The Journal of Phytopharmacology 2015; 4(1): 30-33
Online at: www.phytopharmajournal.com

Research Article

ISSN 2230-480X
JPHYTO 2015; 4(1): 30-33
January- February

Joan Murugi Njagi
Department of Environmental Health, Kenyatta University, P.O. Box 43844-00100 Nairobi, Kenya

Mathew Piero Ngugi
Department of Biochemistry and Biotechnology, Kenyatta University, P.O. Box 43844-00100 Nairobi, Kenya

Cromwell Mwiti Kibiti
Department of Pure and Applied Sciences, Technical University of Mombasa, P.O Box 90420-80100 Mombasa, Kenya

Joseph Ngeranwa
Department of Biochemistry and Biotechnology, Kenyatta University, P.O. Box 43844-00100 Nairobi, Kenya

Wilson Njue
Department of Chemistry, Kenyatta University, P.O. Box 43844-00100 Nairobi, Kenya

Peter Gathumbi
Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi, P.O. Box 29053-00625 Nairobi, Kenya

Eliud Njagi
Department of Biochemistry and Biotechnology, Kenyatta University, P.O. Box 43844-00100 Nairobi, Kenya

Correspondence:
Joan Murugi Njagi
Department of Environmental Health, Kenyatta University, P.O. Box 43844-00100 Nairobi, Kenya

Hypoglycemic effect of *Helichrysum odoratissimum* in alloxan induced diabetic mice

Joan Murugi Njagi, Mathew Piero Ngugi, Cromwell Mwiti Kibiti, Joseph Ngeranwa, Wilson Njue, Peter Gathumbi, Eliud Njagi*

Abstract

Diabetes mellitus is a disease of antiquity with worrying global incidence and prevalence. Conventional management and/or treatment interventions have been hampered by drawbacks like high costs, inaccessibility, likelihood of potential adverse and toxic effects among others. Use of antidiabetic phytodrugs has been on the rise, particularly in the developing countries, perhaps due to cost implications and accessibility advantages. In this study, an aqueous leaf extract of the medicinal plant *Helichrysum odoratissimum* was bioscreened for their hypoglycemic potential in alloxan-induced diabetic mice. Three dose ranges were used viz; 50 mg/kgbwt, 100 mg/kgbwt and 150 mg/kgbwt. Results indicate that the extract appreciably lowered blood glucose level in the diabetic mice. The glucose lowering potency of this extract was dose dependent. Preliminary in vivo toxicity assessment revealed that the plant has no discernible toxicity on the major organs of the study animals. The study results confirm the folklore reports from traditional medical practitioners that the extract has safe bioactivity against diabetes mellitus. It avails an impetus for further bioscreening efforts with a view to the development of more hypoglycemic agents in future.

Keywords: Diabetes mellitus, Diabetic mice, *In-vivo* toxicity, Aqueous leaf extract.

Introduction

Diabetes mellitus is a metabolic disorder in which a relative or absolute deficiency or lack of effect of insulin leads to chronic hyperglycemia with or without glucosuria. It has probably been known to medical science longer than any other ailment, yet, in many respects it is still poorly understood.[1] It is characterized by hyperglycemia (high blood glucose) and occurs because the liver and skeletal muscle cannot store glycogen and the tissues are unable to utilize glucose.[2] This is caused by disturbances in the regulatory systems responsible for the storage and utilization of the chemical energy from food. This includes the metabolism of carbohydrates, fats and proteins, resulting from defects in insulin secretion, insulin action, or both.[3] An estimated 171 million people were suffering from diabetes in 2000, and this number could total 366 million by 2030.[4]

The rising prevalence of diabetes and higher rate of long term complication, especially in will lead to a drastic increase of the burden of diabetes on the health systems.[5] Majority available conventional drugs used for the management of the disease are bedeviled by prohibitive costs, need for expertise in prescription and administration and numerous side effects, which are precursors of complications. For example sulfonylureas and metformin are valuable treatments for hyperglycaemia in NIDDM, but they are often unable to lower glucose concentrations to within the normal range, or to reinstate a normal pattern of glucose homeostasis.[1]

Herbal medications have found profound use in ameliorating diabetic complications. Safe for the fact that their *in vivo* safety still needs to be optimized, these plant derived preparations are cheap and easily accessible. The known use of plants for diabetes dates from the Ebers papyrus of about 1550BC and many traditional plant treatments for diabetes are used throughout the world.[1,6] After the introduction of insulin therapy, the use of traditional treatments for diabetes greatly declined in societies, although some plant extracts are still used as prophylactics and adjuncts to conventional medicine.[1] Against this background, aqueous leaf extracts of *Helichrysum odoratissimum* were assessed for their potential to lower blood glucose levels in alloxan-induced diabetic Swiss albino mice.

