

Ethanol Extract of *Melothria Maderaspatana* Inhibits Glucose Absorption and Stimulates Insulin Secretion in Male C57BL/6 Mice

Ashok Kumar Balaraman^{1*}, Jagadish Singh¹, Tapan Kumar Maity¹, Vaiyaburi Thamil Selvan² and Suresh Babu Palanisamy³

¹Department of Pharmaceutical Technology, Jadavpur University, Kolkata -700 032, India,

²Glenmark Pharmaceutical Ltd, Research and Development Centre, Plot A-607, TTC Industrial Area, MIDC, MAHAPE, Navi Mumbai-400709, India.

³Department of Biotechnology, Anna University of Technology, Tiruchirappalli, Tiruchirappalli – 620024, India.
e-mail: ashokkumarbalaraman@gmail.com

Abstract— A study was planned with the ethanol extract of leaves of *Melothria maderaspatana* (Family: Cucurbitaceae) to check its effects on intestinal glucose absorption and insulin secretion. The results showed that the extract (0.156, 0.312, 0.625, 1.25, 2.5 and 5 mg/mL) demonstrated a significant reduction in glucose absorption in a dose dependant manner. A maximum inhibition was observed at 2.5 mg/mL. The promotion of the extract on insulin secretion was confirmed by incubating β -cell of pancreatic islets and INS-1E insulinoma cells with the extract (1-1000 μ g/mL). Administration of extract at 100 and 200 mg/kg p.o. in an oral glucose tolerance test (OGTT) led to a significant fall in plasma glucose level in 30 minutes after the administration in normal C57BL/6 mice compared to untreated ($p < 0.05$).

Keywords— *M. maderaspatana*; OGTT; acute toxicity; glucose absorption, insulin secretion

I. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder defined by a persistently elevated blood glucose level that leads to complications which could be acute and long term. Acutely, marked hyperglycemia impairs water and electrolyte balance and energy utilization, causing polyuria, polydipsia, dehydration, weight loss, and eventually, cerebral dysfunction and coma [1]. Globally, DM presents enormous and increasingly important public health issues and its prevalence in all age groups was estimated to be 2.8 % (170 million) in 2000 and is expected to be 4.4% (366 million) in 2030 [2]. Diabetes occurrence and associated consequences are found to be higher in countries like India (31.7%), China (20.8%) and USA (17.7 %) and the rate is expected to rise to 79.4 %, 42.3% and 30.3 % by 2030 respectively in the above countries [3]. Among the entire diabetes cases world wide, more than 90 % are type-2 [4] and the overall death rate in people with diabetes is about twice that of people without diabetes [5].

Diabetes, since long, has been treated with plant derived medicines. A number of recent scientific investigations have confirmed the efficacy of many of these preparations, few of which are remarkably effective too. [6, 8]. Cucurbitaceae species, contributing major segment in drug discovery, has been recognized in the empirical control of diabetes species

that have been reported to possess hypoglycemic activity are; *Bryonia alba* L., *Citrullus colocynthis* (L.) Schrad., *Coccinia indica* Wight et Arn., *Cucumis sativus* L., *Momordica charantia* L., *Momordica cymbalaria* Hook., *Momordica foetida* Schumach. Et Thonn., *Tricosanthes dioica* Roxb [9-15].

Melothria maderaspatana (Linn) Cogn. Syn. *Mukia maderaspatana*, *Cucumis maderaspatana* or *Mukia scabella* (Family: Cucurbitaceae) is used as drug in the compound preparation for chronic diseases in which cough is a predominant symptom [16] folklores claim this plant as good diuretic, stomachic, gentle aperients, antipyretic and anti-flatulent [17]. This plant is recommended in southern part of Sri Lanka for the alleviation of various forms of liver disorders [18]. The plant also reportedly found to exhibit anti inflammatory [19] and anti-cancer [20] activities. Ethanol extract obtained from aerial parts of this plant showed a significant anti diabetic, hypolipidemic activities [21] in rat models. Aqueous extract of *M. maderaspatana* was found to exhibit a potent anti-oxidant activity on different in-vitro models [22]. The present study was designed from a muse that extract could reduce plasma glucose level in diabetic animals by inhibition of intestinal glucose absorption, and/or stimulation of insulin secretion.

