American Journal of Advanced Drug Delivery

www.ajadd.co.uk

Original Article

Bermuda Grass (*Cynodon dactylon*) Extracts and its Effect on Lipid Profile Assay of Streptozotocin-induced Wistar Albino rats.

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Date of Receipt- 28/6/2014
Date of Revision- 02/7/2014
Date of Acceptance- 08/7/2014

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ABSTRACT

Aqueous and Ethanolic extracts of *Cynodon dactylon* and their effects on Lipidprofile assay of streptozotocin–induced diabetic Wistar albino rats were investigated. Diabetes mellitus was induced by treatment with streptozotocin. Aqueous and ethanolic extracts of *Cynodon dactylon* at varying concentrations (300mg, 500mg and 700mg/kg body weight) were administered to the diabetic rats. Aqueous and ethanolic extract of herbal preparation of the Bermuda grass (*Cynodon dactylon*) showed a significantly decrease ($p<0.05$) in the lipid profile (cholesterol, triglyceride, and HDL), but the decrease was not progressive, hence they occurred a varying interval. The extract(s) of the grass (i.e. herbal preparation) has no significant ($p>0.05$) effect on LDL within the duration of treatment at different concentrations. The results of this study strongly suggest that *Cynodon dactylon* possess hypolipidemic properties with the exception of LDL.

Keywords: Aqueous, Bermuda, cholesterol, ethanolic, extract, grass, lipoprotein.

Abbreviation: HDL - High density lipoprotein, LDL - Low density lipoprotein, STZ- Streptozotocin, TG- triglycerides.

INTRODUCTION

Medicinal plants and herbs are of great importance to the health of individual and communities. Despite the existence of herbal medicines over many centuries, only relatively small number of plant species has been studied for their application. However, in the recent past, an increasing research evidence is getting accumulated, which clearly indicate the positive role of traditional medicinal plants in the prevention

or control of some metabolic disorders like diabetes, heart diseases, hyperlipidemia and certain types of cancers (Zhang, 1976). One of the great advantages of these medicinal plants is that they are easily available and have moderate side effects (Mehta, 1982).

The erythrocyte cell membrane comprises a typical lipid bilayer, similar to what can be found in virtually all human cells. This lipids bilayer is composed of cholesterol phospholipids in equal proportion by weight. The lipid composition is important as it defines many physical properties such as membrane permeability and fluidity. Additionally, the activity of many membrane proteins is regulated by interaction with lipids in the bilayer (Wan *et al*, 2008). The work is aimed at determining the effect of Bermuda (*Cynodondactylon*) grass on blood lipid profile of diabetic rats.

MATERIALS AND METHODS

Plant collection

The plant material was collected from Igboigbo-Unale in Ibaji Local Government Area, eastern part of Kogi State, Nigeria during dry season (November, 2009). Dirt was removed from the plant parts by rinsing in clean water. The leaves were air – dried for 3 weeks and pulverized using motorized blender into a fine powder of 60 mesh sieve size. The fruit pulp, seed and pericarp were dried in oven at 40°C. The dried samples were then used for the various analyses.

Experimental animals

Wistar albino rats aged (10-20weeks) derived from a colony maintained at the animal house of the department of biochemistry, Choba Park, University of Port Harcourt, Rivers State, Nigeria are used for the experiment. The rats weighing between 100-200g were housed at a temperature of (25 ± 2^{O}C) and were divided into ten (10) groups of 9 animals each. The animals were fed *ad-libitum* with standard commercial laboratory chow (Pfizer feeds Plc., Nigeria) and water throughout the experimental period (21days).

Experiments were performed in accordance with the principles of Laboratory Animal Care.

Preparations of streptozotocin (STZ)

The range of diabetogenic dose of STZ is quite narrow and a light overdose may cause the death of many animals (Lenzen *et al,* 1996).

5g of STZ was dissolved in 100ml of distilled water to give a 5% stock solution of which a single dose of 70mg/kg body weight was injected intraperitoneally on the rats.

Preparation of plant extract

Aqueous extract

Fresh Bermuda grass *(Cynodon dactylon*) was washed with distilled water to remove debris and contaminants after which they were air dried. The grass was homogenized into fine powder and the aqueous extract was prepared by weighing out 200g of pulverized grass into 1.5 litres of distilled water. The resultant mixture was allowed to stand for 24hours with occasional shaking after which it was filtered. The filtrate was evaporated and dried to paste with the aid of a thermostatic water bath at a temperature of 50^{O}C.

