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Hypoglycemic and Hypolipidemic Indices of Ethanolic Leaf Extract of *Nephrolepis Undulata* in Alloxan Induced Diabetic Wistar Rats

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The global incidence of diabetes mellitus continues to present enormous social, economic and health burden. This study was carried out to investigate the hypoglycemic and hypolipidemic property of ethanolic leaf extract of *Nephrolepis undulata* on alloxan induced diabetes in Wistar rats. Fifty nine adult Wistar rats weighing between 120 -150g were used. Twenty four rats were used for toxicity of the plant, which was showed to have an (LD_{50})above 3200mg/kg. And thirty five rats were allotted randomly into 7 groups with 5 rats each(n=5). Groups 1 and 2 severed as the untreated non-diabetic and untreated diabetic controls respectively. Diabetes was induced using40mg/kg single dose injection of alloxan monohydrate and the treatment groups are metformin, insulin and *Nephrolepis undulata*. Weekly Body weight and fasting blood glucose level was checked and increase in body weight was observed in groups treated with metformin, insulin and varying doses of *Nephrolepis undulata*. At the end of the treatment period, laboratory analyses was done and data generated was subjected to statistical analysis using ANOVA and p values < 0.05 was considered significant. Result showed a decrease in serum fasting blood glucose levels, a decrease in TG, LDL and cholesterol levels, with an increase in HDL level across the treatment groups. The result trend observed in this study confirms *Nephrolepis undulata*as a plant with anti-diabetic potentials.

Keywords: Diabetes mellitus (DM), hypoglycaemic, hypolipidaemic, *Nephrolepis undulata.*

Metabolic disorders such as Diabetes mellitus (DM) is associated with insufficient insulin generation by pancreatic cell or disability of cells to respond to/or utilize produced insulin[1,2] One major characteristics of this disorder is hyperglycemia with associated metabolic disturbances in biomolecules such as carbohydrates, fats and protein metabolism[3]. Complications emanating from this disorder include polyuria, polydipsia, ketosis, retinopathy, polyphagia, as well as cardiovascular disorders. Over the past three decades, the prevalence of diabetes globally, has escalated more than twice the initial prevalence rates thereby exceeding far modeled anticipation or projections[4]. Even after improved surveillance, an estimated range of about 1 in 10 adults worldwide

are been affected currently by diabetes[5]. This rise worldwide is associated mainly with type II diabetes with 'metabolic syndrom'- a cluster of metabolic disruptions or disorders that culminate into insulin resistance, hypertension and dyslipidemia[2, 6].

Recently, especially with third world nations like Nigeria, a revamped interest in the application of herbal products in disease management is taken the center stage[7]. This renewed interest might be ascribed to the economic crunch as herbal remedies are not only considered to be efficacious but also cheap. The prototypic molecules of myriads of orthodox drugs are obtained from medicinal plants. An example of such prototype molecule is metformin. Interestingly, over 400 traditionally based plant targeted for diabetes treatments have been reported, although only a handful of these medicinal plants have received clinical trials, obtained scientific recognition and medical evaluation to scrutinize their efficacy. With respect to diabetes, the World Health Organization Expert Committee (WHOEC) has recommended that medicinal claims from traditional herbs be reassessed and investigatedfurther[8].

Prior to commencing this study, literature search revealed sparing scientific information on the anti-diabetic properties of this plant in rats. Hence this study was inspired, to investigate the hypoglycemic potential of *Nephrolepis undulata* and its associated hypolipidemic effect.

MATERIALS AND METHODS

Plant Material

Nephrolepis undulate leaves were air dried at room temperature (28±2ºC) for 5 weeks to a constant weight after which it was grounded with sterilized machine (SBM-2977, OSAKA, JAPAN) and sieved. Fine powder was collected for treatment. Identification of the plant was done by Botany Department, Delta State University

Extract preparation

Three hundred grams (300g) powder was transferred into a conical flask and soaked in 1500ml of absolute ethanol for 72hrs. With the aid of an electrical evaporator (rotary evaporator) conducted at 45ºC, a solvent extract was obtained and immediately transferred into a suitable container. The resultant extract was lyophilized using IIshin freeze dryer (Model No. FD5518, UK). Paste- like slurry concentrate was obtained, kept in an air-tight container and stored at 4^0C in refrigerator before use.