II. MATERIALS AND METHODS

A. Reagents and Chemicals

PGO enzymes, Colloagenase type V, Soya bean trypsin inhibitors, and bovine serum albumin fraction V, were procured from Sigma Chemical Company. Dulbecco modified Eagle medium, RPMI 1640, fetal bovine serum (FBS), and penicillin-streptomycin were obtained from (HiMedia Chemicals) and metformin were obtained from Aventis Pharma Ltd.

B. Preparation of the extract material

Aerial parts of *M. maderaspatana* were collected from Koothiramedu village, Kanchipuram district (Tamil Nadu, India) during October–December. The plant was authenticated by Prof. Jayaraman, Director, National Institute of Herbal Science, PARC, Chennai and a specimen voucher (Ref. No. PARC/2009/260) has been kept there for future

reference. Washed and shade dried plant material was pulverized using a mechanical grinder and kept separately in an air tight container till used further. Pulverized materials of *M. maderaspatana* were soaked in 95% ethanol separately for 72 h (Maceration) with timely shaking and stirring. The extract thus obtained was passed through cotton and the filtrate was concentrated using a rotary vacuum evaporator at 50 °C.

C. Animals

Animals of either sex maintained in an air-conditioned room at 25 ± 2°C and relative humidity of 45-55% under a 12 h light/dark cycle. All the animals had free access to standard diet and water *ad libitum*. The whole experimental protocol was approved by the Institutional Animal Ethics Committee and constituted in accordance with the rules and guidelines of the committee for the purpose of control and supervision on experiments in animals, India.

D. Oral Glucose Tolerance test (OGTT) in normal rats

OGTT was performed with overnight fasted male C57BL/6 mice. In the study, animals were divided into four groups of six in each. Group I received 0.5% methyl cellulose solution (vehicle), group II and III received 100 and 200 mg/kg p.o., extract respectively and group IV received with Metformin 300 mg/kg., p.o. Animals were fed with glucose (2g/kg., p.o) 30 min after the administration of drugs. Blood samples withdrawn by puncturing tail vein at 0, 15,30,60,90,120 min of glucose administration and blood glucose level was determined by using a commercial kit (The Contour Meter) supplied by Bayer Health Care, India. The OGT responses were computed from the area under the curve (AUC) for glucose using Trapezoidal method [23].

E. Effect of *M.Maderaspatana* on glucose absorption from the intestine

The everted sacs of the small intestine were isolated from mice killed by cervical dislocation for glucose absorption experiment. Specifically, the jejunums were removed shortly after death and were placed in a Krebs-Henseleit solution (Krebs-Henseleit solution [g/L]; 6.92 NaCl, 0.35 KCl, 0.29 MgSO₄. 7H₂O, 0.28 CaCl₂, 0.16 KH₂PO₄, and 2.1 NaHCO₃) with oxygen. The jejunums were reverted by using a glass rod and tied at one end with a ligature thread. A Krebs-Henseleit solution with 140 mg/dL glucose was injected into the everted jejunums, and the other ends were tied into jejunal sacs, 1.5 –cm long. The jejunal sacs were incubated in 10 ml of Krebs-Henseleit solution containing glucose (140 mg/dL) with the addition of the plant extract as follows,

Group I: incubated in a Krebs-Henseleit solution with glucose, 140 mg/dL (Negative control)

Group II -VII: incubated in a Krebs-Henseleit solution with glucose, 140 mg/dL, and various concentration of extract at 0.156, 0.312, 0.625, 1.25, 2.5 and 5 mg/mL.

Group VIII: 0.2 mol/L NaF, 0.1 mL (positive control)

The incubation flasks were shaken at 90 oscillations per minute and maintained at a 37° C for 30 minutes with carbogen (95% oxygen and 5% carbon dioxide [CO₂]). After

the incubation, the sacs were dissected, and the solution in each sac was examined for its glucose level [24, 25].

