RESEARCH ARTICLE - OPEN ACCESS

International Research Journal Of Clinical Studies And Pharma Trends DOI: http://dx.doi.org/10.51521/IRJCPT.2021.1110

Contents lists available at bostonsciencepublishing.us

International Research Journal Of Clinical Studies And Pharma Trends

Antidiabetic Antioxidant and Hematological Effect of Ethanolic Extract of Muntingia Calabura on Streptozoticin Induced Diabetic Rats

Giddla Leela Jacob[1], Kudipudi Harinadha Baba[2]

[1] *M.Pharm Assistant Professor, Rajeev Gandhi College of Pharmacy, Andhra Pradesh, India.*

[2]*Principal, Narayana Pharmacy College, Nellore, Andhra Pradesh, India.*

A R T I C L E I N F O

Article history:
Received 24 May 2021
Revised 17 June 2021
Accepted 08 July 2021
Published 20 August 2021

Keywords:
Antidiabetic,
Antioxidant,
Haemotological,
Streptozoticin and
Muntingia Calabura.

A B S T R A C T

Diabetes has emerged as major problem International Diabetes Federation (IDF), there have been an estimated 40 million persons with diabetes in India in 2007 and this number is predicted to rise to almost 70 million people by 2025. Fresh stem bark of muntingia calabura was collected in Tadepalligudem in the plant was identified and authenticated by Dr D.V Swamy Professor in Horticulture Dr YSR Horticulture university. The bark of muntingia calabura was peeled out from stem and was washed cleanly. The bark muntingia calabura was air dried to 10 days for constant weight in room. About 750 grams of bark was collected. Thirty male wistar 200-250 gms rats were randomized in to five groups consisting six animals in each group. Streptozotocin is used as an agent to induce diabetes mellitus by selective cytotoxicity effect on pancreatic beta cells. Thus it affects endogenous insulin release and as a result increases blood glucose level. Streptozotocin was confirmed in this study in relation to the normal control rats. Long term reduction of this parameter may result in internal and external haemorrhage and finally leads to death. However, after plant extract administration, the level of platelet was improved markedly especially at the dose of 100 mg/kg while that of 200 mg/kg did not have strong effect as compared with diabetic untreated rats. This effect indicated the ability of the plant extract to stimulate the biosynthesis of clotting factors due to the presence of active muntingia calabura.

INTRODUCTION

Diabetes has emerged as major problem International Diabetes Federation (IDF), there have been an estimated 40 million persons with diabetes in India in 2007 and this number is predicted to rise to almost 70 million people by 2025 [1]. The countries with the most important number of diabetic people are going to be India, China and USA by 2030. It is estimated that each fifth person with diabetes are going to be an Indian. Due to these sheer numbers, the economic burden thanks to diabetes in India is amongst the very best within the world [2,3]. The real burden of the disease is however thanks to its associated complications which cause increased morbidity and mortality. WHO estimates that mortality from diabetes, heart condition and stroke costs about $210 billion in India within the year 2005 Much of the heart disease and stroke in these estimates was linked The world of diabetes among adults(aged 20-79years)will be 6.4% affecting 285 million adults, in 2010, and will increase to 7.7%, and 439 million adults by 2030 [4,5]. Between 2010 and 2030, there'll be a 69% increase numbers of adults with diabetes in developing countries and a 20% increase in developed countries [6].

Diabetes mellitus is an endocrine, metabolic disorders caused by relative or an absolute lack of insulin.1According to International Diabetes Federation (IDF), worldwide 382 million people were affected by diabetes in 2013 and it's expected to boost to 592 million by 2035 [7]. IDF estimates 65 million diabetic patients in India in 2013and it is expected to cross 109 million by 2030.2In India diabetic patients are increasing day by day may be because of the change in food pattern, i.e. fast food diet intake and change in lifestyle [8]. Management of diabetes is a tough task. The medicines utilized in diabetic treatment are either too costlier or have adverse effects like hypo glycemic coma, insulin resistance, hypersensitivity and metallic taste etc. Hence, within the recent years, herbal compounds are gaining popularity in both developed and developing countries because of their natural origin, low adverse effects. Ethnobotanical information indicates that around

* Corresponding author.
Giddla Leela Jacob, Assistant Professor, Rajeev Gandhi College of Pharmacy, Andhra Pradesh
E-mail address: giddlaleelajacob@gmail.com