Qualitative and quantitative phytochemical screening

Series of chemical tests was conducted on the powdered plants extract to investigate the presence of secondary metabolites such as protein, reducing sugar, cardiac glycoside, steroids, polyphenoils, flavonoids, tannis, alkaloids, saponins and flavonoids respectively, using classic methods of Ojieh *et al.* (2013).

Animals protocol

Fifty-nine apparently healthy adult male Wistar rats of comparable age weighing between 150grams-200grams were used. They were exposed to standard laboratory condition of room temperature (24±2°C) and a relative humidity (46±6%) with 12hours light/dark cycle with adequate ventilation for the experimental time frame. The animals were rested for 10-14 days for acclimatization with access to rat chows and distilled water before being randomly apportioned into groups. Pre-induction serum blood glucose level of the animals was checked using Accuchek glucometer and the animals were handled based on the method stipulated by Ward and Elsea (1997).

Toxicity test (LD_{50} Studies)

Lather dose (LD_{50}) determination was done with 24 Wistar rats were using Lorke's method (1983) by giving different high dose concentration (800mg/kg, 1600mg/kg, 2900mg/kg, 3200mg/kg, 4100mg/kg and 5000mg/kg) to six different groups of six rats each and noting the dose that will produce 50% death. LD_{50} was calculated by = "a×b; where: *a* = least dose that kills a mouse and *b* = highest dose that does not kill any mouse.

Induction of diabetes /Drug reconstitution

Alloxan monohydrateat at a dose of 40mg/kg body weight single intraperitoneal injection was used[12, 13].Metformin was administered to the animals was using the following formula: Rat dose = (human dose/average BW) x 7 and 72 hours after induction, confirmation of diabetes was carried out by random blood glucose level of e"200mg/dl, using the Accuchek glucometer[14].

Duration of the Experiment

Total duration of the experiment was 5 weeks but treatment period was 3 weeks.

Sample collection

Blood glucose level was monitored weekly and at the close of the treatment period,the rats were sacrificed by cervical decapitation. Laparotomy was performed to assess the internal organs and samples collected for analyses. The blood samples were centrifuged at a rate of 4000 rpm for 10minutes and the serum was collected and stored in a refrigerator at 4^{o}C for analysis. Stored plasma sample were analyzed for plasma (HDL)-cholesterol, triglycerides (TG), and total cholesterol (TC) by enzymatic determination, using kits (Randox laboratories Ltd, U.K). Low Density Lipoprotein (LDL) was calculated from the Friedewald formula (LDL= Total cholesterol – [HDL + 0.46 – TG] mmol/l).

Statistical analysis

The results were expressed as Mean ± SD and statistical significance of the treatment effect was analyzed using one way analysis of variance and significance level at p values < 0.05 was considered significant, while p values > 0.05 was considered to be statistically non-significant.

RESULTS

Phytochemical screening of *Nephrolepis undulata* ethanolic extract(quantitative and qualitative) revealed major bioactive agents such as alkaloids, saponnins glycosides, tannins, steroids, flavonoids, fixed oils and fats resins, anthraquinones, proteins and amino acids and phenol.