F. Effect of *M.Maderaspatana* on insulin secretion *in vitro*

i. Isolation of pancreas cells

The splenic pancreas was isolated immediately from the bodies of the mice immediately after their cervical dislocation. The pancreas was cut into small pieces and subjected to an enzymatic digestion medium in a controllable shaker and subjected to a temperature of 37° C for 20 minutes. The digestion medium consisted of Dulbecco modified minimum essential medium supplemented with 1 mg/mL of colloagenase type V, 2mg/mL of soya bean trypsin inhibitor, and 2 % bovine serum albumin fraction V. The digested tissue was centrifuged at 1700 revolutions per minute (rpm) at a temperature pf 4° C for 15 minutes, washed twice in a phosphate buffered saline solutions (pH 7.2), and seeded in culture flasks containing RPMI 1640, supplemented with 10% heat inactivated FBS, 100 U/mL of penicillin, and 100 µg/mL of streptomycin at 37° C in 5% CO₂ incubator [26, 27].

ii. Insulinoma cells

INS-IE cell clusters were cultured in a humidified atmosphere containing 5% CO₂ in a complete medium composed of RPMI 1640 and supplemented with 10 % heat inactivated FBS, 1 mmol/L sodium pyruvate, 50 µmol/L 2-mercaptoethanol, 2 mmol/L glutamine, 10 mmol/L HEPES, 100 U/mL of penicillin, and 100 µg/mL of streptomycin [28].

iii. Insulin secretion

Isolated islets were pre-incubated for 2 h in glucose – free culture medium. After a low-speed centrifugation (1200 rpm for 10 minutes), cells were resuspended in glucose-free Krebs-Ringer bicarbonate HEPES buffer (KRBH) (135 mmol/L NaCl, 3.6 mmol/L KCl, 5 mmol/L NaHCO₃, 0.5 mmol/L NaH₂PO₄, 0.5 mmol/L MgCl₂, 1.5 mmol/L CaCl₂, and 10 mmol/L HEPES, 0.1 % bovine serum albumin, pH 7.4) and divided into 5 groups, with an equal amount of cells in 24-well plates and pre-incubated for 1 h. then, the culture plate was incubated for 1 h with KRBH of 0 mmol/L glucose (control group) or 16.7 mmol glucose (positive control) or the extract (1, 10, and 100 µg/mL). Aliquots were removed from each well, centrifuged, and stored at -20° C for insulin assay by chemiluminescence enzyme immunoassay [26, 28].

III. STATISTICAL ANALYSIS

Data are expressed as the mean ± S.E.M, and the statistical analysis was carried out by one-way ANOVA with Bonferroni post ANOVA test using Graph Pad Insat version 5. The p- value < 0.001 and <0.05 were considered significant

IV. RESULTS

A. Oral glucose tolerance test in normal and diabetic animals

OGTT was performed to estimate the effect of the drug on insulin and β-cell functions of pancreas at glucose load in

normal condition [29]. The level of plasma glucose increases after an oral glucose load to the maximum after 30-90 min and decreased until 120 min with the final downward move of > 0.25 mmol/L. The consumption of *M.Maderaspatana* markedly altered the glucose tolerance in the test animals which can be noticed from the decreased AUC. Compared to the control group, the AUC of glucose in drug treated animals were significantly lowered (Tab.1).

B. Inhibition of glucose absorption from intestine

The measurement of glucose concentration in the sacs after 30 minutes of incubation revealed the inhibiting activity of *M.Maderaspatana* on glucose absorption in a dose response manner (FIG. 1). The maximum inhibition occurred at 2.5 mg/dL dose level ($p < 0.05$), the inhibition effects at doses 2.5 and 5.0 mg/dL were not statistically significant. Sodium fluoride used as reference in this study exhibited a significant effect on reducing glucose absorption.