Materials and Methods

Collection of plant materials

Leaves of *Helichrysum odoratissimum* were collected from their natural habitat Mbeere North district of Embu county, Kenya, based on the folklore reports from the practising herbalists on the medicinal potential of the plant. A taxonomist identified the plant and voucher specimen was deposited at the National Museum of Kenya herbarium. The voucher specimen number of the plant was *Diab 4/06*. The leaves were cut into small pieces and air-dried at 25±3°C away under a shade for three weeks. The dried materials were then crushed into powder by use of an electric mill (Christy and Norris Ltd, England).

Extract preparation

100g of crushed leaf material of *H. odoratissimum* were boiled in one liter of distilled water for two hours with frequent stirring, following which the mixture was cooled at room temperature and then decanted into a dry clean conical flask. After decantation, the leaf extract was filtered with Whatmann no. 1 filter papers using a vacuum pump. The filtrate was then freeze-dried for three days (72 hours). The ensuing powder was stored at 4°C in airtight containers.

Dosage preparation for administration

Normal saline was used as the vehicle for administration. It was prepared by dissolving 0.85 g of NaCl in 100 ml of double-distilled water. Aqueous leaf extract doses were prepared as follows: for 50 mg/kg body weight dose, 12.5 mg was dissolved in 1ml of physiological saline; for 100 mg/kg body weight dose, 25 mg was dissolved in 1 ml of physiological saline; and for 150 mg/kg body weight dose, 37.5 mg was dissolved in 1ml of physiological saline. The study animals were given 0.1 ml of the extract solutions. Insulin was also reconstituted and animals were given 0.1 ml.

Bioassay for hypoglycemic activity

Study animals

Male Swiss albino mice aged 4-5 weeks and weighing 25-30 g were used for the study. They were feeding on the standard mice pellet diet and had access to water *ad libitum*. Permission for handling mice in this study was sought and availed by animal rights agency in Nairobi, Kenya (Ethical reference number: 98/12/2012). The study animals were fasted overnight and allowed free access to water. They were divided into 4 groups of five animals each. Group 1 (Non-diabetic mice) was given 0.1ml of normal saline; Group 2, (diabetic mice) was given 0.1ml of normal saline; Group 3, (diabetic mice) was given insulin at a dose of 1 IU/kg body weight; and Group 4, (diabetic mice) was given aqueous leaf extracts of *Helichrysum odoratissimum* at three dose levels (50 mg/kg body weight, 100mg/kg body weight and 150 mg/kg body weight). Each dose level was given to 4 animals. Group 1 and 2 served as controls, whereas Group 3 served as the reference group. Diabetic condition was induced in mice by intraperitoneal injection of alloxan monohydrate (150 mg/kgbw) 72 hours before the start of the experiment. Before administration of the different treatments the animals were bled and blood glucose level in the animals was measured. This was the initial measurement at time zero. The animals were again bled hourly until the fourth hour.

Blood glucose determination

Blood samples were collected by squeezing the tails of the animals, following which a drop of blood was placed onto a Glucometer strip. The hypoguard machine was used together with GB Supreme blood glucose test strips for blood glucose assay. For all experimental groups, the blood sugar levels were taken at 0 hour and after one hour for four hours.

Preliminary in vivo toxicity analysis

Twenty mice were divided into two groups of ten animals each for preliminary *in vivo* toxicity analysis. The first group was treated with normal saline for thirty days and served as controls. The other group was treated with 450 mg /kg body weight of the extract for thirty days. The animals were observed closely and regularly, and fed on standard mice pellets and water *ad libitum*. Any animal that died or showed signs of death was sacrificed. The animals that survived 30 days of administration were put to sleep using dry ice and sacrificed. The animals were dissected and pieces of pancreas, heart, kidney, muscle and livers were removed and preserved in 10 % formalin for histological preparation and observation, following which they were microscopically examined for pathological changes.

