SP-1

Protective Effect of Gymnema Sylvestre Ethanolic Extracts on Diabetic Dyslipidemia and Cardiac Tissue in High Fat Diet and Diabetic Induced Wistar Rats.

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Abstract: Diabetes mellitus is the major epidemics affecting worldwide. This multifactorial metabolic disorder characterized by the hyperglycemia, increase insulin resistance and diabetic dyslipidemia resulting metabolic and molecular changes eventually contribute diabetic-related vascular complications. Diabetic dyslipidemia may increase the risk of atherosclerosis and cardiovascular disease. *Gymnema Sylvester* is a known anti-diabetic herb and also have the anti-obesity and cardio protective properties. By virtue of their ameliorating effect, the present is designed to evaluate the protective effect of *Gymnema sylvestre* on diabetic dyslipidemia and cardiac tissue under high fat diet and diabetic induced condition. The animals were divided into six groups (n=6). Group 1: normal pellet diet. Group 2: high fat diet (67.5% lard oil, 31% cholesterol, 1% d1-methionine, 0.3% yeast powder and 0.1% NaCl for entire experimental period. Group 3: administered with streptozotocin (40mg/kg b.w., i.p) for 5 consecutive days to establish Type 2 diabetes. Group 4: diabetic induced and *Gymnema* treated (200mg/kg of b.w. for 3 weeks); Group 5: diabetic induced and *Gymnema* treated (400mg/kg of b.w. for 3 weeks); Group 6: diabetic induced and metformin (25mg/kg b.w. for 3 weeks).Both dosages of *Gymnema sylvestre* restored blood glucose level and body weight in diabetic moel as standard diabetic drug metformin. The high dose *Gymnema sylvestre* normalize the serum lipid profile and reduce pathological changes in cardiac muscles than the lower dose of *Gymnema sylvestre* and metformin. In conclusion, *Gymnema sylvestre* restored blood glucose level as standard diabetic drug metformin. However, high dose of *Gymnema sylvestre* restores the lipid abnormalities and reduce pathological changes of cardiac muscles. The bioactive components Gymnemic acid, present in this herbal drug could be responsible for these ameliorating effects.

Keywords: Diabetes, *Gymnema sylvestre*, diabetic dyslipidemia, cardiac muscle.

INTRODUCTION

Diabetes mellitus is one of the major epidemics affecting worldwide. Diabetes mellitus is a multifactorial metabolic disorder recognized by the hyperglycemia as a result of defective in insulin secretion or insulin insensitivity or both. The etiology of diabetes is multifaceted associated with genetic factors, aging, and change in normal lifestyle etc., [1]. Consumption of high calorie, lack of physical activities, and obesity contribute diabetes mellitus ultimately resulting in damage to many tissues. Basically diabetes mellitus is classified into Type1, Type2 and Gestational diabetes. Type 1 diabetes is caused by pancreatic beta cell destruction resulting impairment of insulin secretion. Type 2 diabetes is mainly a result of insulin resistance. Gestational diabetes denotes glucose intolerance with onset or during pregnancy[2].In type 2 diabetes, chronic hyperglycemia, increasing insulin resistance, and diabetic dyslipidemia all contribute to a variety of metabolic and molecular changes that leading to the development of diabetic-related vascular complications[3]. Diabetic dyslipidemia is characterized by elevated levels of total cholesterol, triglycerides (TGL), small dense LDL particles (LDL), and lower levels of high density lipoprotein cholesterol (HDL-C) in the systemic circulation of diabetic patients[4]. These lipid abnormalities may increase the risk of atherosclerosis and cardiovascular disease[5].*Gymnema Sylvester* commonly known as Sirukurunjan (Tamil) is a very popular anti-diabetic plant traditionally used as a sugar destroyer[6] for centuries. This herbal drug is also used in many ailments such as jaundice[7], asthma, bronchitis[8], appetite suppressant [9]and conjunctivitis[10] and other diseases[11, 12]. In addition to theses, the anti-obesity[13] and cardio protective properties[14] of Gymnema *Sylvester* have been reported. In light of the advantages, the present study is designed to evaluate the protective effect of Gymnema sylvestre on diabetic dyslipidemia and cardiac tissue under high fat diet and type 2 diabetic conditions.

