SCIENCEDOMAIN

Seasonal Variation in the Anti-Diabetic and Hypolipidemic Effects of *Momordica charantia* Fruit Extract in Rats

O. T. Kolawole[1*] and A. A. Ayankunle[1]

[1]*Department of Pharmacology & Therapeutics, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.*

ABSTRACT

Aims: To investigate seasonal variation in anti-diabetic and hypolipidemic activities of *Momordica charantia* fruits harvested at different seasons of the year, namely spring, summer, autumn and winter.
Methodology: Air-dried and pulverized fruit samples were extracted by soaking in 70% methanol for 72h. The filtrate was concentrated using rotary evaporator. The yields of spring (MME), summer (JME), autumn (SME) and winter (DME) samples were 8.4, 7.1, 4.8 and 5.1% respectively. For each of the four fruit samples, rats were divided into six groups of six rats each. First group served as normal control (non-diabetic). The remaining five groups were made diabetic by administering alloxan (120mg/kg body weight) intraperitoneally. Second group served as diabetic control. Third, fourth and fifth groups were treated with oral doses of 200, 400 and 600mg/kg body weight of *Momordica charantia* fruit extracts respectively. The sixth group received oral dose of glibenclamide (5mg/kg body weight) which served as the standard drug. These treatments were repeated daily for 28 days.
Results: Treatment with methanol extracts of *Momordica charantia* caused a significant (p<0.01) and dose-dependent changes with respect to blood glucose level and lipid profile in all the four samples, when compared with the untreated animals. The highest activity was observed with spring sample, followed by the summer sample. Autumn and winter samples have more or less similar but lesser effects than summer sample.
Conclusion: The results of this study showed that anti-diabetic and hypolipidemic effects of *Momordica charantia* fruit extract vary during different seasons of the year. The spring sample produced the highest activity. This suggests that the active principles in *Momordica charantia* fruit that are responsible for its antidiabetic and hypolipidemic

_____

*Corresponding author: Email: tymkol@yahoo.co.uk;

activity vary in quantity and/or quality during different seasons of the year and reach the peak during spring.

1. INTRODUCTION

The active principles or constituents (phytochemicals) in medicinal plants are chemical compounds known as secondary plant products. These secondary metabolites are biosynthetically derived from primary metabolism and accumulated by plants in small quantity. Many of these active principles have therapeutic values, and as such, medicinal plants are widely prescribed for the prevention and treatment of various diseases. Plants have been used as source of medicine throughout human history and they continue to serve as the basis for many pharmaceuticals used today (Newman and Cragg, 2007). However climatic conditions may have significant impact on the availability of the active constituents in medicinal plants. Phytochemical constituents of some plants have been shown to vary quantitatively at different seasons of the year. Therefore therapeutic efficacy of medicinal plants is also likely to vary during different seasons of the year (Singh, 2008). For this reason, the quality and uniformity of the chemical constituents and their concentrations in medicinal plants should be taken into consideration when assessing the safety and effectiveness of herbal remedies (Ginsburg and Deharo, 2011).

One of the medicinal plants that have shown promising efficacy in the treatment of diabetes mellitus and other diseases is *Momordica charantia* Linn. (Cucurbitaceae). It is also known as karela or bitter melon. The hypoglycemic activity of *Momordica charantia* has been reported in experimental animals (Grover and Yadav, 2004; Krawinkel and Keding, 2006). Recently, cucurbitane-type triterpenoids were isolated from the stem of *Momordica charantia* and its antioxidant activity was demonstrated (Liu et al., 2010). The impact of different seasons on the hypoglycemic effect of *Momordica charantia* has not been studied. Knowing the season of the year in which the plant has optimum activity will promote its usefulness as anti-diabetic remedy. To fill this gap in literature, the present study investigated the effect of different seasons on the hypoglycemic activity of *Momordica charantia*.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials and Extraction Process