The LD_{50} was estimated to be above 3200mg/kg. The administration of metformin, insulin and extracts of *Nephrolepis undulate* (200, 400 mg and 800 mg/kg) to the diabetic rats did not show significant improvement in body weight at week 1 to 2, while it was significant at week 3 (Table 2). As well,reduction in serum blood glucose levels was not significant at week 1 to 2, but it was significant at week 3 (Table 3). There was a significant increase in the cholesterol, triglycerides and low-density lipoprotein in diabetic rats as compared to control. The administration of metformin, insulin and extracts of *Nephrolepis undulata* resulted in reduction in serum cholesterol,

Table 1. Animal Grouping

Groups	Treatment
1	Diabetes not induced and no treatment done
2	Diabetic rats but no treatment done
3	Diabetic rats treated with Metformin @ 50mg/kg/day
4	Diabetic rats treated with Insulin @ 40ul/day
5	Diabetic rats treated with *Nephrolepis undulate* extract @ 200mg/kg/day
6	Diabetic rats treated with *Nephrolepis undulate* extract @ 400mg/kg/day
7	Diabetic rats treated with *Nephrolepis undulate* extract @ 800mg/kg/day

Table 2. Effect of ethanolic leaves extract of *Nephrolepis undulata* on the body weight (gm) Percentage changes in the body weight of the experimental animals

Groups	Initial Body Weight	Baseline Body weight	Final Body weight	% Δ in initial baseline bwt	% Δ in final baseline bwt
Positive control	140.40±3.70	140.56±3.59	150.94±2.95	09.44±1.32^{b}	7.39±1.27^{b}
Negative control	144.56±3.58	133.58±2.52	118.44±3.00	-22.21±3.11^{a}	-12.87±1.17^{a}
Metformin 50mg/kg	147.42±1.77	140.50±.82	135.96±2.73	-8.64±2.82^{b}	-3.47±1.73^{b}
Insulin	151.48±2.29	141.72±2.28	138.46±3.51	-9.52±1.28^{b}	-2.46±1.10^{b}
Extract 200mg/kg	146.04±1.88	133.20±2.03	140.02±2.70	-4.43±2.09^{b}	4.74±2.30^{b}
Extract 400mg/kg	145.44±2.03	131.78±1.04	137.90±3.36	-5.83±3.83^{b}	4.28±1.70^{b}
extract 800mg/kg	139.20±3.20	131.78±2.47	142.46±3.08	2.25±1.59^{b}	7.45±1.11^{b}

Values are expressed as mean± SD. ANOVA followed by LSD's multiple range tests. Values not sharing a common superscript differ significantly at P<0.05.\

Table 3. Effect of ethanolic leaves extract of *Nephrolepis undulate* on the glucose level (mg/dl) and percentage changes in glucose level of the experimental animals

FBS Groups	Initial Glucose Level 1	Baseline Glucose Level 2	Glucose Level Weeks 3	% Δ in initial and baseline FGL	% Δ in initial and final FGL	% Δ in baseline and final FGL
Positive control	84.40±6.82	87.00±2.05	97.00±3.85	2.89±7.79 [a]	13.05±6.00[a]	10.04±2.01 [a]
Negative control	85.00±3.65	298.60±38.85	600.00±12.10	69.30±4.49[b]	85.83±0.61[b]	50.23±6.48[b]
Metformin	87.20±3.92	317.40±12.53	124.00±4.60	72.40±1.50 [a]	28.96±5.44 [a]	-157.02±11.76 [a]
Insulin	87.60±2.87	314.00±21.31	148.40±7.56	71.59±2.24 [a]	40.45±3.17 [a]	-113.98±18.47[a]
Extract 200mg/kg	91.20±2.78	295.00±30.98	166.20±7.00	67.57±3.64 [a]	44.58±3.49 [a]	-77.00±15.07 [a]
Extract 400mg/kg	79.20±5.20	303.60±54.72	161.40±4.19	68.54±7.98 [a]	50.05±3.90 [a]	-86.94±34.80 [a]
Extract 800mg/kg	88.60±3.01	402.80±30.93	115.80±5.40	77.43±2.04 [a]	22.52±5.62 [a]	-248.07±20.56 [a]

Values are expressed as mean± SD. ANOVA followed by LSD's multiple range tests. Values not sharing a common superscript differ significantly at $P<0.05$.

triglycerides and low-density lipoprotein while increasing serum concentration of high density lipoprotein(Table 4).