800 medicinal plants having hypo glycemic or anti diabetic potential [9]. Herbal plants are abundant in India .Hence the search for safer and effective anti diabetic agents has become the current research area. It's a well-established fact that diabetes is a risk factor for cardio vascular disease. While micro vascular complications of diabetes include nephropathy and retinopathy, macro vascular complications resulting in atherosclerotic cardiovascular disease such as coronary artery disease, cerebrovascular disease and peripheral vascular disease are the cause of death in the diabetic population. The Diabetes Control and Complications trial (DCCT) demonstrated that tight control of blood sugar is effective in reducing clinical complications significantly, but even optimal control of blood sugar could not prevent complications suggesting that alternative treatment strategies are needed [10]. Since numerous studies demonstrated that oxidative stress, mediated mainly by hyperglycemia-induced generation of free radicals, contributes to the development and progression of diabetes and related contributions, it became clear that ameliorating oxidative stress through treatment with antioxidants could be an efficient strategy for reducing diabetic complications [10].

An ethno botanical study was carried on the medicinal plants often used for the management of diabetes in Andhra Pradesh by traditional healer's .muntingia calabura the one of the used by the traditional healers for diabetes. Even though medicinal plants are widely used, the effective treatment of the disease has not been verified with scientific standards. Only a couple of plants used for diabetes in traditional medicine are scientifically audited in vivo.

MATERIALS AND METHODS:

Plant Material:

Fresh stem bark of muntingia calabura was collected in tadepalligudem in the plant was identified and authenticated by Dr D.v swamy professor in horticulture Dr YSR Horticulture university.

Preparation of Plant Extract:

The bark of muntingia calabura was peeled out from stem and was washed cleanly. The bark muntingia calabura was air dried to 10 days for constant weight in room. About 750 grams of bark was collected. It was collected in the month of March. It was then collected and then pulverized using an electric blender. It's blended into a coarse powder form .The coarse powder was then poured into a conical flask for maceration.

As solvent ethanol was used and kept aside for one week .stirring of the solvent in the conical flask should be done frequently .Its then filtered through good whatmann filter paper then we can find brown colour Solvent. This is kept for distillation at 60-80 temperature. Then it's observed with thick residue after the evaporation of the ethanol that residue is kept for one month in desiccators in order to remove the moist content in the extract. 750 gms: 6.5% yield.

RESULTS

Experimental design:

Thirty male wistar 200-250 gms rats were randomized in to five groups consisting six animals in each group.

Group 1: Normal control.

Group 2: Diabetic animals received o.5 ml of distilled water daily.

Group 3: Diabetic rats treated with 100 mg per kg.

Group 4: Diabetic rats treated with 200 mg per kg.

Group 5: Diabetic rats treated with glibenclamide only.

Animals:

Male wistar rats weighing 200 -250 grams were purchased from mahaveer animal house of Hyderabad. Animals are kept in college animal house which was well ventilated house conditions.[temperature -28-27c]photo period 12hrs light 12hrs night cycle. Humidity 45%-50% the animals were allowed for free access of water for 20 days.

Induction of diabetes in rats:

STZ [2-deoxy-2-(3-methyl-3-nitrosourea) 1-D-glucopyranose] is a broad-spectrum antibiotic. Diabetes was induced in overnight fasted male wistar rats by a single intraperitoneal injection of freshly prepared solution of streptozotocin 60mg/kg in o.1m citrate buffer [PH 4.5] 15 min after the intraperitoneal administration of nicotamide 110mg/kgprepared in normal saline. Diabetes was confirmed in the animals by elevated plasmaglucose levels.after 24 hrs of administration.

Blood sample collection method:

While handling the head with the left hand with the help of the index finger the eye was pressed just behind the angle of the jaw resulting in the engorgement of the retro orbital plexus. Then tip of the capillary was inserted at the medical canthus into the retro-orbital plexus. Capillary tube: 1ml (bore size).

The animal was restrained (unanaesthetised) in such a way that loose skin of the neck was tightened with gentle rotation by the other hand As the vessels are ruptured, blood wells up in the peri-orbital space The tip of the capillary was then slightly withdrawn, so that the bloodflows into the capillary, which was collected in microcentrifuge (1 ml)tube containig small quantity of potassium oxalate and sodium flouride as anticoagulant.

Preparation of Serum:

Blood was collected through blood sample was collected in EDTA sample bottles. For haematological analysis

Results of blood glucose levels in diabetic rats:

Effect of muntingia calabura bark extract on blood glucose levels in diabetes induced rats was given in Table 1. Effect of ethanolic extract of muntingia calabura comparing with control in blood glucose levels was given Table 2.

Evaluation of anti-oxidant activity (In vitro): in muntingia calabura.