MATERIALS AND METHODS

Adult male Wistar albino rats weighed about 140 to 160g were used for this study, and the animals were maintained under controlled conditions of a 12:12 hour light/dark cycle with a temperature of 22-25 degrees with a relative humidity of 50-60%. Animals were allowed access standard rat pellet diet and drinking water ad libitum. This study protocol was conducted in accordance with the standard of the Institutional Animal Ethical Committee (CPCSEA no. SU/CLATR/IAEC/XI/100/2018).

Experimental protocol

The animals were divided into six groups and each group consists of six animals. Group 1 is the control animals fed with normal pellet diet. Remaining groups received a high-fat diet, for the first 14 days. Group 2 animals were fed with high fat diet (67.5% lard oil, 31% cholesterol, 1% d1-methionine, 0.3% yeast powder and 0.1% NaCl for entire experimental period (42 days). Group 3 administered with streptozotocin (40mg/kg b.w., i.p) for 5 consecutive days to establish Type 2 diabetic model. The Group 4 and Group 5 were diabetic induced animals and treated with ethanolic extract of *Gymnema sylvestre* by gavage at low dosage (200mg/kg of b.w. for 3 weeks) and high dosage (400mg/kg of b.w. for 3 weeks) respectively. Group 6 is positive control animal, induced Type 2 diabetes with Streptozotocin and treated with metformin (25mg/kg b.w. for 3 weeks).During

the experimental period, the animal weight and the blood glucose level were carefully monitored and recorded. The glucose level was diagnosed by collecting the blood sample from the tail vein and determined by using a glucose analyzer with a glucose strip inserted in the glucometer. At the end of the experimental period, the animals were sacrificed under deep anesthesia by over dose of ketamine (i.p.). The organs such as the liver, kidney, pancreas, heart, and aorta were dissected and weighted immediately. The tissue was fixed in 10% formalin for histological analysis. The animals were trans-cardially perfused using 10% formals saline. Organs were dissected out and post fixed 10% formalin for histological studies.

Serum lipid profile

Blood was collected in EDTA coated test tubes to prevent clotting of blood. These tubes were centrifuged at 2500 rpm for 15 min for separation of plasma. Blood was also collected in separate tubes and was allowed to clot for 20 min for serum separation. The clotted blood was centrifuged at 2500 rpm for 15 min for separation of serum. The separated serum samples were subjected to serum lipid profiles such as HDL (High-density lipids), LDL(low-density lipids), VLDL(very low-density lipids),TGL (Triglycerides) and Total cholesterol. The test samples and standards samples were fed into the autoanalyser (Hitachi 912) after programming for cholesterol. All procedures were performed following the manufacturer's instructions. The results are printed out. The cholesterol levels were expressed as mg /dl.

