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Hypoglycaemic Properties of Aqueous Extracts of Anacardium occidentale, Moringa oleifera, Vernonia amygdalina and Helianthus annuus: A Comparative Study on Some Biochemical Parameters in Diabetic Rats

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ABSTRACT: *Several phytomedicine have been reported to possess antidiabetic and hypoglycaemic potentials. However, their relative activities are not being looked into. The aim of this study was to determine the hypoglycaemic effects and compare some biochemical parameters of aqueous extracts of four plants; Anacardium occidentale stem bark (CS), Moringa oleifera (ML), Vernonia amygdalina (BL) and Helianthus annuus seeds (SF) on alloxan induced diabetic rats. The preliminary phytochemical screening reveals the presence of alkaloids, tannins, saponins, cardiac glycosides, terpenes, steroids, phenol and resins in all extracts. Diabetic was induced by interperitonial injection of alloxan monohydrate while treatment was done for 14 days. Significant reduction ($p<0.05$) was recorded in glucose level of the alloxan induced diabetic rats treated with CS, ML, BL and SF, expressing their hypoglycaemic potentials and competing favourably with the reference drug metformin.Treatment of experimental rats with 150 mg/kg bwt. of aqueous extracts significantly decrease ($p<0.05$) the levels of Low Density Lipoprotein (LDL), Triglyceride (TG) in all extracts treated and cholesterol except for BL when compare with the diabetic control while High Density Lipoprotein (HDL) was significantly increased ($p<0.05$) in all extract treated rats. Aqueous extracts also significantly ($p<0.05$) alter serum concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in a similar manner with metformin treated group. Result showed that hypoglycaemic potential was in the order BL > ML > SF > CS.*

KEYWORDS: *Anacardium occidentale, Biochemical parameters, Helianthus annuus, Hypoglycaemic properties, Moringa oleifera, Vernonia amygdalina*

I. INTRODUCTION

The practise of traditional herbal medicine is as old as man and has being practised by many age long civilization; used in treatment and prevention of various diseases and illness affection people and animals. Different civilizations possess its own technique of traditional medicine employed in the treatment of these ailments. The technique usually requires using plant parts, e.g. seeds, berries, roots, leaves, bark or flowers to prepare decoctions, syrups, extracts or tinctures for medicinal purposes [1-3]. Many of these medicinal plants and herbs are part of our diet as spices, vegetables and fruits [4]. The use of a combination of these medicinal plants is a technique which works by exploiting various reactive ingredients possessed by plants, which may also work together to bring about a beneficial effect of medicinal value.

Diabetes mellitus is a global metabolic epidemic affecting essential biochemical activities in almost every age group [4] and also the most severe metabolic pandemic of the 21st century, affecting essential biochemical activities in almost every cell in the body [5]. Several breakthroughs have been recorded in the treatments of diabetes which include the use of exogenous insulin [6] and hypoglycaemic agents like sulphonylureas and biguanides [7]. However, these breakthroughs still have their short comings such as adverse health effects [8]. Different plant constituents are now being discovered as natural hypoglycaemic agents with potent activity for diabetics and its complications, they are considered free from side effects than synthetic ones and are less toxic, relatively cheap and popular [9]. Over 800 plants have been reported to possess antidiabetics properties [10], they include *Anacardium occidentale, Moringa oleifera, Vernonia amygdalina.* However, in this recent review by Preethi, 2013 [10], *Helianthus annuus* was not listed in over 800 medicinal plants with antidiabetics properties. This study was design to investigate the hypoglycaemic properties of *Helianthus annuus* and compare it with that of *Anacardium occidentale, Moringa oleifera, Vernonia amygdalina* and also compare their effects on other Biochemical parameters in alloxan induced diabetic rats.

Vernonia amygdalina is commonly called bitter leaf (BL) because of the characteristic odour and astringent bitter taste of the leaf. The plant is widely distributed in west coast of Africa where it grows wild and as a domestic browse plant [11]. This plant has however been named by different ethnics around the world: 'Ewuro' in Yoruba; 'Etidot' in Ibibio; 'Onugbu' in Igbo; 'Ityuna' in Tiv; 'Oriwo' in Edo and 'Chuwaka' in Hausa [12]. The aqueous leaf extract have been shown to possess hypolipidemic effects respectively on diabetic and non-diabetic rats [13]. Its protective role on the kidneys [14] and livers [15] of alloxan-induced diabetic rats has also been documented.

