



BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 9(10), 2014 [423-428]

Hypoglycemic and antioxidant activities of *stachytarpheta jamaicensis* ethanolic leaves extract on alloxan-induced diabetic sprague dawley rats

M.H.Wan Rozianoor*, Y.Nurol Eizzatie, S.Nurdiana

Faculty of Applied Sciences, Universiti Teknologi MARA, 40450, Shah Alam, Selangor, (MALAYSIA)

E-mail: rozianoor@salam.uitm.edu.my

ABSTRACT

The aim of this study was to determine the hypoglycemic and antioxidant activities of *Stachytarpheta jamaicensis* ethanolic leaves extract. Measurement was done on the fasting blood glucose level and the catalase activity in alloxan-induced male Sprague Dawley rats. 150 mg/kg of alloxan was injected to each rat to create diabetic condition. Sixteen rats were divided into 4 groups and each group was consisted of four rats. Group I, Group II, Group III and Group IV received saline, no treatment, *Stachytarpheta jamaicensis* extract, and metformin (standard drug) respectively. The amount of dose of plant extract from Oral Glucose Tolerance Test (OGTT) was determined as 200 mg/kg for each rat. After 14 days of treatment, diabetic rats administered with plant extract marked significant reduction ($p < 0.05$) in blood glucose level which was to 6.7 mmol/L as well as improvement in catalase activity which was 0.027 $\mu\text{mole}/\text{min}/\text{mg pr}$. While for diabetic rats treated with metformin, the fasting blood glucose level also reduced but not as much as diabetic rats treated with the plant extract which was reduction to 21.3 mmol/L and the catalase activity for diabetic rats treated with metformin were slightly increased which was 0.019 $\mu\text{mole}/\text{min}/\text{mg pr}$. This finding suggests that *S. jamaicensis* gave promising result in lowering the blood glucose level and improved the catalase activity. In line with the GC-MS analysis, the presence of genipin and linolenic acid might be the contributing properties for hypoglycemic and antioxidant activities for *S. jamaicensis*.

© 2014 Trade Science Inc. - INDIA

KEYWORDS

Stachytarpheta jamaicensis;
Hypoglycemic;
Antioxidant;
Diabetes;
Sprague dawley rats.

INTRODUCTION

Diabetes is a disease that is characterized by rapid increase of glucose level in blood. The incidence of this disease had increase all over the world especially in Asia^[1]. There are two type of diabetes which are type I and type II. Type I diabetes or also known as insulin

dependent diabetes mellitus is caused by lack in insulin secretion, while type II diabetes is caused whether resistance toward insulin by a target cell or due to abnormalities in β cell function in pancreas^[2].

This endocrine disease can cause macrovascular complications such as stroke, heart attack, and peripheral vascular diseases^[3,4] which lead to the fatality for

FULL PAPER

type II diabetes^[5]. Other complications caused by diabetes are adult blindness, gangrene, and kidney failure^[6]. The common symptoms experienced by a person that developed diabetes are polyuria, polydipsia, weight loss, blurred vision and fatigue. Polyuria is a kind of disease with frequent urination that is caused by the presence of glucose in the urine^[7].

The reaction of host defence such as macrophages, neutrophils and other cells of the immune system require free radical compounds in order to defend the body system from pathogen. However, excessive production of free radicals or also known as oxidative stress can cause cell death and tissue injury. In order to avoid overproduction of free radicals, the body system needs to be equipped with antioxidant to neutralize the free radicals molecule^[8]. Among the examples of antioxidant that resides within the human body are superoxide dismutase, catalase and glutathione peroxidase. In diabetic patient, the production of free radicals was produced within the beta cell of pancreas and usually associated with diabetic complications^[9].

Stachytarpheta jamaicensis or also known as 'selasih dandi' is believed to have the ability to lower the blood glucose level and is traditionally used by the locals. It is a weedy plant that grows annually and can grow about 60 to 120 cm tall. *S. jamaicensis* has smooth stem with slightly woody at the base. Its flower can be reddish purple to deep blue in colour. Recent study has reported the use of *S. jamaicensis* as an analgesic, anthelmintic, antacid, and anti-inflammatory^[10]. However, the study on *S. jamaicensis* as having hypoglycemic and antioxidant activities is not very common. Hence, this study is believed to be the first attempt to determine the effectiveness of *S. jamaicensis* as a plant based medicine especially in lowering down the blood glucose level and increasing the catalase activity in alloxan-induced diabetic.

