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The Effect of Solvent Extracts of *Parimari microphylla* on Metabolites of Alloxan-Induced Diabetic Rats

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Abstract: The effect of solvent extracts (ethanol and normal saline) of the seed of *Parimari microphylla* was investigated on alloxan induced diabetic rats. Rats were administered orally with 500 mg kg^{-1} body weight of the extracts over a period of five weeks. Plasma glucose, total lipids and cholesterol levels of the animals were monitored throughout the period of the experiment. Also determined were the tissue cholesterol, total lipids, pyruvate and glycogen. The results shows high level of plasma glucose, total lipids and cholesterol i.e., hyperglycemia, hypperlipidemia and hypercholesterolemia, respectively in all the diabetic treated rats except those administered with the ethanolic extract of *P. microphylla* (EEPM) which reduced the plasma glucose, total lipids and cholesterol of the diabetic animals significantly ($p<0.05$). Also the elevated tissue pyruvate, total lipids and cholesterol in the diabetic rats were equally reduced significantly ($p<0.05$) by EEPM and their reduction is favourably compared to that of the control. EEPM increased the reduced tissue glycogen significantly ($p<0.05$). These results show that the EEPM possesses antidiabetic properties which can normalise all the alterations associated with metabolites in diabetic animals.

Key words: *Parimari microphylla*, hyperglycemia, diabetic rats, hyperlipidemia Antidiabetic properties, hypercholesterolemia

INTRODUCTION

Diabetes mellitus is a metabolic disorder in which glucose in the blood cannot be taken up by the cells hence their switching over to different nutrients such as fats and protein to generate energy. This will result to metabolic inbalance of virtually all the nutrients in the body (Valentine, 1992). Diabetes is caused by absolute or relative deficiency in the actions of insulin and possibly abnormally high amount of glucagons and other insulin-antagonizing substances such as growth hormones, amines and carticolamines (Stephen *et al*., 2005).

There are two major types of diabetics mellitus; the first occurs as a result of impairment of β-cells of the pancreas to secrete insulin leading to reduced insulin secretion referred to as Insulin Dependent Diabetes Mellitus (IDDM) or type 1. The disease could also occur when the insulin receptions on the surface of peripheral cell membranes are resistant to the circulating insulin called non Insulin Dependent Diabetes Mellitus (NIDDM) or type 2. The disease is characterized by fasting hyperglycemia, glycosuria and osmotic diuresis. Hunger, thirst and loss of weights are other manifestations.

Diabetes mellitus is the leading cause of adult blindness and renal failure, heart attacks and stroke (Mollar and Flier, 1991). About 60 to 70% of diabetic patients have mild to severe nerve damage

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resulting in slowed digestion, impaired sensation in the feet and hands and erectile dysfunction. Diabetes mellitus is a major public health problem affecting the whole world. It has been ranked the world's fastest growing metabotic disorder with an average annual growth of 1 to 2% (Olapade, 1995; Mitchell, 2005).

This consistent increase in the incidence of diabetes mellitus has spurred researchers in search of remedies and better treatment. The disease is not yet curable through orthodox medicine. The non-pharmacological means (diet and exercise) and/or the pharmacological means (insulin and oral hypoglycemics) are used merely for the management of the disease. The limitations in these management methods necessitate a search for alternatives among the arsenal of herbs available for man use (Trease and Evans, 1996).

The seeds of *Parimari microphylla* belongs to the family Chrysobalanaceae and is claimed good for all obstruction of the internal organs, arrest vomiting and allay nausea. It alleviates mostly all circulatory problems and used in the treatment of dysentery. The seed has influence on the activities of the pancreas and beneficiary effects on the glandular systems, the extract of the seeds has been claimed to be helpful in the treatment of diabetic mellitus. The objective of this research among others therefore is to authenticate the anti-diabetic properties of *P. microphylla* using alloxan induced diabetic rats.