Data analysis

In the *in vivo* hypoglycemic assays, unpaired student's t-test was used to evaluate the significance between means of extract treated animals and the diabetic control, reference control and the normal control. The data was represented as means ± SEM. $P<0.05$ was considered statistically significant. Minitab statistical computer package was used.

Results and Discussion

Effect of *Helichrysum odoratissimum* on blood glucose in alloxan-induced diabetic mice

In the 1st hour, the aqueous leaf extracts of *Helichrysum odoratissimum* at the three dose ranges did not significantly lower blood glucose levels (Table 1; Figure 1). In the 2nd hour, the three dose levels lowered the blood sugar levels by 30%, 54%, and 54%, respectively. At this hour, the extract lowered blood glucose levels to normal, but not as effectively as insulin ($^{a}p<0.05$; $^{b}p<0.05$). In the 3rd hour, the percent reduction of blood glucose level in the three dose levels was 26%, 62%, and 70%, respectively. The 50 and 100 mg/kg body weight dose range lowered the blood glucose level as they did in the 2nd hour (Table 1). On the other hand, the 150 mg/kg body weight dose range lowered blood sugar level to below normal and as effective as insulin (Figure 1). In the fourth hour, the extract continued producing the hypoglycemic activity in a dose dependent manner by 38%, 67% and 75%, respectively. At this point, the 100 and 150 mg/kg body weight dose ranges lowered blood glucose levels to below normal and the hypoglycemic potential of the dose of 150 mg/kg body weight was comparable to that of insulin. The 50 and 100 mg/kg body weight doses significantly lowered blood glucose levels, but not as effectively as insulin.

Similar work carried out by[7] demonstrated hypoglycemic activity in alloxan-induced diabetic mice on administration of aqueous leaf extracts of five Kenyan plants.

The leaf extracts of *H. odoratissimum* showed a non-dose dependent response. This trend was equally seen by[5], who demonstrated non-dose dependence hypoglycemic effects of aqueous stem bark extracts of *K. squarossa* in alloxan-induced diabetic mice. This observation could suggest that the extract could have been actively absorbed in the cell system.

Previous studies done with ethanolic seed extracts of *Luffa aegyptiaca* and leaves of *Carissa edulis* have been shown to significantly lower blood glucose levels in streptozotocin-induced diabetic rats.[5,8,9] [9]showed that ethanolic leaf extracts of *Cassia kleinii* could lower blood glucose in streptozotocin-induced diabetic rats.[9,10]

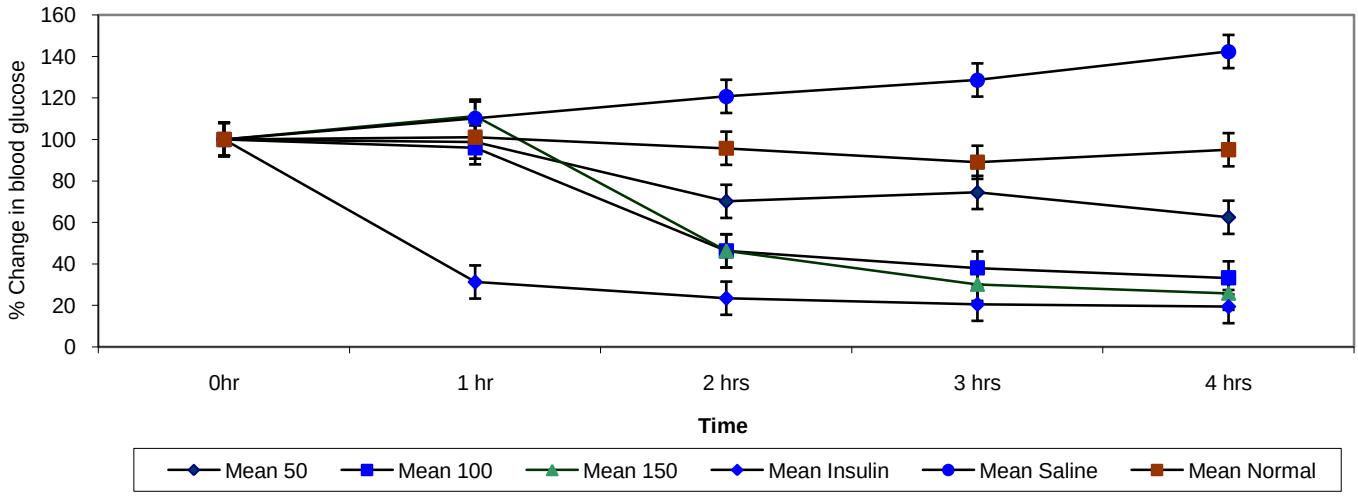
The mode of action of the extract may have by insulinomimetism as is the mode of action of some oral hypoglycaemics a biguanids, which relieve hyperglycemia by lowering hepatic gluconeogenesis, upregulating skeletal muscle glucose uptake, and limiting plasma triacylglycerols.[11] This was also proposed by[7] in the studies of hypoglycemic medicinal plants in Kenya. Further, the extract is thought to have ameliorated the blood glucose levels in alloxan-induced diabetic mice by stimulating glucose catabolic enzymes and inhibiting gluconeogenic enzymes. This has previously been seen