EXPERIMENTAL

Chemicals and apparatus

Glucose, metformin, alloxan monohydrate, saline, ethanol, phosphate buffer, and hydrogen peroxide were obtained commercially and of analytical grade. The apparatus used were Accu Check Glucometer, conical flask, measuring cylinder, blade, muslin cloth, spec-

trophotometer, centrifuge, electronic balance, analytical balance, rotary evaporator, dissecting set, Schott bottle, pH meter, gavage and syringe.

Collection of plant materials and preparation of extracts

The leaves of *Stachytarpheta jamaicensis* were collected from Kota Bharu, Kelantan, Malaysia and the plant material was authenticated by Assoc. Prof. Dr. Norrizah Jaafar Sidek, a plant taxonomist from Universiti Teknologi MARA, Malaysia. The plant leaves were washed and oven dried at temperature between 40°C to 60°C for one week. The dried leaves were coarsely powdered with blender. 10g of the leaf powder was homogenized in 100ml of 70% ethanol and was then stored in an oven for 48 hours with initial stirring. The extract was filtered using Whatman No.1 filter paper and concentrated in vacuum at temperature below 40°C using rotary evaporator^[11]. The final dark semi-solid extract was then transferred into a plastic vial and kept at 4 °C prior to use.

Determination of bioactive compounds

GC-MS analysis was performed using an Agilent-5973 network system. A mass spectrometer with an ion trap detector in full scan mode under electron impact ionization (70 eV) was used. The chromatographic column used for the analysis was an HP-5 capillary column (30 m × 0.32 mm i.d., film thickness of 0.25 µm). Helium was used as a carrier gas at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230°C. With the column held initially at 60°C for 2 min, 1 µL of essential oil solution in hexane (HPLC grade) was injected and analyzed, and then the temperature was increased to 240°C with a 3°C/min heating ramp.

Oral glucose tolerance test (OGTT)

The rats were divided into three groups (n=3) and allowed for 12 hours fasting. Administration of *S. jamaicensis* extract was then taken place at 100, 150 and 200 mg/kg for each group respectively^[12]. The blood was drawn at the interval of 30, 60, 90, and 120 minutes and was tested using glucometer.

Determination of fasting blood glucose level

The rats were fasted for 12 hours before obtaining

the blood sample. The blood was drawn from the tail by prick method and was then tested using Accu-Check glucometer^[13].

Induction of diabetes on rats

Sixteen male Sprague Dawley rats weighing between 150g to 250g were selected for this study. The rats were administered intraperitoneally with alloxan monohydrate in physiological saline pH 7.4 by using syringe after the 24-hours of fasting to promote diabetic condition. The amount of alloxan used for single injection was 150 mg/kg. After three days of injection, the blood was obtained to determine the glucose level by using glucometer strip. The rats with constant high glucose levels between 3.10 to 3.66 g/L were considered as diabetic rat and further used in the experimental study^[11].

Experimental design

The rats were divided into 4 groups and each group consisted of four rats. Group I (normal control) consisted of normal rats received saline, group II (diabetic control) consisted of diabetic rats received no treatment, group III consisted of diabetic rats treated with the *S. jamaicensis* extract, and group IV consisted of diabetic rats treated with metformin (standard drug). The rats were kept under normal laboratory condition in animal room of Faculty of Applied Sciences. They were fed with standard pellet and water supplied *ad libitum* throughout the experimental period. In group III, the dose of *S. jamaicensis* extract used was 200 mg/kg. The amount of dosage for each rat was based on the formula below:

Total body weight (gm) X dosage (gm) = require dosage (gm)

