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Comparative Studies of the Aqueous Extracts of *OcimumGratissimum, Aloe Vera, Brassica Oleracea*and *Ipomoea Batatas* on Some Biochemical Parameters in Diabetic Rats

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Abstract:*The current orthodox treatment modalities for diabetic mellitus have many setbacks including undesirable side effects and the high cost of long term treatment. The aim of this study was to determine the anti-diabetic effectsand compare some biochemical parameters of four plants; Ocimumgratissimum (OG),Aloe vera (AV), Brassica oleracea(BO) and Ipomoea batatas(IB) on alloxan induced diabetic rats. The preliminary phytochemical screening reveals the presence ofalkaloid, tannin, cardiac glycoside, resin and steroids in all four plants. Diabetic was induced by interperitonial injection of alloxan monohydrate while treatment was done for 21 days. Diabetic rats had significant increased (P<0.05)cholesterol, triglyceride (TG), Low Density Lipoprotein (LDL) and artherogenic index (AI). Treatment of experimental rats with 150 mg/kg bw. of aqueous extractsof plants significantly decrease (P<0.05) the levels of cholesterol and LDL in OG and BO treated groups when compare with the control. Computed artherogenic index was significantly reduced (P<0.05) in AV treated group when compared to the control. Aqueous extracts also significantly (P<0.05) alter serum concentrations of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in a similar manner with metformin treated group. Result showed that decrease in plasma glucose was in the order IB>OG>BO>AV. All plant extracts possess antidiabetic, anti-hyperlipidemic effects and were able to ameliorate the weight loss observed in diabetic rats.*

Keywords:*Aloe vera,Brassicaoleracea,Comparativestudies,Diabetics,Ipomoeabatatas,Ocimumgratissimum*

I. Introduction

Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic values. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of this isolation were based on the uses of the agent in traditional medicine. The plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world inhabitant relying mainly on traditional medicines for their primary health care [1].

In Nigeria and many other countries of the world, plant parts or herbal preparations are used as herbal drug for the treatment of various ailments. In herbal practices, the practitioners claim that plant parts possess various phytochemicals which exhibit diverse pharmacological and biological responses and diversities [2]. It is also a common practice to use several combinations of herbs (Polyherb) in the treatment of a single disease, a reason why we choose to compare theeffects of the aqueous extract of four plants namely; *Ocimumgratissimum, Aloe vera, Brassica oleracea* and *Ipomoea batatas* on some biochemical parameters in diabetic rats.

Several plants have being used traditionally in the treatments of diabetics (Type 1 and Type 2) throughout the world, being a common disease and affecting the citizens of both developed and developing countries[3] it's therefore necessary to document several plants with anti-diabetic activities for easy identification and choice of usage.

Ocimumgratissimum(Lamiaceae)is a common plant in the Savannah and coastal areas of Nigeria, the plant is called "effinrin-nla" by the Yorubas, "Ahuji" by the Igbos, while in the Northern part of Nigeria, the Hausas call it "Daidoya"[4]. The plant is used in the treatment of epilepsy, high fever and diarrhoea [4]. Its traditional uses, morphology, phytochemistry, antihypertensive effect, hepatoprotective effects and more importantly for the purpose of our study, its anti-diabetic activities has been documented in a review by Prabhu*et al*.,[5]. Mohammed *et al.*, [6] reported the antidiabetic effect of aqueous leave extracts at a dosage of 250, 500 and 1000 mg/kg body weight with 500 mg/kg significantly lowering blood glucose level.

Aloe vera(barbadensis)belongs to the family *Xanthorrhoeaceae*, it's a popular plant, planted in gardens and around the household for its therapeutic purpose and as supplements in skin care. *Aloe vera* has been used as an anti-inflammatory natural agent, for treatment of ulcers, hepatitis and for wound healing[7]. Its anti-diabetic effect has been reported by Noor *et al.*, [8].

Brassica oleracea(*Brassicaceae*) is a biannual plant with great importance as human food crop plant. It has been reported that the ethanolic extract has hepatoprotectiveproperties[9]. *Ipomoea batatas* (*Convolvulaceae*) has been reported to contain antioxidativecomponents[10] and its white skinned tubers alsoposses' antidiabetic properties [11].In a review on Diabetes and medicinal plants by Kavishankar*et al*., [3], of the 136listed medicinal plants, *Brassica oleracea* and *Ipomoea batatas* were not included, making its necessary to document theirantidiabetic effects. The present study compares the hypoglycemic potentials of the leaves of these plants;*Ocimumgratissimum*(OG),*Aloe vera*(AV), *Brassica oleracea*(BO) and *Ipomoea batatas*(IB)at 150 mg/Kg bw. with metformin as a reference drug at 14.2 mg/Kg bw.

