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## Ameliorative Effects of Methanol Extract of *Englerina Drummondii* (Mistletoe) Leaves On Diabetes Mellitus-Induced Hyperglycaemia and Testicular Dysfunction In Male Wistar Rats

Ologhaguo M. Adienbo\*, Hart, Victor Opuada, Adienbo, Ologhaguo Macstephen  
Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Port Harcourt, Nigeria.

### ABSTRACT

Diabetes mellitus affects more than 100 million worldwide, with a soaring prevalence. Prolonged diabetes results in reproductive dysfunction. Mistletoe is widely used in ethnomedicine, including treatment of infertility. This study is aimed at evaluating effect of *Englerina drummondii* (mistletoe) leaves on testicular function in diabetic male rats. Methanol Extract of *Englerina drummondii* Leaves (MEDL) was obtained by soxhlet extraction. Thirty male Wistar rats (170–200 g) were grouped into 6 (A – F; n=5). Diabetes Mellitus (DM) was induced in groups D, E and F by administration of 250mg/kg fructose and High Fat Diet (HFD) for 6 weeks. Thereafter, rats in all groups were treated orally for 56 days with either MEDL or Distilled Water (DW) as follows: Group A (control) 1ml DW; B (500mg/kg MEDL), C (250mg/kg MEDL), D (DM + 250mg/kg MEDL), E (DM + 500mg/kg MEDL), F (DM + 1 ml DW; served as diabetic control). Fasting Blood Glucose (FBG) measured on weeks 0, 2, 4, 6 and 8. After treatment, rats were sacrificed, blood collected and serum used for testosterone; epididymal semen for sperm analysis. Results show that MEDL significantly ( $P<0.05$ ) reduced FBG in groups D and E ( $3.98\pm 0.21$  and  $4.04\pm 0.29$ mmol/L respectively), compared to group A ( $3.94\pm 0.14$ mmol/L). Treatment with MEDL to diabetic rats significantly ( $P<0.05$ ) increased sperm count, viability, and testosterone, while reducing sperm abnormality, compared to diabetic control. It was concluded that *Englerina drummondii* leaves possess anti hyperglycemic effects, and ameliorates testicular dysfunction in diabetic rats.

**Keywords:** *Englerina drummondii*, mistletoe, Diabetes mellitus, Sperm quality, testicular dysfunction.

\*Corresponding Author Email: [ologhaguo.adienbo@uniport.edu.ng](mailto:ologhaguo.adienbo@uniport.edu.ng)

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## INTRODUCTION

Diabetes mellitus is the most common endocrine disorder that affects more than 100 million (6% population) people worldwide. It is caused by the deficiency or ineffective action of the hormone insulin produced by the pancreas, which results in an increase in the concentrations of glucose in the blood (Deshmukh and Jain, 2015)<sup>1</sup>. Because of the tremendous increase in prevalence of diabetes mellitus, with a demographic transition in its epidemiology in recent years, populations previously unaffected or minimally affected by it are now reporting soaring prevalence figures (Uloko, *et al.* 2018)<sup>2</sup>.

The rate of infertility is a serious problem among couples in Nigeria. It is estimated that 56 % of infertile couples of childbearing age seek medical attention. Amidst these couples, 10% - 30% of infertility cases are attributed exclusively to the male (Temidayo and Stefan, 2018)<sup>3</sup>. Male infertility is a secondary complication that usually arises from diabetes mellitus, as studies have shown that diabetes mellitus affects male fertility by causing deleterious effects in the endocrine control of spermatogenesis, and in spermatogenesis itself (Rato *et al.* 2013; Condorelli, *et.al*, 2018)<sup>4,5</sup>.

Increased fructose consumption causes metabolic syndrome disorders such as insulin resistance, elevated triglycerides and oxidative stress (Nakagawa *et.al.* 2006)<sup>6</sup> which is evident in diabetes mellitus. This is because of fructose's unique metabolism that results in intracellular ATP depletion, uric acid generation, endothelial dysfunction, oxidative stress, and lipogenesis (Havel, 2005; Segal *et.al.* 2007)<sup>7,8</sup>.