MATERIALS AND METHODS

Extract Source and Preparation

The plant materials (seeds of *P. microphylla*) were purchased on 10th August 2006, at Ikare local market in Akoko N/E local Government area of Ondo State, Nigeria. They were air dried for two weeks at room temperature so as to preserve its basic biochemical components. They were later milled into powder. One hundred gram of the powder was separately soaked in 500 mL of 2% ethanol (w/v) and normal saline (0.9% NaCl w/v) for 48 h. The resulting mixtures were vigorously shaken for 6 h using Wrist Action shakers for thorough extractions. The extracts were separately filtered and the residue in each case were dried and re-weighed to know the quantity extracted. The filtrates contained 700 mg mL^{-1} in the case of normal saline and 750 mg cm^{-3} in the case of ethanolic extract.

Source of Animals and Feeding Trials

Thirty rats with average weight ranging between 105±3.75 to 130±4.35 g were arranged in 6 groups designated A to F with each group containing five rats. The rats were fed on Purina chow and water *ad libitum* throughout the period of the experiment. Animals in groups B to F were made diabetic by a single intraperitoneal injection of 80 mg kg^{-1} body weight of alloxan in distilled water. Blood glucose levels more than 180 mg dm^{-1} was chosen as an indication criterium for diabetes. The grouping and treatments of the animals were as follows:

Group A: Non-diabetic rats on distilled water and normal diet designated as NDDW otherwise know as control

Group B: Diabetic rats on 2% ethanolic extracts (500 mg kg^{-1} b.wt.) of *P. microphylla* designated as DEEPM

Group C: Diabetic rats on normal saline extracts (500 mg kg^{-1} b.wt.) of *P. microphylla* designated as DNEPM

Group D: Diabetic rats on 2% ethanol designated as DETH

Group E: Diabetic rats on normal saline designated as DNOS

Group F: Diabetic rats on distilled water designated as DDSW

EEPM: Ethanolic extracts of the seed of *Parimari microphylla*

Oral intubation was employed to administer the various extract to the animals. Each rat was treated as appropriate with the extracts on daily basis for a period of five weeks. Plasma glucose level of the rats was monitored throughout the period of the experiment. Also determined were the plasma cholesterol, total lipids, tissue glycogen and pyruvate. At the end of the 5th week the animals were sacrificed and dissected. The liver, kidney and heart were homogenized and used for the experiment removed. The method of Trinder (1969) was used for the determination of plasma glucose and cholesterol. Glycogen concentration in tissue homogenates was determined by the method of Jemmyn (1975), while pyruvate and total lipids were determined by the methods of Oser (1963) and Strova and Markarova (1989), respectively.

Statistical Analysis

Duncan Multiple Range Test (DMRT) was used for the analysis and interpretation of results obtained in this experiment. Groups with $p<0.05$ were taken to be statistically significant.

RESULTS AND DISCUSSION

There were numerous changes in metabolites that associates with diabetes as shown in this present study. Table 1 shows changes in plasma glucose concentrations of alloxan-induced diabetic rats treated with solvent extracts of the seed of *P. microphylla* over a period of five weeks, all the diabetic treated rats presented elevated plasma glucose. From the result, it is only the plasma glucose level of the group placed on ethanolic extract of *P. microphylla* (DEEPM) that had their blood glucose reduced significantly ($p<0.05$) when compared to all other diabetic treated rats. Also, of all the diabetic treated rats, the glycogen level of both the liver and kidney of DEEPM increased significantly ($p<0.05$) to almost the level of the control (Table 2) while the total pyruvate level of rats in this group (DEEPM) reduced significantly in the liver and kidney when compared to diabetic rats in other treated groups except the control (Table 2, 3).