Moringa oleifera have been used to combat malnutrition, especially among infants and nursing mothers[16]. *Moringa* leaves (ML) can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value[17]. Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers, and immature pods acts as cardiac and circulatory stimulants, possess antihypertensive, cholesterol lowering, antioxidant, anti-diabetic and hepatoprotective properties[18].

Anacardium occidentale is popularly called cashew (CS). Nutritionally, the commercial importance of *V. amygdalina* is due to its richness in nutrients that consists of 47% fat, 21% protein and 22% carbohydrate, vitamins and all essential amino acids especially thiamine [19]. The plant also exhibit hypoglycaemic and hypotensive properties [20,21].

Helianthus annuus seed is the fruit of the sunflower (SF), the seeds are excellent source of dietary fibre, essential fatty acid, some amino acids and vitamins[22]. *H. annuus* are rich in minerals and cholesterol-lowering phytosterols [23].Luka *et al*., 2012 [24] has also reported the hypoglycaemic properties of *H. annuus*. Currently, several hypoglycaemic agents and insulin are used in the treatment of diabetes mellitus [25], it's however, imperative to document their relative anti-diabetic and hypoglycaemic activities for easy identification and choice of usage.

II. MATERIALS AND METHODS

2.1 Collection of plant materials

The plant materials;*Anacardium occidentale* (cashew stem bark), *Moringa oleifera l*eaf, *Vernonia amygdalina* (Bitter leaf) and *Helianthus annuus* (sunflower seeds) used for this study were obtained from Jos, Plateau state and identified at the Department of Botany, University of Jos before usage.

2.2 Experimental rats

The experiment animals used for this study were locally bred Wister strain (*Rattus norvegicus*) of both sexes from the animal house of university of Jos, Nigeria. Rats are of average weight 174±15 g. The animals were maintained under standard environmental conditions, had free access to food (Grand Cereal products, Jos, Nigeria) and water *ad libitum.* Seven groups of five rats each were randomly distributed in cages and acclimatized for 7 days.

2.3 Preparation of plant extracts

Briefly; the plant materials were rinsed with tap water and dried by spreading under the shade until a constant weight was obtained. The plant materials were then pulverized into powder. 50 grams of each leaves was soaked separately in 100mL of boiled distilled water and agitated intermittently for 24 hours. They were then filtered using fine sieve to obtain the aqueous extracts in each case. The extracts were allowed to dry in an oven dryer at 50^{o}C to obtain the crude extracts. The extracts were stored in an air tight container and were later reconstituted in distilled water to give the required dose of 150 mg/kg bwt.which was administered during the study.

2.4 Experimental design

Group 1: Normal control (Positive control) (NC)
Group 2: Diabetic control (Negative control) (DC)
Group 3: Diabetic group, received 14.2 mg/kg bwt.of Metformin (MT)
Group 4: Diabetic group, received 150 mg/kg bwt.of Cashew stem bark extract (CS)
Group 5: Diabetic group, received 150 mg/kg bwt.of Moringa Leaf extract (ML)
Group 6: Diabetic group, received 150 mg/kg bwt.of Bitter leaf extract (BL)
Group 7: Diabetic group, received 150 mg/kg bwt.of Sunflower seed extract (SF)
Each group consist of five animals (n = 5).

2.5 Treatment of experimental animals

Groups 1 and 2 received 0.2 mL of distilled water per day. Group 3 received Metformin (Jiangsu Ruinian Qianjin Pharmaceuticals Ltd, China) at 14.2 mg/kg bwt.per day. The extracts (150 mg/kg bwt.) were orally administered once daily for a period of fourteen (14) days to Groups 4 – 7. Blood glucose levels were

taken at 7days interval using One Touch® Glucometre (Life scan Inc. 1995, Milpitas California 95305, USA) to check progress of treatment.

