Journal of Medical and Applied Biosciences *Volume 4, September 2012*

www.cenresinpub.org
ISSN 2277-0054

EFFECTS OF BI-HERBAL ETHANOLIC EXTRACT OF PHYLLANTHUS NIRURI AND MORINGA OLIEFERA ON THE PLASMA GLUCOSE LEVEL AND HEMATOLOGICAL PARAMETERSOF STREPTOZOTOCIN-INDUCED DIABETIC ALBINO RATS

Ezeonwu, Valentine Ugonna
Department of Biochemistry
Modibbo Adama University of Technology

ABSTRACT

Bi-ethanolic extract of *phyllanthus niruri* whole plant and *Ocimum gratissimum* leaves was evaluated for anti-diabetic activities in STZ-induced diabetes as well as its implications on some hematological parameters of the diabetic animals. Ethanolic extract from both herbs at dose levels of 150mg/kg, 200mg/kg and 300mg/kg orally administered once lowered blood sugar level of the animals within 2hours and 3hours after administration. The extracts showed significant reduction in the sugar levels of the diabetic animals as well as prevented weight loss due to diabetes in a dose dependent manner with 500mg/kg showing highest activity. The hematological studies of the animals after 14days of administration with the extracts showed no significant difference in the WBC, PCV and HB levels of all treated groups when compared with the control. The bi-herbal extract therefore not only possesses hypoglycemic property but also do not alter the hematological composition of the animals.
Keywords*: Phyllanthus niruri, Moringa oleifera, Hyperglycemia, Hematological parameters, STZ*

INTRODUCTION

Several herbs and their derivatives are known to have anti-diuretic properties. Phyllanthus niruri, a member of the euphobiaceae is a widely spread tropical plant commonly found in coastal areas that grows 40-70cm tall is a herb proven to have a wide range of therapeutic effects (Ezeonwu, 2011). Among these therapeutic effects include antidiuretic activity (Nwanjo, 2006), immune-stimulating effects (Adedapo et al; 2005) Hepatoprotective and antioxidant role as reported by Lee (2006) Chatterjee and Sil(2006). Phyllanthus niruri acts as a hypolipemic agent (Khanna et al, 2002), anti-lithic agent (Barros, 2003) (Freitas et al, 2002) anti-viral (Naik and juvekar, 2003) (lam, 2006) and anti-malarial properties (subeki 2005) (cimanga 2004). The plant is reported to have a very rich phytochemistry among which include flavonoids, alkaloids, glycosides, saponins and tannins (Ezeonwu, 2012). Phyllanthus niruri has been attributed to conflicting reports of toxicity. Antifertility activities have been associated with this plant in both male and female albino rats (Ezeonwu, 2011; rainforest-database.com) just as seen in many plants with anti-malarial property. Ezeonwu (2012) in a study to examine the implications of the herb on hematological parameters reported though that P. niruri did not affect WBC and PCV levels, it however lowered the HB level in all treated groups in a dose dependent manner. The plant has been reported to induce progressive weakness as observed across a group during treatment (Ezeonwu, 2011) though no reason was given for such observation. Moringa oleifera popularly known as the drumstick tree belong to the family moringaceae as the only member of that family. This is a plant that has been adjudged as one of the best plants with countless nutritional and

therapeutic benefits. Some authors have called this plant the miracle tree. Amongst these benefits reported are anthelmintic, antibiotic (Marcu, 2004), antimicrobial activities (Tilza et al; 2010), Hepatoprotective and antioxidant properties (Patel et al, 2008). Both plants have individually been reported to have hypoglycemic properties with a suggestion that P.niruri may be much more active; a bi-herbal treatment of P.niruri and M. oleifera in CCl_4-induced liver damage in albino rats shows potency in reversing the liver damage (Ezeonwu, 2012). However, there has been no documentation reporting the combination of both plants in the treatment of hyperglycemia. This study tries to investigate the activity of the bi-ethanolic extract of both herbs on STZ-induced diabetes as well as the hematological state of the treated groups.