II. Materials and Methods

2.1 Collection of plant materials

Theplant materials; *Ocimumgratissimum* (scent leaf), *Aloe vera*, *Brassica oleracea* (cabbage) and *Ipomoea batatas*(sweet potatoleaf) 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 (*Rattusnorvegicus)* of both sexes(since diabetics affect males and females) from the animal house of university of Jos, Nigeria. Rats are of body weight between 130 – 220 g. The animals were maintained under standard environmental conditions, had free access to food (Grand Cereal products, Jos, Nigeria) and water *adlibitum*. Seven groups of five rats each were randomly distributed in cages and acclimatized for 7 days.

2.3 Preparation of plant extracts

The plants (leaves) material were rinsed with tap water and dried by spreading under the shade until a constant weight was obtained. The plant materials were thenpulverized into powder. 50grams of each leaves was soaked separatelyin 100ml of boiled distilled water and agitatedintermittently 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 extract was stored in an air tight container and was later reconstituted in distilled water to give the required dose of 150 mg/kg b.wt which was administered during the study.

2.4 Experimental design

Group 1: Normal control (positive control)
Group 2: Diabetic control (negative control)
Group 3: Diabetic group, received 14.2 mg/kg bw of Metformin
Group 4: Diabetic group, received 150mg/kg bw of scent leaf extract
Group 5: Diabetic group, received 150mg/kg bw of *Aloe vera* extract
Group 6: Diabetic group, received 150mg/kg bw of Cabbage extract
Group 7: Diabetic group, received 150mg/kg bw of Sweet potato leaf extract
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 RuinianQianjin Pharmaceuticals Ltd, China) at 14.2 mg/Kg bw per day. The extracts (150 mg/kg bw) were orally administered once daily for a period of twenty one (21) daysto 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 150mg/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 ≥250mg/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 from orbital plexus venous into centrifuge tubes

and were allowed to clot for about 45 minutes, after which they were spun at 3000rpm 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 by Harborne[12], Trease& Evans [13] and Sofowora[14].

2.9 Assay of biochemical parameters

Activities of alkaline phosphatase (ALP) were determined by the method of Wright *et al*., [15] while the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed by the method of Reitman & Frankel, [16]. Total cholesterol concentration in the serum was assayed by the method of Fredrickson *et al*., [17] while serum HDL-cholesterol concentration was determined using the method described by Albers *et al*., [18]. Serum triglyceride concentration was determined by the method of Hainline*et al*., [19]. The atherogenic index was calculated by finding the ratio of the serum Total cholesterol concentration to serum HDL-cholesterol concentration.

Statistical analysis

Data were presented as Mean ± SD of 5 replicates and were analyzed using Duncan multiple range test 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

3.1 Phytochemical screening

The phytochemical screening of the aqueous extract of *Ocimumgratissimum,Aloe vera, Brassica oleracea* and *Ipomoea batatas*indicated the presenceof alkaloid, tannin, cardiac glycoside, resin and steroids in all four plants. Flavonoids were not detected in OG, saponins and balsams were not detected in BO and IB, while phenols were only absent in BO (TABLE 1).

3.2 Body weight

Control groups had significant increase ($P<0.05$) in final weight while diabetic rats had significant decrease ($P<0.05$) in final weight when compared to the initial weights. The reference drug metformin had a similar increase compared to the control as their weight significantly increase ($P<0.05$) as seen on the last day of the experiment. OG, BO and IB showed decrease in weight which was however not significant ($P>0.05$) when compared to the initial weights. AV showed a significant difference ($P<0.05$) from its initial weight (Fig. 1).

3.3 Glucose level

Control rats maintained a blood glucose level below 100 mg/dl throughout the period of the study, while diabetic rats exhibited a gradual increase in plasma glucose to about 500 mg/dl. However, metformin and extracts treated groups showed various patterns in decreasing blood glucose levels with OG and IB competing favourably with the reference drug while AV showed the best response in the decreasing plasma glucose (Fig. 2).