Mistletoe has been used historically in medicine for its supposed value in treating arthritis, high blood pressure, epilepsy and infertility (Watson, 2001)<sup>9</sup>. Although there are several species of mistletoe varying widely in toxicity to humans (Judd, 2002)<sup>10</sup>, however there is paucity of information on the effects of the specie, *Englerina drummondii* (Balle ex R.M. Polhill & D. Wiens), on male reproductive functions, especially in a diabetic state. The aim of this study was to investigate the effect of the methanol extract of *Englerina drummondii* (mistletoe) leaves on sperm parameters in high fat diet and fructose-induced diabetic testicular dysfunction in male *wistar* rats.

## MATERIALS AND METHOD

### Plant Collection and Extraction

Fresh leaves of the *Englerina drummondii* (mistletoe) plant were harvested from a local farm in Bori, Rivers state, Nigeria. The plant identification was done at the University of Port Harcourt Herbarium (UPH/P/200). The leaves of the plant were air dried and pulverized into coarse powder form and kept at room temperature in an air tight container pending soxhlet extraction. Methanol Extract of *Englerina drummondii* Leaves (MEDL) was obtained by

soxhlet extraction. Three kilograms (3kg) of the powdered leaves was carefully packed in a soxhlet extractor and covered with cotton at the top. Then 200ml of methanol was poured in a round bottom flask for each pack and fixed in a heating mantle. Temperature was set at 50°C and the methanol was heated to evaporation and the extract collected and poured into an evaporating dish then left to cool.

### **Experimental animals**

Thirty (30) adult male Wistar rats weighing between 170-200g were used in this study. The rats were obtained from the Animal Unit of the Department of Human Physiology, University of Port Harcourt. The rats were acclimatized for two weeks and were housed in conventional wire mesh cages under standard laboratory conditions and were allowed free access to water and food *ad libitum* throughout the duration of the study; except when the rats were fasted for 12 hours prior to Fasting Blood Glucose (FBG) check.

### **Induction of Diabetes mellitus**

Type 2 Diabetes Mellitus was induced in some of the rats by the administration of 250mg/kg BW of fructose by gavage (Villegas, *et.al.* 2018)<sup>11</sup> and High Fat Diet (HFD) for 6 weeks prior to the commencement of treatment. The animals were placed on this diet immediately after their two weeks acclimatization. The HFD composed of 30% sucrose solution and a fifty-fifty percentage mixture of their normal feed and animal fat. The fructose solution was prepared daily for the 6 weeks using distilled water. Diabetes mellitus was confirmed in the animals after 6 weeks and only animals with a minimum FBG level of 7.0 mmol/L and above, which is often used as the diagnostic value for diabetes (American Diabetes Association, 2010; Rhoades, 2012)<sup>12</sup>, were selected for the study.

### **Experimental Design**

Thirty (30) adult male Wistar rats were selected into 6 groups (A – F, n = 5). The group A served as control and received a placebo of 1ml of distilled water. Groups B and C received 500mg/kg and 250mg/kg Methanol Extract of *Englerina drummondii* Leaves (MEDL) respectively. Animals in groups D, E and F received 250mg/kg of fructose (Villegas, *et.al.* 2018)<sup>11</sup> and High Fat Diet (HFD) for 6 weeks to induce Diabetes Mellitus in rats prior to the commencement of treatment. Thereafter, the Diabetic Rats (Db) in groups D and E were administered with 250mg/kg and 500mg/kg MEDL respectively. Group F served as the diabetic control and received a placebo of 1ml of distilled water. All extract treatment and placebos were administered to the rats daily by gavage throughout the duration of the study. The duration of treatment with MEDL lasted for 8 weeks (56 days) so as to cut across the spermatogenic cycle of *Wistar* rats (Rodrigues, *et.al.* 2016)<sup>13</sup>. Fasting blood glucose test was done before the commencement of treatment (week 0) and at every two week interval (week 2, week 4, week 6 and week 8) during treatment, using a glucometer with blood obtained from the tail of the

rats.