C. Insulin secretion

The extract of *M. maderaspatana* at 1, 10, and 100 µg/mL concentration apparently induced insulin secretion from the isolated islets after a static incubation for 1 h (FIG. 2). However the inter-dose statistical significance was not different. Thus, the doses were increased to 10,100, and 100 µg/mL, for the study with INS-1E. The data obtained here showed a similar effect that was significantly exerted on insulin secretion. Moreover, it was shown that inducing effect of insulin secretion appeared to be dose dependent, the highest inhibition on glucose absorption was noticed at highest dose used (1000 µg/mL) (FIG.3)

V. DISCUSSION

The change in plasma glucose levels in response to an oral glucose load has long been used as clinical procedure for the diagnosis of DM and in preclinical trials to evaluate the efficacy of hypoglycemic agents. In this study, the extract of leaves of *M. maderaspatana* was administered to non-diabetic animals exhibited some glucose lowering activity, specifically within an hour after the glucose load, this kind of changes were not noted in the non treated normal groups which received only the vehicle during the study. However, the effect was not as persistent as in the case treated with the standard drug metformin (Tab.I).

In our earlier investigation with this plant, ethanol extract of leaves of *M. maderaspatana* had been studied to possess a significant anti-diabetic and hypolipidemic activities in STZ induced diabetic animals [21]. The present study was designed to check if the leaves have ability to act on inhibition of glucose absorption through intestine and enhance insulin secretion in *invitro* models. [24, 25]. The ethanol extract inhibited glucose absorption in a dose dependant manner. This suggests that the postprandial glucose reduction of *M. maderaspatana* could be partially due to the inhibition of sugar absorption in the small intestine.

The insulinotropic effect observed in this study was assessed by using isolated pancreatic islets and INS-1E Insulinoma cells. Notable effect on glucose induced insulin

secretion was found from isolated islets. The extract at concentrations 1, 10, and 100 µg/mL did show a dose dependent response on insulin secretion. Glucose inhibitory action and insulin secretory action of *M. maderaspatana* together with its effect on oral glucose tolerance test suggests that the extract could act by stimulating insulin secretion beside its effect on increased lipid level *in vivo*. Along with the mechanism concerned to specific target (organ or tissue), the extract has been shown to have a preventive effect against oxidative damage that could promote the anti-diabetic ability of body [22].

Postprandial hyperglycemia plays a major role in both micro and macro vascular complications during diabetes [30]. The results associated with the current study indicate that anti-diabetic activity of the extract could be related to the inhibition of glucose absorption and enhancement of insulin secretion. The postprandial glucose reducing effect of *M. maderaspatana* may be able to effectively decrease the risk of developing type-2 diabetes among patients with impaired glucose tolerance. The extract could also act as an adjunct to insulin in keeping the postprandial glucose level in control in type-2 diabetes patients [31].

Though the major components responsible for the observed intestinal glucose inhibitory and insulin secretory activity were not isolated from *M. maderaspatana*, the phytochemical screening of the leaves extract revealed the presence of Glycoside, flavonoid and phenolics. The observed beneficial anti-diabetic activity of *M. maderaspatana* may be due to the presence of high content of flavonoid as it is proved to protect cells from oxidative, stress-mediated cell injury [32]. And the presence of glycosides may also influence the insulin secretion as well [33]. Thus these chemical substances may account for the observed anti-diabetic effects in the study. However, a separate study is required to be carried out with isolated constituent from this plant to confirm it.

VI. STATEMENT OF CONFLICT OF INTEREST

There is no conflict of interest found

ACKNOWLEDGEMENT

The first author of this paper is grateful to University Grants Commission (UGC), New Delhi, India for financial support. The authors also thank the authority of Jadavpur University, Kolkata, India for providing the facility to carry out this project work.

REFERENCES

- [1] Carla J. Greenbaum and Leonard C. Harrison. (2008) Diabetes: translating research into practice, Informa Health care, New York, London. P.1-2.
- [2] Vivian A. Fonseca. (2006) Clinical Diabetes: translating research into practice. Saunders – an imprint of Elsevier. P. 2-3 (Chapter 1).
- [3] Sarah Wild, MB Bchir, Gojka Roglic, Ander Green, Richard Sicree and Hilay King, Global.(2004) Prevalence of Diabetes; Estimates for the year of 2000 and projection for 2030, Diabetes care, 27 (5), 1047-1053
- [4] C.H. Laua, C.M. Chanb, Y.W. Chanb, K.M. Laua, T.W. Laua, F.C. Lama, W.T. Lawa,C.T. Chec, P.C. Leunga, K.P. Funga,b, Y.Y. Hob,