The study was conducted between March, 2010 and January, 2011. Fruits of *Momordica charantia* were collected from Apete in Ibadan, Nigeria and authenticated in the herbarium of Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria, where voucher specimen was deposited (Voucher sample no.: FHI 107759). The samples were collected during spring (March) when temperature was 28°C and rainfall was 294mm, summer (June) when temperature was 22°C and rainfall was 836mm, autumn (September) when temperature was 25°C and rainfall was 575mm and winter (December) when temperature was 32°C and rainfall was 180mm. The fresh fruits were washed with clean water to remove particles and debris and then dried in the shade for one week. The dry samples were blended into fine powder using electric blending machine. One hundred and fifty grams (150g) portion of each

of the four samples were separately extracted in 70% methanol by soaking in the solvent over a period of three days. The extracts were then filtered using Whatman filter paper (No. 1). The filtrates were then concentrated in vacuum at 40°C using a rotary evaporator. The solid samples obtained were designated MME (spring sample), JME (summer sample), SME (autumn sample) and DME (winter sample). The yields of the samples were 8.4, 7.1, 4.8 and 5.1% respectively. These solid samples were stored at 4°C in a refrigerator for the study.

2.2 Experimental Animals

Male Wistar rats weighing between 150 and 200g were obtained from the Animal House of the College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The animals were kept in a well- ventilated and hygienic environment, with free access to standard feed pellets and water *ad libitum*. The ethical guideline and procedure for handling experimental animals were followed in the study (NIH, 1985).

2.3 Induction of Diabetes Mellitus

Rats were allowed to fast for 24h after which they were given 120mg/kg body weight of alloxan monohydrate (Sigma, U.S.A) intraperitoneally as a single dose to induce diabetes (Trivedi et al., 2004).The rats were kept on 5% glucose solution to prevent hypoglycemia. A week after the administration of alloxan, the fasting blood glucose levels of the rats were measured and rats with blood glucose >200mg/dl were considered diabetic and were used for the experiments.

2.4 Experimental Procedure

Six groups of animals were used for the experiments (6 rats per group). Group I served as the normal control (non-diabetic). Groups II -VI were alloxan-diabetic rats. They were treated as follow:

Group I (normal control) = distilled water daily.
Group II (diabetic control) = a single dose of alloxan (120mg/kg) and daily dose of distilled water.
Group III = a single dose of alloxan + 200mg/kg fruit extract daily.
Group IV = a single dose of alloxan + 400mg/kg fruit extract daily.
Group V = a single dose of alloxan + 600mg/kg fruit extract daily.
Group VI = a single dose of alloxan + 5mg/kg glibenclamide daily.

This daily treatment was repeated for 28 days. Animals were sacrificed on the 28th day (2hr after the last treatment) by cervical dislocation. Blood was collected and serum was separated by centrifugation at 3000 rpm for 10 min. Fasting blood glucose levels were determined using One Touch Basic Blood Glucose Monitoring System (LifeScan Inc., Milpitas, California, U.S.A). Total plasma cholesterol (TC), triglycerides (TG) and high density lipoprotein-cholesterol (HDL-c) were determined by enzymatic assay method using analytical kits (Biolabo SA, Maizy, France). Very low density lipoprotein-cholesterol (VLDL-c) and low density lipoprotein-cholesterol (LDL-c) were calculated using Friedewald's formula (Friedewald et al., 1972). Atherogenic index (AI) was calculated using the formula: AI = LDL-c / HDL-c (Abbot et al., 1988) and coronary risk index (CRI) was obtained by the formula: CRI = Total cholesterol / HDL-c (Alladi et al., 1989).

2.5 Statistical Analysis

The values obtained from the study were expressed as mean ± standard error of mean (SEM).Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. Probability (p) values<0.05 were considered significant.

3. RESULTS AND DISCUSSION

3.1 Effects of Extracts on Fasting Blood Glucose Level

Administration of the extracts for 28 days produced a dose-dependent decrease of fasting blood glucose with all the four samples of *Momordica charantia* fruit investigated. The decrease was significant (p<0.01) with 200, 400 and 600mg/kg body weight of the extracts from spring and summer samples. The effect was comparable with that produced by the standard drug, glibenclamide. The reduction in blood glucose level was not as pronounced with the extracts from autumn and winter samples (table 1-4). The anti-diabetic activity of the four samples was in the following order: MME > JME > SME ≤ DME.

