International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-3, 2014 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Antihyperglycemic Activity of *Mentha piperita* Ethanol Leaves Extract on Streptozotocin Induced Diabetic Rats

G. Chandirasegaran, C. Elanchezhiyan*, S. Suhasini, A. Babby

Department of Zoology, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India.

Manuscript No: IJPRS/V3/I3/00344, Received On: 17/07/2014, Accepted On: 27/07/2014

ABSTRACT

Mentha piperita (Pepper mint) has been used medicinally for thousands of years. In the present study the antihyperglycemic effect of *Mentha piperita* was investigated experimentally. The diabetes was induced by intra peritoneal injection single dose of Streptozotocin (STZ) (50mg/kg b.w). After three days (72hr) of induction of diabetes, the diabetes animals were treated with ethanolic extract of *Mentha piperita* (300mg/kg b.w). The *Mentha piperita* treated diabetic rats significantly decreased the level of blood glucose and creatinine as well as increased level of insulin, glycogen, body weight. These finding demonstrated that *Mentha piperita* possess antihyperglycemic activity against STZ induced diabetic rats. The antidiabetic effects of *Mentha piperita* was compared with standard reference drug glibenclamide.

KEYWORDS

Mentha piperita, Antihyperglycemic, Streptozotocin, Glibenclamide

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by the classical symptom of hyperglycemia, which in turn leads to various acute and chronic complications if left untreated and may cause massive damage to the renal, cardio vascular, retinal and neurologic systems. It occurs as a result of a relative or an absolute lack of insulin, or its action on the target tissue, or both[1]. During diabetes mellitus (DM) persistent hyperglycemia causes an increased production of free radicals via autoxidation of glucose[2]. Diabetes mellitus affects more than 10% of the population and is the fifth most common cause of death worldwide. Currently more than 170 million people worldwide are affected by this metabolic syndrome and this figure is expected to be double by the year 2025[3]. The general population is increasingly using herbal medicines as dietary supplements to relieve and treat many different human disorders[4].

Mentha piperita L (Peppermint) is a medicinally important plant that belongs to the family Labiatae[5]. Peppermint (*Mentha*×*piperita*,) also known as *M. balsamea* Willd. Known to be hybrid mint, a cross between water mint and spearmint. *Mentha piperita* (L) (family Labiatae; genus *Mentha*) is reported to be commonly used in the treatments of loss of appetite, common cold, bronchitis, fever, nausea, vomiting[6], spasmodic responses[7] and antimicrobial and antioxidant activities[8]. It is also used for culinary purposes. There is evidence that it has an antimicrobial and antioxidant activities[9] and is commonly used as vegetable. Previously some biological and pharmacological activities of this plant have been reported[10]. Peppermint is also found to

***Address for Correspondence:**
Dr. C. Elanchezhiyan
Associate Professor, Department of Zoology
Annamalai University, Annamalai Nagar
Chidambaram – 608 002, Tamil Nadu, India.
E-Mail Id: chezhiyan6@gmail.com

have antiviral activity. It is virucidal against influenza, herpes and other viruses[11]. STZ is widely used to induce diabetes in experimental animals by causing the selective destruction of pancreatic β-cells that secrete insulin[12]. The present study was designed to investigate the antihyperglycemic activity of ethanolic extract of *Mentha piperita* in STZ induced diabetic rats.

MATERIALS AND METHOD

Chemicals

Streptozotocin (STZ) was purchased from Sigma–Chemical Co, Bangalore. All other chemicals and reagents used for this study were analytical grade.

Collection and Preparation of Plant Material

The leaves of *Mentha piperita* were collected from the natural habitats of Tanjore, Tamil Nadu, India. The leaves were washed thoroughly for 3 times in running tap water to remove soil particles and adhered debris and finally with sterile distilled water. The leaves were cut, shade dried, ground into fine powder and stored in air tight plastic container until use.