Determination of catalase activity

After 14 days of treatment, the liver of the rats were obtained. The liver was cleaned with chilled 1.15 % of potassium chloride (KCL) solution (pH 7.4). 50 % of homogenate was prepared within 0.15 mM phosphate buffer (pH 7.0). After that, the homogenate was centrifuged at 3500 x g at 4°C for 10 minutes. The supernatant was taken and was used to analyse the catalase activity. The catalase activity was measured by adding 0.1 ml of the supernatant into a cuvette which contains 1.9 ml of 50 mM phosphate buffer (pH 7.0) followed

by 1.0 ml of freshly prepared 30 mM H₂O₂. The catalase activity was measured using spectrophotometer at wavelength of 240 nm. The blank used composed of 2.9 ml phosphate buffer and 0.1 ml homogenate. The catalase activity was calculated using the formula as below^[14]:

$$\frac{[(Abs\ t - Abs\ b)/5 \times vol(3) \times dil(2)]}{40\ mg\ pr}$$

Where:

Abs t = absorbance of sample /min

Abs b = absorbance of blank /min

Vol = volume of the reaction medium (3ml)

Dil = dilution of supernatant (2ml)

40 = value of the extinction coefficient

Statistical analysis

The quantitative measurement was made on four animals in each group. The mean and variance of each analysis was calculated and the standard deviation from the mean was determined. Data that has been obtained were subjected to ANOVA via SPSS software 18th edition and paired T-Test with the level of significance at p<0.05.

RESULTS & DISCUSSION

Oral glucose tolerance test analysis

The glucose tolerance test was conducted to determine the glycemic control of *S. jamaicensis* extracts after the treatment. Figure 1 depicts the blood glucose level for three different dosages of *S. jamaicensis* extracts administered on 3 groups of diabetic rats (n=3). The rats administered with *S. jamaicensis* extract at 200 mg/kg indicated gradual decrease of blood glucose level as compared with rats that treated with *S. jamaicensis* extract at 100 and 150 mg/kg.

Rats treated with *S. jamaicensis* extract at 100 mg/kg shows fluctuation in blood glucose level, while rats administered with 150 mg/kg *S. jamaicensis* extract indicates increment in blood glucose at 30 minutes interval but decrement at the following intervals. According to^[15], oral glucose tolerance test was accepted as a standard to diagnose diabetes and was performed to determine the suitable dose of plant extract to be given towards diabetic rats within 14 days of treatment^[1].

FULL PAPER

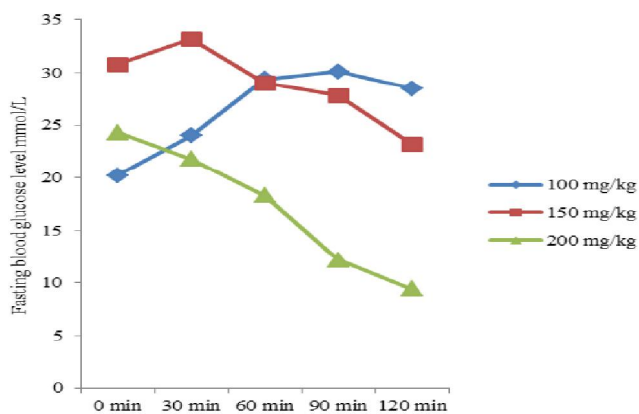


Figure 1 : The oral glucose tolerance after the treatment with *S. jamaicensis* extract at 100, 150, and 200 mg/kg. The data were calculated as mean \pm SEM (n=3).

TABLE 1 : GC-MS analysis of *Stachytarpheta jamaicensis* ethanolic leaves extract

No	Retention time	Area %	Library/ID	Quality	Common name
1	13.581	12.4	Cyclopenta [c] pyran-4-carboxylic acid, 7-methyl-, methyl ester	94	Genipin
2	17.386	11.3	n-Hexadecanoic acid tridecanoic acid	96 91	Palmitic acid Tridecanoic acid
3	18.800	13.2	Phytol	93	Phytol
4	19.097	15.7	9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z), cis,cis,cis-7, 10, 13-hexadecatriena 9,12,15-octadecatrien-1-ol	91 91 90	Linolenic acid, methyl ester Hexadecatrienoic acid Linoleyl alcohol