Table 1. Effects of *Momordica charantia* methanol extract (spring sample) on blood glucose level and lipid profile

Group	Blood glucose level(mg/dl)		Lipid profile (mg/dl)			
	Alloxan induction	Day 28	TG	TC	HDL	LDL
I	82±2.2	80±3.1	62±1.6	74±1.3	29±1.9	32±2.1
II	241±2.9	223±2.6	132±0.2	113±2.2	14±2.4	72±1.2
III	237±2.4	139±2.2**	118±2.5	96±1.6	19±0.9	53±1.7
IV	246±1.4	130±2.8**	102±1.8*	88±3.1*	22±1.3*	45±2.6*
V	240±2.9	118±1.6**	73±2.5**	80±2.7*	28±1.4**	37±2.1**
VI	243±1.3	126±1.6**	71±2.8**	78±1.9**	30±1.0**	33±2.5**

*n=6; *p<0.05, **p<0.01 compared with the untreated diabetic animals*

Table 2.Effect of *Momordica charantia* methanol extract (summer sample) on blood glucose level and lipid profile

Group	Blood glucose level(mg/dl)		Lipid profile (mg/dl)			
	Alloxan induction	Day 28	TG	TC	HDL	LDL
I	86±1.3	84±1.6	67±1.3	78±2.6	24±0.8	40±1.2
II	228±2.7	219±3.2	126±2.9	122±2.6	17±1.5	79±1.8
III	239±2.7	157±1.6**	114±3.6	110±3.5	20±2.0	67±1.4
IV	220±2.3	120±1.1**	105±2.4*	10±1.0*	23±2.4	60±2.1*
V	225±0.9	108±2.2**	85±1.8*	98±2.7*	25±2.3*	55±1.1*
VI	244±2.2	125±1.9**	62±2.5**	95±0.5**	35±2.0**	48±1.6**

*n=6; *p<0.05, **p<0.01 compared with untreated diabetic animals*

Table 3. Effect of *Momordica charantia* methanol extract (autumn sample) on blood glucose level and lipid profile

Group	Blood glucose level (mg/dl)		Lipid profile (mg/dl)			
	Alloxan induction	Day 28	TG	TC	HDL	LDL
I	84±2.1	83±2.0	73±1.3	75±2.3	27±1.8	33±1.5
II	252±1.6	250±2.9	134±2.1	125±2.7	15±2.1	83±2.3
III	248±3.1	205±1.7	127±3.2	115±0.8	16±1.4	73±2.2
IV	247±1.6	172±2.5*	115±3.0*	109±1.7*	18±2.4	68±1.4*
V	238±2.4	150±2.7**	93±1.6*	100±1.2*	21±2.5*	59±0.4*
VI	248±1.9	127±2.2**	70±2.6**	92±1.8**	33±3.0**	45±1.5**

*n=6; *p<0.05, **p<0.01 compared with untreated diabetic animals*

Table 4. Effect of *Momordica charantia* methanol extract (winter sample) on blood glucose level and lipid profile

Group	Blood glucose level (mg/dl)		Lipid profile (mg/dl)			
	Alloxan induction	Day 28	TG	TC	HDL	LDL
I	89±1.4	88±1.0	66±1.3	74±1.6	26±0.8	35±1.8
II	243±2.4	245±2.8	139±3.0	129±2.1	16±2.0	84±0.8
III	240±1.9	199±3.1	131±0.4	119±2.5	18±2.6	75±1.4
IV	251±1.7	179±0.8*	120±1.3*	112±2.4*	20±1.2	68±2.2
V	244±3.1	158±2.6*	99±1.8*	102±2.5*	23±1.0*	59±0.9*
VI	239±2.3	121±1.7**	81±2.1**	94±2.3**	37±2.7*	40±1.8*