MATERIALS AND METHODS

Animals

Twenty-fouralbino rats (200-250g) of either sex obtained from the animal house of the department of Medical Laboratory Science, University of Maiduguri were used for this study. The animals were housed in metal cages. They were allowed to acclimatize to the new environment for 3days before the commencement of study. Throughout the period of the experiment the animals were fed normal feed.

Preparation of Plant Extracts

Phyllanthus niruri whole plant and Moringa oleifera leaves were collected from the field. Both plant materials were shade dried for 12days before powdering using manual grinder. Equal amount of both plants (500g each plant) was sieved and extracted with 90% (v/v) ethanol as solvent by hot percolation for 8hours in soxhlets apparatus at 60^0C. The solvent was evaporated under controlled temperature. Appropriate weights of the residue were prepared to obtain the various concentrations used for the study.

Diabetics Inducement

After 3days of acclimatization, the rats were subjected to 12hours fast. With the use of a single intraperitoneal injection of freshly prepared STZ at a dose of 65mg/kg body weight, diabetes was induced. After 2days of administration, FBS were measured in the rats and animals with concentrations above 240mg/dl were classified diabetic.

Experimental Design

Six experimental groups of four albino rats were used in the experiment. Each group was treated and fed as follows.

Group A: served as the control and received nothing but normal feed and water

Group B: Received a single injection of STZ

Group C: Received a single injection of STZ before receiving 150mg/kg of the bi-ethanolic extract.

Group D: Received a single injection of STZ before receiving 200mg/kg of the bi-ethanolic extract.

Group E: Received a single injection of STZ before receiving 300mg/kg of the bi-ethanolic extract.
Group F: Received a single injection of STZ before receiving 500mg/kg of the bi-ethanolic extract.

Blood Collection

For FBS estimation which was carried out after a 24hour fast on all animals, blood was collected 2hours and 3hours after a single administration of the extracts through tail puncture respectively. At the end of the 2weeks treatment, the animals were sacrificed 18hours after the last dosage. Whole blood was collected by cardiac puncture and collected into heparinized tubes for hematological studies.

Fasting Blood Sugar and Hematological Analysis

Fasting Blood Sugar estimation was carried out on the first day of treatment of the animals with the extract after 2hours and 3hours respectively, while hematological analysis was carried out after 14days of treatment with the extract. One touch automated Glucometer (product of Lifescan Company) was used for estimation of the FBS levels. The counting of the White Blood Cells was done using the method described by Heiserman (2004). The Sahli's hemoglobinometer was used in Hb estimation while the macro hematocrit method described by Dacie and Lewis (2001) was used in Packed Cell Volume test.

Statistical Analysis

The results were expressed as mean ± standard error of mean (SEM). Statistical analysis of the data was done using student T-test

Results

Fasting blood Sugar Levels and Body Weight

Table I: Effects of bi-herbal ethanolic extract of phyllanthus niruri and Moringa oleifera on the plasma glucose level and body weight of streptozotocin-induced diabetic albino rats

Group	Treatment	Mean Fasting Blood Sugar Level (After 2hours) (mg/dl)	Mean Fasting Blood Sugar Level (after 3 hours) (mg/dl)	Mean body weight change (g)
A	Normal Control	56.55 ± 3.05	54.31 ±2.41	1.04 ±0.03
B	Diabetic Control	158.48 ± 6.08	155.17 ±4.12	2.10 ±1.01
C	Sample Treatment	161.37 ± 5.03	139.16 ±7.78*	0.13 ±0.55
D	Sample Treatment	133.32 ± 6.06*	108.03 ±3.31*	2.07 ±0.73
E	Sample Treatment	114.44 ± 4.21*	101.42 ±3.09*	1.11 ±0.02
F	Sample Treatment	99.03 ± 2.39*	81.95 ±6.13*	2.18 ±0.65

* ($P<0.05$) significantly different from Diabetic control

HEMATOLOGY

Table II shows the effect of the bi-herbal extract on the hematological parameters