Preparation & Purification of Plant Extract

100g powder were refill in a Soxhlet apparatus and extracted with the solvent of ethanol. The crude extract were collected and filtered by Whatman No 1 filter paper. The purified extract was used for further analysis.

Experimental Animal

Male Wistar albino rats (180-220 g) were obtained from Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram, Tamil Nadu, India. They were maintained at standard laboratory conditions and fed commercial pellet diet. Deionized distilled water was provided *ad libitum*. The animals were housed in polycarbonate cages in an animal room with 12 hr day – night cycle. They were acclimatized to laboratory condition at least one week before experiment. All the animal experimentation was approved by Institutional Animal Ethical Committee (IAEC-889/2013).

Induction of Experimental Diabetes

Diabetes was induced in rats by streptozotocin injection through intraperitoneal (I. P) at a dose of (50 mg/kg b.w) dissolved in 0.1 M cold citrate buffer (pH = 4.5)[13]. The rats were allowed to drink 5% glucose solution over night to overcome the drug- induced hypoglycemia. The blood glucose levels reach above 250 mg/dl on the third day after injection of streptozotocin was considered as diabetic rats. Then the treatment was started on the fifth day after injection of streptozotocin and it was considered as first day of treatment.

Experimental Design

All animals were randomly divided into four groups with six animals in each group

Group 1: Normal rats

Group 2: Diabetic control rats

Group 3: Diabetic rats given ethanolic extract of *Mentha piperita* (300 mg/kg of body weight)

Group 4: Diabetic rats given standard drug glibenclamide (600 µg/kg of body weight).

Biochemical Analysis

The animals were sacrificed at the end of experimental period by decapitation. Blood was collected, serum separated by centrifugation at 3000 g for 10 minutes. Glucose content was estimated by 0-Toluidine method[14]. Insulin level was assayed by Enzyme Linked Immunosorbant Assay (ELISA) kit[15]. Glycosylated hemoglobin (HbA1c) estimation was carried out by colorimetric method of[16] Liver glycogen[17]. Creatinine[18]. Body weight was determined by observing the initial (0 day) and final body weight (45^{th}) day of all the groups were observed.

Statistical Analysis

All data were expressed as means ± SEM data were assessed by the method of analysis of ANOVA followed by student t-test $P<0.05$ were considered as statistically significant.

RESULTS

Ethanolic extract of *Mentha piperita* treated with STZ induced diabetic rats that possess antidiabetic effect.

Body Weight

In the present study diabetic rats show decrease in body weight during the experimental period. Oral treatment of ethanolic extract of *Mentha piperita* significantly improved the body weight as compared to diabetic rats (Table.1).

Blood Glucose and Plasma Insulin

STZ induced diabetic rats the glucose level was significantly increased and decreased plasma insulin levels compared with control rats (Table.2). After treatment with leaves ethanolic extract of *Mentha piperita* and glibenclamide for 45 days the values were reached near to normal level as that of control rats.

Glycosylated Hemoglobulin and Creatine Level

Glycosylated hemoglobin and creatinine levels were significantly increased in STZ induced diabetic rats as compared to control rats. The ethanolic extract of *Mentha piperita* reverted the glycosylated hemoglobin and creatinine level near to normal range.

Glycogen Level

The liver glycogen level was decreased in diabetic rats as compared to control rats (table.2). The diabetic rats treated with ethanolic extract of *Mentha piperita* brought to normal level as compared to control levels.

Table 1: Effect of *Mentha piperita* on body weight in streptozotocin induced diabetic rats

Groups	Initial body weight (g)	Final body weight (g)
Control	179.16±6.75	184.33±6.26
Diabetic	168.50±6.18	127.50±4.48
D+ *M. piperita* (300 mg/kg)	175.50±8.29	160.50±8.62
D+ Glibenclamide (600µg/kg)	174.66±5.72	170.16±5.81

Table 2: Effect of *Mentha piperita* on biochemical parameters in streptozotocin induced diabetic rats