An aliquot of the extract was prepared by dissolving 7g, 5g and 3g in 50ml of distilled water respectively to form three (3) concentrations and stored at 4^{O}C, which served as stock crude drug.

Ethanol extract

Fresh Bermuda grass (*Cynodon dactylon*) was washed with distilled water to remove debris and contaminants, after which they were dried. The dried grass was homogenized into fine powder. 200g of powdered grass was soaked in one (1) liter of absolute ethanol and the resultant mixture was

allowed to stand for 24hours with occasional shaking, after which it was filtered. The filtrate was evaporated, first with a rotary evaporator and dried to paste with the aid of a thermostatic water bath at 45^{O}C.

An aliquot of the extract was prepared by dissolving 7g, 5g and 3g in 50ml of distilled water respectively to form three (3) concentrations and stored at 4^{O}C, which served as stock crude drug.

Blood lipid profile

This is the result of a blood test that measures levels of lipids or fats, including cholesterol and triglycerides. Factors, such as age, sex, genetic influence lipid profile (Robert, 2006).

Certain aspects on one's lifestyle, including diet, level of physical activity, level of diabetes and control and smoking status, also affect lipid profile. Some medical conditions can also raise or lower cholesterol and triglyceride levels (Robert, 2006).

A lipid profile is a direct measure of three blood components: cholesterol, triglycerides and high density lipoproteins (HDL).

Triglycerides

Triglycerides are esters of trihydric alcohol glycerol with 3 long chain fatty acids. They are partly synthesized in the liver and partly ingested in food. The determination of triglyceride is utilized in the diagnosis and treatment of patients with diabetes mellitus, nephrosis, liver obstruction, lipid metabolism disorders and numerous other endocrine diseases.

The triglycerides were determined after enzymatic hydrolysis with lipases (Wahlefeld, *et al*, 1974). Indicator is quinonemine formed from hydrogen peroxide. 4-amino antipyrene and 4-chlorophenol under the catalytic influence of peroxidase was measured at 540nm using UNISPEC 22D+ spectrophotometer.

Reaction principle

Triglycerides + 3H2O $\xrightarrow{\text{lipase (LPL)}}$ glycerol + fatty acid

Glycerol + ATP $\xrightarrow[Mg2+]{GK}$ glycerol-3-phosphate +ADP

Glycerol-3-phosphate+O_2 $\xrightarrow{GPO}$ Dihydroxyacetone phosphate + H_2O_2

H_2O_2 + 4-aminoatipyrine + P-chlorophenol $\xrightarrow{PoD}$ 4- (p-benzoquinone-monolmino)-phenazone + 2H_2O + HCl

Method

Methods for triglycerides determination generally involve enzymatic or alkaline hydrolysis of triglycerides into glycerol and free fatty acids followed by either chemical or enzymatic measurement of the glycerol released.

This method is based on the work by Wahlefeld, 1974 using a lipoprotein lipase from micro-organisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide product then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dyestuff (Trinder endpoint reaction).

Test procedure

20µl of standard and test were mixed with 2000µl of reagent and incubated at 20-25^{O}C i.e. room temperature for 5 minutes. Absorbance was read at 540nm and calculated.

Calculation:

$$\frac{\text{A sample mmol/l} \quad \text{X conc. of standard (2.28)}}{\text{A standard}}$$

Cholesterol

The major constituents of plasma lipids are cholesterol and triglycerides. Cholesterol is an important constituent of cell

membrane and a precursor for the synthesis of bile salts and steroid hormones. Cholesterol is synthesized in the liver and transported in the blood mainly in the form of LDL and HDL. In blood, cholesterol is present in free as well as esterified form. Free cholesterol and cholesterol received from its esters after enzymatic hydrolysis are oxidized enzymatically. The indicator, quinoneimine is from ydrogenperoxidase and 4-amino antipyrene in the presence of phenol and peroxidase.

Reaction principle

Cholesterol ester $+H_20 \xrightarrow{\text{cholesterol esterase}}$ Cholesterol + fatty acid

Cholesterol $+ O_2 \xrightarrow{\text{cholesterol oxidase}}$ Cholesterol-3-one $+H_2O_2$

H_2O_2 + 4-amino antipyrene + phenol $\xrightarrow{\text{peroxidise}}$ quinoeimine + H_2O

Cholesterol esters in serum are hydrolyzed by cholesterol esterase. The free cholesterol is then oxidized by cholesterol oxidase to the corresponding ketone liberating hydrogen peroxide, which is then converted to water and oxygen by the enzyme, peroxidase. Para aminophenazone (4-aminophenazone) takes up the oxygen and together with phenol forms a pink coloured quinoneimine dye, which can be measured at 515nm/ yellow green filter.