Fasting blood glucose analysis

The effects of *S. jamaicensis* ethanolic leaves extract on fasting blood glucose level in alloxan-induced diabetic rats were shown in Figure 2. The diabetic rats treated with *S. jamaicensis* extract reduced the blood glucose by 75.7 % after 14 days of treatment as compared with diabetic rats that were treated with metformin which indicates 35.5 % of reduction in blood glucose. The group treated with *S. jamaicensis* extract exhibited significant reduction ($p < 0.05$) of fasting blood glucose level when analysed using paired T-Test. Blood glucose level reduced to 6.7 mmol/L as compared with untreated diabetic group which was only to 26.7 mmol/L. In two weeks' time, this *S. jamaicensis* extract was able to reduce the blood glucose level to normal condition as according to^[17], fasting blood glucose for diabetes is considered as ≥ 7.0 mmol/L.

S. jamaicensis extract was able to reduce the blood glucose in diabetic rats most probably because of the present of genipin and linolenic acid. Based on the GC-MS analysis, the percentage of genipin within

Gas chromatography- mass spectrometry (GC-MS) analysis

S. jamaicensis extract was analysed using GC-MS to identify compounds that are present within the plant extract. GC-MS is one of the analytical tools used to analyse the complex mixture. GC-MS has variety analytical functions used to trace toxic leftover and pesticide within soil and water samples, analysis of forensic evidence and drug samples, as well as analysis in quality control of food and pharmaceutical product industries^[16]. TABLE 1 shows the bioactive compounds present in the *S. jamaicensis* ethanolic leaves extract.

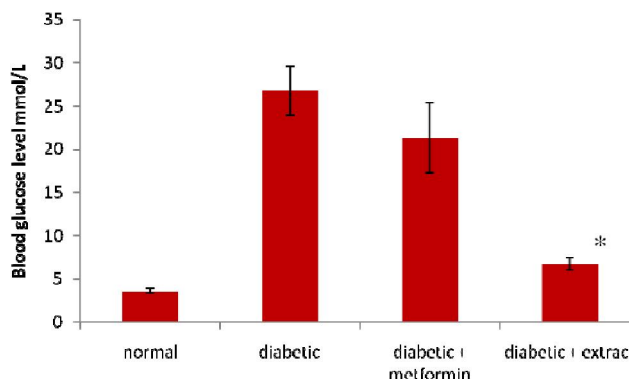


Figure 2 : Fasting blood glucose level in alloxan induced diabetic male rats after 14 days. Data was expressed as mean \pm SEM, (n=4). * $p < 0.05$ compared to corresponding value for diabetic group when analysed using paired T-Test.

the plant extract was 12.4 %, while linolenic acid was 15.7 %. Reported by^[18], genipin was able to improve beta cell dysfunction by intensifying mitochondrial membrane potential, escalates ATP levels and stimulates insulin secretion. Other than that, according to^[19], polyunsaturated fatty acid such as linolenic acid can escalate the number of insulin receptor and insulin action. Therefore, this polyunsaturated fatty acid can improve

regulation of the glucose in a person with diabetic. Additional to this, dietary consists of long-chain polyunsaturated fatty acid can prevent cardiovascular diseases as well as hyperglycemia^[20].

Liver catalase activity

Figure 3 shows the catalase activity within four different groups of rats. Normal group indicates the highest activity of catalase which was 0.033 $\mu\text{mole}/\text{min}/\text{mg}$ pr as compared with the other three groups. Catalase catalysed the reduction of hydrogen peroxide into water and oxygen^[19]. There was an increment of catalase activity within diabetic group treated with *S. jamaicensis* which was 0.027 $\mu\text{mole}/\text{min}/\text{mg}$ pr as compared with untreated diabetic group which was 0.017 $\mu\text{mole}/\text{min}/\text{mg}$ pr. Diabetic group treated with metformin also shows the increment in catalase activity which was 0.019 $\mu\text{mole}/\text{min}/\text{mg}$ pr but slightly lower compared to rats treated with *S. jamaicensis* extract.

However, analysis of variance (ANOVA) conducted showed that there was no significant difference in catalase activity between all four groups since $p > 0.05$. Reduction in catalase activity within untreated diabetic group is believed to be caused by the consistent increase in the blood glucose level. Prolong hyperglycemia condition within diabetic rats deteriorates the activity of catalase that promotes the production of free radicals^[21].