2.6 Induction of diabetics

Diabetics mellitus was induced in animals by single intraperitonial injection of 150 mg/kg body weight of alloxan monohydrate (Sigma, St. Louis, USA) suspended in normal saline, after an overnight fasting. 48 hours later, diabetics were confirmed using One Touch® Glucometre. Animal with fasting blood glucose level ≥250 mg/dL were considered diabetic and included in the study.

2.7 Collection of samples

At completion of the 14 days treatment, the rats were subjected to an overnight fast prior to collection of blood samples. The rats were anesthetized at the time of sacrifice by been placed in a seal cotton wool soaked in diethyl ether inhalation jar. Blood samples were collected into centrifuge tubes and allowed to clot for about 45 minutes, after which they were spun at 3000 rpm for 5 minutes, the serum collected were transferred into bijou bottle using pasture pipette and kept for analysis.

2.8 Phytochemicals

Phytochemical tests were carried out using standard procedures [26-28].

2.9 Assay of biochemical parameters

Biochemical parameters assayed include alkaline phosphatase[29], alanine aminotransferase [30],aspartate aminotransferase [30] and Lipid profile [31-33].

2.10 Statistical analysis

Data were presented as Mean ± SD of 5 replicates and were analyzed using DMRT following one-way analysis of variance (ANOVA) using SPSS 16.0 computer software package (SPSS Inc., Chicago, U.S.A). Differences at $p<0.05$ were considered significant.

III. RESULTS

Preliminary screening of aqueous extracts of BL, ML, CS and SF revel the presence of several phytochemical principles. Alkaloid, tannins, saponins, cardiac glycosides, terpenes and steroids, phenol and resins were all detected. Flavonoids were not detected in BL while balsams were not detected in BL and ML (Table 1).

Significant increases ($p<0.05$) were observed at the end of the period of administration in initial weights of NC and MT, while a significant decrease ($p<0.05$) were observed in DC and CS. However, there was no significant effects ($p>0.05$) observed in final weight of CS, ML and SF when compared to their initial weights (Table 2).

Extracts expressed different activities in their ability to lower plasma glucose level. Diabetic rats had a steady increase in plasma glucose to about 500 mg/dL on day 14. All extracts were able to reduce these increases to below 160 mg/dL and competed favourably with the reference drug metformin (Table 3).

Significant increase ($p<0.05$) were observed in CHOL, TG, and LDL of diabetic rats with significant decrease in HDL when compared to normal control. These values where however reversed by aqueous extracts of CS, ML, BL and SF at the 14th day of administration. Values obtained were also in line with that obtained from the reference drug metformin (Table 4).

Serum levels of ALP were significantly increased ($p<0.05$) in all administered extracts when compared to normal control. However, significantdecreases ($p<0.05$) were observed in serum levels of ALT and AST when compared to diabetic control (Table 5).

Table 1: PreliminaryPhytochemical Screening of Aqueous Extracts of*Vernoniaamygdalina, Moringaoleifera*, Cashew Stem Bark and Sunflower Seed

Phytochemicals	Cashew Stem Bark	*Moringa oleifera*	Bitter leaf	Sunflower Seed
Alkaloid	+	+	+	+
Flavonoid	+	+	-	+
Tannins	+	+	+	+
Saponins	+	+	+	+
Cardiac glycosides	+	+	+	+
Terpens and Steroids	+	+	+	+
Balsam	+	-	-	+
Phenol	+	+	+	+
Resins	+	+	+	+

KEY:+ = Detected - = Not detected

Table 2:Effects of Aqueous Extracts on Weight of Experimental Rats

Groups	Initial (g)	Final (g)
Normal control	125.97±07.08[a]	180.23±11.38[b]
Diabetic control	182.50±10.14[a]	132.75±04.99[b]
Metformin	159.00±04.00[a]	185.35±04.73[b]
Cashew stem bark	164.75±07.32[a]	162.25±02.22[a]
Moringa leaf	174.00±16.15[a]	163.50±13.00[a]
Bitter leaf	189.75±10.81[a]	166.75±04.57[b]
Sunflower seed	191.00±06.38[a]	192.00±07.16[a]