Cholesterol estimation

Enzymatic colorimetric test for cholesterol with lipid clearing factors.

Procedure

20µl of sample and standard were added to 200µl of reagent. It was mixed and incubated at 37^{OC} in a water bath for 5minutes or at room temperature at 20-25^{O}C for 10minutes. The spectrophotometer/filter photometer was set to zero using blank at 510nm/yellow green filter and the absorbance of standard test and QC were measured against reagent blank.

Calculation:

$$\frac{\text{A sample X 5.17 (mmol/l)}}{\text{A standard}}$$

High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL)

High density lipoprotein reagent was used. Test-tubes were labeled as blank, standard and test. 0.5ml (500µl) of HDL reagent was pipetted into all the tubes. The 0.2ml (200µl) of the sample was added into the blank and the cholesterol standard. Then centrifuge for 10 minutes at 4,000rpm.

Then another test-tube was labeled as blank, standard and test. 1.0ml (100µl) of cholesterol reagent pipetted into all the tubes, 0.1ml (100µl) supernatant into the respective tubes, allow to stand for 10minutes at room temperature (25^{O}C), zero the spectrophotometer with the blank, and the absorbance test was read and recorded in mmol/l at 500nm.

LDL was calculated by using the formula; T.C – HDL + 0.46 × T.G

RESULTS

See Table No. 1, 2, 3 and 4.

DISCUSSION

It has been demonstrated and observed that most herbal formulations are generally less toxic, free from adverse side effects associated with synthetic ones, readily available and cheap (Mitra *et al*, 2000; Bhattacharya *et al*, 1997 and Annapurna, *et al*, 2001).

The use of streptozotocin for the induction of diabetes mellitus was attributed to the work of Ferreira, *et al,* 2002, which was confirmed by the evaluation of fasting blood glucose concentration of experimental rats administered with 70mg/kg body weight of streptozotocin.

The periodic (weekly) effects of aqueous and ethanolic extracts of *Cynodon dactylon*on Triglycerides showed an elevated level, greater than those of the normal and diabetic control rats when compared statistically. This elevated and statistical significant ($p<0.05$) levels observed (table 1) is a clear indication that in severe insulin deficiency, there is accelerated lipolysis which result in elevated serum triacylglycerol levels.

The rise in cholesterol level of diabetics was shown in the fasting blood glucose levels of the diabetic control and treated rats. Acetyl-CoA is channeled to cholesterol synthesis as a result of impaired fatty acid synthesis. The TCA cycle only mobilizes little of the acetyl-CoA and the remainder is been converted to β-hydroxybutyrate, acetoacetate and acetone.

However, a reduction in cholesterol concentration following treatment with *Cynodon dactylon* was observed indicative of clear hypolipidemic effects. Values for high density lipoprotein (HDL) were observed to be stable in normal and diabetic control rats as well as DTR on 300mg, 500mg aqueous extract and 300mg ethanolic extracts with the other groups showing a significant ($p<0.05$) rise in HDL, though with the expectation of Daonil which show a significant ($p<0.05$) decrease in HDL (see table 2).

The values obtained for low density lipoprotein (LDL) across groups showed no significant increase ($p>0.05$) though with exception of DTR on 300mg ethanolic extract, when compared to normal control rats (NCR), indicating that Daonil, Glucophage and the herbal preparation (*Cynodon dactylon*) has a lipolipidemic activity (table 3).

Conclusively, results obtained from the analysis revealed that administration of *Cynodon dactylon*, Daonil and Glucophage led to a significant interaction. The study shows that the grass *Cynodon dactylon* demonstrate clear hypolipidemic effects on streptozotocin-induced wistar albino rats and this may be of immense benefit in management of type I (IDDM) diabetes mellitus and its associated vascular complications, hence could be recommended as remedy for diabetes mellitus.