*n=6; *p<0.05, **p<0.01 compared with untreated diabetic animals*

3.2 Effects of Extracts on Lipid Profile

With repeated administration of glibenclamide and the extracts for 28 days, there was a dose-related reduction in the serum concentrations of total cholesterol (TC), triglyceride (TG) and low density lipoprotein cholesterol (LDL-c) and an elevation in the concentration of high density lipoprotein cholesterol (HDL-c). These changes were significant ($p<0.01$) especially with the spring sample which produced the highest activity that was similar to that of glibenclamide. With 600mg/kg body weight of the extracts, the atherogenic index (AI) for spring, summer, autumn and winter samples were 1.3, 2.1, 2.8 and 2.6 respectively. For glibenclamide, AI was 1.2. The corresponding coronary risk index (CRI) for spring, summer, autumn and winter samples were 2.8, 3.8, 4.6 and 4.4 respectively. CRI was 2.6 with glibenclamide.

In all the four samples tested, *Momordica charantia* fruit extracts produced a dose-dependent reduction in fasting blood glucose. This is consistent with many other reports on the hypoglycemic effect of the plant (Sathishsekar and Rajasekaran, 2007; Miura et al., 2001). Diabetes mellitus is usually associated with derangement in lipid metabolism resulting in hyperlipidemia (Odetola et al., 2006). The increase in cholesterol levels observed in diabetes mellitus is a consequence of accelerated fatty acid oxidation to acetyl coA which is involved in cholesterol synthesis (Adeneye and Olagunju, 2009). Since insulin/glucagon ratio

is low in diabetes mellitus, the function of lipoprotein lipase in clearing VLDL-cholesterol from the blood is compromised (Harris and Crabbs, 1982) and this leads to hypercholesterolemia. This certainly contributes to the development of cardiovascular diseases. The extracts of *Momordica charantia* reversed this situation in a dose-dependent manner. However, the extract of spring and summer samples showed more anti-diabetic effects than those of autumn and winter samples. Variation in the quantity or quality of the active compounds in *Momordica charantia* is most likely the cause of the significant differences observed in the hypoglycemic activities of the four samples of the plant. The prevailing environmental factors during different seasons of the year could directly or indirectly affect the availability of some precursors that the plant needs for the biosynthesis of the active ingredients which are responsible for its biological activities (Osadebe et al., 2008).

Several studies have investigated the effect of temperature changes on the production of secondary metabolites (Gairola et al., 2010). Many of these studies have reported that changes in temperature during different seasons of the year is one of the major environmental factors that can affect the amount of secondary metabolites and other bioactive compounds that medicinal plants produce (Salick et al., 2009). Generally, an increase in temperature stress leads to increased production of secondary metabolites (Loreto et al., 2006). This is because under such condition, plant growth is inhibited more than photosynthesis and more of the carbon fixed is made available for the production of these bioactive constituents (Mooney et al., 1991). Both high temperature and extremely low temperature can lead to this situation (Kala, 2009).

The extract of *Momordica charantia* fruits harvested in spring exerted the highest anti-diabetic effect probably because the active compound was at the peak concentration around that season of the year. Some earlier studies on other plants have reported seasonal changes in their pharmacological activities. For example, *Rosa damascene* was demonstrated to possess relatively low levels of antioxidant activities in spring and strong activities in autumn (Bahareh et al., 2010). *Macleaya microcarpa* is another plant that was shown to demonstrate seasonal variation of its bioactive alkaloid contents (Pencikova et al., 2011). Central nervous system activity of essential oil from *Ocimum gratissimum* also varies depending on the time of the year the plant is harvested (Freire et al., 2006).

The results of the present study showed that *Momordica charantia* is another medicinal plant that exhibits seasonal variation in its biological activity. Therefore to get optimal anti-diabetic effect, it seems best to harvest the fruit of *Momordica charantia* in spring or summer. We do not fully understand the mechanism by which *Momordica charantia* produces its anti-diabetic effect, but it has been reported to possess insulinomimetic effects (Fernandes et al., 2007). The fruit extract of bitter melon was shown to increase glucose uptake and it also up-regulated Glut-4, a glucose transporter (Kumar et al., 2009; Shih et al., 2009). Phytochemical constituents like p-insulin, charantin and visine are suspected to be responsible for its anti-diabetic property (Rao et al., 2001).

