Effect of *Mikania micrantha* leaf extract on the level of blood glucose and hepatic glycogen in the normal and alloxan-induced diabetic rats

W.H.Wan Nurhayati*, T.Norli Arlizan, S.Nurdiana
Faculty of Applied Sciences, Universiti Teknologi MARA,40450, Shah Alam, Selangor, (MALAYSIA)
E-mail: wannurhayati@salam.uitm.edu.my

ABSTRACT

The present study investigates the hypoglycaemic effect of *Mikania micrantha* leaf extract in normal and alloxan induced diabetic rats. Male Spargue Dawley rats were used in the study. Oral glucose Tolerence tests (OGTT) was conducted in non-diabetic and alloxan induced diabetic rats using orally administered glucose followed by either the leaf extract or subcutaneous injection of metformin. The fasting blood glucose level ranged from 5.15 ± 0.58 mmol/L. The effect of daily oral administration of aqueous extract of *Mikania micrantha* leaves for a period of 20 days was studied on blood glucose level and hepatic glycogen content in alloxan-induced diabetic rats. Blood glucose level of diabetic rats treated with aqueous extract of *Mikania micrantha* showed significant reduction ($p<0.05$) as compared to non- diabetic group. The amount of hepatic glycogen in diabetic rats also was decreased. The observation of this study indicates that the effect of metformin was far more noticeable in diabetic rats. In conclusion, *Mikania micrantha* has a beneficial effect in the treatment of diabetes mellitus and can form a part of therapy in its management.   © 2013 Trade Science Inc. - INDIA

INTRODUCTION

Diabetes is a main chronic disease in the world which threatens human health. It is a result from the shortage of insulin secretion. It is one of the oldest diseases affecting millions of the people all over the world[1]. It is clinically divided into two main types which are, Type I and Type II. Type I diabetes, once known as juvenile diabetes or insulin-dependent diabetes, is a chronic condition in which the pancreas produces little or no insulin and a hormone needed to allow sugar to enter the cells to produce energy. Type II diabetes, which is far more common, occurs when the body becomes resistant to the effects of insulin or does not make enough insulin[2].

The long term complication of diabetes mellitus includes nephropathy which can lead to kidney failure or irreversible end-stage kidney disease, retinopathy with potential loss of vision, amputation, cardiovascular symptoms and sexual dysfunction[3]. Diabetes can be diagnosed by performing test to measure the blood glucose level such as hemoglobin (A1C) test, fasting blood glucose which is a blood sample will be taken after an overnight fast and also oral glucose test tolerance (OGTT) in which measures the body's ability to metabolize glucose, or clear it out of the bloodstream.

In 1995 it was estimated that around 135 million people were affected from this disease and it was expected to affect 300 million by the year 2025[4]. Man-

Full Paper

agement of diabetes without any side effect is still a challenge to the medical community. For treatment of diabetes, several drugs such as biguanides, sulfonylurea and thiazolidenediones are presently available to reduce hyperglycemia in diabetes mellitus but they have side effects[5]. The alternative drug is continuously searched to overcome the side effects problems, and the medical plants may provide the useful sources of a new oral hypoglycemic compound for the diabetic therapies.

There are many management options for diabetes which include diet and administration of insulin or hypoglycemic agents. However, these methods may not be promising to cure diabetes. Besides, these approaches also may not be affordable for patients in developing countries due to socio-economic conditions[6]. Over a few decades, the reputation of the herbal remedies has increased due to its therapeutic value and safety. According to[7], since ancient times, plants have been a source of medicine and it is widely used today regardless of the fact that their biologically active compounds are unknown, due to minimal adverse effect and low costs.

Many herbals have been described for the treatment of diabetic in ancient literature. For example are Cinnamon (*Cinnamomum zeylanicum*), Cumin seeds (*Cuminum cyminum*), Curry leaves (*Murraya koenigii*), Fenugreek seeds (*Trigonella foenumgraecum*) and many more[8]. They have shown to have hypoglycemic action in both animals and humans[9]. In this case, even though *Mikania micrantha* has been report elsewhere can treat the diabetes, but this plant has not been subjected to scientific investigation. Recently, a chemical component of Mikania micrantha has been evaluated by previous researcher in several studies such as antimicrobial[10], allelopathic contents, phenolics contents and some more. This plant is containing several components which have been proven to have anti-diabetic effect. *Micania micrantha* contain terpenes, flavonoids and alkaloids which are antioxidants. They may prevent the progressive impairment of pancreatic beta-cell function due to oxidative stress and may thus reduce the occurrence of type II diabetes. This study revealed the anti-diabetic agents of *Mikania micrantha* leaf extract on alloxan-induced diabetic rats and the result was evaluated through the two parameters which are plasma glucose and hepatic glycogen content. Finally, this study was beneficial for

prospective study for the new alternative agent in treatment of diabetes.