- and C.B.S. Laud. (2007) Pharmacological investigations of the anti-diabetic effect of Cortex Moutan and its active component paeonol, *Phytomed*, 14, 778–784.
- [5] National Diabetes Fact sheet. (2005) Centers for Disease Control and Prevention. Available at <http://www.cdc.gov/diabetes/pubs/estimates.htm>.
- [6] Bailey. C.J. and Day. C. (1989) Traditional plant medicines as treatment for diabetes, *Diabetes care*, 12, 553-564.
- [7] John B. Taylor and David J. Triggle. (2006) *Comprehensive Medicinal Chemistry-II. Volume 1. Global Perspective*, Text Book, Elsevier, p. 357.
- [8] Marles, R.J., Farnsworth, N.R. (1995) Antidiabetic plants and their active constituents, *Phytomed*, 2, 133-169.
- [9] Kameshwara Rao B., M.M. Kesavalu, CH. Apparao. (2001) Antihyperglycemic activity of *Momordica cymbalaria* in alloxan diabetic rats, *Journal of Ethnopharmacol*, 78, 67-71.
- [10] Atta-Ur-Rahman, Zaman, K. (1989) Medicinal plants with hypoglycemic activity, *Journal of Ethnopharmacol*, 26, 1 -55.
- [11] Ivorra, M.D., Paya, M., Villar, A. (1989) A review of natural products and plants as potential antidiabetic drugs, *Journal of Ethnopharmacology*, 27, 24-275.
- [12] Marles, R.J., Farnsworth, N.R. (1995) Antidiabetic plants and their active constituents, *Phytomed*, 2, 137-189.
- [13] Roman-Ramos, R., Alarcon-Aguilar, F.J., Flores-Saenz, J.L. (1995) Antihyperglycemic effect of some edible plants, *Journal of Ethnopharmacol*, 48, 25-32.
- [14] Abdel-Hassan, I.A., Abdel-Barry, J.A., Tariq Mohammeda, S. (2000) The hypoglycemic and antihyperglycemic effect of *Citrullus colocynthis* fruit aqueous extract in normal and alloxan diabetic rabbits, *Journal of Ethnopharmacol*, 71, 325-330.
- [15] Jayasooriya, A.P., Sakono, M., Yukizaki, C., Kawano, M., Yamamoto, K., Fukuda, N. (2000) Effects of *Momordica charantia* powder on serum glucose levels and various lipid parameters in rats fed with cholesterol-free and cholesterol-enriched diets, *Journal of Ethnopharmacol*, 72, 331-336.
- [16] Kirtikar KR, Basu BD. (1975) *Indian medicinal plants*, 2nd ed., Vol III. New Delhi, India, International book distributors, 1161-1162
- [17] Nadkarani KN. (1971) *Indian Matria Medica*, Bombay, Prakashan Pvt Ltd. 820. The wealth of India, Publication and information directorate, New Delhi: C.S.I.R., 336.
- [18] K.A.P.W. Jayatilaka, M. Ira Thabrew and D.J.B. Perera. (1990) Effect of *Melothria maderaspatana* on carbon tetrachloride induced changes in rat hepatic microsomal drug-metabolizing enzyme activity, *Journal of Ethnopharmacol*, 30, 97-105.
- [19] Ramakrishnamacharya C.H., Krishanaswamy M.R., Bhima Rao R, Viswanathan S. (1996) Anti-inflammatory efficacy of *Melothria maderaspatana* in active rheumatoid arthritis, *Clin. Rheumatol*, 12, 214-215.
- [20] Hussein A.S. M., Kingston D.G.I. (1982) Screening of the medicinal plants used in Sudan folk medicine for anti-cancer activity (II), *Fitoterapia*, 53, 119-123.
- [21] Ashok Kumar Balaraman, Jagadish Singh, Sasmita Dash, Tapan Kumar Maity. (2010) Antihyperglycemic and hypolipidemic effects of *Melothria maderaspatana* and *Coccinia indica* in streptozotocin induced diabetes in rats. *Saudi Pharmaceutical Journal*. 18, 3, 173-178.
- [22] Boobalan Raja, Kodukkur Viswanathan Pugalendi. (2009) Evaluation of antioxidant activity of *Melothria maderaspatana* in vitro. *Cent. Eur. J. Biol*, 5,2, 224-230.
- [23] Shirwaikar A, Rajendran K, Barik R. (2006) Effect of aqueous bark extract of *Garuga pinnata* Roxb. In streptozotocin-nicotinamide induced type -2 diabetes mellitus. *J Ethnopharmacol*, 107, 285-90
- [24] Wilson TH, Wiseman G. The use of sacs if everted small intestine for the study of the transference of substances from the mucosal to the serosal surface. *J. Physiol* 1954: 123: 116-25
- [25] Nagler R, Forrest WJ, Spiro HM. Effect of glucogon on transfer of glucose, utilizing everted sacs of small intestine. *Am J Physiol* 1960: 198: 1323-5
- [26] Latha M, Pari L. Sitasawad S, Bhone R. Insulin-secretagogue activity and cytoprotective role of the traditional antidiabetic plant *Scoparia dulcis* (sweet broomweed). *Life Sci*. 2004 : 75: 2003-14
- [27] Shewade YM, Umrani M, Bhone RR. Large-scale isolation of islets by tissue culture of adults' mouse pancreas. *Transplant proc*. 1999: 31: 1721-3
- [28] Merglen A, Theander S, Rubi B, Chaffard G, Wollheim CB< Maechler P. Glucose sensitivity and metabolism-secretion coupling studied during two-year continuous culture in INS-IE Insulinoma cells. *Endocrinology* 2004: 145: 667-78
- [29] Otto Tschritter, Andreas Fritsche, Fatemeh Shirkavand, Fausto Machicao, Hans Haring, Michael Stumvoll. Assessing the shape of the glucose curve during an Oral Glucose Tolerance Test, *Diabetes Care*. Apr 2003, 26: 4-5.
- [30] Ceiello A, The possible role of postprandial hyperglycemia in the pathogenesis of diabetic complications, *Diabetologia* 2003: 46: M9-M16.
- [31] Wayer C, Gottlieb A, Kim DD, Lutz K, Schwartz S, Gutierrez M, et al. Pramlintide reduces postprandial glucose excursions when added to regular insulin lisproin subject with type-1 diabetes: a dose-timing study. *Diabetes Care* 2003: 26: 3074-9.
- [32] Edi A, Eidi M, Sokhtheh M. Effect of fenugreek (*Trigonella foenumgraecum* L) seeds in serum parameters in normal and Streptozotocin induced diabetic rats. *Nutr Res* 2007: 27: 728-33.
- [33] Seetharam YN, Chalageri G, Setty SR, Bheemachar. Hypoglycemic activity of *Abutilon indicum* leaf extract in rats. *Fitoterapia* 2002:73:156-9.