Groups	Glucose (mg/dl)	Insulin (µU/ml)	Liver glycogen (g/100gm)	Glycosylated hemoglobin (HbA1C) %	Creatinine (mg/dl)
Control	88.67±1.15	16.09±1.11	26.39±1.36	4.14±2.35	0.56±1.04
Diabetic	212.14±1.13	10.19±0.22	10.67±1.38	7.04±0.57	2.28±0.18
D + *M. piperita* (300 mg/kg)	111.00±6.70	13.00±1.90	16.42±0.72	5.12±0.52	0.90±0.04
D + Glibenclamide (600 µg/kg)	92.20±0.97	15.10±1.51	21.37±1.62	4.55±0.42	0.71±0.06

DISCUSSION

STZ-induced hyperglycemia is a useful experimental model for studying antihyperglycemic activity. Because of its structural features, STZ gets selective entry into the β cells of the islets of Langerhans via the low affinity glucose transporter GLUT2 in its plasma membrane and causes destruction of β cells, which leads to a reduction in insulin release, which in turn results in a rise in blood glucose concentration, i.e. hyperglycemia[19]. The glibenclamide is a standard antidiabetic drug, used to compare the antihyperglycemic property in experimental rats. Glibenclamide have been involved in stimulating insulin secretion from pancreatic-cells principally by inhibiting ATP sensitive KATP channels in the plasma membrane[20].

In the present study ethanolic extract of *Mentha piperita* showed antihperglycemic effect on STZ induced diabetic rats. STZ-induced diabetic rats treated with the extract showed a decrease in the glucose level and increase in insulin level in blood by regeneration of pancreatic β-cells to near normal as compared to standard drug glibenclamide treated rats. The results of this study indicate that ethanolic extract of *Mentha piperita* stimulates increased insulin secretion from either the remnant β-cells or induce the regeneration of damaged β –cells.

Induction of diabetes with STZ is associated with the characteristic loss of body weight, which is due to the increased muscle wasting and loss of tissue proteins[21]. STZ induced diabetic rats treated with extract reversed the weight loss as compared to standard drug glibenclamide.

The excess glucose presents in the blood during diabetes, react with hemoglobin and form glycosylated hemoglobin. In poorly controlled diabetes, there is upsurge in the glycosylation of some proteins including hemoglobin. Glycosylated hemoglobin develops reduced affinity for oxygen which contributes to long-term complications of diabetes[22]. STZ induced diabetic rats treated with extract showed significant decrease in glycosylated hemoglobin indicated that the efficiency of *Mentha piperita* in glycemic control.

The creatinine values recorded in all the four groups are shown in Table-2. Diabetic control rats recorded a progressive increase in creatinine levels when compared to the normal rats indicating renal damage. Diabetic rats treated with extract showed a decreased creatinine levels as compared to diabetic rats.

CONCLUSION

From this study we can conclusively state that *Mentha piperita* leaf extract has beneficial effects on STZ induced diabetic rats. Further studies should be carried out on this plant in order to understand its mechanism of action.

ACKNOWLEDGEMENTS

Authors are very much thankful to University Grants Commission (UGC) - New Delhi, Govt. of India for funding support of this work. We also thank Department of Zoology (UGC-SAP Sponsored) for providing facility and encouragement.

REFERENCES

1. Marles R. J., and Farnsworth N. R. (1995). Antidiabetic plants and their active constituents, *Phytomedicine*, 2(2), 137-189.
2. Wolff, S. P., and Dean, R. T. (1987). Glucose autoxidation and protein modification. The potential role of "autoxidative glycosylation" in diabetes. *Biochem. J*, 245, 245-250.
3. Boyle, J. P., A. A. Honrycult, K. M. Narayan, T. J. Hoerger, L. S. Geiss, H. Chen and T. J. Thompson (2001). Projection of diabetes burden through 2050. Impact of changing demography and disease prevalence in the US. *Diabetes Care*, *24*(11), 1936-1940.
4. Cutler HG (1995). Natural product flavor compounds as potential antimicrobials, insecticides and medicinal, *Agro. Food Ind. Hi-Tech*, 6: 19-23.
5. Kirethekar, Basu, I. (1985). *Indian Medicinal Plants*. 714-716.