EXPERIMENTAL

Preparation of aqueous extract of *Mikania micrantha*

The collected leaves of *Mikania micrantha* (Asteraceae) were washed, weighed and dried in oven. Upon drying, dried leaves were blended into powder form, then 100 g of the dried leaves powder was mixed with 1000 ml of ethanol and distilled water. The extract had been concentrated by evaporation at room temperature. It has been filtered and centrifuged at 3000 rpm for 20 min. Supernatant obtained was concentrated using a rotary evaporator at 40°C (BUCHI Rotavapor R-200) and freeze-dried to yield yellowish dark green powder of *Mikania micrantha* crude ethanolic extract. The extracts were stored at -20°C until needed in the experiment.

Experimental design and procedure

Adult male of Sprague Dawley rats weight between 150-300 g were choosed. The rats were randomly divided into four groups (Group 1, Group, Group 3 and Group 4) of four animals each group. Group 1 was kept as normal control, group 2 was kept as diabetic control. While Group 3 and 4 were kept as metformin treated group and *Mikania micrantha* treated group respectively. Group 4 were given orally aqueous leaf extract of *Mikania micrantha* at dose levels of 150 mg/kg of body weight. All of the groups were fed and drinking water was provided *ad libitum.* Figure 3.1 summarized the experimental design involved in this study.

Induction of diabetes animals

The rats were induced by intraperitoneal injection of alloxan monohydrate in physiological saline at a dose of 150 mg/kg of body weight. The diabetic state has been confirmed after 72 hours of alloxan injection. The rats with fasting blood glucose higher than 150 mg/dl was selected for the study.

Determination of fasting blood glucose level

Blood was obtained from tail vein of rats fasted

Full Paper

overnight (24 hours) and fasting blood glucose concentration was measured on day 0 (before the start of treatment) and day 20 (end of treatment) using AccuChek® Performa glucometer.

Hepatic glycogen measurement

Liver glycogen content was determined according to the method[11]. The frozen liver (50 mg) was hydrolysed with 2 ml 30% KOH for 15 min in boiling water bath (100°C). Liver hydrolysate was cooled and added with 2.4 ml of 95% ethanol. The mixture was incubated overnight at 4°C and then centrifuged at 3000 rpm for 15 min. Supernatant was discarded and the tubes were allowed to drain in inverted position for 10 min to obtain glycogen pellet. Then, the glycogen pellet was dissolved in 1 ml of distilled water via vigorous shaking, followed by the addition of 5 ml of anthrone reagent (0.2% anthrone, 72% (v/v) H_2SO_4) with the tubes placed in cold water to prevent overheating. After cooling, the reaction mixture was heated in boiling water bath (100°C) for 15 min, and then cooled under running tap water. Absorbance was then read at 620 nm wavelength using spectrophotometer. Glucose that was dissolved in saturated benzoic acid solution was used as standard and glycogen content was expressed as milligram of glycogen per gram of wet liver tissue.

Statistical analysis

The quantitative measurement has been made on six 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 with level of significance at $p<0.05$.

RESULTS & DISCUSSION

Effect of *Mikania micrantha* and metformin on blood glucose level

Changes in fasting blood glucose level in diabetic rats treated with *Mikania micrantha* and metformin was presented in TABLE 1. Change in glucose was calculated as (Glucose level on day 15 after treatment-glucose level on day 3 after alloxan injection). No significant change in fasting blood glucose level was observed in the normal control during the treatment pe-

riod. The fasting blood glucose level ranged from 5.15 ± 0.58 mmol/L to 5.20 ± 0.50 mmol/L. Significant ($p<0.05$) increase in blood glucose level from 23.65 ± 3.67 to 33.35 ± 2.35 was observed in diabetic rats. Oral administration of *Mikania micrantha* in diabetic rats at dose 150 mg/kg body weight for 20 days revealed a significant reduction from 26.75 ± 4.16 to 25.25 ± 6.12 in blood glucose level ($p<0.05$).