TABLE I. EFFECT OF ETHANOL EXTRACT OF *M.MADERASPATANA* ON PLASMA GLUCOSE TOLERANCE ABILITY OF MALE C57BL/6 MICE

Groups (mg/kg p.o.)	Area Under Curve (AUC; y=0)
Vehicle control (0.5% methyl cellulose)	16712.83±259.14
<i>M.maderaspatana</i> 100	14225.17±163.44*
<i>M.maderaspatana</i> 200	12724.00±82.87*
Metformin 300	12091.5±174.38*

*P<0.001 – analysis done bonferroni test

TABLE II. EFFECTS OF AN ETHANOL EXTRACT OF *M. MADERASPATANA* ON MOUSE INTESTINAL GLUCOSE ABSORPTION.

Group	Glucose concentration inside the sacs (mg/dL)	% Inhibition
Control	421.8 ± 0.663	0.00
<i>M.maderaspatana</i> , 0.156 mg/mL	415.8 ± 0.663	1.42
<i>M.maderaspatana</i> , 0.313 mg/mL	401.4 ± 0.510	4.84
<i>M.maderaspatana</i> , 0.625 mg/mL	383.4 ± 1.122	9.10
<i>M.maderaspatana</i> , 1.25 mg/mL	356.6 ± 0.510	20.44
<i>M.maderaspatana</i> , 2.5 mg/mL	295.2 ± 0.583	30.01
<i>M.maderaspatana</i> , 5.0 mg/mL	298.8 ± 0.860	29.16
Sodium Fluoride	294.8 ± 0.374	30.11

All the values were significant ($p < 0.05$) compared with control.