Values are mean ± S.D for five determination (n=5), Values with different letter superscripts are significantly different ($p<0.05$)

Table 3: Effects of Extracts on Glucose Levels of Experimental Rats

Groups	Glucose Levels (mg/dL)			
	Initial	Day 1	Day 7	Day 14
Normal Control	96.35±00.72	83.78±02.83	93.01±04.07	92.59±00.98
Diabetic Control	84.00±03.74	292.25±06.70	390.50±14.89	495.50±05.45
Metformin	82.47±02.54	397.05±05.30	290.53±04.98	124.52±03.92
Cashew Stem Bark	72.00±14.21	350.75±98.06	254.75±10.24	159.00±27.39
Moringa Leaf	74.25±06.18	410.25±49.07	188.75±42.11	101.88±02.95
Bitter Leaf	63.50±03.42	325.25±19.62	268.75±13.40	83.25±22.31
Sunflower Seed	85.25±03.78	414.50±89.76	283.50±57.20	152.70±87.85

Values are mean ± S.D for five determination (n=5)

Table 4: Effects of Extracts on Lipid Profile

Groups	Lipid Profile (mmol/L)			
	CHOL	HDL CHOL	TRIGLYCERIDE	LDL CHOL
Normal Control	2.20±0.08[a]	0.50±0.07[a]	0.90±0.08[a]	0.80±0.06[a]
Diabetic Control	2.50±0.14[b]	0.44±0.02[b]	2.68±0.07[b]	1.52±0.02[b]
Metformin	2.07±0.61[abc]	0.95±0.37[ac]	0.52±0.06[c]	0.91±0.12[a]
Cashew Stem Bark	2.05±0.31[a]	1.10±0.07[c]	1.70±0.67[d]	0.17±0.06[c]
Moringa Leaf	1.64±0.14[c]	1.09±0.09[c]	0.93±0.14[d]	0.23±0.04[c]
Bitter Leaf	2.45±0.13[b]	1.75±0.22[d]	0.67±0.08[c]	0.33±0.19[cd]
Sunflower Seed	2.09±0.18[a]	1.28±0.15[c]	1.03±0.19[d]	0.35±0.08[d]

Values are mean ± S.D for five determination (n=5), Values with different letter superscripts are significantly different ($p<0.05$)

Table 5: Effects of Extracts on Some Marker Enzymes

Groups	Marker Enzymes (IU/L)		
	ALP	ALT	AST
Normal Control	218.00±00.82[a]	123.33±1.25[a]	274.33±01.25[a]
Diabetic Control	354.50±15.09[b]	99.58±5.97[b]	356.85±11.71[b]
Metformin	248.00±08.64[a]	42.63±3.63[c]	52.47±02.52[c]
Cashew Stem Bark	306.50±61.27[b]	41.75±5.60[c]	105.55±07.23[d]
Moringa Leaf	389.00±05.23[b]	20.13±2.09[c]	60.58±04.12[d]
Bitter Leaf	454.50±77.90[b]	27.30±4.70[d]	42.53±05.81[c]
Sunflower Seed	391.00±21.32[b]	33.50±3.11[c]	84.85±29.68[d]

Values are mean ± S.D for five determination (n=5), Values with different letter superscripts are significantly different ($p<0.05$)

IV. DISCUSSION

The most routine and biochemical marker used in the diagnosis and progress monitoring during management of diabetes mellitus in clinical and experimental settings is blood and/or serum glucose concentration [34]. Blood glucose concentrations were measured in this study and results showed significant reduction ($p<0.05$) in blood glucose of diabetic rats treated with aqueous extracts of *V.amygdalina* and*M.oleifera,* Cashew stem bark and Sunflower seed. Extracts also competed favourably with the reference drug metformin. However, bitter leaf was better in expressing this hypoglycaemic potential followed by moringa leaf, metformin, sunflower and the cashew stem back respectively. Factors responsible for weight lost in diabetes include proteolysis, lipolysis and acute fluid loss [35] as observed in table 2. Aqueous extracts from CS, ML and SF reverse these lost in weight observed in the diabetic rats.However, BL could not produce the same effect on the weight of the experimental rats.