Group	Treatment	Mean PCV Level (%)	Mean WBC count X ($10^3/mm^3$) cells	Mean HB count (g/dl)
A	Normal Control	42 ± 4.11	3.64 ± 0.38	13.01 ± 1.12
B	Diabetic Control	41 ± 3.98	3.72 ± 0.55	12.83 ± 0.43
C	Sample Treatment	44 ± 3.62	3.56 ± 0.04	13.12 ± 0.76
D	Sample Treatment	39 ± 5.19	3.18 ± 0.18	12.44± 0.66
E	Sample Treatment	42 ± 4.33	3.30 ± 0.91	12.02 ± 0.18
F	Sample Treatment	40 ± 4.76	3.69 ± 1.01	12.93± 1.37

DISCUSSION

In the present study, the bi-herbalethanolic extract of phyllanthus niruri and Moringa oleifera was investigated for hypoglycemic activity in STZ-induced diabetes as well as implications on the hematological parameters. The oral administration the extract significantly reduced the FBS of 24hours fasted diabetic rats. Both plants have been shown to be rich in polyphenols, flavonoids and alkaloids (Ezeonwu, 2012). Some plants with phytochemicals such as alkaloids have been reported to have hypoglycemic activity (Bever and Zahad, 1979). The hypoglycemic activity of the bi-herbal extract may be associated with flavonoids and alkaloids since phenolic compounds have been reported to possess anti-diabetic effects (Farjou et al, 1987; Wegner and Fintelmann). Streptozotocin has always been used to induce diabetes in experimental animals. The mechanisms have been suggested that STZ enters the β-cells through GLT2 transporter causing alkylation of DNA. Szkudelski (2001) reported that this DNA damage induces activation of poly ADP-ribosylation process that is more important for the diabetogenicity of STZ than DNA damage itself, by a very complex mechanism he suggested STZ leads to free radical production and damages β-cells by necrosis hence. The mechanism by which this extract reduces the plasma glucose level is unclear, however, it is suggestive that the extract stimulates insulin secretion by increasing responsiveness of β-cells to both glucose and non-glucose secretagogues resulting in more insulin being released at all blood glucose concentration. Though the animals were fasted for 24hours before FBS estimation, the reduction in glucose level cannot be ascribed to food withdrawal since from the table, the values of the test group and the controls show otherwise. The effect of the extract on the hematological parameters investigated showed there was no appreciable change on the WBC count, HB and PCV estimation as the values obtained fell within the reference range for rats as reported in Pass and Freeth (1993). Since all the hematological parameters considered in this study were not affected by the extract, it suggests that the extract may not have any negative implications and can be said to not possess any toxicity potential at the treated doses and considering other publications about the bi-herbal extract. Also, a significant observation in this study was the absence of any physical behavioral changes during the study and the hypothesis that blood sugar level may not have any relationship to WBC count in healthy or diabetic animals except another disease condition is present. Finally, this study shows hypoglycemic activity of bi-ethanolic extract of two herbs with no hematological implications. This study initiates the combination possibility of both

plant extracts in the management of diuretic conditions. The underlying mechanism stated is clearly suggestive as studies are required to substantiate this claim.

REFERENCES

Ezeonwu, V.U. (2011). *Antifertility Activity of Aqueous Extract of Phyllanthus niruri in Male Albino Rats.*The Internet Journal of Laboratory Medicine. Volume 4 Number 2

Nwanjo, H.U. (2006). *Studies on the effect of aqueous extract of phyllanthus niruri on the plasma glucose level and some hepatospecific markers in diabetic Wister rats*. The Internet Journal of laboratory Medicine. Volume 2 Number 2

Adedapo, A. A., Asegbayibi, A. Y., Emikpe, B.O. (2005). *Some clinic-pathological changes associated with the aqueous extract of the leaves of phyllanthus niruri in rats*. Interscience. Journal.**40** (1): 119-122.

Lee, C.Y., Peng, W.H., Cheng, H.Y., Chen, F.N., Lai, M.T., Chiu, T.H. (2006). *Hepatoprotective effect of phyllanthus niruri on acute liver damage induced by carbon tetrachloride*. Am. J. China Med. **34** (3): 471-482.