REFERENCES

1. Annapurna, A., Kanaka, Mahalakshimi, D., Murali, and Krishna, K. (2001). Anti-Diabetic activity of a polyherbal preperation (Tinture of Punchparna) in normal and diabetic Rats. *Ino. Exp. Biol.* 39: 500-502.
2. Bhattacharya, S. K., Satyan, K. S. and Chakibarti, A. (1997). Effects of Trasina on Ayurvedic herbal formulation of pancreatic islet superoxide dismutase activity in hyperglycemia. *Indian J. Exp. Biol*. 35: 297-229.
3. Cameron, M. J., Arreaza, M. A., Grattan, M., Meagher, C., Sharif, S., Bundick, M. D., Stricter, R. M., Cook, D. N. and Delovitch, T. L. (2000). Differential expressions of CC Chemokines and CCR5 Receptor in the Pancreas in association with progression to type I diabetes. *J. Immunol* 165:1102-1110.
4. Chopra, R.N., Nayar, S.L. and Chopara, I.C. (1999). Council of Scientific and Industrial Research (CSIR), 1st Edn., *Council of Scientific and Industrial Research* (CSIR), New Delhi, pp: 88.
5. Ferreira, L. D., Huey, P. U., Pulford, B. E., Inshii, D. N. and Eckel, R. H. (2002). Sciatic Nerve lippoprotein lipase is reduced in STZ-induced diabetes and corrected insulin. Endocrinology 143:1213-1217.
6. Iwase, M., Nunoi, K., Sadosshima, S., Kikuchi, M. and Fujishima, M. (1989). Liver, Kidney, and Islet cell tumors in spontaneously hypertensive and Normostentive Rats treated with

streptozotocin. Tohoku J. *Exp. Med*. 159:83-90.
7. Jarald, E.E., S.B. Joshi and D.C. Jain, 2008. Antidiabetic activity of aqueous extract and non polysaccharide fraction of *Cynodon dactylon* Pers. *Ind. J. Exp. Bio*., 46: 660-667.
8. Mehta, K.C. (1982). Indian herbal drugs in the treatment of diabetics. Current medical practice, 26: 305 – 308.
9. Zhang, X. (1976). Traditional medicine and WHO, World health, the magazine of World Health Organization 49th year No.2.4–5.

Table 1. Triglycerides Values (mmol/l) of Normal and Diabetic Controls/Diabetic test rats treated with standard drugs as well as aqueous and ethanolic extracts of Bermuda grass

GROUPS	WEEK 1	WEEK 2	WEEK 3
NCR	0.75 ± 0.07^{a}	1.35 ± 0.21^{a}	1.2 ± 0.28^{a}
DCR	0.7 ± 0.41^{a}	1.55 ± 0.35^{b}	1.2 ± 0.00^{a}
DTR on Daonil	0.5 ± 0.00^{a}	1.55 ± 0.07^{b}	1.4 ± 0.28^{b}
DTR on Glucophage	0.8 ± 0.42^{c}	1.1 ± 0.14^{b}	1.5 ± 0.42^{d}
DTR on 300mg Aq. Extract	0.55 ± 0.71^{a}	1.0 ± 0.14^{a}	1.6 ± 1.6^{b}
DTR on 500mg Aq. Extract	0.7 ± 0.14^{c}	1.1 ± 0.00^{b}	1.4 ± 0.14^{d}
DTR on 700mg Aq. Extract	0.55 ± 0.35^{a}	1.3 ± 0.35^{b}	1.0 ± 0.00^{b}
DTR on 300mg Et. Extract	1.05 ± 0.5^{a}	1.6 ± 0.28^{b}	1.45 ± 0.14^{b}
DTR on 500mg Et. Extract	1.0 ± 0.28^{c}	1.3 ± 0.35^{b}	1.55 ± 0.35^{d}
DTR on 700mg Et. Extract	0.95 ± 0.21^{e}	0.95 ± 0.07^{w}	1.35 ± 0.5^{c}

Results are mean ± S.D of triplicate determination.
Values in the same column with different superscript letter are statistically significant at 95% confident level ($p \leq 0.05$).