DISCUSSION

The inability of some synthetic drugs with antioxidant effect such as butylated hydroxytoluene (BHT) in effectively managing diabetic mellitus has opened a new channel for local herbal investigation[15]. Diabetic mellitus which was once alien to developing nations is now a dreaded menace requiring urgent attention. Local herbs have been and are still being investigated in this regard to combat associated symptoms or manage this scourge tag diabetes[16]. It is on this premise that *Nephrolepisundulate* was investigated to x-rayit's hypoglycemic and hypolipidemic usefulness in medicine.

Phytochemical analysis of *Nephrolepis undulata*e thanolic extracts as observed from this work, revealed myriads of bioactive substances which is in consonant with documented literatures for this class of plant[17].

The median lethal dose (LD_{50}) was calculated to be above 3200 mg/kg. Clarke and Clarke, 1979, substantiated that any substance whose LD_{50} is above 1000 mg/kg is regarded as relatively safe. Similarly, WHO (1991) cogitated extracts or bio agents with LD_{50} above 3000 mg/kg as essentially safe.

Metformin as previously studied, increased insulin sensitivity of peripheral tissues by elevating glucose uptake by the same tissues. This increase glucose uptake is enhanced through translocation of glucose transporters in adipose and muscle tissue[12]. Furthermore, other investigations on metformin intake reveals that metformin elicits the inhibition of free fatty acids release into the circulation through suppressed activities of hormone-sensitive lipase with simultaneous increase clearance from circulation. The antidiabetic activity of *Nephrolepis undulata* ethanolic extract was dose dependent which might be attributed to the fact that the ethanolic extract promotes insulin secretion via closure of K^+-ATP channels, membrane depolarization and Ca^{2+} influxstimulation, which functions initially as a key step in insulin secretion. Contextually, a number of local herbs have been reported to possess

insulin stimulatory and antidiabetic effects[20], however their performance level is a function of the bioactive components present. Bioactive ingredients such as flavonoids, sterols, alkaloids, triterpenoids and phenolics are bioactive agents with known antidiabetic principles[21].In the alloxan induced diabetic rats, agents like flavonoids are scientifically perceived to attenuate and regenerate damaged beta cells[22]. Phenols more so, have shown some level of effectiveness asanti-hyperglycemic agents.

Results obtained from this study as it relates hypoglycemia seems to align with foregoing reports by Asuquo et al. (2010) who demonstrated the hypoglycemic effect of the *Nephrolepis undulata* extract. It has also been demonstrated that blood glucose reducing drugs acts by stimulating insulin synthesis and/or secretion of insulin fromâ-cells of the pancreatic islets. In addition, they may also increase sensitivity of receptors to insulin, insulinase inhibiting effect, and most pertinently stimulation of glucose uptake from peripheral tissues[12]. In spite of these demonstrations, the mechanism of action of this extract still remains to be explored. However, observations from previous studies indicated that intervention sites in the biochemical process of diabetes are diverse and multidimensional. Furthermore, the extract derived from local herbs or indigenous plants have been reported to attenuate carbohydrate absorption in the GIT thereby preventing progressive entry of carbohydrate into the blood and inhibit sudden elevation of blood glucose after food consumption[12].

A commonly recognized complication of DM is hyperlipidemia which is characterized by elevated levels of cholesterol, triglycerides, phospholipids and other lipoproteins[24].This is so since insulin activates lipoprotein lipase and hydrolysis of triglyceride under normal condition. Nevertheless, lipoprotein lipase in diabetic state is inactive due to insulin deficiency thereby producing hypertriglyceridemia. Metformin (50mg/kg), insulin (40µl/kg) and ethanolic extract of *Nephrolepis undulata (*200, 400 mg and 800 mg/kg) reduces triglycerides in serum of alloxan-induced diabetic rats. Previous literatures have indicated that Metformin or exogenous insulin administration may inhibit endogenous glucose production or act as an impediment with respect to gastrointestinal glucose absorption[25].