1. Evaluation of DPPH
2. Evaluation of ABTS
3. Evaluation of SOD was calculated by T-paired test

Days	Normal	Control	Standard	100mg	200mg
0	86.66±1.687	225.767±5.797	217.33±5.965	225.767±5.79	219.500±5.79
7	86.733±1.715	206.33±5.220	158±2.701	182.167±1.75	148.317±5.94
14	85.467±0.648	176.272±4.546	123.00±1.390	145.67±2.61	106.00±1.03

Table 1: Effect of muntingia calabura bark extract on blood glucose levels in diabetes induced rats.

Days	Diabetic control	100mg	200mg
0	225.767±5.797	225.767±5.797	219.500±11.069
7	206.33±5.220	181.167±1.759	148.317±5.945
14	176.272±4.56	145.667±2.616	106.00±1.03

Table 2: Effect of ethanolic extract of muntingia calabura comparing with control in blood glucose levels.

Compound	IC50(µg/ml)	Standard	IC50(µg/ml)
GLJ	45.17	Gallic acid	45.17
GLJ	45.17	Vitamin C	1.722

Table 3: Evaluated parameters of GLJ with Standard Gallic acid and Vitamin C.

Parameters	Normal	Diabetes	100mg muntingia calabura	200mg muntingia calabura	Standard
Hb	14.5±0.00	12.667±0.00	13.23±0.43	13.483±0.683	13.483±0.683
RBC	4.500±0.00	3.90±0.00	3.98±0.893	4.067±0.267	4.067±0.267
WBC	8225.00±275.0	6450±0.00	7158.33±841.667	740.33±1091	740.33±1091
Platelet	3.90±0.00	2.3±0.00	4.308±1.808	4.475±1.642	4.475±1.642
PCV	42.00±0.739	39.0±0.00	39.58±0.917	39.583±0.917	39.583±0.917
MCV	95.1±0.00	93.767±0.00	94.60±0.167	39.583±0.917	39.583±0.917
MCH	87.767±0.00	29.250±0.00	31.80±2.533	31.950±2.533	31.950±2.533
MCHC	34.13±0.00	30.50±0.00	31.583±2.917	31.583±2.717	31.583±2.717

Table 4: Determination of Hematological Parameters.

4. Mean and SEM VALUES are calculated

Evaluated compound GLJ with different standard solutions were given in Table 3.

Determination of Hematlogical Parameters:

These include haemoglobin ,[Hb] packed cell volume[PCV],mean corpuscular volume [mcv] mean corpuscular haemoglobin [MCH] mean corpuscular haemoglobin concentration [MCHC] redcell distribution width [RCDW] whitebloodcells [WBC] neutrophils, monocytes, lymphocytes, eosinophills, basophils, and platelet were also analysed and represented in Table 4.

DISCUSSION:

Streptozotocin is used as an agent to induce diabetes mellitus by selective cytotoxicity effect on pancreatic beta cells. Thus it affects endogenous insulin release and as a result increases blood glucose level [12]. The continuous administration of ethanolic extract of muntingia calabura bark at 200 mg per kg or glibenclamide for 14 days significantly reduced the blood glucose concentration in STZ induced diabetic rats. The plant extract (200 mg/kg) showed a comparable activity with the glibenclamide treated groups.

Glibenclamide is standard antidiabetic drug that stimulates insulin secretion from beta cells of islets of Langerhans. The probable mechanisms of action of the plant extract at higher dose could be linked to potentiation of insulin from beta cells or by increasing peripheral glucose uptake [13] the assessment of haematological parameters could be used to reveal the deleterious effect of foreign compounds including plant extracts on the blood constituents of animals. They are also used to determine possible alterations in the levels of biomolecules 222such as enzymes, metabolic products, haematology, normal functioning and histo morphology of the organs.

The occurrence of anaemia in diabetes mellitus has been reported due to the increased non-enzymatic glycosylation of RBC membrane proteins [14,15]. Oxidation of these proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to haemolysis of RBC [16]. In this study, the RBC membrane lipid peroxide levels in diabetic rats were not measured. However, there d blood cells parameters such as Hb, MCHC, MCH, PCV,MCV and were studied to investigate the beneficial effect of muntingia calabura extract on the anaemic status of the Oyedemi S.O et al./Asian Pacific Journal of Tropical Biomedicine (2011)353-358357diabetic rats. The levels of RBC, Hb, haematocrit, LUC andMCHC in the diabetic animals were drastically reduced which may be attributed to the infections on the normal body systems. This observation agrees with report of Baskaret all [17] who reported anti hyperglycemic activity of ethanolic bark extract of Rubia cordifolia in streptozotocin-induced diabetic rats.

