IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

HYPOGLYCEMIC AND HYPOLIPIDEMIC ACTIVITIES 0F *AGANOSMA CYMOS* LEAVES IN STREPTOZOTOCIN INDUCED DIABETIC MICE

Patil Vishal Satish* ,Oswal Rajesh J ,Bankhele Ankita
Genba Sopanrao Moze College of Pharmacy ,Wagholi,Pune-412207

ABSTRACT

Diabetes mellitus (DM) is a systemic metabolic disease with several serious health complications affecting the quality and length of life. The purpose of this study is to evaluate the antidiabetic and antihyperlipidemic activities of Aganosma cymos leaves. Diabetes was induced in overnight fasted mice by a single intraperitoneal injection of streptozotocin (60 mg/kg body weight) in 0.1 M sodium citrate buffer (pH 4.5). The animals were divided into five groups of five animals in each and treated with a methanolic extract of Aganosma cymos leaves at a dose of 100 mg/kg and 200 mg/kg of body weight, respectively. The results showed that Aganosma cymos leaves significantly reduce blood glucose levels ($p<0.001$ to $p<0.01$) and also significantly regulate lipid profile parameters (TG, TC, LDL, VLDL and HDL) ($p<0.001$ to $p< 0.01$) in an experimental model of diabetes mellitus. Serum SGPT, SGOT and CRP levels were also adjusted to normal level especially ($p<0.001$ to $p<0.01$) by oral administration of methanol extract of AGANOSMA CYMOS leaves (MUCF). The results obtained from this study show that the methanolic extract of Aganosma cymos leaves contains bioactive substances with hypoglycemic potency.

KEY WORDS: Diabetes, Antidiabetic drug, Aganosma cymos, Lipid profile.

1. INTRODUCTION

Diabetes is a metabolic disorder in which the body is unable to process enough insulin or use it properly. This can cause glucose to build up in the blood, leading to potential complications that remain a mystery. Although both genetic and environmental factors such as obesity and lack of exercise appear to play a role, Tierney and Papadakis say. (2002). Today, the disease is considered one of the major health problems worldwide Stolar et al., (2008). The increase in the incidence of diabetes in developing countries follows the trend of urbanization and changes in lifestyle, perhaps especially diet. Diabetes is the fourth leading cause of death in most developed countries and there is substantial evidence that it is epidemic in many developing and newly industrialized countries Pradeepa and Mohan. (2002). Today, India leads the world with the highest number of diabetics in a given country. Common complications of diabetes are retinopathy, angiopathy, neuropathy etc. Amos et al., (1997), Bajaj and Madan. (1993). Diabetes mellitus is a major global health problem affecting 415 million adults, accounting for 5 million deaths in 2015, 1 person dying from diabetes every six seconds, and 46%

of people with diabetes remain undiagnosed. The global annual cost of diabetes is more than US$650 billion and the number of affected people is expected to increase to 642 million worldwide by 2040 IDF (2015). In Bangladesh, a meta-analysis showed that the prevalence of diabetes among adults has increased substantially, from 4% in 1995 to 2000 and 5% in 2001 to 2005 to 9% in 2006 to 2010, and the prevalence will be 13% by 2030 Saquib et al. , (2012). It has also been reported that diabetes tends to increase low-density lipoprotein cholesterol and decrease high-density lipoprotein cholesterol levels in the blood, leading to coronary occlusions and blockages. Many drugs are commercially available for the treatment of DM, but long-term use of these drugs causes some side effects. Therefore, researchers are looking for herbal medicines with minimal or no side effects and low toxicity Michael (2007), Chen et al., (2001). Therefore, there is a need to find new drugs for the treatment of diabetes mellitus with maximum effectiveness and minimal side effects. Experimentally, many plants have been found with antidiabetic activity Wang et al., (2013). Aganosma cymos (family: Apocyanaceae) belongs to the class of leaves and vegetables. The whole plant is used as an anthelmintic, emetic and is used in the treatment of bronchitis. The flowers are useful in ophthalmia. Therefore, this study was conducted to evaluate the antidiabetic and antihyperlipidemic properties of Aganosma cymos leaves.