Among the treated groups only DEEPM that showed significant reduction ($p<0.05$) in plasma total lipid levels (Table 4). In the same vein, the total lipids of the liver, kidney and heart of DEEPM also shown a significant ($p<0.05$) reduction when compared to other diabetic treated rats (Table 5). The plasma cholesterol level of all the diabetic rats were elevated but those in the group administered

Table 1: Plasma glucose concentration (mg dm^{-1}) of alloxan-induced diabetic rats administered solvent extracts of the seed of *P. microphylla* over a period of five weeks

	Week				
Animal groups	1	2	3	4	5
NDDW	82±3.46	86±5.16	85±4.60	92±3.41	96±4.20^{a}
DEEPM	258±8.42	200±4.55	180±4.42	132±4.32	100±5.28^{a}
DNEPM	262±6.48	248±8.55	255±6.42	246±4.55	242±7.32^{b}
DETH	256±6.34	262±7.42	248±8.55	230±6.43	253±6.46^{b}
DNOS	250±6.81	236±6.85	246±6.84	230±6.18	248±5.33^{b}
DDSW	240±6.41	240±5.86	248±4.67	248±4.67	244±5.33^{b}

Each value represent a mean of five determinations±SEM. Tabulated values with different letter(s) are significantly ($p<0.05$) different

Table 2: Glycogen concentration (mg kg^{-1} wet weight) in the liver and kidneys of alloxan-induced diabetic rats administered solvent extracts of the seed of *P. microphylla* over a period of five weeks

Animal groups	Liver glycogen	Kidney glycogen
NDDW	5.28±0.74^{a}	3.22±0.09^{a}
DEEPM	5.00±0.72^{a}	3.12±0.08^{a}
DNEPM	2.41±0.62^{b}	1.22±0.06^{b}
DETH	2.62±0.43^{b}	1.04±0.04^{b}
DNOS	1.78±0.62^{b}	1.26±0.07^{b}
DDSW	1.66±0.74^{b}	0.96±0.04^{b}

Each value represent a mean of five determinations±SEM. Tabulated values with different letter(s) are significantly ($p<0.05$) different

Table 3: Pyruvate concentration (mg/100 kg) in the liver and kidneys of alloxan-induced diabetic rats administered solvent extracts of the seed of *P. microphylla* over a period of five weeks

Animal groups	Liver pyruvate (mg/100 kg)	Kidney Pyruvate (mg/100 kg)
NDDW	68.42±4.22^{a}	46.24±3.62^{a}
DEEPM	73.36±5.32^{a}	50.22±4.33^{a}
DNEPM	112.24±6.42^{b}	87.26±5.61^{b}
DETH	115.48±7.46^{b}	89.46±6.68^{b}
DNOS	114.66±6.64	88.52±5.92^{b}
DDSW	115.84±7.22	86.33±5.22^{b}

Each value represents a mean of five determinations±SEM. Tabulated values with different letter(s) are significantly ($p<0.05$) different

Table 4: Plasma total lipid concentration (mg dL^{-1}) of alloxan-induced diabetic rats administered solvent extracts of the seed of *P. microphylla* over a period of five weeks

	Week				
Animal groups	1	2	3	4	5
NDDW	148±5.68	152±6.46	140±6.55	153±73	154±72^{a}
DEEPM	256±6.84	275±7.84	186±6.64	155±6.46	157±5.84^{a}
DNEPM	264±6.99	278±6.88	296±7.43	343±7.84	478±9.52^{a}
DETH	258±6.56	288±7.46	345±8.44	420±6.92	520±6.84^{a}
DNOS	250±7.48	272±6.84	356±7.33	434±7.35	548±7.42^{b}
DDSW	260±6.87	280±7.99	375±8.46	368±7.33	552±9.82^{b}

Each value represents a mean of five determinations±SEM. Tabulated values with different letter(s) are significantly ($p<0.05$) different

Table 5: Total lipids concentration (mg g^{-1}) in selected tissues of alloxan-induced diabetic rats administered solvent extracts of the seed of *P. microphylla* over a period of five weeks