The alterations of these parameters are well known to cause anaemic condition in man [18] Following plant extract administration, the level of RBC and its related indices were appreciably improved especially at200 mg/kg. This gives an indication that the plant extract may contain some phytochemicals that can stimulate the formation or secretion of erythropoietin in the stem cells of the animals. Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells [19] The stimulation of this hormone enhances rapid synthesis of RBC which is supported by the improved level of MCH and MCHC [20].

These parameters are used mathematically to define the concentration of haemoglobin and to suggest the restoration of oxygen carrying capacity of the blood. Though, the action mechanism of this plant is not investigated in this study. However, it may be attributed to the ability of plant extract to lower lipid peroxidation level that causes haemolysis of erythrocytes [21] Previous study on this plant revealed the presence of flavonoids proanthocyanidins, tannins, phenols and flavonols in this plant.

These compounds have been reported to possess strong antioxidant capacity therefore, could inhibit per oxidation of polyunsaturated fatty acids in the cell membrane and haemolysis of red blood cells in the diabetic animals reported by Torell and Faure et all [22,23] Streptozotocin is a well known chemical that suppresses the immune system by damaging WBC and certain organs in the body [24]. The intraperitoneal injection of streptozotocin into rats significantly

reduced the WBC count and its differentials such as basophils, monocytes, eosinophils, lymphocytes and neutrophils.

The reduction of these parameters could be linked to suppression of leucocytosis from the bone marrow which may account for poor defensive mechanisms against infection [25] Consequentially, they might have effects on the immune system and phagocytic activity of the animals [22] The white blood counts and its related indices were significantly restored to near normal after plant extract administration at both doses. The presence of some phytochemicals with ability to stimulate the production of white blood count in the extract could be responsible for the observed result in the treated rats. The extract at both dosages significantly improved the levels of WBC, monocytes, lymphocytes, eosinophils and neutrophils as compared with glibenclamide treated group. However, the extract did not have any significant effect on basophils in this study. Platelet aggregation ability has been shown in diabetic patient with long term poor glycaemic control due to lack or deficiency of insulin Platelets known as thrombocytes help to mediate blood clotting, which is a meshwork of fibrin fibres. The fibres adhere to any vascular opening and thus prevent further blood clot. It plays a crucial role in reducing blood loss and repairing of vascular injury [26] the reduction of platelets levels in diabetic rats induced with muntingia calabura.

CONCLUSION

Streptozotocin was confirmed in this study in relation to the normal control rats. Long term reduction of this parameter may result in internal and external haemorrhage and finally leads to death. However, after plant extract administration, the level of platelet was improved markedly especially at the dose of 100 mg/kg while that of 200 mg/kg did not have strong effect as compared with diabetic untreated rats. This effect indicated the ability of the plant extract to stimulate the biosynthesis of clotting factors due to the presence of active muntingia calabura.

ACKNOWLEDGEMENT

All thanks and praises to god Almighty or his countless, abundant and never ending blessings in completing this work. It is a proud privileged honor or us to express our hatful thanks and gratefulness to all the persons who backed us directly or indirectly throughout this research work as magnitude.