TABLE 1 : Effect of *Mikania micrantha* leaf extract on blood glucose level (mmol/l) before treatment and after treatment

Groups	Blood glucose level at day 3 after Alloxan monohydrate induction (mmol/L)	Blood glucose level At Day 20 *Mikania micrantha* and metformin treatment (mmol/L)
Normal Control	5.15 ± 0.58	5.20 ± 0.50
Diabetic Control	23.65 ± 3.67	33.35 ± 2.35
Metformin treatment	27.52 ± 1.57	22.06 ± 1.49
Mikania micrantha treatment	26.75 ± 4.16	25.25 ± 6.12

Each value is mean ± SD of four samples; Value were significant at p<0.05

The present work was designed to investigate the hypoglycemic effects of *Mikania micrantha* extract in normal and alloxan-induced diabetic rats. Alloxan monohydrate has been shown to destruct β cells of pancreas producing hyperglycemia. In this experiment, the diabetes was characterized by presence of sugar in urine and hyperglycemia. Blood analysis was done to observe changes in blood glucose level of the rats before and after 20th day treatment. There was no significant different in the blood glucose level ($p>0.05$) of a normal group because no treatment was given. The mean blood glucose level remained almost similar which was 5.15 ± 0.58 mmol/L and 5.20 ± 0.50 mmol/L. There was no significant decrease in blood glucose level of diabetic control with mean values of 33.35 ± 2.35 as compared with the mean values of 22.06 ± 1.49 of metformin treatment group and *Mikania micrantha* treatment group with the mean value 26.75 ± 4.16 mmol/L.

On the 20th day, there was significant ($p<0.05$) decrease in blood glucose level of metformin treatment group and *Mikania micrantha* group with the mean values of 22.06±1.49 and 25.25±6.12 respectively. The outcome of the present study showed that after 20 days of treatment with *Mikania micrantha*, diabetic rats showed significant reduction effect ($p<0.05$) on their blood glucose level. In all rats that were fed with

Full Paper

Mikania micrantha leaf extract the blood glucose level decreased significantly on 20th day indicating a positive effect of *Mikania micrantha* leaf extract in reducing diabetes. This effect may be due to stimulation of β cells of pancreas to increased release of insulin or through any other mechanism involving glucose utilization. Similar results were also been reported by[12], who showed the beneficial effects of *Mikania micrantha* extract. The control group, a group without any treatment, consistently showed increased in plasma glucose level.

Furthermore, the observation in this study indicated that the hypoglycaemic effect of metformin was far more noticeable in diabetic rats. Metformin has been suggested to partly reduce blood glucose via increases in glucose uptake by cells[13].

Effect of *Mikania micrantha* and metformin on hepatic glycogen and liver weight

Liver plays a vital role in regulation of blood glucose level and hence it is of interest to study the role of *Mikania micrantha* on hepatic glycogen content. In this study, there was a marked reduction in the level of liver glycogen and increased activity of glycogen phosphorylated in diabetic rats. The decrease in glycogen content of liver observed in the present study is probably due to the lack of insulin in the diabetic state. This prevention of glycogen depletion in the liver might possibly be due to stimulation of insulin release[14]. The result of the liver glycogen content was shown in the TABLE 2.

TABLE 2 : Comparison of the liver weight and hepatic glycogen in control non diabetic, alloxan diabetic and alloxan-diabetic rats orally treated with *Mikania micrantha* leaf extract

Group	Liver weight (g 100g^{-1} b.wt)	Hepatic glycogen (mg/gm wet tissue)
Normal control		
Diabetic control	12.88 ± 0.79	53.72 ± 2.40
Diabetic treated with	9.55 ± 0.56	22.50 ± 0.60
Mikania micrantha	8.68 ± 0.45	42.20 ± 2.30
Diabetic treated with metformin	6.98 ± 0.27	38.45 ± 2 .67

Each value is mean ± SD of four samples; Value were significant at p<0.05

The hepatic glycogen levels showed a significant decreased in diabetic rats compared with those in the controls. The rats receiving extract at 150 mg/kg b.w. showed the significantly highest levels of hepatic glycogen concentration (p<0.05). The liver weights of alloxan-diabetic rats were lower than those of non-diabetic rats. This was perhaps due to the reduced body weight and decreased hepatic glycogen concentration. Despite an increase in hepatic glycogen of alloxan-diabetic rats orally treated with *Mikania micrantha* leaf extract, the liver weight and hepatic glycogen concentration did not return to levels seen before induction of diabetes. This perhaps might be due to short duration of experimental period. The mechanism through which the plant extracts increased hepatic glycogen cannot be explained by the results of the present study. However, they partly attribute this finding to increases in cellular glucose uptake induced by the leaf extract which has been speculated to contain flavonoids and coumarins that preserve cell function by protect against the progression of insulin resistance[15]. Insulin regulates activities of enzymes involved in hepatic glycogenesis and gluconeogenesis to maintain blood glucose homeostasis[16].

CONCLUSIONS

Our study indicates that *Mikania micrantha* has a beneficial effect in the treatment of diabetes mellitus and can form a part of therapy in its management. After 20 days of treatment with *Mikania micrantha*, diabetic rats showed significant reduction effect (p<0.05) on their blood glucose level with the mean values of 22.06±1.49 and 25.25±6.12 respectively. This finding may form a basis for the inclusion of *Mikania micrantha* as a spice in dietary management of diabetes mellitus. But, the possible mechanism involved in the short term hyperglycaemia effect of the leaf extract cannot be established by the current study and remain speculative. This suggests is open for further investigation.