Chatterjee, M. and Sil P.C. (2006) .*Hepatoprotective effect of aqueous extract of phyllanthus niruri on nimesulide-induced oxidative stress in vivo*. Indian Journal of Biochemistry and Biophysics **43** (5): 299-305.

Khanna, A.K., Rizvi F., Chander R. (2002). *Lipid lowering activity of phyllanthus niruri in hyperlipemic rats*. Journal Ethnopharmacol **82** (1): 19-22.

Barros, M.E. (2003). *Effects of an aqueous extract of phyllanthus niruri on calcium oxalate crystallization in vitro*. Urology research **30** (6) 374-379.

Freitas, A.M., Schor, N., Boim, M.A. (2003).*Effect of phyllanthus niruri on urinary inhibitors of calcium oxalate crystallization*. BJU Int. **89** (9): 829- 834.

Naik, A.D and Juvekar, A.R. (2003).*Effects of alkaloidal extract of phyllanthus niruri on HIV replication*. Indian Journal of Medical Science **57** (9): 387-393.

Lam, C.Y., Leung, K.T., Law, P.T., Lee, S.M., Chan, H.L., Fung, K.P., Ooi, V.E., Waye, M.M. (2006). *Antiviral effect of phyllanthus niruri ethanolic extract against Hepatitis B virus*. Journal of cell Biochemistry.**97** (4): 765-812.

Subeki, S. (2005).*Anti-babesial and antiplasmodial compounds from phyllanthus niruri.* J. Nat. Prod. **68** (4) 537-539.

Cimanga, R.K. (2004). *In vitro antiplasmodial activity of callus culture extracts and fractions from fresh apical stems of phyllanthus niruri*. J. Ethnopharmacol. **95** (2-3): 399-404.

Ezeonwu, V.U. (2012) *Phytochemistry and some hematological implications of the oral Administration of aqueous whole plant extract of Phyllanthus niruri in albino rats*. Advanced Laboratory Medicine international. 2(3) 96-101

Ezeonwu, V.U. (2012) *Hepatoprotective activity of bi-ethanolic leaf extract of phyllanthus Niruri and Moringa oleifera in carbon tetrachloride induced liver damage in albino rats.* Asian Journal of biochemical and pharmacological research Issue 2 Volume 3, 33-38

Marcu, M.G. (2004). *Ideal food for obese and malnourishment* PP 67-89

Tilza, I.B; Sanni, S; Zakari, A.I; Sanni, F.S; Muhammed, T; Joseph, M.B. (2010). *In vitro antimicrobial activity of water extract of Moringa oleifera leaf stalk on bacteria normally implicated in eye disease*. Academia arena 2 (6) 80-82

Patel, M.B; Vaghela, K; Anand I; Patel, C. (2008).*A hepatoprotective activity of Moringa oleifera fruit on isolated rat hepatocytes*. Annal Biochem 2-5

Wegner, T and Fintelmann, V. (2001).*Flavonoids and bioactivity*. Wien med wochenschr 149: 241-247

Heiserman L. David (2004) *Methods of hematology*; Sweethaven publishing Services

Dacie, J.V and Lewis, S.M. (2001).*Practical Hematology*. 11thed, Longman Group. Ltd. Hong Kong. Pp. 11-17

Bever, B.O and Zahad, G.R. (1979) *plants with oral hypoglycemic action.*Jour. Crude Res.17: 139-196

Farjou, I.B; Al-Ani, M; Guirges, S.Y. (1987) *Lowering of blood glucose in diabetic rabbits by Artemisia extract.* Journal of faculty Med. Baghdad. 92: 137-141

Szkudelski S. (2001) *The mechanism od Aloxan and STZ action of β-cells of the rat pancreas* Pub. Med. Gov. 50 (6): 537-546

Pass, D and Freeth, G (1993). *The rat*. ANZCCART news 6 (4): 1-4