From this study, it was evident that none of the four samples tested was completely devoid of these active principles, thus the fruits can be harvested for its hypoglycemic benefits all year round. However, along with other factors such as geographical location and methods of collection and storage, environmental factors prevailing during different seasons of the year may affect the quality and uniformity of the chemical constituents and their concentrations in *Momordica charantia* fruits. This knowledge will help in cultivating the plant in a way that will ensure sustained and reliable sources of plant material which will contain the desired and expected amounts of pharmacologically active constituents. It was estimated that 80% of the

population of developing nations rely on herbal drugs for their primary health care needs including the treatment of diabetic mellitus (Shrestha and Dhillions, 2003). Medicinal plants are also major potential source of bioactive compounds for drug development (Gangwar et al., 2010). Therefore there is the need to initiate further studies that will address the effects of climate change on the production of secondary metabolites in *Momordica charantia* so as to ensure effective use of the plant for curing diabetes mellitus and other diseases.

4. CONCLUSION

The results of this study showed that anti-diabetic and hypolipidemic effects of *Momordica charantia* fruit extract vary during different seasons of the year. The spring sample produced the highest activity. This suggests that the active principles in *Momordica charantia* fruit that are responsible for its antidiabetic and hypolipidemic activity vary in quantity and/or quality during different seasons of the year and reach the peak during spring.

ACKNOWLEDGEMENTS

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Abbott, R.D., Wilson, P.W., Kannel, W.B., Castelli, W.P. (1988). High density lipoprotein-cholesterol, total cholesterol screening and myocardial infarction. The Frmingham Study Arterosclerosis, 8, 207-211.

Adeneye, A.A., Olagunju, J.A. (2009). Preliminary hypoglycemic and hypolipidemic activities of the aqueous seed extract of *Carica papaya* Linn. in Wistar rats. Biology and Medicine, 1(1), 1-10.

Alladi, S., Shanmugasundaram, K.R. (1989). Induction of hypercholesterolemia by supplementing soy protein with acetate generating amino-acids. Nutrition Reports International, 40(5), 893-899.

Bahareh, K., Tabaei-Aghdaei, S.R., Matinizadeh, M., Mousavi, A., Jafari, A., Mirjani, L. (2010). Seasonal changes in leaf and twig antioxidant systems in Damask rose (*Rosa damascena* Mill.). J. Med Plants Res., 4(23), 2574-2583.

Fernandes, N.P.C., Lagishetty, C.V., Panda, V.S., Naik, S.R. (2007).An experimental evaluation of the antidiabetic and antilipidemic properties of a standardized *Momordica charantia* fruit extract.BMC Complement Altern. Med., 7, 29.

Freire, C.M.M., Marques, M.O.M., Costa, M. (2006). Effects of seasonal variation on the central nervous system activity of *Ocimum gratissimum* L. essential oil. J. Ethnopharmacol, 105, 161 -166.

Friedwald, W.T., Levy, R.I., Fredickson, D.S. (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of preparative ultracentrifuge. Clin. Chem. 18, 499-502.

Gairola, S., Shariff, N.M., Batt, A., Kala, C.P. (2010). Influence of climate change on production of secondary chemicals in high altitude medicinal plants: Issues needs immediate attention. J. Med Plants Res., 4(18), 1825-1829.

Gangwar, K.K., Deepali, Gangwar R.S. (2010). Ethnomedicinal plant diversity in Kumaun Himalaya of Uttarakhand, India. Nat. Sci., 8(5), 66-78.

Ginsburg, H., Deharo, E. (2011). A call for using natural compounds in the development of new antimalarial treatments – an introduction. Malaria Journal, 10(1), 1 -12.

Grover, J.K., Yadav, S.P. (2004).Pharmacological action and potential uses of *Momordica charantia* – a review. J. Ethnopharmacol, 93, 123 – 132.

Harris, R.A., Crabbs, D.W. (1982). Metabolic interrelationships. In: Textbook of Biochemistry with Clinical Correlations. Ed. Delvin T.M, New York, John Wiley and Sons Inc. pp.531-559.