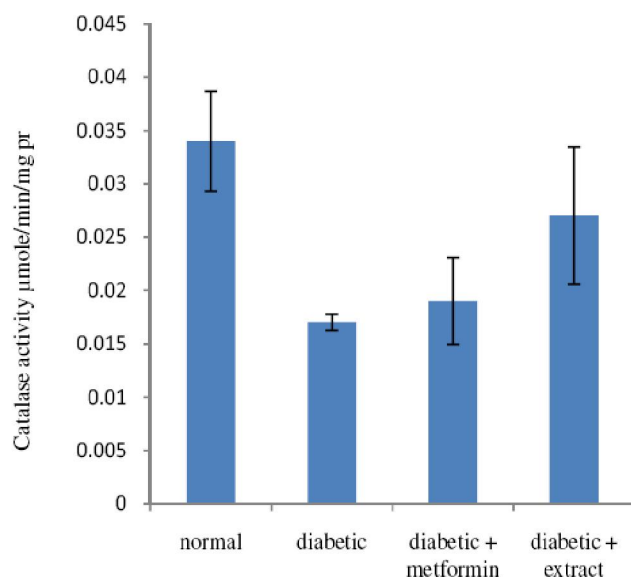


Figure 3 : Liver catalase activity in four different groups of experimental rats. The data were expressed as mean \pm SEM (n=4), result was not significant where $p > 0.05$

Free radical is a dangerous molecule since it can attack the living cell causing damage to the DNA of the cell by breaking down the DNA, ultimately leads to damaging protein product and causing cytotoxicity. In contrast, low blood glucose will enhance the catalase activity. The ability of *S. jamaicensis* to reduce the blood glucose level in diabetic rats was expected to give high catalase activity. The increasing in catalase activity within the diabetic rats treated with *S. jamaicensis* could be due to the presence of linolenic acid. Apart from being able to enhance the secretion of insulin, it is also of great importance in contributing to the antioxidant activity as reported by^[19].

CONCLUSION

This study shows that *S. jamaicensis* ethanolic leaves extract gave promising result in enhancing hypoglycemic and antioxidant activities as this plant extract able to lowering the blood glucose of male diabetic rats to 6.7 mmol/L, and increased the catalase activity to 0.027 $\mu\text{mol}/\text{min}/\text{mg}$ pr. The most probably bioactive compounds contributing to this properties were genipin and linolenic acid which indicated by GC-MS analysis. However, further phytochemical screening should be done in order to confirm the exact properties responsible for these activities as well as the mechanism involved.

ACKNOWLEDGEMENTS

This project was financially supported by Faculty of Applied Sciences, Universiti Teknologi MARA and RIF Grant (600-RMI/DANA 5/3RIF (65/2012)).

REFERENCES

- [1] R.K.Gupta, A.N.Kesari, P.S.Murthy, R.Chandra, A.Tandon, G.Watal; Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of *Annona squamosa* L. in experimental animals, *Journal of Ethnopharmacology*, **99**, 75-81 (2005).
- [2] T.S.Frode, Y.S.Medeiro; Animal models to test drugs with potential antidiabetic activity, *Journal of Ethnopharmacology*, **115**, 173-183 (2008).
- [3] B.Balkau, A.Forhan, D.Simon, E.Eschwege; HbA1c as a diagnostic tool for diabetes, *International Con-*

