International Food Research Journal 20(2): 613-616 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

# *In vivo* antidiabetic and acute toxicity of spray-dried *Vernonia amygdalina* water extract

[1]Yeap, S.K., [2]Liang, W.S., [3]Beh, B.K., [4]Ho, W.Y., [4]Yousr, A.N. and [4*]Alitheen, N.B.

*[1]Institute of Biosciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia*
*[2]Spektra Biotek Sdn Bhd, Kampung Melayu Subang, 40150 Shah Alam, Selangor, Malaysia*
*[3]Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia*
*[4]Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia*

**Article history**

*Received: 7 May 2010*
*Received in revised form: 5 November 2012*
*Accepted: 9 November 2012*

**Keywords**

Antidiabetic
acute toxicity
*in vivo*
*Vernonia amygdalina*

**Abstract**

The spray-dried *Vernonia amygdalina* water extract was evaluated for antidiabetic effect using normoglycaemic, glucose induced hyperglycaemic and streptozotocin induced diabetic mice. This effect was compared with an oral dose of *Momordica charantia*. Besides, acute toxicity of the extract was also evaluated at concentration 2000 and 5000 mg/kg body weight. The extract was able to reduce blood glucose level in glucose and streptozotocin induced hyperglycaemic mice without causing hypoglycemic effect on fasting normoglycaemic mice. Moreover, mice appeared to be normal and no mortality was observed in the acute toxicity study after treated with up to 5000mg/kg of extract. These results indicated that the spray-dried *Vernonia amygdalina* water extract was a potential antidiabetic agent which does not induce hypoglycemic and acute toxicity on normal subject.



## Introduction

Diabetes mellitus is one of the main causes of serious maladies in the 21st century. The world population of diabetic mellitus in year 2008 was approximate 150 million and the population of this pandemic was expected to double by year 2025 (Tan *et al*., 2008). Malaysia contained the fourth highest number of diabetic patients in Asia (approximate 800,000 in year 2007 and expected to reach 1.3 million in year 2010 (Lee and Loh, 2010). Type 2 diabetic, normally associated with obesity and was commonly accepted to be induced by high calorie diet, less exercise following by insulin resistance. This type of diabetic is the major contributor of diabetic incident as compared to genetically inherited type 1 diabetic (Viollet *et al*., 2009). *Vernonia amygdalina* (Family: Compositae) is a soft wooded shrub that traditionally used to treat diabetes. Studies have shown that various extracts of *V. amygdalina* have possessed hypoglycemic effect on alloxan monohydrate and streptozotocin induced diabetic animals (Nwanjo, 2005; Igbakin and Oloyede, 2009). However, the hypoglycemic effect of spray-dried water extract on diet induced diabetes is still remaining unknown. Thus, this study is to verify the preservation on antidiabetic effect of spray-dried water extract especially on diet induced diabetes.

## Materials and Methods

### *Reagents*

Streptozotocin (STZ), Glucose and Glucose (GO) assay kit were purchased from Sigma (USA). *Momordica charantia* was purchase from INS (Malaysia). High fat diet contained about 18.3% carbohydrate, 30% of lipid, 20% protein and 31.7% of other ingredients.

### *Plant material*

Leaves of *V. amygdalina* were collected on the herbal plantation in Kuala Selangor, Malaysia on June 2008 and were identified by science officer Lim Chung Lu (Kepong, Selangor) from Forestry Division, Forest Research Institute Malaysia (FRIM). The voucher number of *V. amygdalina* is FRIM 43216. The leaves of the plant was air-dried

*Corresponding author.
Email: *noorjahan@biotech.upm.edu.my*

in shade and finely powdered. The leaf extract was prepared by keeping 1g of leaf powders soaked in 80 mL of deionised water (60°C) for 2 hours. Then, the extract was filtered with Whatman filter paper no 1 (Millipore, Malaysia), mix with maltodextrin (ratio 1:1) and subjected to spray dry at an air pressure of 1.4 bar and operating temperature of 145ºC (yield 25%, w/w). The spray dried powder was store at 4°C.