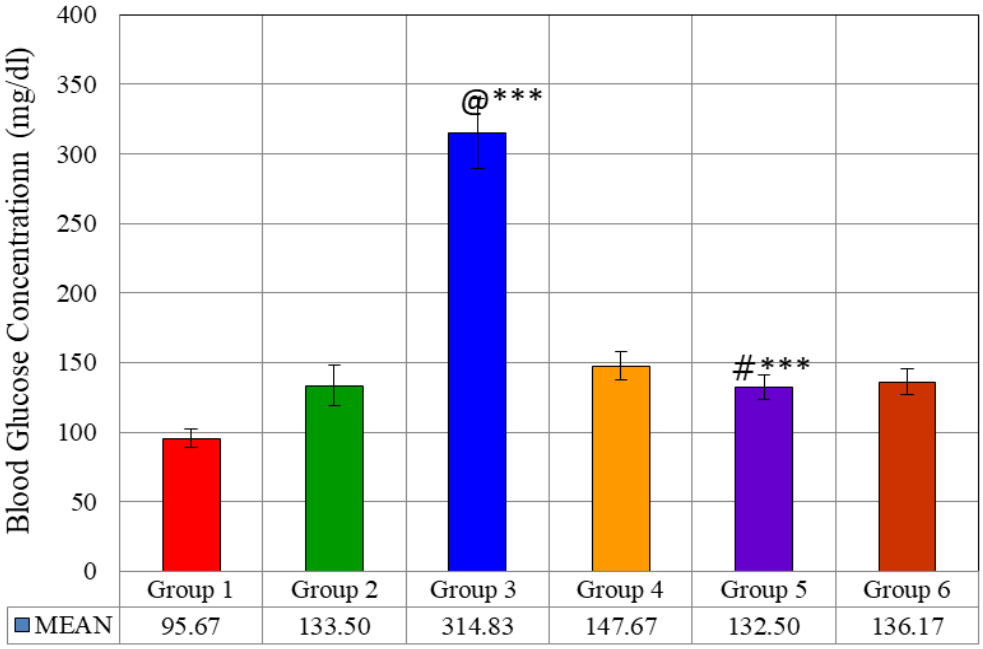
Histological study

Formalin fixed cardiac tissue were processed for routine histology and the sections were taken at 5 µm thickness using rotary microtome and stained with haematoxylin and eosin[15].

STATISTICAL ANALYSIS

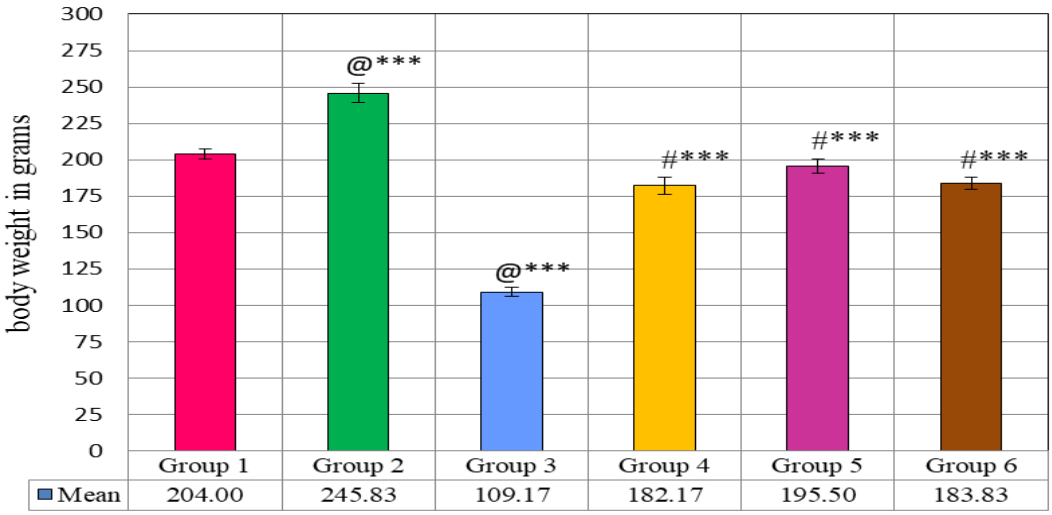
Data were analyzed using Microsoft Excel (Version 2003) and SPSS (SPSS, Version 25 Inc., IBM) software. The values were expressed as the Mean ± SE. One-way ANOVA was performed in SPSS, the level of significance was determined with a "Tukey's posthoc" test and P Values <0.05 was considered as statistically significant.

Blood glucose level



Graph 1 demonstrates blood glucose level control and experimental groups. and each column represents Mean and error bar represents Standard error of mean (n = 6 animals each) with * P<0.05, ** P<0.01 and *** P<0.001 sgnificance. @ - compared with control; #- compared with streptozotocin-induced diabetes. Group 1 - control; Group 2 - High fat diet; Group 3 - streptozotocin-induced diabetes; Group 4 - streptozotocin-induced diabetes + *Gymnema sylvestre* 200 mg/kg; Group 5 - streptozotocin-induced diabetes + *Gymnema sylvestre* 400 mg/kg; Group 6- streptozotocin-induced diabetes + Metformin.

Body Weight of the Animals



Graph 2 illustrates body weight of control and various experimental groups. The values are presented as Mean ± SEM (n=6 animals each) with * P<0.05, ** P<0.01 and *** P<0.001 significance. @ - compared with control; #- compared with streptozotocin-induced diabetes. Group 1 - control; Group 2 - High fat diet; Group 3 - streptozotocin-induced diabetes; Group 4 - streptozotocin-induced diabetes + *Gymnema sylvestre* 200 mg/kg; Group 5 - streptozotocin-induced diabetes + *Gymnema sylvestre* 400 mg/kg; Group 6- streptozotocin-induced diabetes + Metformin.