3.4 Lipid profile

Total cholesterol was significantly increased ($P<0.05$) in diabetic rats. Metformin, AV and IB significantly reverse these increases in total cholesterol while OG and BO significantly reduced ($P<0.05$) total cholesterol when compared to the control.

Significant decrease and increase in HDL and TG respectively were observed only in diabetic control group while a significant increase ($P<0.05$) in LDL was observed in diabetic control and IB treated group. However, OG and BO showed significant decrease ($P<0.05$) with AV and metformin having values comparable to the control groups.

Computed artherogenic index (CHOL/HDL) had a significant increase ($P<0.05$) only in diabetic control while a significant decrease was observed in AV treated group (TABLE 2).

3.5 Liver enzyme

Results of ALP shows a significant increase ($P<0.05$) in all treated groups with metformin having the least value of 248±8.64 U/L, while BO has the highest value of 473±63.64 U/L. Significant decreases ($P<0.05$)

were observed in all treated groups for ALT and AST except for diabetic rats which had a significant increase (P<0.05) of 356.85±11.71 U/L when compared to the control (TABLE 3).

Table 1: Phytochemical screening of *Ocimumgratissimum*, *Aloe vera*, *Brassica oleracea* and *Ipomoea batatas* Water Extracts

Phytochemical	*Ocimumgratissimum*	*Aloe vera*	*Brassica oleracea*	*Ipomoea batatas* (leaf)
Flavonoids	–	+	+	+
Saponins	+	+	–	–
Alkaloids	+	+	+	+
Phenols	+	+	–	+
Resin	+	+	+	+
Tannins	+	+	+	+
C. Glycosides	+	+	+	+
Steroids	+	+	+	+
Balsam	+	+	–	–

Key + = Present
– = Absent

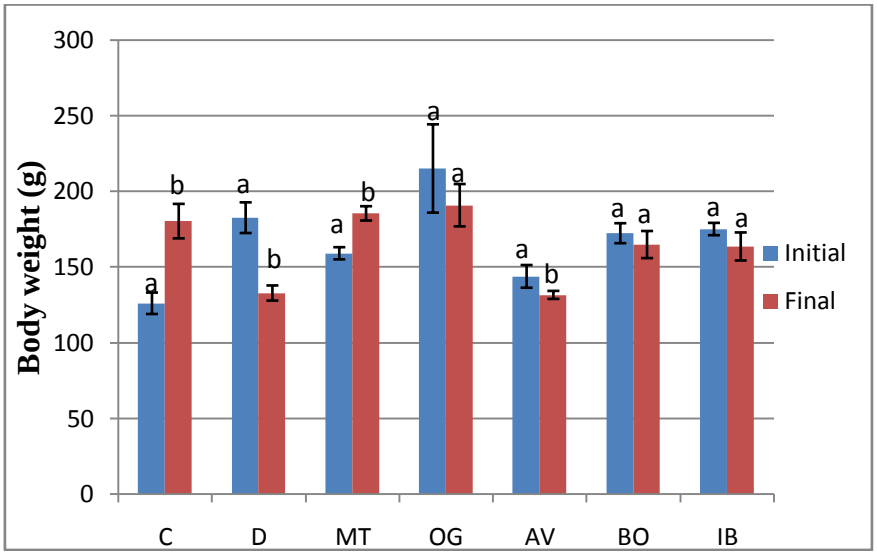

Figure 1: Effect of extracts on body weight

Values are means ± SD, n = 5. Values with different letter superscripts are significantly different (P<0.05).

C = Normal Control, D = Diabetic Control, MT = Metformin, OG = *Ocimumgratissimum,* AV= *Aloevera, BO = Brassica oleracea* and *IB = Ipomoea batatas*