*Animals*

A total 30 male of Imprinting Control Region (ICR) mice, 5-8 weeks old, weight between 20-25 g were used in all experiments. The animals were purchased from the Animal House, University Putra Malaysia. The animals were housed under standard conditions at room temperature and fed with standard pellets and tap water. This work had been approved by the Animal Care and Use Committee, University Putra Malaysia (UPM).

*Acute toxicity tests*

A separate experiment was carried out to study the acute toxicity of spray-dried *V. amgydalina* water extract on mice (8 weeks old). Normal healthy male mice was randomly divided into three group which feed with the vehicle-treated "control" group (Phosphate buffer saline) and two concentration of extract-treated "experimental" groups, totally making up to 3 groups of 10 animals per each group. Extract (2000 and 5000 mg/kg body weight) and vehicle (PBS) control was separately administered orally to the mice. All the mice were allowed access to food and water. Behaviour changes and mortality were observed and recorded over a period of 24 hours. All animal was further incubated until day 14.

*Hypoglycemic and oral glucose tolerance test*

Diabetic mice were induced by either intraperitoneal injection of streptozotocin (STZ, 200 mg/kg body weight) or high fat diet on the mice (initial 6 weeks old and fed with high fat diet that contained 18.3% carbohydrate, 30% lipid, 20% protein and 31.7% of other ingredients (Liu *et al*., 2010) for 1 month). Two weeks later, blood was collected through lateral tail vein and the blood glucose level was monitored. Only mice with blood glucose over 200 mg/dL were considered diabetic and selected for the glucose tolerance test.

In the hypoglycemic and glucose tolerance test, mice were randomly divided into 12 groups with each contain 8 animals. Groups 1 to 4 contained all normal mice (hypoglycemic study), Group 5 to 8 with high fat induced diabetic mice and Group 9 to 12 were STZ induced mice. Mice were fasted for 18 hours prior the test and blood glucose level at time zero was determined prior to the administration of vehicle (PBS) control or extract treatment. Animals in group 1, 5 and 9 were given 1X PBS (0.1mL). For group 2, 6 and 10, *Momordica charantia* (INS, Malaysia) (24µIU/kg/0.1mL of plant insulin which was detected through serum radiation immunoassay) was given as positive control. Group 3, 7 and 11 and group 4, 8 and 12 were given spray-dried *V. amygdalina* water extract at 10 and 50 mg/kg/0.1mL, respectively. Animals in group 1 to 4 were orally given vehicle or extract at time zero while animals in group 5 to 12 were orally given vehicle or extract together with 1g/kg body weight of glucose solution after the blood glucose measurement at time zero. Blood glucose for animals in all groups was further monitor at 30, 60, 90 and 180 minutes after the vehicle and extract administration. Blood samples were collected from the vein of the mice and the blood glucose level was tested with GO assay kit (Sigma, USA) according to the manufacturer's protocol. The glucose level was expressed in percentage (%) change from the initial glycaemia using the following formula:

$$\% \text{ glycaemia change} = \frac{G_x - G_o}{G_o} \times 100$$

$G_o$ is the glycaemia value at time zero after overnight fasting; $G_x$ is the glycaemia value at x minutes after vehicle or extract administration.

*Statistical analysis*

Experimental results are expressed as mean ± SD in triplicate. The data were analysed by ANOVA ($p<0.05$) and means separated by Duncan's multiple range tests (by SPSS version 13 software).

## Results

Acute toxicity studies revealed that non-toxic nature of the spray-dried V. amgydalina water extract on normal healthy male mice even up to 5000 mg/kg body weight. No mortality was observed in the extract-treated mice and behaviour of all the experimental groups appeared the same and normal up to day 14.