Serum lipid profile

	Group I	Group II	Group III	Group IV	Group V	Group VI
HDL (mg/dl)	12.5 ± 0.43	16 ± 0.58 #***	10.83 ± 0.48@*	13.5 ± 0.76#*	13.33 ± 0.49#*	13.17 ± 0.60
LDL (mg/dl)	26.41 ± 2.94	38.89 ± 2.00	30.88 ±3.55	28.27 ± 4.64	28.42 ± 4.03	27.83 ± 2.87
VLDL (mg/dl)	6.77 ± 0.49	18.14 ± 3.17@**	11.44 ±1.95	9.89 ± 1.73	8.79 ± 0.88	9.47 ± 1.42
TGL (mg/dl)	32.82 ± 2.76	61.33 ± 9.84	59.37 ±10.89	41.12 ± 5.08	42.77 ± 3.94	46.18 ± 7.63
Total Cholesterol (mg/dl)	51.57 ± 3.14	77.43 ± 7.65	64.29 ± 8.21	49.53 ± 5.90	54.65 ± 3.74	51.67 ± 6.69

Table illustrates serum lipid profile of control and various experimental groups. The values are presented as Mean ± SEM (n=6 animals each) with * P<0.05, ** P<0.01 and *** P<0.001 significance. @ - compared with control; #- compared with streptozotocin-induced diabetes. Group 1 - control; Group 2 - High fat diet; Group 3 - streptozotocin-induced diabetes; Group 4 - streptozotocin-induced diabetes + *Gymnema sylvestre* 200 mg/kg; Group 5 - streptozotocin-induced diabetes + *Gymnema sylvestre* 400 mg/kg; Group 6- streptozotocin-induced diabetes + Metformin.

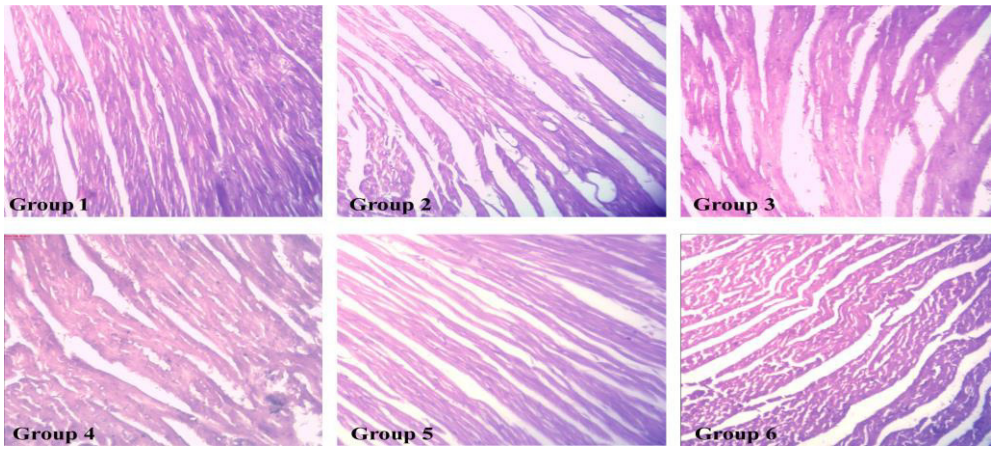


Fig. 1 Photomicrograph showing Histology of cardiac muscle stained with hematoxylin and Eosin. Magnification: 10X. Group 1 - control; Group 2 - High fat diet; Group 3 - streptozotocin-induced diabetes; Group 4 - streptozotocin-induced diabetes + *Gymnema sylvestre* 200 mg/kg; Group 5 - streptozotocin-induced diabetes + *Gymnema sylvestre* 400 mg/kg; Group 6- streptozotocin-induced diabetes + Metformin.