Table 2. Cholesterol assay values (mmol/L) for different groups

GROUPS	WEEK 1	WEEK 2	WEEK 3
NCR	2.1 ± 0.20^{a}	2.4 ± 0.14^{a}	2.8 ± 0.0^{a}
DCR	2.25 ± 0.50^{c}	1.95 ± 0.07^{b}	1.95 ± 0.07^{b}
DTR on Daonil	2.8 ± 0.99^{b}	4.5 ± 0.21^{c}	1.65 ± 0.21^{a}
DTR on Glucophage	1.6 ± 0.42^{a}	1.6 ± 0.0^{a}	2.21 ± 0.07^{c}
DTR on 300mg Aq. Extract	2.15 ± 0.35^{b}	2.3 ± 0.0^{b}	2.05 ± 0.07^{b}
DTR on 500mg Aq. Extract	1.5 ± 0.0^{a}	2.0 ± 0.57^{d}	1.9 ± 0.14^{c}
DTR on 700mg Aq. Extract	2.25 ± 0.50^{c}	1.8 ± 0.28^{b}	1.85 ± 0.35^{ab}
DTR on 300mg Et. Extract	2.65 ± 1.06^{d}	3.3 ± 0.57^{e}	1.7 ± 0.14^{b}
DTR on 500mg Et. Extract	2.6 ± 0.00^{b}	1.8 ± 0.35^{a}	2.3 ± 0.71^{a}
DTR on 700mg Et. Extract	2.55 ± 0.64^{b}	2.25 ± 0.35^{a}	2.9 ± 0.71^{b}

Results are mean ± S.D of triplicate determination.
Values in the same column with different superscript letter are statistically significant at 95% confident level ($p \leq 0.05$).

Table 3. High Density Lipoprotein (HDL) assay values (mmol/L) for different groups

GROUPS	WEEK 1	WEEK 2	WEEK 3
NCR	2.1 ± 0.0^{a}	2.15 ± 0.35^{a}	2.05 ± 0.21^{a}
DCR	2.1 ± 1.14^{a}	1.90 ± 0.42^{a}	2.0 ± 0.00^{a}
DTR on Daonil	2.05 ± 0.49^{b}	2.25 ± 0.21^{b}	0.95 ± 0.21^{a}
DTR on Glucophage	1.85 ± 0.71^{a}	1.65 ± 0.21^{a}	2.35 ± 0.21^{c}
DTR on 300mg Aq. Extract	1.85 ± 0.35^{a}	2.0 ± 0.14^{b}	2.0 ± 0.28^{b}
DTR on 500mg Aq. Extract	1.80 ± 0.00^{a}	1.85 ± 0.71^{b}	2.05 ± 0.071^{c}
DTR on 700mg Aq. Extract	1.60 ± 0.28^{b}	1.55 ± 0.71^{a}	2.15 ± 0.071^{c}
DTR on 300mg Et. Extract	1.65 ± 0.071^{b}	1.95 ± 0.21^{c}	2.05 ± 0.78^{d}
DTR on 500mg Et. Extract	1.55 ± 0.071^{e}	1.30 ± 0.0^{a}	2.0 ± 0.28^{f}
DTR on 700mg Et. Extract	1.85 ± 0.21^{c}	1.65 ± 0.21^{b}	2.35 ± 0.21^{d}

Results are mean ± S.D of triplicate determination.
Values in the same column with different superscript letter are statistically significant at 95% confident level ($p \leq 0.05$).

Table 4. Low Density Lipoprotein (LDL) assay values (mmol/L) for different groups

GROUPS	WEEK 1	WEEK 2	WEEK 3
NCR	3.35 ± 0.71^{e}	0.90 ± 0.14^{a}	1.50 ± 0.57^{a}
DCR	0.55 ± 0.35^{a}	0.7 ± 0.57^{b}	0.6 ± 0.57^{a}
DTR on Daonil	0.35 ± 0.07^{b}	0.5 ± 0.42^{c}	0.25 ± 0.71^{a}
DTR on Glucophage	0.45 ± 0.071^{ab}	0.45 ± 0.21^{a}	0.4 ± 0.28^{a}
DTR on 300mg Aq. Extract	0.45 ± 0.07^{a}	0.55 ± 0.35^{b}	0.75 ± 0.35^{c}
DTR on 500mg Aq. Extract	0.30 ± 0.0^{a}	0.65 ± 0.49^{c}	0.45 ± 0.35^{b}
DTR on 700mg Aq. Extract	0.65 ± 0.49^{c}	0.95 ± 0.64^{f}	0.3 ± 0.14^{a}
DTR on 300mg Et. Extract	0.4 ± 1.77^{d}	2.95 ± 1.77^{c}	0.65 ± 0.64^{a}
DTR on 500mg Et. Extract	1.5 ± 0.42^{a}	1.2 ± 0.141^{a}	1.25 ± 0.92^{a}
DTR on 700mg Et. Extract	1.0 ± 0.57^{a}	1.0 ± 0.42^{a}	1.5 ± 1.13^{a}

Results are mean ± S.D of triplicate determination.
Values in the same column with different superscript letter are not statistically significant at 95% confident level ($p \geq 0.05$).