Ramkumar KM, Rajaguru P, Latha M, Ananthan R. Ethanol extract of Gymnema montanum leaves reduces glycoprotein components in experimental diabetes. *Nut Res* 2007; 27: 97-103
9. Gul Memon A, Rahman A, Ahmed N. Serum glycoproteins in diabetic and non- diabetic patients with and without cataract. Pak *J Ophthalmol* 2008; 24: 3.
10. Neerati P, Barla R, Bedada S. Influence of diabetes mellitus on P-glycoprotein function in rat intestine. *Pharmacologia* 2011; 2(10): 293-8
11. Sulaiman GM, Al-Amiery AAH, Mohammed AA, Al-Temimi AA. The effect of cherry sticks extract on the levels of glycoproteins in alloxan-induced experimental diabetic mice. *Ann Clin Lab Sci Winter* 2012; 42: 34-41.
12. Suganya S, Narmadha R, Gopalakrishnan VK, Devaki K. Hypoglycemic effect of Costus pictus D. Don on alloxan induced type 2 diabetes mellitus in albino rats. *Asian Pac J Trop Biomed*, 2012; 2(2): 117-23

13. Sasaki T, Matsy S, Sonae A. Effect of acetic acid concentration on the colour reaction in the o-toluidine boricacid method for blood glucose estimation. RinshoKagaku 972; 1: 346-53.
14. Natelson S, Scott ML, Beffa C. A rapid method for theestimation of urea in biologic fluids. *Am J Clin Pathol*, 1951; 21: 275-81
15. Brod J, Sirota JH. The renal clearance of endogenous creatinine in man. *J Clin Invest* 1948; 27: 645-54
16. Niebes P. Determination of enzymes and degradation products of glycosaminoglycan metabolism in the serum of healthy and various subjects. *Clin Chim Acta* 1972;42: 399–408.
17. Elson LA, Morgon WTJ. Colorimetric method for the determination of glucosamine and galactosamine. *Biochem J* 1993;27: 1824.
18. Welmer HE, Moshine JR, Sailcin D. Distribution of glycoproteins in normal human plasma. Am. Rev.Tuberculosis 1952;68: 594.
19. Dische Z, Shettles LB. Special colorimetric reaction of methyl pentoses and a spectrophotometric micro method for their determination. *J BiolChem* 1948;175: 595.
20. Lenzen S. The mechanisms of alloxan and streptozotocin-induced diabetes. *Diabetologia* 2008; 51(2): 216-26.
21. Suresh Kumar RB, Kar B, Dolai N, Bala A, Kanti P. Evaluation of antihyperglycemic and antioxidant properties of Streblus asper Lour against streptozotocin–induced diabetes in rats. *Asian Pac J Trop Biomed* 2012; 2(2): 139-43
22. Toft I, Jenssen T. Type 2 diabetic patients have increased gluconeogenic efficiency to substrate availability, but intact autoregulation of endogenous glucose production. Scand *J Clin Lab Invest* 2005; 65(4): 307-20
23. Parillo F, Arias MP, Supplizi AV. Glycoprofile of the different cell types present in the mucosa of the horse guttural pouches. *Tissue Cell* 2009; 41(4): 257-65.
24. Ciftci G, Yarim GF. Evaluation of IGF-I levels and serum protein profiles of diabetic cats and dogs. *J Vet Sci* 2011; 12(4): 325-31.
25. Buse MG. Hexosamines, insulin resistance, and the complications of diabetes: current status. *Am J Physiol Endocrinol Metab* 2006; 290: E1-E8.
26. Marshall S. Role of insulin, adipocyte hormones, and nutrientsensing pathways in regulating fuel metabolism and energy homeostasis: A nutritional perspective of diabetes, obesity, and cancer. *Sci STKE* 2006; 346: re7.
27. Wellen KE, Lu C, Mancuso A, Lemons JM, Ryczko M, Dennis JW, et al. The hexosamine biosynthetic pathway couples growth factor-induced glutamine uptake to glucose metabolism. *Genes Dev* 2010; 24(24): 2784-99.
28. Lebensohn N, Re A, Carrera L, Barberena L, D'Arrigo M, Foresto P. Serum sialic acid, cellular anionic charge and erythrocyte aggregation in diabetic and hypertensive patients. Medicina (B Aires) 2009; 69(3): 331-4.
29. Saravanan G, Ponmurugan P, Senthil Kumar GP, Rajarajan T. Antidiabetic effect of S-allylcysteine: Effect on plasma and tissue glycoproteins in experimental diabetes. *Phytomedicine* 2010; 17(14): 1086-9.
30. Goldberg S, Harvey SJ, Cunningham J, Tryggvason K, Miner JH. Glomerular filtration is normal in the absence of both agrin and perlecan-heparan sulfate from the glomerular basement membrane. *Nephrol Dial Transplant* 2009; 24(7): 2044-51.
31. Ghassan MS, Ahmed AHA, Abbas AM, Ali AAT. The effect of cherry sticks extract on the levels of glycoproteins in alloxaninduced experimental diabetic mice. *Ann Clin Lab Sci Winter* 2012; 42: 34-41.