6. Akdogan, M., Kilinç, I., Oncu, M., Karaoz, E., Delibas, N. (2003). Investigation of biochemical and histopathologi-cal effects of *Mentha piperita* L. and *Mentha spi-cata* L. on kidney tissue in rats. *Human and Experimental Toxicology*, *22*(4), 213–19.
7. Lu, M., Battinelli, L., Daniele, C., Melchioni, C., Salva-tore, G., Mazzanti, G. (2002). Muscle relaxing activity of Hyssopus officinalis essential oil on isolated in-testinal preparations. *Planta Medic*., *68* (3), 213–16.
8. Romero-Jimenez, M., Campos-Sanchez. J., Analla, M., Munoz-Serrano, A., Alonso-Moraga, A. (2005). Genotoxicity and anti-geno toxicity of some traditional medicinal herbs. *Mutation Research*. *585*(1-2), 147–55.
9. Mimica-Dukic, N., Bozin, B., Sokovic, M., Mihajlovic, B., Matavulj, M. (2003). Antimicrobial and antioxidant activities of three *Mentha* species essential oils. *Planta Medica*., *69*(5), 413–19.
10. Zheng, W., Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry*, *49*(11), 5165-5170.
11. Mohsenzadeh, M. (2007). Evaluation of antibacterial activity of selected Iranian essential oils against Staphylococcus aureus and Escherichia coli in nutrient broth medium. *Pak J Biol Sci*. *10*(20), 3693-3697.
12. Akosy, N., Vural, H., Sabuncu, T., Akosy, S. (2003). Effect of melatonin on oxidative-antioxidative status of tissues in streptozotocin-induced diabetic rats. *Cell Biochem Funct*, *21*, 121-125.
13. Bandaranayke, W. M. (2002). Bioactivities, bioactive compounds and chemical constituents of mangrove plants. *Wetlands Ecology Management*, *10*, 421-52.
14. Sasaki, T., Matsy, S., & Sonae, A. (1972). Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose estimation. *Rinsho Kagaku*, *1*, 346-353.
15. Maruthupandian, A., Mohan, V. R. (2011). Antidiabetic, Antihyperlipidaemic and Antioxidant activity of *Pterocarpu marsupium* Roxb. in alloxan induced diabetic rats. *International Journal of Pharm Tech Research*, *3*, 1681-1687.
16. Sekar, N., Kanthasamy, S., William, S., Subramanian, S., Govindasamy, S. (1990). Insulin action of vanadate in diabetic rats. *Pharmacological Research*, *22*, 207-217.
17. Shirwaikar, A., Rajendran, K., Punitha, I. S. R. (2005). Antidiabetic activity of alcoholic stem extract of Coscinium fenestratum in streptozotocin-nicotinamide induced type2 diabetic rats. *J. Ethnopharmacology*, *97*, 369-374.
18. Folin, O., and Wu, h. (1919). A System of blood Analysis. *J. Biol Chemistry*. *38*(1), 81-110.
19. Elsner, M., Guldbakke, B., Tiedge, M., Munday, R., & Lenzen, S. (2000). Relative importance of transport and alkylation for pancreatic beta cell toxicity of streptozotocin. *Diabetologia*, *43*, 1528-1533.
20. Warnick, G. R., Nguyan, T., Albers, A. A. (1985). Comparison of improved precipitation methods for quantification of high density lipoprotein cholesterol. *Clinical Chemistry*, *31*, 217-224.
21. Chatterjea MN, Shinde R(2002). Text book of medical biochemistry (317), New Delhi: *Jaypee Brothers Medical Publishers.*
22. Bhattaram, V. A., Ceraefe, M., Kohlest, C., Vest, M., Deundorf. (2002). Pharmacokinetics and bioavailability of herbal medicinal products, *Phytomedicine*, *9*, 1-36.