RESULTS

The present study showed a significant increase (P<0.001) of pre-prandial blood glucose level in streptozotocin induced diabetic group (Group 3) and marginally increased in high fat diet group (Group 2). Both dosages of Gymnema sylvestre ethanolic extract treatment (Group 4 and Group 5) were restoring blood glucose level in diabetic induced animals as standard diabetic drug metformin group (Group 6). However the high dose of Gymnema treatment showed remarkable reduction of glucose level (P<0.001) compared to the lower dose of Gymnema [Graph1]. The body weight of the group2 animals was consistently increased (P<0.05) after high fat diet. Whereas in group 3 streptozotocin-induced diabetic animals exhibited abrupt reduction in body weight (P<0.001) compared with control group. Conversely with both the lower and higher dosages of Gymnema sylvestre, (Group 4 and Group 5) the body weight of the animals is conserved (P<0.001). Similar outcomes were observed in Metformin treated group (Group 6)as in Gymnema sylvestre treatments [Graph 2]. The panel of serum lipid profile such as HDL, LDL, VLDL, TGL and Total cholesterol demonstrated a marked elevation in group 2 following high fat diet than the streptozotocin-induced diabetes (Group 3). Although HDL level was significantly reduced in diabetic induced animal when compared with control animals. In both lower and higher dosage of Gymnema treatment showed noticeably increased HDL level in Group4 and Group 5. Despite the recovery of other lipid profile parameters such as LDL, VLDL, TGL, and total cholesterol levels was observed in the Gymnema-treated groups, they did not show statistically significance. Nonetheless long-term Gymnema treatment might produce statistically meaningful results [Graph 2]. The histological investigation of the cardiac muscles [Fig.1] revealed that severe pathological changes in streptozotocin induce diabetic animals. Disarrangement of cardiac muscles fiber, alteration of the shape and size of the nucleus were observed in diabetic animals. These degenerative changes were remarkably reduced in higher dose of *Gymnema sylvestre* treatment that displays normal pattern of myofibril and nucleus arrangement than the lower dose of *Gymnema* and metformin treated groups. However lower dose of *Gymnema* and metformin treated groups the pathological changes were improved compare to diabetic induces animals. The high fat diet group showed similar changes as in diabetic induces group, in addition to that deposition of adipose cells occurs.

DISCUSSION

The decrease in blood glucose level following *Gymnema* treatment clearly indicates the hypoglycemic effect of this herbal drug. The previous experiments also stated that high concentration of *Gymnema* leaves extract reduces the glucose level in albino Wistar rat[16]. The present investigation, the high fat diet group shows increasing body weight and lipid profile panel (HDL, LDL, VLDL, TGL and Total cholesterol). Conversely, streptozotocin-induced diabetic animals' exhibit reduced body weight, increased lipid profiles panel. Interestingly, the *Gymnema* administration normalized these abnormalities. The ameliorating effect of *Gymnema sylvestre* could be due to the presence of active constituents Gymnemic acids and saponin components. Supporting to our statement, Ramesh et al., (2014) described anti-obesity and antidiabetic properties of the Gymnemic acid and it was suggested that *Gymnema* inhibit of triglycerides accumulation in the muscle and liver, along with reduction fatty acid accumulation in the blood circulation eventually decreases body weight. An earlier study also indicated that Gymnema treatment reduced body weight body mass index, and increased VLDL levels in diabetic subjects without affecting insulin secretion or sensitivity[17]. Similar results were observed in patients with impaired Glucose tolerance following Gymnema treatment[18]. Abnormalities of lipid profile levels in diabetic condition (Diabetic dyslipidemia) could be due to reduced turnover of these plasma lipids resulting accumulation in blood circulation. These Diabetic dyslipidemia might be either caused by adipose tissue dysfunction[19] and/or peripheral effect of insulin on adipose tissue[20]. The adipocytes secrete insulin-sensitizing adipokines such as adiponectin and leptin[21]. Adiponectin improves insulin sensitivity and control dyslipidemia through activating AMPK and also increasing fatty acid (FA) oxidation [22, 23]. Leptin is a crucial regulator of energy homeostasis and it is widely recognized that the lower level of leptin increases lipolysis [24, 25]. *Gymnema sylvestre* could ameliorate metabolic imbalance by modulating leptin and adiponectin levels in white adipose tissue[19].The higher levels of total cholesterol, LDL, and triglycerides and lower levels of HDL often associated with an atherogenic pattern of risk factors in pre-diabetic condition[26]. Moreover lipid abnormalities (low HDL, small dense LDL, and elevated triglycerides) increased risk of Coronary Heart disease in Type 2 Diabetes[27]. These investigations are consistent with the current study, which found that streptozotocin-induced diabetic rats had increased lipid profiles and severe histopathological changes in cardiac muscle. However these degenerative changes were remarkably normalized in higher dose of *Gymnema sylvestre* treatment than the lower dose of *Gymnema* and metformin treated groups. On the basis of the results, it is proposed that the higher dosage of ethanolic extract of *Gymnema sylvestre* regularize the lipid abnormalities and also reduce pathological changes of cardiac muscles consequently restore normal histology. The ameliorating effect of *Gymnema sylvestre* could be due to the presence of active constituents Gymnemic acids.