by[12], who observed the same mode of action by *C. roseus* in streptozotocin-induced diabetic rats.

Table 1: Effects of *H. odoratissimum* extract on blood glucose levels in alloxan induced diabetic mice (mg/dl)

	Treatment	0 hr	1 hr	2 hr	3 hr	4 hr
Normal	Saline	67.5±4.0	68.0± 2.9	63.8±2.7	59.5±1.6	63.8± 2.4
Diabetic	Saline	155.8±11.0	171.0±9.4	188.0±14.3	200.3±14.8	221.8± 15.9
Diabetic	Insulin 1IU/kgbw	191.0±7.5	59.5±1.9	44.8±2.4	39.3±2.4	37 .0± 1.4
Diabetic	*H.odoratissimum* 50mg/kgbw	121.0±12.1	122.3±23.8^{b*}	83.5±10.0^{ab}	93.3±21.9^{ab}	77.3± 4.3^{ab*}
Diabetic	*H.odoratissimum* 100mg/kgbw	175.0±24.9	170.5±29^{b*}	79.8±10.0^{ab}	58.8±3.6^{ab}	54.5± 2.7^{ab*}
Diabetic	*H.odoratissimum* 150mg/kgbw	150.5±5.4	167.0± 4.6^{b*}	69.8±8.1^{ab}	45.5±3.6^{a*}	38.8± 1.1^{a*}

*P<0.05 with respect to normal control; ^{a}P<0.05 with respect to diabetic control; ^{b} P<0.05 with respect to insulin. The data was analyzed using Student's 't'– test. All the results were expressed as mean ± SEM. All blood glucose levels were recorded in mg/dl.



*P<0.05 with respect to normal control; aP<0.05 with respect to diabetic control; b P<0.05 with respect to insulin. The data was analyzed using student's t– test

Figure 1: Percentage reduction in blood glucose by varying doses of *H. odoratissimum* in diabetic mice

Preliminary in vivo toxicity analysis

Animals treated with the leaf extract of *Helichrysum odoratissimum* showed mild perivascular inflammation in the kidneys, but the renal cells were intact (Figure 2). The spleen tissue cells were intact apart from mild lymphoid depopulation. The liver had no signs of pathology, the hepatocytes were intact, but there was mild perihepatitis. The heart muscle had no pathology.

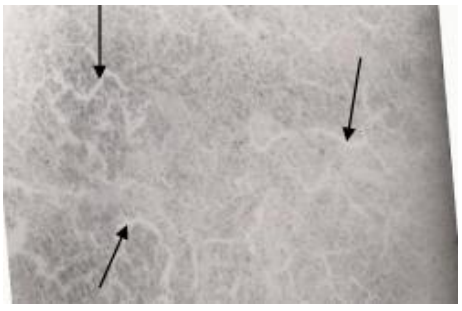


Figure 2: Histological section of a spleen of a mouse treated with an aqueous stem bark extract of *Helichrysum odoratissimum* (450 mg/kgbw). Reduction in lymphoid population manifested by absence of follicles (arrows) Exposure time: 30 days. Magnification: X100