This study also looked into the phytochemicals present in these plant extracts as shown in table 1. Alkaloid, tannins, saponins, cardiac glycosides, terpenes and steroids, phenol and resins were all detected in all four extracts while flavonoids was not detected in BL, balsams were not detected in BL and ML. Secondary metabolites of plants such as some of the once listed above possess some alpha-glycosidase inhibitors and competitively inhibit intestinal brush border enzymes with an eventual reduction in digestion and absorption of carbohydrates from the gut-postprandial hyperglycaemia, hence resulting in an effective glucose control [36]. The role of tannins has been discoursed in this light [37]. A positive correlation has also been indicated between the presence in plants of flavonoids, glycosides and phytosterols with hypoglycaemic and anti-hyperglycaemic actions[38].*V. amygdalina* has an astringent taste, which affects its intake [39].The bitter taste is due to anti-nutritional factors such as alkaloids, saponins, tannins and glycosides detected in the sample [39].

Effects of extracts on lipid profile are as presented in table 4. Each plant showed a significant reduction ($p<0.05$) in the lipid profile parameters (total cholesterol, triglyceride and low density lipoprotein) when compared with the alloxan-induced diabetic control except for Better leaf which had no significant effect in total cholesterol level. Extracts also significantly ($p<0.05$) increase HDL when compared to diabetic control. The high values for lipid profile parameters such as CHOL, LDL and TG observed in the alloxan-induced diabetic control group could be partly due to increased intestinal cholesterogenesis, which is due to high activity of 3-hydroxyl-3-methylglutamyl-CoA reductase in the intestine of the alloxan-induced diabetic rats [40], the rate limiting enzyme in the biosynthesis of cholesterol and also increased availability of acetyl-CoA resulting from increased fat oxidation in diabetes mellitus. It has been suggested that the increase in triglyceride of diabetic animals may be due to insulin deficiency which results in hyperglycaemia; fatty acids from adipose tissues are mobilized for energy purpose and excess fatty acids are accumulated in the liver, which are converted to triglyceride [41]. Research shows that insulin increases the number of LDL receptor, so chronic insulin deficiency might be associated with a diminished level of LDL receptor which leads to their high levels in serum or plasma thereby increasing LDL particles and result in the increase in LDL-cholesterol value in diabetes mellitus. Increased triglycerides, LDL and total cholesterols indicate high risk of atherosclerosis. They are used to evaluate the risk of heart disease. All extracts could have a positive effect on insulin levels as they significantly lower serum LDL cholesterol.

The effects of aqueous extracts on liver enzymes of alloxan-induced diabetic rats are as presented in table 5. The levels of AST, ALT and ALP have been reported to increase in alloxan-induced diabetic rats [42,43], as they were also reported in this study except for ALP. There was a significant reduction ($p<0.05$) in the serum level of AST and ALT by each plant extract, when compared with the alloxan-induced diabetic control. This suggests that aqueous extracts may have ameliorated the drug-induced damage to the liver cells as observed in diabetic untreated rats. Histopathological studies of CS has reveal that its hexane extract significantly reduce accumulation of mucopolysacchsarides in the kidneys of diabetic animals and had no nephrotoxic potential, suggesting the safety of CS [44].Certain fraction of BL has also been reported to improving renal and hepatic functions due to its effects on levels of ALT, AST and ALP. These observations are consistent with an earlier report on hepatoprotective potentials of leaf extracts of *V. amygdalina* in mice [45,46]. On the other hand, aqueous extracts of ML and oils from SF seed are said to be relatively safe when administered orally and authorized as food supplements respectively [47,48].

V. CONCLUSION

Results from the study reveal the hypoglycaemic properties of aqueous extracts of *Anacardium occidentale, Moringa oleifera, Vernonia amygdalina and Helianthus annuus*. The hypoglycaemic effect was in the order BL > ML > SF >CS.*Moringa oleifera* and *Vernonia amygdalina* competed favourably with the reference drug metformin. All extracts were able to improve the lipid profile of diabetic rats. All extracts except bitter leaf were also able to prevent the weight lost observed in diabetic untreated rats.

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