FULL PAPER

- gress Series, **1253**, 19-26 (2003).
- [4] D.K.Patel, S.K.Prasad, R.Kumar, S.Hemalatha; An overview on antidiabetic medicinal plants having insulin mimetic property, *Asian Pasific Journal of Tropical Biomedicine*, 320-330 (2012).
- [5] R.A.Defronzo, M.A.Ghani; Assessment and treatment of cardiovascular risk in prediabetes: impaired glucose tolerance and impaired fasting glucose, *The American Journal of Cardiology*, **108(3s)**, 3-24 (2011).
- [6] R.J.Marles, N.R.Fransworth; Antidiabetic plants and their active constituents, *Phytomedicine*, **2(2)**, 137-189 (1995).
- [7] D.W.Guthrie, R.A.Guthrie; The diabetes sourcebook. (5th Ed). McGraw-Hill, New York, 3-5 (2003).
- [8] P.Rosen, P.P.Nawroth, G.King, W.Moller, H.J.Tritschler, L.Packer; The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a congress series sponsored by unesco-mcbsn, the American diabetes association and the German diabetes society, *Diabetes Metabolism Research and Review*, **17**, 189-212 (2001).
- [9] R.Gupta, A.K.Sharma, M.C.Sharma, R.S.Gupta; Antioxidant activity and protection of pancreatic β -cells by embelin in streptozotocin-induced diabetes, *Journal of Diabetes*, **4**, 248-256 (2012).
- [10] M.Idu, E.K.I.Omogbai, G.E.Aghimien, F.Amaechina, O.Timothy, S.E.Omonigho; Preliminary phytochemistry and antimicrobial properties of *Stachytarpheta jamaicensis* (Linn.) Vahl. Stem, *Research Journal of Medicinal Plant*, **1(4)**, 149-153 (2007).
- [11] M.Tailang, B.K.Gupta, A.Sharma; Antidiabetic activity of alcoholic extract of *Cinnamomum zeylanicum* leaves in alloxan induced diabetic rats, *People's Journal of Scientific Research*, **1**, 9-12 (2008).
- [12] Shirwaikar, K.Rajendran, C.D.Kumar, R.Bodla; Antidiabetic activity of aqueous leaf extract of *Annona squamosa* in streptozotocin-nicotinamide type 2 diabetic rats, *Journal of Ethnopharmacology*, **91**, 171-175 (2004).
- [13] M.Bhat, S.K.Kothiwale, A.R.Tirmale, S.Y.Bhargava, B.N.Joshi; Antidiabetic properties of *Azadirachta indica* and *Bougainvillea spectabilis*: in vivo studies in murine diabetes model. *Evidence-Based Complementary and Alternative Medicine*, **2011**, 1-9 (2009).
- [14] Mekail, Z.A.Sharafaddin; A study of the activity of catalase and glutathione s-transferase in weanling and adult rats intoxicated with diazinon, carbaryl and lambda-cyhalothrin, *J. Duhok Univ*, **12(1)**, 138-145 (2009).
- [15] R.Williams, W.Herman, A.L.Kinmonth, N.J.Wareham; The evidence base for diabetes care, John Wiley & Sons Inc, USA., 27-29 (2002).
- [16] D.C.Smith, S.Forland, E.Bachanos, M.Matejka, V.Barrett; Qualitative analysis of citrus fruits extracts by GC/MS: an undergraduate experiment, *Chem. Educator*, **6**, 28-31 (2001).
- [17] Y.Heianza, S.Hara, Y.Arase, K.Saito, K.Fujiwara, H.Tsuji, S.Kodama, S.D.Hsieh, Y.Mori, H.Shimano, N.Yamada, K.Kosaka, H.Sone; HbA1c 5.7- 6.4% and impaired fasting plasma glucose for diagnosis of prediabetes and risk of progression to diabetes in Japan (topic 3): a longitudinal cohort study, *Lancet*, **378**, 147-154 (2011).
- [18] R.A.A.Muzarelli; Genipin-crosslinked chitosan hydrogels as biomedical and pharmaceutical aids, *Carbohydrate Polymers*, **77**, 1-9 (2009).
- [19] G.Kaithwas, D.K.Majumdar; In vitro antioxidant and in vivo antidiabetic, antihyperlipidemic activity of linseed oil against streptozotocin-induced toxicity in albino rats, *Eur. J. Lipid Sci. Technol*, **114**, 1237-1245 (2012).
- [20] S.A.Syadati, A.Mirzaei-Aghsaghali, H.Fathi, J.Davuodi; Importance essential fatty acids (n-6 and n-3) in animal nutrition: I: ruminant, *Scholars Research Library*, **3(2)**, 1161-1176 (2012).
- [21] M.Kanter, O.Coskun, A.Korkmaz, S.Oter; Effects of *Nigella sativa* on oxidative stress and β -cell damage in streptozotocin-induced diabetic rats, *The Anatomical Record Part A*, **279**, 685-691 (2004).