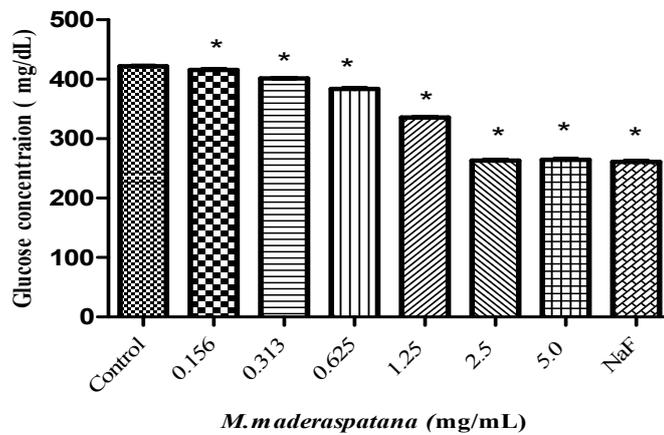


Figure 1. Effects of an ethanol extract of *M. maderaspatana* on intestinal glucose absorption. Values are given as mean ± SEM of each group (n=5). Data were analyzed using one way ANOVA followed by Bonferroni's multiple comparison test ($*p < 0.001$)

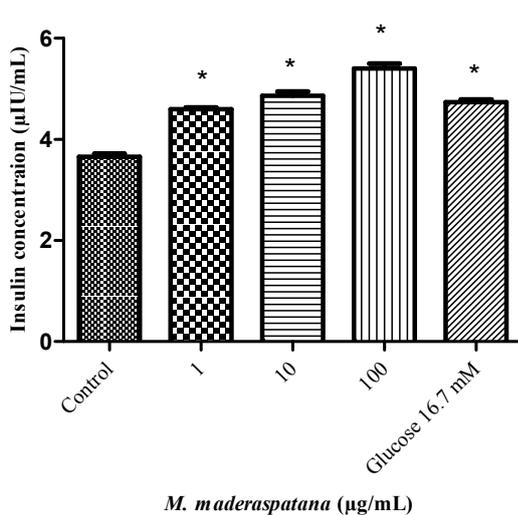


Figure 2. Effect of ethanol extract of *M. maderaspatana* on insulin secretion from the isolated mice splenic pancreatic islets. Values are given as mean ± SEM (n=5). Data were analyzed using one way ANOVA followed by Bonferroni's multiple comparison test ($*p < 0.001$)

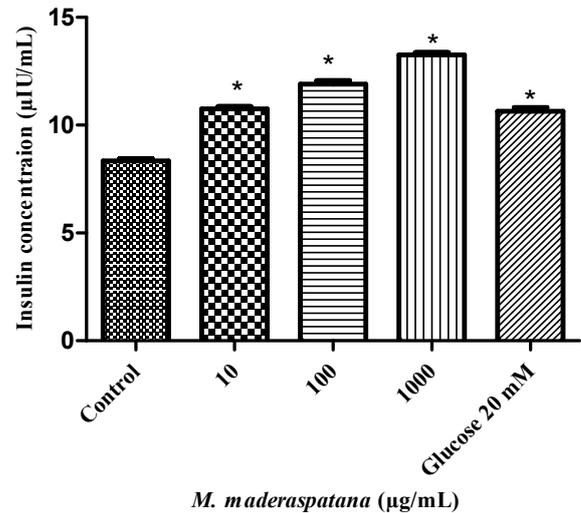


Figure 3. Effects of an ethanol extract of *M. maderaspatana* on insulin secretion from INS-IE Insulinoma cell clusters. Values are given as mean ± SEM (n=5). Data were analyzed using one way ANOVA followed by Bonferroni's multiple comparison test ($*p < 0.001$)