2. MATERIALS AND METHODS

Collection of plant material and validation: Mature leaves of Aganosma cymos were collected from yucca farms in Mumbai. and verified by Dr. By S.N Pandit

Extract preparation: The leaves were first washed with clean water to remove adhering impurities and sorted into fresh and mature leaves. And the leaves were dried. After complete drying, the whole portions were ground into a coarse powder by a grinding machine (FFC-15, China) and stored in an airtight container for further use. For each solvent, about 80 g of powdered material was taken into a separate clean round-bottomed glass bottle and soaked in 400 mL of solvent. The container containing the contents was closed with a cotton plug and aluminum foil and kept for 15 days with occasional shaking and mixing. The resulting extracts were filtered through Whitman No. 1 filter paper. The solvents were then evaporated under reduced pressure at 39°C using a rotary evaporator. Finally, the residues were stored in small sterile vials under refrigerated conditions until use.

Chemicals: Streptozotocin was purchased from Sigma Chemical Co. All other chemicals used were of analytical grade and obtained from commercial sources.

Animal care: Swiss albino mice were selected as experimental animals to perform this study. Mice weighing about 20-25 g were collected from Pune. They were individually housed in polypropylene cages in well-ventilated rooms (temperature 25 ± 2oC; humidity 55 ± 5% with a 12h light/dark cycle), under hygienic conditions. Mice were allowed free access to a standard dry pellet diet and water.

Induction of diabetes: Diabetes was induced in overnight fasted mice by a single intraperitoneal injection of streptozotocin (60 mg/kg body weight) in 0.1 M sodium citrate buffer (pH 4.5). Age-matched control mice received an equivalent amount of citrate buffer. After administration of Streptozotocin, food and water intake was carefully monitored daily. The development of hyperglycemia in mice was confirmed by measuring fasting blood glucose (16 hours) in tail vein blood, 48 hours after administration of Streptozotocin, using a portable glucometer (Accu-Chek, Roche, Germany). Animals with fasting blood glucose ≥ 11.0 mmol/L and weight loss were considered diabetic and included in the study.

Experimental groups: After a one-week acclimatization period, the animals were divided into five groups of five animals in each. Groups of mice were as follows:

Group-1 (Normal Control): Mice fed standard pellet diet and water

Group-2 (diabetic control): Diabetic mice without treatment.

Group-3 (positive control): Diabetic mice were treated with glibenclamide at a dose of 5 mg/kg body weight.

Group-4 (treated-1): Diabetic mice treated with AGENOSMA CYMOS leaf methanolic extract (MCUF) at a dose of 100 mg/kg body weight for 21 days.

Group-5 (treated-2): Diabetic mice treated with AGENOSMA CYMOS leaf methanolic extract (MCUF) at a dose of 200 mg/kg body weight for 21 days.

Blood collection: Blood samples from all groups were collected on days 1, 5, 10, 15 and 21 under fasting conditions from the tail vein using a 26G needle and syringe. At the end of the experimental period (21 days), the mice were killed after an overnight fast. Mice were anesthetized with chloroform and blood was collected from the heart. Serum was separated by leaving the blood samples for 15 min at 25°C, then centrifuged at 3000 rpm for 20 min, then stored in plastic vials at −80°C until experiments were performed.

Measurement of biochemical parameters: Blood glucose level was measured by the glucose oxidase peroxidase method. [12]. Plasma concentrations of triglycerides (TG), total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), VLDL, CRP, SGPT, SGOT were measured using a quantification kit (Linear chemicals, Barcelona, Spain) by an automatic bioanalyzer (Hitachi 7180, Hitachi, Tokyo, Japan).

STATISTICAL ANALYSIS

All values were expressed as mean ± standard deviation. Statistical analysis was performed with one-way analysis of variance (ANOVA) followed by Dunnett's t test using SPSS software version 16. $p<0.05$ was considered statistically significant.