CONCLUSION

The present study concluded that the high fat diet and low multiple dose of streptozotocin induces type 2 diabetes mellitus and cause degenerative changes in cardiac muscle. Both dosages of *Gymnema sylvestre* restored blood glucose level as standard diabetic drug metformin. Interestingly the high dose ethanolic extract of *Gymnema sylvestre* normalize the lipid abnormalities and also reduce pathological changes of cardiac muscles than the lower dose of *Gymnema sylvestre* and metformin. The bioactive components Gymnemic acid, present in this herbal drug could be responsible for these ameliorating effects. However, prospective studies would help to identify more the therapeutic information of *Gymnema sylvestre on* type 2 diabetes associated dyslipidemia cardiac pathophysiology.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Barbara Mlinar, Janja Marc, Andrej Janež , Marija Pfeifer (2007) Molecular mechanisms of insulin resistance and associated diseases Clinica Chimica Acta 375:20–35. doi:10.1016/j.cca.2006.07.005.
2. Zubin Punthakee, Ronald Goldenberg, Pamela Katz (2018) Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome. Can J Dia betes 42:S10–S15.
3. M. Y. Donath, "Targeting inflammation in the treatment of type 2 diabetes: time to start," Nature Reviews Drug Discovery, vol. 13, no. 6, pp. 465–476, 2014.
4. Bishwajit Bhowmik, Tasnima Siddiquee, Anindita Mujumder, Faria Afsana, Tareen Ahmed, Ibrahimu A. Mdala, Nayla Cristina do V. Moreira, Abul Kalam Azad Khan , Akhtar Hussain , Gerd Holmboe-Ottesen and Tone Kristin Omsland (2018) Serum Lipid Profile and Its Association with Diabetes and Prediabetes in a Rural Bangladeshi Population. *Int. J. Environ. Res. Public Health* 15:1944-1956. doi:10.3390/ijerph15091944
5. Bruno Vergè (2015) Pathophysiology of diabetic dyslipidaemia: where are we? Diabetologia 58:886–899, DOI 10.1007/s00125-015-3525-8
6. Najafi, S., Deokule, SS., (2011) Stuies on Gymnea sylvestre – a medicinally important plant of the family of Asclepiaaceae. Trakia J.Sci. 9(2), 26-32.
7. Di Fabio G, Romanucci V, Zarrelli M, Gioano M and Zarrelli A (2013) C-4 gem- dimethylated Oleanesof Gymnema sylvestre and their pharmacological activites Mol. 18 (12);14892-14919.
8. Pragya Tiwari, B.N.Mishra, and Neelam S.Sangwan (2014) Phytochemical and Pharmacological Properties of Gymnema sylvestre: An Important Medicinal Plant. BioMed Research International http://dx.doi.org/10.1155/2014/830285.
9. Sujin RM. (2008) Anti-diabetic effect of Gymnema sylvestre (asclepiadaceae) powder in the stomach of rats. Ethnobot Leafl 2008; 153.
10. Parijat Kanetkar, Rekha Singhal an Madhusuan Kamat (2007) Gymnema sylvestre J.Clin. Biochem.Nutr., 41,77-81
11. Beverly C. David, G. Sudarsanam (2013) Antimicrobial activity of Gymnema sylvestre (Asclepiadaceae) Journal of Acute Disease 222-225.
12. Rongzi Li, Yuxian Zhang, Suhail Rasool, Thangiah Geetha, and Jeganathan Ramesh Babu (2019) Effects and Underlying Mechanisms of Bioactive Compounds on Type 2 Diabetes Mellitus and Alzheimer's Disease. *Oxidative Medicine and Cellular Longevity https://doi.org/10.1155/2019/8165707*.