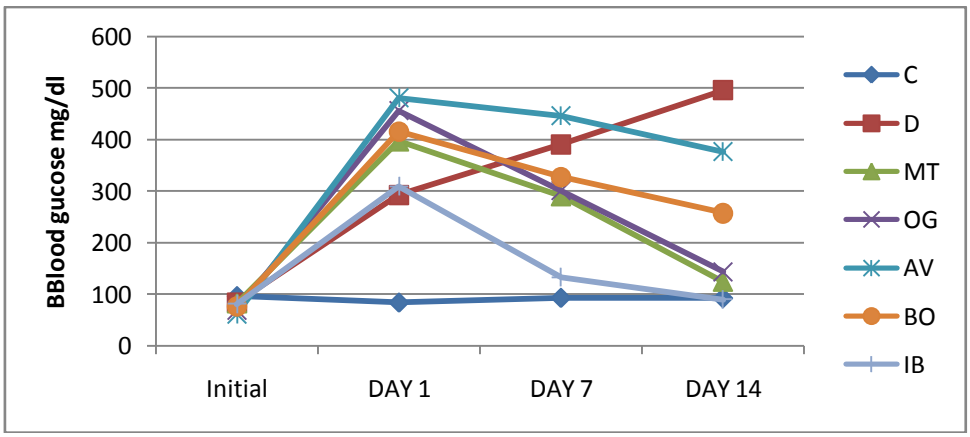

Figure 2: Effect of extracts on glucose level of alloxan-induced diabetic rats

Values are mean, n=5

C = Normal Control, D = Diabetic Control, MT = Metformin, OG = *Ocimumgratissimum,* AV= *Aloevera, BO = Brassica oleracea* and *IB = Ipomoea batatas*

Table 2: Effect ofextracts on lipid profile level of alloxan-induced diabetic rats

	CHOL **(mmol/L)**	HDL **(mmol/L)**	TG **(mmol/L)**	LDL **(mmol/L)**	AI
Normal Control	2.20±0.08[a]	0.50±0.08[a]	0.90±0.08[ab]	0.80±0.08[a]	2.34±0.06[a]
Diabetic Control	2.50±0.41[b]	0.44±0.02[b]	2.68±0.07[c]	1.52±0.02[b]	5.04±0.27[b]
Metformin	2.07±0.61[a]	0.95±0.37[a]	0.52±0.06[a]	0.91±0.12a	2.09±0.35[a]
Ocimumgratissimum	1.65±0.13[c]	0.87±0.88[a]	0.46±0.09[a]	0.43±0.05[c]	1.99±0.31[a]
Aloe vera	2.20±0.22[a]	1.22±0.07[a]	1.06±0.10[b]	0.66±0.15[a]	1.50±0.02[c]
Brassica oleracea	1.74±0.13[c]	0.92±0.12[a]	0.67±0.07[ab]	0.55±0.08[c]	1.79±0.34[a]
*Ipomoea batatas*leaf	2.10±0.70[a]	1.05±0.08[a]	1.57±0.19[b]	1.00±0.71[ab]	2.02±0.43[a]

Values are means ± SD, n = 5. Values in each column with different letter superscripts are significantly different (P<0.05).

CHOL = Total cholesterol; HDL = HDL- cholesterol; TG = triglyceride; LDL = LDL- cholesterol; AI = Artherogenic index

Table 3: Effect of extracts onsome liver enzymes of alloxan-induced diabetic rats

	ALP (U/L)	**ALT (U/L)**	**AST (U/L)**
Normal Control	218.00±0.82[a]	123.33±1.25[a]	274.33±1.25[a]
Diabetic Control	354.50±15.09[b]	99.58±5.97[b]	356.85±11.71[b]
Metformin	248.00±8.64[c]	42.63±3.63[c]	52.47±2.52[c]
Ocimumgratissimum	407.00±10.86[d]	16.60±2.27[d]	47.20±9.63[c]
Aloe vera	449.50±9.47[e]	33.30±4.74[c]	90.40±2.34[d]
Brassica oleracea	473.00±63.64[e]	39.88±4.21[c]	92.85±11.13[d]
*Ipomoea batatas*leaf	453.25±58.09[e]	37.73±2.72[c]	95.58±0.66[d]

Values are means ± SD, n = 5. Values in each column with different letter superscripts are significantly different (P<0.05)

IV. Discussion

Alloxan monohydrate is known to induce diabetes by partial destruction of pancreatic beta cells of the islet of langerhan[20, 21] ,this result in depletion of insulin levels and hyperglycemiaresults, leading to type 1 diabetes mellitus. The alloxan treated rats therefore represents a good laboratory model for insulin dependent diabetics mellitus (IDDM). The classical symptoms of diabetes includespolyurea, polydipsia, polyphagia and weight loss[22].The wetness and odour of the cages containing the alloxan-induced diabetic rats observed during the first five to eight days of the study was due to the polyuria which is a symptom of acute diabetics mellitus.Proteolysis, lipolysis and acute fluid loss during diabetics may also be responsible for weight loss [23]as observed in Fig. 1. Aqueousextracts from OG, BO and IB significantly (P<0.05) reverse these lost in weightobserved in diabetic rats.