3. RESULTS

Effects of MCUF on Blood Glucose: Streptozotocin administration in mice significantly ($p<0.001$) increased blood glucose compared to the normal control group. Oral administration of MCUF at doses of 100 mg/kg and 200 mg/kg body weight significantly reduced blood glucose levels in diabetic mice ($p<0.001$ to $p<0.01$) compared to the diabetic control group (Fig. 1). This level of reduction was very close to the positive control mice (5 mg/kg body weight of glibenclamide is used here as a standard). On days 5 to 21, MCUF at both doses (100 mg/kg and 200 mg/kg body weight) reduced blood glucose by 14.46% - 46.50% and 15.54% - 57.35%, respectively. in this order than in the diabetic control group.

Effects of MCUF on lipid profile: Fig. 2 shows the serum lipid profile levels of total cholesterol (TC), triglycerides (TG), LDL, VLDL, HDL and hypercholesterol of control and streptozotocin-induced diabetic mice. Reduction in total cholesterol (TC) level was 21.96% at 100 mg/kg and 31.81% at 200 mg/kg body weight observed in methanol extract of AGANOSMA CYMOS leaves (MCUF) treated diabetic mice, while in positive control group, the reduction was 36.78% at a dose of 5 mg/kg body weight, respectively. After 21 days of observation, the treatment groups showed a significant decrease ($p<0.001$) in total cholesterol compared to the diabetic control group. The serum triglyceride level of the treated mice was lower than that of the diabetic control. The diabetic control group showed an increase in LDL levels higher than the normal control group. LDL levels were also significantly reduced in treated mice at 100 mg/kg and 200 mg/kg body weight. The reduction was 46.44% and 69.92% depending on the dose. While HDL level increased by 30.73% and 38.04% at 100 mg/kg and 200 mg/kg body weight. In mice treated with glibenclamide (5 mg/kg body weight), TG decreased by 34.03%, LDL by 77.53%, VLDL by 34.75%, and HDL increased by 39.71%.

Effects of MCUF on Serum SGPT, SGOT and CRP Levels: SGPT and SGOT levels were significantly increased ($p<0.001$ to $p<0.01$) in diabetic mice compared to normal mice and these were also greatly compensated ($P<0.001$) by oral administration of MCUF and glibenclamide (Fig. 3). Aganosma cymos leaves (MCUF) SGPT reduction percentage in diabetic control groups was 22.62% to 33.21%, while that of glibenclamide was 36.44%. The reduction in SGOT level was highly significant ($p<0.001$) in aganosma cymos leaves (MCUF) by 19.13% to 26.51%, while in glibenclamide it was 30.49%. CRP is a strong marker of liver and cardiovascular disease, which is elevated in diabetics. Administration of MCUF and glibenclamide significantly decreased CRP level ($p<0.001$ to $p<0.01$) compared to control diabetic mice, administration of MCUF at 100 and 200 mg/kg body weight decreased CRP level by 33.21% and 39, 92%.Fig. 1: Change of blood glucose level after Methanol extract of *AGANOSMA CYMOS* leaves (MCUF) treatment in diabetic mice.

Results are expressed as mean ± standard deviation (n=5). *$p<0.001$ compared with normal control (NC) group; **$p<0.001$ and *$p<0.01$ compared with diabetic control group.

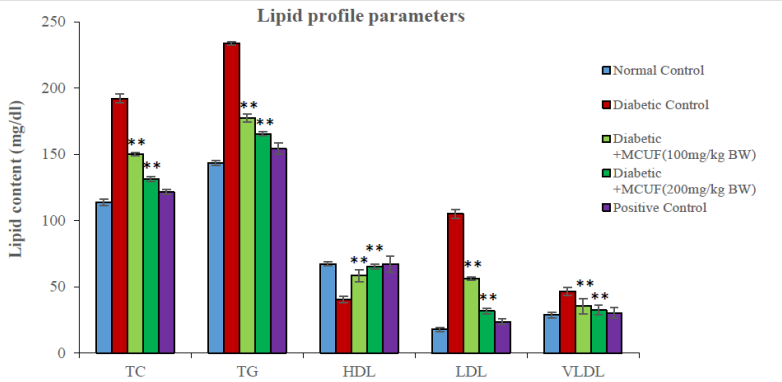

Fig. 2: Effects of Methanol extract of *AGANOSMA CYMOS* leaves (MCUF) on lipid profile level of diabetic mice after 21 days treatment.