The hypoglycaemic effect on fasting normal mice is shown in Figure 1. Fasting blood glucose of mice treated with PBS fluctuated during 180 minute monitoring period. Both *M. charantia* and *V. amygdalina* extract caused a significant raise of blood glucose at 30 minute time point of the test. After that, the blood glucose level of all extracts treated mice steadily revert back to initial glucose level. Increase of blood glucose of mice treated with 10 mg/kg V. amygdalina was significantly less when compared

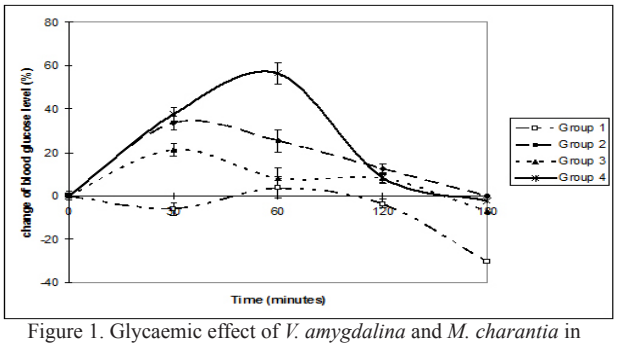


Figure 1. Glycaemic effect of *V. amygdalina* and *M. charantia* in fasting normal mice

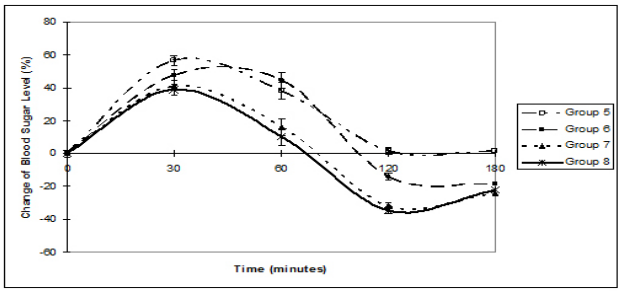


Figure 2. Effect of *V. amygdalina* and *M. charantia* on oral glucose tolerance in fasting diet induced diabetic mice

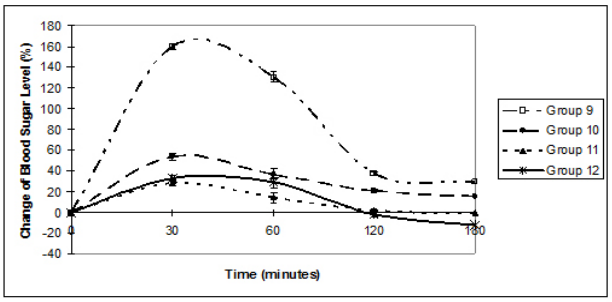


Figure 3. Effect of *V. amygdalina* and *M. charantia* on oral glucose tolerance in fasting STZ induced diabetic mice

with mice treated with 50 mg/kg *V. amygdalina* and *M. charantia*.

Glucose induced less blood sugar raised on *V. amygdalina* treated diet induced diabetic mice compared to *M. charantia* treated and control diabetic mice (Figure 2). The peak blood glucose concentration then fell to the initial blood glucose much faster on mice treated with *V. amygdalina* and remain lower then the initial blood glucose during the 180 minute testing period.

Figure 3 showed that both *V. amygdalina* and *M. charantia* significantly maintained the blood glucose level of STZ induced diabetic mice than untreated group (Group 9). Blood glucose of untreated mice rose from initial level to the peak value at 30 minutes time point and steady at 40% rose after 120 minute of oral glucose tolerance test. None of the groups were observed with drop of glucose level lower than initial.

## Discussion

In this study, *V. amgydalina* did not induce any mortality up to 5g/kg b.w. on mice. Traditionally, *V. amygdalina* was consumed as vegetable as a source of protein in Africa (Ndaeyo, 2007). The acute toxicology study has confirmed that it can be classified as safe to be consumed.