The results of this study revealed the presence of several phytochemicals. Analysis of these plants extracts indicated appreciable amount of alkaloid, tannin, cardiac glycoside, resin and steroids in all plant materials. However, flavonoids were not detected in OG, saponins and balsams were not detected in BO and IB, while phenols were only absent in BO (TABLE 1). Phytochemicals screening of OGand BO were similar to the once reported by Prabhu*et al*., [5] and Ahmed *et al*., [9] respectively.

Over 150 plants extracts and some of their active principles include flavonoids are known to be used for the treatment of diabetes [24]. Moreover, tannin contains drugs demonstrated antidiabtetic activity [25].The fact that plants are endowed with a variety of phytochemicals or bioactive compounds that are thought to act on a variety of targets by various modes and mechanisms [26, 27] make these plants and their phytochemical components a source of anti-hyperglycemic agents.Saponin, flavonoids and tannins are reported to exhibit various hypergycemiceffects, increasing pancreatic secretions of insulin and may also participate in increased peripheral uptake of glucose [28, 29].

The antidiabtetic test shows that the 150mg/kg of these plants extracts *Ocimumgratissimum,Aloe vera, Brassica oleracea*and *Ipomoea batatas* reduced the blood glucose level in the treated diabetic animals when

compared with the diabetic control. Each groups of plants extracts had a significantly reduced level of glucose unlike the increased level of glucose observed in the diabetic animals. OG, MT, and IB competed in a likely manner, reducing the plasma glucose levelto below 250 mg/dl. BO was however better in reducing the glucose levels compared to AV.

The results of the lipid profile showed that the 150mg/kg of these plants extracts *Ocimumgratissimum,Aloe vera, Brassica oleracea*and *Ipomoea batatas*significantly ($P<0.05$) reduced the total cholesterol, triglyceride and low density lipoprotein, compared to the diabetic control. The high density lipoprotein which was reduced in the diabetic control was significantly increased ($P<0.05$) in each group of rats that was administered with *Ocimumgratissimum,Aloe vera, Brassica oleracea*and *Ipomoea batatas* plants extracts. The major complications in diabetes include aberrant lipid metabolism and vascular wall function. Since alterations in serum lipid profile are known in diabetes which is likely to increase the risk of coronary heart disease, a reduction in serum lipids particularly total cholesterol, triglyceride and low density lipoprotein level should be considered as a beneficial in long term prognosis of diabetic patients. Many plants extracts have the potential therapeutic value in combating multifactorial artherosclerotic disorder[30], which are parts of the major complications of diabetes. Reducing the risk of arthrosclerosis will thus lead to the development of effective and better management of hyperlipedemia. All plant extracts administered reduced the risk of atherosclerotic as observed by the computed AI. The best being AV and the least was IB. The atherogenic index (total cholesterol/HDL-cholesterol) is a reliable and strong indicator of cardiovascular diseases. Myocardial infarction increases considerably when the ratio is higher than 5 [31]. The ratios observed in this study were less than half of this value in all administered extracts and were not significantly different ($P>0.05$) when compared to the control except for AV which was significantly reduced (TABLE 2), suggesting that the extracts may not predispose subjects to coronary heart disease, thereby not giving rise to further cardiovascular complications during the diabetic treatment.

This study reveals that the plants extracts (*Ocimumgratissimum,Aloe Vera, Brassica oleracea*and *Ipomoea batatas*)at 150 mg/kg bw. Significantlyreduced ($P<0.05$) the levels of aspartate aminotransferase and alanine aminotransferase when compared with the diabetic control where cytosolic leakages of AST and ALP were observed.ALP was increased in the diabetic control as well as each group of rats that were administered with all plants extracts, it was however reduced in metformin treated group.During hepatic damage, cellular enzyme like AST, ALT and ALP present in the liver cell may leak into the serum resulting to increase their concentrations [32]. The results obtain in this study are similar to the reference drug, this may be due to the protective effect of extracts on liver cells.

Conclusion

It is evident from this studies that all aqueous plant extracts possess antidiabetic and anti-hyperlipidemic effects. The extracts were also able to ameliorate the effects of weight loss as observed in diabetic rats. The hypoglycemic effect was in the order IB >OG >BO >AV. The aqueous extracts may be completely safe at the dosage administered due to their effects on assayed marker enzymes. Further studies areon-going in our laboratory to document their possible synergetic or antagonistic effects.

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