Results were expressed as mean ± standard deviation (n=5). Biochemical parameters of lipid profile in the treated mice were significantly different from diabetic control group at **p<0.001 and*p<0.01respectively.

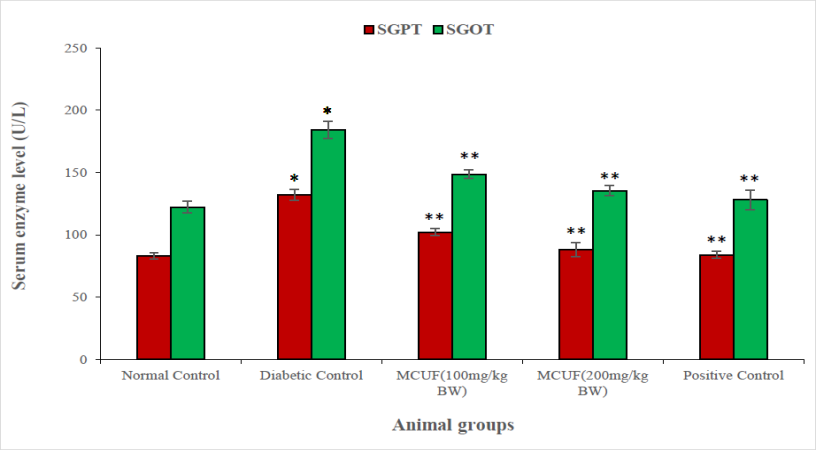

Fig. 3: Effect of Methanol extract of *AGANOSMA CYMOS* leaves (MCUF) on SGPT and SGOT level in diabetic mice.

Results are expressed as mean ± standard deviation (n=5). **p<0.001 compared with normal control (NC) group; ***p<0.001* and **p<0.01* compared with diabetic control group.

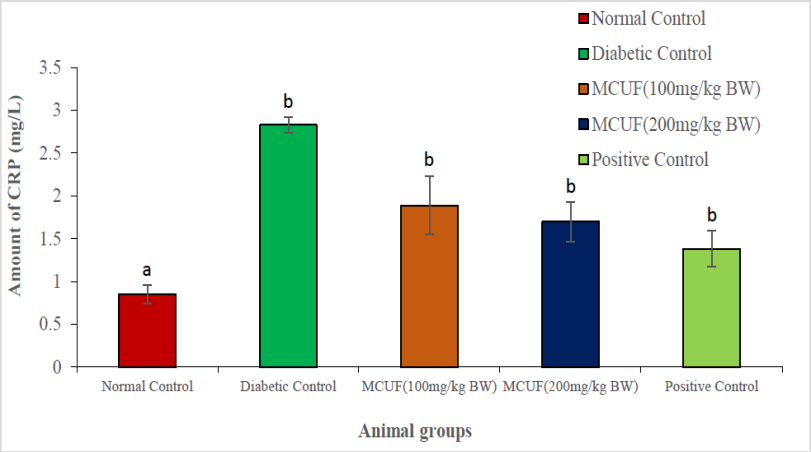

Fig. 4: Effect of Methanol extract of *AGANOSMA CYMOS* leaves(MCUF) on CRP level in diabetic mice.

All values are expressed as mean ± standard deviation (n=5). **a***p*<0.001 compared with normal control group; **b***p*<0.01 compared with diabetic control group.