ACKNOWLEDGEMENT

Full Paper

REFERENCES

[1] B.Andallu; Control of hyperglycemic and retardation of cataract by mulberry (*Morus indica. L*) leaves in streptozotocin diabetic rats, Ind J Exp Biol., **40**, 791–795 **(2002)**.

[2] H.S.Jun, H.Y.Bae, B.R.Lee, K.S.Koh, Y.S.Kim, K.W.Lee et al.; Pathogenesis of non-insulin dependent (type II) diabetes mellitus (NIDDM)-genetic predisposition and metabolic abnormalities, Adv Drug Deliv Rev., **35**, 157-77 **(1999)**.

[3] E.A.Omar, A.Kam, A.Alqahtani, V.Li, V.Razmovski-Naumovski, S.Nammi, K.Chan, B.D.Roufogalis, G.Q.Li; Hebal Medicines and nutraceuticals for diabetic vascular complications: mechanism of action and bioactive phytochemicals, Curr Pharm Desx., **16(34)**, 3776-807 **(2010)**.

[4] H.King, R.E.Aubert, W.H.Herman; Global burden of diabetes 1995–2025: prevalence, numerical stimates and projection, Diabetes Care, **21**, 1414–1431 **(1998)**.

[5] J.P.Liu, M.Zhang, W.Y.Wang, S.Grimsgaard; Chinese herbal Medicines for type- II Diabetes mellitus, Cochrane Database Syst Rev., **3**, 3642 **(2004)**.

[6] M.Ducorps, W.Ndong, B.Jupwo, G.Belmejdoub, C.Thiolet, H.Mayaudon, B.Bauduceau; Diabetes in Cameroon: classification difficulties in Africa, Medecine Tropicale, **56**, 264-270 **(1996)**.

[7] J.K.Grover, S.Yadev, V.Vats; Medicinal plants of India with antidiabetic potential, J. Ethnopharmacol., **81(1)**, 81-100 **(2002)**.

[8] B.Joseph, D.Jini; Insight into the hypoglycaemic effect of traditional indian herbs used in the treatment of diabetes, Research Journal of Medicinal Plant, **5**, 352-376 **(2011)**.

[9] G.B.Kavishankar, S.Lakshmidevi, M.Mahadeva, H.S.Murthy, P.Prakash, S.R.Niranjana; Diabetes and medicinal plants, Int J Pharm Biomed Sci., **2**, 65-80 **(2011)**.

[10] H.Huang, W.Ye, X.Wei, C.Zhang; Allelopathic potential of sesquiterpene lactones and phenolic constituents from *Mikania micrantha* H.B.K., Biochemical Systematics and Ecology, **36**, 867-871 **(2009)**.

[11] K.C.Ong, H.E.Khoo, Effects of myricetin on glycemia and glycogen metabolism in diabetic rats, Life sciences, **67**, 1695-1705 **(2000)**.

[12] A.Silva-Santos, A.M.S.Antunes, H.R.Bizzo, C.A.D'Avila, L.C.Souza-Santos; The application of essential oils and terpenics/ terpenoids compounds in the field pharmaceutic and cosmetic through the knowledge registered in patents, Revista Brasileira de Farmacognosia., **14**, 48-50 **(2004)**.

[13] G.Zhou, R.Myers, L.Chen, X.Shen, F.Melody, J.Wu, J.Ventre, T.Doebber, N.Fujii, N.Musi, M.F.Hirsman, L.J.Goodyear, D.E. Moller; Role of AMP-activated protein kinase mechanism of metformin action, Journal of Clinical Investigation, **108**, 1167-1174 **(2001)**.

[14] S.Chakrabarti, T.K.Biswas, B.Rokeya, L.Ali, M.Mosihuzzaman, N.Nahar, A.K.A.Khan, B.Mukherjee; Advanced studies on the hypoglycemic effect of *Caesalpinia bonducella* in type I and type II diabetes in Long Evans rats, J.Ethnopharmacol, **84**, 41-46 **(2003)**.

[15] P.C.Hollman, M.B.Katan; Health effects and bioavailability of dietary flavonoids, Free Radic Res., **31**, 75-80 **(1999)**.

[16] P.V.Rao, S.Pugazhenthi, R.L.Khandelwal; The effect os STZ-induced diabetic rats and insulin supplementation on expression of the glycogen phosphorylase gene in rat liver, Journal on Biological Chemistry, **270**, 24955-24960 **(1995)**.