Conflict of interest

No

Funding

Self

REFERENCES

1. Ramachandran A, Snehalatha C, Mary S, Mukesh B, Bhaskar AD, Vijay V; Indian Diabetes Prevention Programme (IDPP). The Indian Diabetes Prevention Programme shows that lifestyle modification and metformin prevent type 2 diabetes in Asian Indian subjects with impaired glucose tolerance (IDPP-1). Diabetologia 2006;49:289–297CrossRefPubMedGoogle Scholar
2. Wang Y, Mi J, Shan XY, Wang QJ, Ge KY. Is China facing an obesity epidemic and the consequences? The trends in obesity and chronic disease in China. Int J Obes (Lond) 2007;31:177–188CrossRefPubMedGoogle Scholar
3. Siegel K, Narayan KM, Kinra S. Finding a policy solution to India's diabetes epidemic. Health Aff (Millwood) 2008;27:1077–1090Abstract/FREE Full TextGoogle Scholar
4. International Diabetes Federation. IDF Diabetes Atlas. Epidemiology and Mobidity. In: International Diabetes Federation. Available from http://www.idf.org/. Accessed on 1 March 2011Google Scholar
5. Malik VS, Popkin BM, Bray GA, Després JP, Willett WC, Hu FB. Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. Diabetes Care 2010;33:2477–2483Abstract/FREE Full Text Google Scholar
6. Hu FB, Li TY, Colditz GA, Willett WC, Manson JE. Television watching and other sedentary behaviors in relation to risk of obesity and type 2 diabetes mellitus in women. JAMA 2003;289:1785–1791CrossRefPubMedWeb of Science Google Scholar
7. Bruin JE, Petre MA, Raha S, Morrison KM, Gerstein HC, Holloway AC. Fetal and neonatal nicotine exposure in Wistar rats causes progressive pancreatic mitochondrial damage and beta cell dysfunction. PLoS ONE 2008;3:e3371CrossRefPubMedGoogle Scholar
8. Zhang P, Zhang X, Brown J, et al. Global healthcare expenditure on diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010;87:293–301CrossRefPubMedWeb of Science Google Scholar
9. Chan JC, Malik V, Jia W, et al. Diabetes in Asia: epidemiology, risk factors, and pathophysiology. JAMA 2009;301:2129–2140CrossRefPubMedWeb of Science Google Scholar
10. Yang W, Lu J, Weng J, et al.; China National Diabetes and Metabolic Disorders Study Group. Prevalence of diabetes among men and women in China. N Engl J Med 2010;362:1090–1101CrossRefPubMedWeb of Science Google Scholar
11. Schulze MB, Hoffmann K, Manson JE, et al. Dietary pattern, inflammation, and incidence of type 2 diabetes in women. Am J Clin Nutr 2005;82:675–684; quiz 714–715Abstract/FREE Full TextGoogle Scholar
12. Krishnaveni M, Ravi D. Qualitative and Quantitative Study of Phytochemicals in Muntingia calabura L. Leaf and Fruit. World J. Pharm. Res. 2014; 3(6):1687–1696.
13. Buhian WPC, Rubio RO, Valle Jr, DL. and Martin-Puzon JJ. Bioactive metabolite profiles and antimicrobial activity of ethanolic extracts from Muntingia calabura L. leaves and stems. Asian Pac. J. Trop. Biomed. 2016; 6(8):682–685.
14. Mahmood ND. et al. Muntingia calabura: a review of its traditional uses, chemical properties, and pharmacological observations. Pharm. Biol. 2014; 52(12):1598–1623.
15. Sufian AS, Ramasamy K, Ahmat N, Zakaria ZA. & Yusof MIM. Isolation and identification of antibacterial and cytotoxic compounds from the leaves of Muntingia calabura L. J. Ethnopharmacol. 2013: 146(1):198–204.
16. Sridhar M, Thirupathi K, Chaitanya G, Kumar BR, Mohan GK. Antidiabetic Effect of Leaves of Muntingia calabura L., in Normal and Alloxan-Induced Diabetic Rats. Pharmacologyonline.2011; 2:626–632.
17. Sani MH, Zakaria ZA, Balan T, Teh LK. & Salleh M Z. Antinociceptive activity of methanol extract of Muntingia calabura leaves and the mechanisms of action involved. Evidence-Based Complement. Altern. Med. 2012; 1-10.
18. Herlina H, Amriani A, Solihah I & Sintya R. Antidiabetic Activity Test of Ethanolic Seri Leave's (Muntingia Calabura L.) Extract in Male Rats Induced by Alloxan. Sci. Technol. Indones. 2018; 3(1):7–13.
19. Azwanida, N. N. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants; 2015; 4(3):3–8.
20. Zhang M, Lv XY, Li J, Xu ZG. & Chen L. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. Exp. Diabetes Res. 2008; 1-9.
21. Yusoff N. et al. Nypa fruticans Wurmb. Vinegar's Aqueous Extract Stimulates Insulin Secretion and Exerts Hepatoprotective Effect on STZ-Induced Diabetic Rats. Nutrients. 2017; 9(9):925.

22. Tripathy D. et al. Insulin secretion and insulin sensitivity in relation to glucose tolerance: lessons from the Botnia Study. Diabetes. 2000; 49(6):975–980.

23. Nijveldt RJ. et al. Flavonoids: a review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr. 2001; 74(4):418–425.

24. Luzi, L. & Pozza, G. Glibenclamide: an old drug with a novel mechanism of action? Acta Diabetol. 1997; 34(4):239–244.

25. Sheppard DN. & Robinson KA. Mechanism of glibenclamide inhibition of cystic fibrosis transmembrane conductance regulator Cl– channels expressed in a murine cell line. J. Physiol. 1997; 503(2):333–346.

26. Kamalakkannan N. & Prince PSM Antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin-induced diabetic wistar rats. Basic Clin. Pharmacol. Toxicol. 2006. 98(1):97–103.