Animal groups	Liver	Kidney	Heart
NDDW	125±4.68^{a}	92±4.88^{a}	46±2.84^{a}
DEEPM	130±6.42^{a}	96±5.22^{a}	50±3.11^{a}
DNEPM	256±5.82^{b}	164±6.21^{b}	98±5.46^{b}
DETH	260±4.86^{b}	165±4.36^{b}	100±4.82^{b}
DNOS	258±5.46^{b}	160±3.64^{b}	96±3.42^{b}
DDSW	262±6.48^{b}	162±5.84^{b}	99±4.52^{b}

Each value represents a mean of five determinations±SEM. Tabulated values with different letter(s) are significantly ($p<0.05$) different

Table 6: Plasma cholesterol concentration (mg L^{-1}) in alloxan-induced diabetic rats administered solvent extracts of the seed of *P. microphylla* over a period of five weeks

	Week				
Animal groups	1	2	3	4	5
NDDW	125±5.22	130±6.82	120±6.44	135±7.43	130±6.22^{a}
DEEPM	192±6.88	210±7.92	170±6.32	145±6.48	136±5.88^{a}
DNEPM	180±6.42	220±6.78	238±7.44	252±6.88	274±7.32^{b}
DETH	186±5.26	225±5.42	230±6.44	240±5.36	267±6.45^{b}
DNOS	194±5.51	230±6.47	244±7.40	256±5.86	279±6.44^{b}
DDSW	190±6.22	236±5.85	248±6.99	260±6.82	280±4.44^{b}

Each value represents a mean of five determinations±SEM. Tabulated values with different letter(s) are significantly ($p<0.05$) different

the ethanolic extract had their cholesterol level drastically reduced to almost the level of the control (NDDW) (Table 6). There was a significant ($p<0.05$) increase in cholesterol concentration in the liver, kidney and heart of all the diabetic treated rats except DEEPM groups which presented reduced cholesterol levels in all the organs (Table 7).

In diabetes, the tissue chemistry is affected (Floukes, 1971; WHO, 1985), which also has consequential effects on their metabolic activities. This also results in changes in key metabolites (biomolecules) either at the tissue and or plasma level(s). In this study, increased level of plasma glucose otherwise known as hyperglycemia, an important feature of diabetics was confirmed (Table 1). Hyperglycemia in diabetes has earlier been supported by the report of several researchers particularly Ayetobi (1995), Osadebe and Ajali (2001) and Mitchell (2005).

Table 7: Total cholesterol concentration (mg g^{-1}) in selected tissues of alloxan-induced diabetic rats administered solvent extracts of the seed of *P. microphylla* over a period of five weeks

Animal group	Liver	Kidney	Heart
NDDW	30±2.46[a]	21±3.62[a]	9.0±1.24[a]
DEEPM	34±3.14[a]	24±2.66[a]	11.0±1.84[a]
DNEPM	68±4.21[b]	32±2.48[b]	20.0±2.11[b]
DETH	72±4.24[b]	36±3.24[b]	22.0±3.11[b]
DNOS	73±3.24[b]	34±2.66[b]	23.0±4.31[b]
DDSW	74±3.46[b]	33±3.48[b]	21.0±4.81[b]

Each value represents a mean of five determinations ±SEM. Tabulated values with different letter(s) are significantly ($p<0.05$) different

The status of hyperglycemia in the diabetic rats as achieved in this study may be attributed to complete absence or relative deficiency of insulin. When insulin is absent or deficient; glucose will not be utilized appropriately by the peripheral tissues, there will be increased gluconeogenesis and glycogenolysis resulting in elevated plasma glucose level (Omoruyi and Adamson, 1989; Mahpara *et al*., 2006). However, the reduction in the plasma glucose level by the ethanolic extract of *P. microphylla* may be attributed to the bioactive ingredients in the plant extracts which may have insulin-like properties that promote glucose absorption, transportation and utilization by peripheral tissues.