13. Ramesh Pothuraju, Raj Kumar Sharma,Jayasimha Chagalamarri,Surender Jangra,Praveen Kumar Kavadi (2014) A systematic review of *Gymnema sylvestre* in obesity and diabetes management 94(5): 834-840.
14. Vinay Kumar, Uma Bhandari, Chakra Dhar Tripathi, Geetika Khanna (2012) Evaluation of antiobesity and cardioprotective effect of Gymnema sylvestre extract in murine model. Indian Journal of Pharmacology, 44 (5):607-613.
15. Bancroft's Theory and Practice of Histological Techniques: (2012) edited by S. Kim Suvarna, Christopher Layton, John D Bancroft. Elsevier Health - UK; 7 edition (26 October 2012).
16. Y.sugihara (2000) antihyperglycemic effect of gymnemic acid iv,a compound derived from gymnema sylvestre in streptozotocin diabetic mice,journal of asian national prouct), research .vol 2 no4 pg 321-327.
17. Laura Y Zuñiga [I], Manuel González-Ortiz [I], Esperanza Martínez-Abundis (2017) Effect of Gymnema sylvestre Administration on Metabolic Syndrome, Insulin Sensitivity, and Insulin Secretion. J Med Food, 20(8):750-754. doi: 10.1089/jmf.2017.0001.
18. Luis A Gaytán Martínez [I], Luis A Sánchez-Ruiz [I], Laura Y Zuñiga [I], Manuel González-Ortiz [I], Esperanza Martínez-Abundis [I] Effect of *Gymnema sylvestre* Administration on Glycemic Control, Insulin Secretion, and Insulin Sensitivity in Patients with Impaired Glucose Tolerance. J Med Food 2021 Jan;24(1):28-32. doi: 10.1089/jmf.2020.0024.
19. Hyeon-Jeong Kim, Sanghwa Kim, Ah Young Lee, Yoonjeong Jang, Orkhonselenge Davaadamdin, Seong-Ho Hong, Jun Sung Kim and Myung-Haing Cho. (2017)The Effects of *Gymnema sylvestre* in High-Fat Diet-Induced Metabolic Disorders The American Journal of Chinese MedicineVol. 45, No. 04, pp. 813-832 https://doi.org/10.1142/S0192415X17500434.
20. Oldberg, I.J. Diabetic Dyslipidemia: Causes and Consequences. JCEM 2001, 86, 965–971.
21. Sethi JK, Vidal-Puig AJ. Thematic review series: Adipocyte biology. Adipose tissue function and plasticity orchestrate nutritional adaptation. J Lipid Res 2007;48:1253-62.
22. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002;8:1288-95.
23. Yamauchi T, Kamon J, Waki H, Imai Y, Shimozawa N, Hioki K, et al. Globular adiponectin protected ob/ob mice from diabetes and ApoE deficient mice from atherosclerosis. J Biol Chem 2003;278:2461-8.
24. Qureshi, K. and G.A. Abrams. Metabolic liver disease of obesity and role of adipose tissue in the pathogenesis of nonalcoholic fatty liver disease. World J. Gastroenterol. 13: 3540–3553, 2007.
25. Mopuri, R., M. Ganjayi, K.S. Banavathy, B.N. Parim and B. Meriga. Evaluation of anti-obesity activities of ethanolic extract of Terminalia paniculata bark on high fat diet-induced obese rats. BMC Complement Altern. Med. 15: 76, 2015.
26. Haffner SM, Mykkanen L, Festa A, Burke JP, Stern MP: Insulin-resistant predia□betic subjects have more atherogenic risk factors than insulin-sensitive predi□abetic subjects: implications for prevent□ing coronary heart disease during the prediabetic state. Circulation 101:975– 980, 2000
27. Ronald M. Krauss, MD (2004) Lipids and Lipoproteins in Patients With Type 2 Diabetes Diabetes Care 27(6):1496–1504.