Previous study has showed that *V. amygdalina* aqueous extract was able to induced hypoglycemic effect on non-diabetic rat (Taiwo *et al*., 2009). However, in this study, both *V. amygdalina* and *M. charantia* were observed with raised glucose level in fasting normal mice. This may be due to the present of maltodextrin which was added as carrier during spray drying process of *V. amygdalina*. For *M. charantia*, maltodextrin is a common bulking agent in preparing supplement capsule. Thus, it is observed that higher concentration of *V. amygdalin*a extract induced higher level of glucose rise compared to untreated normal mice. Diabetes mellitus is a pathologic condition cause by severe metabolic imbalances and non-physiologic changes due to oxidative stress in pancreas islets which was created by persistent and chronic hyperglycemia and thus damages the pancreatic β cells (Arulselvan and Subramanian, 2007). Unlike some of the anti-diabetic drug, such as tolbutamide, which reduces blood glucose through increasing insulin secretion and sensitivity, V. amydalina and *M. charantia* was able to reduce blood glucose level on both diet induced and STZ induced diabetic mice. Sesquiterpene lactones and the bitter principles of the extract were proposed to have contributed in stimulation of insulin sensitisation that caused hyperglycemic effect (Osinubi, 2007). STZ caused cytotoxic effect on pancreatic β cells which further induced insulin dependent diabetes. This study also showed that the secondary metabolites in *V. amygdalina* may also replace insulin which cannot being produced in STZ treated mice to metabolise glucose. Moreover, the sesquiterpene lactones, bitter principles and phenolic compounds in *V. amygdalina* were also believed to initiate the beta cell regeneration through their antioxidant activity. Thus, *V. amygdalina* was suitable to be used in managing both type I and type II diabetes mellitus.

## Conclusion

This study has suggested that spray-dried *V. amygdalina* aqueous extract was suitable for managing diabetes. Furthermore, it was also safe to be consumed by normal subject without inducing severe hypoglycemic and toxic side effect. Further

study on long term administration on type I and type II diabetes management should be carried out in future.

## References

Arulselvan, P. and Subramanian, S.P. 2007. Beneficial effects of Murraya koenigii leaves ono antioxidant defense system and ultra structural changes of pancreatic β-cells in experimental diabetes in rats. Chemico-Biological Interactions 165: 155-164.

Igbakin, A.P. and Oloyede, O.B. 2009. Comparative studies on the hypoglycaemic, hypoproteinaemic, hypocholesterolaemic and hypolipidaemic properties of ethanolic and normal saline extracts of the root of *Vernonia amygdalin*a in diabetic rats. Advances in Environmental Biology 3: 33-38.

Liu, X.P., Cao, Y., Kong, H.-Y., Zhu, W.-F., Wang, G.-F., Zhang, J.-J., Qiu, Y.-C. and Pang, J.-X. 2010. Antihyperglycemic and antihyperlipidemic effect of *Uraria crinita* water extract in diabetic mice induced by STZ and food. Journal of Medicinal Plants Research 4: 370-374.

Ndaeyo, N.U. 2007. Assessing the contributions of homestead farming to food security in a developing economy: A case study of Southeastern Nigeria. Journal of Agriculture and Social Sciences 3:11-16.

Nwanjo, H.U. 2005. Efficacy of aqueous leaf extract of *Vernonia amygdalina* on plasma lipoprotein and oxidative status in diabetic rat models. Nigerian Journal of Physiological Sciences 20:39-42.

Osinubi, A.A.A. 2007. Effects of *Vernonia amygdalina* and chlorpropamide on blood glucose. Medical Journal of Islamic World Academy of Sciences 16:115-119.

Lee, Y.P. and Loh, F.F. 2010. Sweet malady: Diabetes among children on the rise. Star 24/7/2010 Page 1.

Taiwo, I.A., Odeigah, P.G.C. and Ogunkanmi, L.A. 2009. The glycaemic effects of *Vernonia amygdalina* and *Vernonia tenoreana* with tolbutamide in rats and the implications for the treatment of diabetes mellitus. Journal of Scientific Research and Development 11: 122-130.

Tan, M.J., Ye, J.M., Turner, N., Hohnen-Behrens, C., Ke, C.Q., Tang, C.P., Chen, T., Weiss, H.C., Gesing, E.R., Rowland, A., James, D.E. and Ye, Y. 2008. Antidiabetic activities of triterpenoids isolated from bitter melon associated with activation of the AMPK pathway. Chemistry and Biology 15: 263-273.

Viollet, B., Lantier, L., Devin-Leclerc, J., Hebrard, S., Amouyal, C., Mounier, R., Foretz, M. and Andreelli, F. 2009. Targeting the AMPK pathway for the treatment of type 2 diabetes. Front Bioscience 14: 3380-3400.