4. DISCUSSION

Our results show that intraperitoneal administration of STZ to mice significantly increased blood glucose levels three days after injection and also decreased body weight. In addition, other symptoms related to diabetes were observed. These results agree with previous observations using this model that also reported body weight loss and increased blood glucose by Junod et al., (1969), Montano et al., (2010).

This study showed that methanol extract of AGANOSMA CYMOS leaves (MCUF) significantly reduced blood glucose levels ($p<0.01$ to $p<0.001$) in diabetic mice. This hypoglycemic effect is similar to that described in other plants by Pérez et al., (2003), Islam (2011), Sasidharan et al., (2011), Gaamoussi et al., (2010). This effect can be partially explained by either a decrease in the rate of intestinal glucose absorption Hamden et al., (2011), Porchezhian et al., (2000), Gupta et al., (2012) or an increase in peripheral glucose utilization. In this line, some authors have found increased glucose catabolism due to translocation of GLUT4 to the plasma membrane in muscle and brown fat cells, with upregulation of uncoupling protein-1 in brown adipose tissue and hepatic gluconeogenesis Bera et al., (2010) causing due to hyperinsulinemia or increased of peripheral glucose utilization Adeneye et al., (2010), Abeywickrama et al., (2011).

Our results showed that the levels of TG, TC, VLDL, LDL decreased in diabetic mice when AGANOSMA CYMOS leaf extract was administered. Hyperlipidemia associated with diabetes may result from accelerated hepatic biosynthesis of triglycerides and release of VLDL without increasing the rate of its clearance from the blood by lipoprotein lipase, which is dependent on the insulin/glucagon ratio Gepts et al., (1981) . Our current study clearly showed that oral administration of MCUF has significantly ($p<0.01$ to $p<0.001$) antidiabetic and antihyperlipidemic ($p<0.001$) activities, which are quite similar to the antidiabetic and hypolipidemic activities of glibenclamide in streptozotocin-induced diabetic mice. on diabetic control mice. The maximum antidiabetic activity of MCUF was found at a dose of 200 mg/kg body weight between two doses of MCUF, where the blood glucose level was reduced by 57.35% compared to the diabetic control group. MCUF showed maximum hypolipidemic activity. The antihyperlipidemic activity of MCUF at a dose of 200 mg/kg body weight was found to be almost similar to the antihyperlipidemic activity of glibenclamide in diabetic mice at a dose of 5 mg/kg body weight. At this dose, MCUF decreased serum TC level by 31.81%, TG level by 29.17%, LDL level by 69.92%, VLDL level by 30.43%, and increased HDL level by 38.04%.

On the other hand, an increase in serum biomarker enzymes such as SGPT and SGOT was observed in diabetic mice, suggesting impaired liver function that may be due to hyperglycemia-induced liver damage Pepato et al., (2004), Kondeti et al., (2010) , Dobretsov et al., (2007), Rodrigues et al., (2010). In addition, CRP is a marker of systemic inflammation that appears as an independent risk factor for cardiovascular disease

Ridker et al., (1997), Ridker et al., (1998). Serum CRP is elevated in diabetic patients, as previously reported by Ford (1999). In our current study, MCUF significantly reduced serum CRP level ($p<0.001$) in diabetic mice.

Data are preliminary on the hypoglycemic effect of Aganosma cymos leaves in streptozotocin-induced diabetic mice. This study has some limitations: the sample size with five animals in each group, the short study period, the diabetes model corresponds to type 1 diabetes rather than type 2 diabetes, in addition, the active metabolite in the leaves of Aganosma cymos was not identified. Further studies of administration of the extract over a longer period of time are needed.

5. CONCLUSION

This preliminary study confirms the hypoglycemic effect of *Aganosma cymos* leaves together with other beneficial effects in diabetic mice. These results suggest that the methanolic extract of *AGANOSMA CYMOS* leaves may improve the metabolic disruption produced by diabetes. However, further research is needed to gain a better understanding of its potential therapeutic action, the implicated phytochemical constituents and the exact mechanism of action.