Reduction in glycogen levels in the liver and kidney of diabetic rats (Table 2) as observed in this study is also in conformity with the documentation of Newsholme and Start (1976). Low level of tissue glycogen in diabetic animals is also attributable to insulin deficiency which results in reduction in glucose uptake by the peripheral tissues, this inturn will lead to decrease in glycogen synthesis in the glycogenic tissues (Labib, 1995). In diabetic, there is also increased level of glucargon (insulin antagonistic hormone) which enhances glycogen degradation in both the liver and the kidney, where, it is usually stored (Nerfinder *et al*., 1976).

Elevation of pyruvate concentration in the kidney and liver of diabetic rats as observed in this present study (Table 3), is in agreement with the report of Smith *et al*. (1983) and Williams (1989). Increase in tissue pyruvate levels of diabetic animals is also associated with insulin deficiency. This results in increase in glucose synthesis from non-carbohydrate precursors (Eastham, 1985) which would increase pyruvate level being an intermediate biomolecule in gluconeogenesis.

The increase in glycogen concentrations and decrease in pyruvate levels in tissue of diabetics rats by the ethanolic extract of *P. microphylla* (EEPM) in this study may be associated with the bioactive component of the plant extract which may possess insulin-like actions such as enhancement of glucose uptake by the peripheral tissues, activation of glycogenesis and inhibition glycogenenolysis and gluconeogenesis.

High levels of total lipids (hyperlipidemia) and cholesterol (hypercholesterolemia) in the plasma of diabetic rats as observed in this study (Table 4, 6) have also been documented (Mitchell, 2005). As indicated for plasma, high levels of total lipids and cholesterol were also observed in the tissue of diabetic rats (Table 5, 7). This conformed with the earlier report of West *et al*. (1966) and Smith *et al*. (1983). Insulin normally function to decrease lipolytic activity in adipose tissue, promote uptake of fatty acids and chylomicrons from the plasma by peripheral and adipose tissues and also enhancement of lipogenesis and lipid storage in the tissues (Mitchell, 2005). Deficiency of insulin in diabetics results in reduced clearance of lipids from the plasma after fatty meal and increased lipolytic activity leading to the release of fatty acids to the plasma and decrease lipogenesis. These result to a high level of lipid and fatty acids in the plasma, a condition known as hyperlipidaemia (Wills, 1986). Plasma hyperlipidaemia results in the deposition of lipid in tissues such as liver, kidney and heart leading to tissue lipidoses (Stroev, 1986).

Also, excess acetyl-CoA produced in diabetics is channeled towards the production of cholesterol in the liver which is released into plasma leading to hypercholesterolaemia (Earle, 1990). This also

results in the deposition of cholesterol in other tissue such as the kidney and heart as shown. The ability of EEPM to reduce the levels of plasma total lipid and cholesterol together with tissue cholesterol and lipid in diabetics may be due to bioactive ingredients present in the plant extract. EEPM may possess, among others, insulin-like properties that can decrease lipolytic activity, enhance lipogenesis and storage and possess cholesterol-lowering action.

This study revealed that in diabetic rats there was plasma hyperglycaemia, hyperlipidaemia, hypercholesterolaemia and gross alteration in other metabolites such as glycogen and pyruvate in the plasma and other tissues. It also showed that the ethanolic extract of the seed of *P. microphylla* was able to significantly ($p<0.05$) increase or reduce concentration of all these metabolites to the normal level and at the same time normalize the various metabolic alterations associated with diabetics in both the plasma and tissue levels. The mechanism by which EEPM was able to exhibit its antidiabetic properties is yet to be elucidated but it contains bioactive ingredient which exhibit this insulin-like properties. The bioactive ingredient of the EEPM when identified, isolated and administered in a purer form may have better antidiabetic properties. However, to achieve optimal glucose control without undue risk of abnormally lowering sugar levels, patients with diabetes should monitor their blood glucose regularly. In conclusion, the solvent extract of the *P. microphylla* can ameliorate some of the complication caused by alloxan-induced diabetes.

It is recommended that further study should be done on the seed of *P. microphylla* to isolate, identify, purify and characterized the bioactive ingredient in the seed that possess antidiabetic properties.

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