The mechanism of action of *Nephrolepis undulata ethanolic* extract at varying doses of 200, 400 mg/kg and 800 mg/kg appear uncertain. Notwithstanding, possible hypothesis have been postulated relating to the extract enhancing enzyme activity associated with bile acid synthesis and its excretion thereby decreasing serum cholesterol and triglycerides. In addition, the lipid attenuating effect of the ethanolic extract is suggestive of the activities of saponins, flavonoids, triterpenoids, steroids, glycosides and other phenolic compounds deposited in the plant. Bioactive agents like saponins has demonstrated immense significance as hypotensive, antihyper cholesterol and cardiac depressant properties[26, 27], while ingredient like triterpenoids with insulin-like activity is observed to normalize the rate of lipogenesis.

Table 4. Effect of Ethanolic leaves extract of *Nephrolepis undulata* on serum cholesterol, triglycerides and high-density lipoprotein and low-density lipoprotein alloxan induced diabetic rats

Groups	Cholesterol	Triglyceride (mg/dl)	HDL	LDL
Positive control	138.06±6.09^{a}	156.82±11.21^{a}	49.32±5.08^{a}	128.18±4.14^{a}
Negative control	191.38±12.48^{b}	359.37±107.48^{b}	22.03±5.54^{b}	190.10±9.58^{b}
Metformin	157.02±9.43^{a}	219.98±7.22^{a}	42.37±4.62^{a}	136.11±7.43^{a}
Insulin	160.45±14.31^{a}	161.57±11.93^{a}	33.56±2.44^{d}	137.41±6.56^{a}
Extract 200mg/kg	171.94±10.83^{d}	204.73±44.56^{d}	35.70±3.90^{d}	151.65±7.87^{c}
Extract 400mg/kg	140.55±12.97^{a}	162.98±12.74^{a}	41.80±1.10^{a}	138.34±1.45^{a}
Extract 800mg/kg	112.84±3.33^{c}	123.09±2.24^{c}	50.16±5.30^{a}	137.44±6.81^{a}

Values are expressed as Mean± SD. ANOVA followed by LSD's multiple range tests. Values not sharing a common superscript differ significantly at $P<0.05$.

Also, insulinotropic effect of flavonoids has been demonstrated to activate normoglycemia, while phenolic compounds are associated with lipid lowering ability[28].

The low concentrations of HDL and high concentration of LDL observed in diabetic rats compared to control rats in this study is in agreement or consistent with reports of several other studies[24, 29, 30]; demonstrating that a rise in glucose level on induction of diabetes, results in a corresponding increase in plasma lipids. It has been reported that elevated serum lipids in diabetes is due to the increased mobilization of free fatty acids from peripheral fat depots as a result of inhibition of the hormone sensitive lipase[31]. The excess fatty acids produced are converted into phospholipids and cholesterol, which together with excess triacylglycerols formed at the same time in the liver are discharged into the blood in form of lipoproteins. Thus, the marked hyperlipidemia observed in diabetic rats may be regarded as a consequence of uninhibited actions of lipolytic hormones in fats depots[32]. Treatment of diabetic rats with the extracts of *Nephrolepis undulata* caused a significant ($P < 0.05$) decrease in serum and liver lipids, showing its hypolipidemic effect.

The results of this study support earlier reports and could be related to the presence of alkaloids, saponins, flavonoids and polyphenols known to reduce serum lipid level in animals[33].

CONCLUSION

In conclusion, its worthy of note that the hallmark of the 21st century is the concerted effort in discovering compounds with the efficacy in managing chronic metabolic diseases, diabetes inclusive. This search has resulted in a more intense studying of traditional claims of potent herbs, leading to the discovery of new brand of drugs. With the immense therapeutic prospects of *Nephrolepis undulata* as applied by local herbal merchandise, this study has been able to establish that *Nephrolepis undulata* has both hypoglycaemic and hypolipidaemic properties.

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