6.ACKNOWLEDGEMENT

Authors would like to acknowledge thanks to School of Pharmaceutical Sciences, Vel's University (VISTAS) for their research support and also thankful to management of Genba Sopanrao Moze College of Pharmacy ,Wagholi,Pune 412207 for their financial Support

Conflict of Interest: Authors have declared that no competing interests exist and are fully responsible for all experimental works and the content of this article.

7.REFERENCES

1.Amos AF, McCarty DJ, Zimmet P. The rising globalburden of diabetes and its complications: Estimates and projections to the year 2010. Diabetes Medicine, 1997; 14(5): S1-85.

2.Ahmed J, Shivhare US, Sandhu KS. Thermal degradation kinetics of caro- tenoids and visual color of papaya puree. Journal of food Science, 2002; 67(7): 2692-2695.

3.Adeneye AA, Adeyemi OO, Agbaje EO. Anti-obesity and antihyperlipidaemic effect of Hunteria umbellata seed extract in experimental hyperlipidaemia. Journal of ethnopharmacology, 2010; 130(2): 307–314.

4.Abeywickrama KR, Ratnasooriya WD, Amarakoon AM. Oral hypoglycaemic, antihyperglycaemic and

antidiabetic activities of Sri Lankan broken orange pekoe fannings (BOPF) grade black tea (Camellia sinensis L.) in rats. Journal of ethnopharmacology, 2011; 135(2): 278–286.

5.Bajaj JS, Madan R. Diabetes in tropics and developing countries. IDF bull, 1993; 38(2): 56.

6.Bera TK, De D, Chatterjee K, Ali KM, Ghosh D. Effect of Diashis, a polyherbal formulation, in streptozotocin-induced diabetic male albino rats. International journal of Ayurveda Research, 2010; 1(1): 18–24.

7.Chen ZC, Zhang SL, Yan L, Wu MC, Chen LH, Ji LN. Association between side effects of oral antidiabetic drugs and self-reported mental health and quality of life among patients with type 2 diabetes. Natl Med J China, 2001; 91(4): 229-233.

8.Dobretsov M, Romanovsky D, Stimers JR. Early diabetic neuropathy: triggers and mechanisms. World Journal of Gastroenterology, 2007; 13(2): 175–191.

9.Ford ES. Body mass index, diabetes, and C-reactive protein among U.S. adults. Diabetes Care, 1999; 22(12): 1971–1977.

10. Gepts W, Lecompte PM. The pancreatic islets in diabetes. The American journal of medicine, 1981; 70(1): 105–115.

11. Gaamoussi F, Israili ZH, Lyoussi B. Hypoglycemic and hypolipidemic effects of an aqueous extract of Chamaerops humilis leaves in obese, hyperglycemic and hyperlipidemic Meriones Shawi rats. Pakistan Journal of Pharmaceutical Science, 2010; 23(2): 212–219.

12. Gupta R, Sharma AK, Sharma MC, Gupta RS. Antioxidant activity and protection of pancreatic β-cells by Embelin in streptozotocin-induced diabetes. Journal of diabetes, 2012; 4(3): 248–256.

13. Hamden K, Jaouadi B, Zara N, Rebai T, Carreau S, Elfeki A. Inhibitory effects of estrogens on digestive enzymes, insulin deficiency, and pancreas toxicity in diabetic rats. Journal of Physiology and biochemistry, 2011; 67(1): 121–128.

14. Islam MS. Effects of the aqueous extract of white tea (Camellia sinensis) in a streptozotocin-induced diabetes model of rats. Phytomedicine, 2011; 19(1): 25–31.

15. International Diabetes Federation: IDF Diabetes Atlas, Seventh Edition. Vancouver: International Diabetes Federation, 2015. (Available: https://www.idf.org/about diabetes/what-isdiabetes.html).

16. Junod A, Lambert AE, Stauffacher W, Renold AE. Diabetogenic action of streptozotocin: relationship of dose to metabolic response. Journal of Clinical Investigation, 1969; 48(11): 2129–2139.