That no discernible pathological lesions were seen in mice administered with high doses of the leaf extracts of *H. odoratissimum* is a welcome indication that the extract is a safe herbal alternative in management of diabetes mellitus. The inflammation observed at the site of injection may simply be attributed to drug induced reactions.[13]

Conclusion

This study has impeccably demonstrated safe use of leaf extracts of *H. odoratissimum* in management of diabetes mellitus in alloxan-induced diabetic mice. Further studies on this plant will be necessary using the oral route of administration since pharmacological hypoglycemic is administered orally. Further, the use of organic extracts would also reveal classes of organic secondary metabolites in this plant that may be hypoglycemic. Nevertheless, the desired objective of this study was accomplished

Acknowledgements

This study was undertaken in Kenyatta University and the University of Nairobi, for which reason they are hereby acknowledged. Moral and material support availed by the Late Solomon Buleti, Mr. Mugeki, Mrs. Rose Gitari and Mr. Jackson Gachoka is acknowledged with gratitude.

Conflict of interest

No conflict between the authors.

References

1. Abdulgader DH. Plant extracts as treatment for diabetes mellitus. 1996, PhD thesis.

2. Piero MN, Njagi JM, Kibiti CM, Ngeranwa JJN, Njagi ENM, Njue WM, Gathumbi PK. Herbal management of diabetes mellitus: a rapidly expanding research avenue. International Journal of Current Pharmaceutical Research, 2012; 4(2):1-4.

3. Murugi NJ, Piero MN, Kibiti CM, Ngeranwa JJN, Njagi ENM, Njue WM, Maina D, Gathumbi PK. Hypoglycemic effects of *Caesalpinia volkensii* on alloxan-induced diabetic mice. Asian Journal of Pharmaceutical and Clinical Research, 2012; 5(2):69-74.

4. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care, 2004; 27:1047–1053.

5. Murugi NJ, Piero MN, Kibiti CM, Ngeranwa JJN, Njagi ENM, Njue WM, Maina D, Gathumbi PK. Evaluation of antidiabetic effects of *Kleinia squarrosa* on alloxanized diabetic mice. Asian Journal of Biochemical and Pharmaceutical Research, 2012; 2(2):54-60.

6. Bailey CJ, Day C. Traditional plant medicines as treatments for diabetes. Diabetes Care, 1989; 12:553-564.

7. Piero MN, Njagi JM, Kibiti CM, Ngeranwa JJN, Njagi ENM, Njue WM, Maina D, Gathumbi PK. Hypoglycemic activity of some kenyan plants traditionally used to manage diabetes mellitus in Eastern Province. Journal of Diabetes and Metabolism, 2011; 2:155.

8. El-Fiky FK, Abou-Karam MA, Afify EA. Effect of *Luffa aegyptiaca* (seeds) and *Carissa edulis* (leaves) extracts on blood glucose level of normal and streptozotocin diabetic rats. Journal of Ethnopharmacology, 1996; 50(1):43-47.

9. Babu V, Gangadevi T, Subramoniam A. Antidiabetic activity of ethanol extract of *Cassia kleinii* leaf in streptozotocin-induced diabetic rats and isolation of an active fraction and toxicity evaluation of the extract. Ind J Pharmacol, 2003; 35:290-296.

10. Piero MN. Hypoglycemic effects of some Kenyan plants used in management of diabetes mellitus in eastern province, MSc. thesis, 2006, Kenyatta University, Kenya.

11. Scott RV. Diabetes Mellitus, Type 2 - A Review. 2004.

12. Singh SN, Vats P, Suri S, Shyan R, Kumria MML, Ranganathan S, Sridharan K. Effects of an antidiabetic extract of *Catharanthus roseus* on enzyme activities in streptozotocin induced diabetic rats. Journal of Ethnopharmacology, 2001; 76:269-277.

13. Paumgartten FJ, Delgado IF, Alves EN, Nogueira AC, Defaris RC, Neubert D. Single dose toxicity study of beta-myrcene, a natural analgesic substance. Brazilian Journal of Medical Biology Reviews, 1990; 32:837-839.