Kala, C.P. (2009). Medicinal plants conservation and enterprise development. Med. Plants, 1(2), 79-95.

Krawinkel, M.B., Keding, G. (2006). Bitter gourd (*Momordica charantia*): A dietary approach to hyperglycemia. Nutr. Rev., 64, 331 – 337.

Kumar, R.S., Balaji, T.S., Uma, Sehgal, P.K. (2009). Fruit extracts of *Momordica charantia* potentiate glucose uptake and up-regulate Glut-4, PPARγ and P13K. J. Ethnopharmacol, 126, 533-537.

Liu, C., Yen, M., Tsang, S., Gan, K., Hsu, H., Lin, C. (2010). Antioxidant triterpenoids from the stem of *Momordica charantia*. Food Chem. Toxicol, 11, 751 – 756.

Loreto, F., Barta, C., Brilli, F., Nogues, I. (2006). On the induction of volatile organic compound emissions by plants as consequence of wounding or fluctuations of light and temperature. Plant Cell Environ., 29, 1820-1828.

Miura, T.C., Itoh, N., Iwamoto, M., Kato, M., Kawai, S.R., Park, I. Suzuki. (2001). Hypoglycemic activity of the fruit of the *Momordica charantia* in type 2 diabetes in mice. J. Nutr. Sci. Vitaminol., 47, 240-344.

Mooney, H.A., Winner, W.E., Pell, E.J. (1991).Response of plants to multiple stresses. Academic Press, San Diego, California, USA.

Newman, D.J., Cragg, G.M. (2007).Natural products as sources of new drugs over the last 25 years. J Nat Prod., 70, 461 – 477.

NIH. (1985). Principles of Laboratory Animal Care. No. 85-23 revised.

Odetola, A.A., Akinloye, O., Egunjobi, C., Adekunle, W.A., Ayoola, A.O. (2006). Possible antidiabetic and anthyperlipidemic effect of fermented *Parkia biglobosa* (JACQ) extract in alloxan- induced diabetic rats. Clin. Exp. Pharmacol. Physiol, 33, 808-812.

Osadebe, P.O., Dieke, C.A., Okoye, F.B.C. (2008). A study of the seasonal variation in the antimicrobial constituents of the leaves of *Loranthus micranthus* sourced from *Percea americana*. Res. J. Medicinal Plant, 2, 48-52.

Pencikova, K., Urbanova, J., Musil, P., Taborska, E., Gregorova, J. (2011). Seasonal variations of bioactive alkaloid contents in *Macleaya microcarpa* (Maxim) Fedde. Molecules, 16(4), 3391-3401.

Rao, B.K., Kesavulu, M.M., Apparao, C. (2001). Anti-hyperglycemic activity of *Momordica cymbalaria* in alloxan diabetic rats. J. Ethnopharmacol, 78, 67-71.

Salick, J., Fangb, Z., Byg, A. (2009). Eastern Himalayan alpine plant ecology, Tibetan ethnobotany, and climate change. Global Environ. Change, 19(2), 147-155.

Sathishsekar, D., Rajasekaran, S. (2007). Protective role of *Momordica charantia* seeds extract on membrane bound ATPase and lysosomal hydrolases in rats with streptozotocin diabetes. J. Plant Sci., 2, 293-301.

Shih, C., Lin, C., Lin, W., Wu, J. (2009). *Momordica charantia* extract in insulin resistance and skeletal muscle Glut 4 protein in fructose-fed rats. J. Ethnopharmacol, 123, 82 – 90.

Shrestha, P.M., Dhillion, S.S. (2003). Medicinal plant diversity and use in the highlands of Dolakha district, Nepal. J. Ethnopharmacol, 86, 81-96.

Singh, A. (2008). A note on variation of active principles in Indian medicinal plants and TIM formulations. Ethnobotanical Leaflets, 12, 603 – 606.

Trivedi, N.A., Mazumdar, B., Bhatti, J.J., Hemavathi, K.G. (2004). Effect of shilagit on blood glucose and lipid profile in alloxan induced diabetic rats. Indian J Pharmacol., 36, 373-376.