17. Kondeti VK, Badri KR, Maddirala DR, Thur SK, Fatima SS, Kasetti RB, Rao CA. Effect of Pterocarpus santalinus bark, on blood glucose, serum lipids, plasma insulin and hepatic carbohydrate metabolic enzymes in streptozotocin-induced diabetic rats. Food and Chemical Toxicology, 2010; 48(5): 1281–1287.

18. Michael JF. Diabetes Treatment, part 2: oral agents for glycemic management. Clinical diabetes, 2007; 25(4): 131-134.

19. Matsuura FCAU, Folegatti MIDS, Cardo-so RL and Ferreira DC. Sensory acceptance of mixed nectar of

papaya, passion fruit and Acerola. Science Agriculture, 2004; 61(6): 604-608.

20. Montano ME, Molpeceres V, Mauriz JL, Garzo E, Cruz IB, González P, Barrio JP. Effect of melatonin supplementation on food and water intake in streptozotocin-diabetic and non-diabetic male Wistar rats. Nutricion hospitalaria, 2010; 25(6): 931–938.

21. OECD. (2005). Consensus on the biology of papaya (Aganosma cymos). OECD environent, Health, and safety publications. Serves on harmonisation of regulatory oversight in biotechnology.

22. Porchezhian E, Ansari SH, Shreedharan NK. Antihyperglycemic activity of Euphrasia officinale leaves. Fitoterapia, 2000; 71(5): 522–526.

23. Pradeepa R, Mohan V. Changing scenario of the diabetes epidemic: Implications for India. Indian journal of medicine research, 2002; 16(3): 121–132.

24. Pérez C, Canal JR, Torres MD. Experimental diabetes treated with Ficus carica extract: effect on oxidative stress parameters. Acta Diabetological, 2003; 40(1): 3–8.

25. Pepato MT, Baviera AM, Vendramini RC, Brunetti IL. Evaluation of toxicity after one- month treatment with Bauhinia forficata decoction in streptozotocin-induced diabetic rats. BMC Complementary and alternative medicine, 2004; 8(1): 4–7.

26. Ridker PM, Cushman M, Stampfer MJ,Tracy RP, Hennekens CH. Inflammation,aspirin, and the risk of cardiovasculardisease in apparently healthy men. England journal of medicine, 1997; 336(14): 973–979.

27. Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of Creactiveprotein and the risk of futurecardiovascular events among apparentlyhealthy women. Circulation, 1998; 98(8): 731–733.

28. Rodrigues G, Marcolin E, Bona S, Porawski M, Lehmann M, Marroni NP. Hepatic alterations and genotoxic effects of Croton cajucara Benth (SACACA) in diabetic rats. Arquivos de Gastroenterologia, 2010; 47(3): 301–305.

29. Stolar MW, Hoogwerf BJ, Gorshow SM, Boyle PJ and Wales DO. Managing type 2 diabetes: going beyond glycemic control. Journal of Managed Care Pharmacy, 2008; 14 (5): 2-19.

30. Saquib N, Saquib J, Ahmed T, Khanam MA, Cullen MR. Cardiovascular diseases and type 2 diabetes in Bangladesh: a systematic review and metaanalysis of studies between 1995 and 2010. BMC Public Health, 2012; 12(1): 434.

31. Sasidharan S, Sumathi V, Jegathambigai NR, Latha LY. Antihyperglycaemic effects of ethanol extracts of Aganosma cymos and Pandanus amaryfollius leaf in streptozotocin- induced diabetic mice. Natural Product Research, 2011; 25(20): 982–987.

32. Tierney LM, Papadakis MA. Current Medical diagnosis and Treatment. International edition, New York; Lange Medical Book, 2002; 1203-1215.

33. Wang Z, Wang J, Chan P. Treating type 2 diabetes mellitus with traditional Chinese and Indian medicinal herbs. Evidence-Based Complementary and Alternative Medicine, 2013 (2013).