### Samples Collection

Twenty-four hours after last treatment, the rats were anaesthetized with 50 mg/kg sodium thiopental intraperitoneally. Blood was collected via cardiac-puncture and serum was obtained for hormonal assay. Semen examination was carried out using a modified method of Zemjanis (1970)<sup>14</sup> as described by Olufisayo and Oluremi (2008)<sup>15</sup>. Semen obtained from the epididymis by gentle pressure was placed on a pre-warmed slide. A drop of sodium citrate buffer (2.9%) was added to the semen and cover slip applied to evaluate motility under x40 of microscope. The semen sample was also stained with Eosin-Nigrosin to evaluate the ratio live to dead cells. This sample was used to estimate sperm abnormalities. The epididymis was then submerged in a graduated test-tube containing 5 ml of formol saline. The volume of semen was evaluated as the measure of displacement of formol saline. The entire epididymis was then crushed in formol saline and this mixture was used to evaluate spermatozoa concentration using the improved Neubauer haemocytometer.

### Data Analysis

Data were analyzed using SPSS (version 23) and Microsoft excel. Results obtained were expressed as Mean  $\pm$  SEM. Differences in mean were determined using Analysis of Variance (ANOVA) with multiple comparisons while Significant level was set at  $P < 0.05$ .

## RESULTS AND DISCUSSION

Table 1 shows that, at commencement of the treatment (week 0), all diabetes-induced rats in groups D, E and F showed significant ( $P < 0.05$ ) increase in Fasting Blood Glucose level, when compared to those in the normal control group. However, from week 2 through week 8, a reduction in the FBG was observed in the extract-treated diabetic rats (groups D and E), which was statistically not significant ( $P > 0.05$ ), when compared to the normal control; while the untreated diabetic rats remained hyperglycaemic with FBG significantly high, compared to the normal control group.

**Table 1: Effect of *Englerina drummondii* leaf extract on Fasting blood glucose in diabetic male rats**

Groups (n = 5)	FBG (mmol/L)				
	Week 0	Week 2	Week 4	Week 6	Week 8
Group A (Control)	3.88 $\pm$ 0.16 (3.40 / 4.40)	4.36 $\pm$ 0.29 (3.70 / 5.10)	4.06 $\pm$ 0.32 (3.30 / 5.20)	4.06 $\pm$ 0.29 (3.50 / 4.90)	3.94 $\pm$ 0.14 (3.70 / 4.40)
Group D (Db + 250mg/kg MEDL)	7.82 $\pm$ 0.17* (7.20 / 8.20)	4.24 $\pm$ 0.38 (3.40 / 5.50)	4.14 $\pm$ 0.24 (3.60 / 5.00)	4.10 $\pm$ 0.28 (3.40 / 4.80)	3.98 $\pm$ 0.21 (3.30 / 4.40)
Group E (Db + 500mg/kg MEDL)	7.66 $\pm$ 0.21* (7.30 / 8.20)	4.26 $\pm$ 0.29 (3.40 / 5.10)	4.36 $\pm$ 0.28 (3.70 / 5.20)	4.18 $\pm$ 0.32 (3.10 / 5.00)	4.04 $\pm$ 0.29 (3.10 / 4.80)
Group F (Db Control)	7.92 $\pm$ 0.20* (7.40 / 8.30)	6.74 $\pm$ 0.31* (5.80 / 7.60)	6.96 $\pm$ 0.13* (6.60 / 7.30)	6.78 $\pm$ 0.36* (5.70 / 7.70)	6.90 $\pm$ 0.22* (6.30 / 7.60)

Values are presented as Mean  $\pm$  S.E.M (Minimum value / Maximum value). \*Indicates significant difference ( $P < 0.05$ ) compared to normal control. <sup>a</sup>Indicates significant difference ( $p < 0.05$ ) compared to Diabetic Control.

Also, the sperm parameters (Table 2), show significant ( $P < 0.05$ ) decrease in sperm motility, viability and count respectively, in the untreated diabetic control group, when compared with the normal control group. Similarly, animals treated with the extract only (groups Band C), showed significant ( $P < 0.05$ ) increase in sperm motility, viability and count respectively, when compared with rats in the untreated diabetic control group. However, the extract-treated diabetic rats (groups D and E) had significant ( $P < 0.05$ ) reduction in sperm motility, viability and count respectively, when compared with the normal control group; while the sperm viability and motility were significantly increased in the diabetic rats treated with 250mg/kg of the extract (group D). In addition, sperm abnormality was significantly ( $P < 0.05$ ) increased in both the treated and untreated diabetic rats, compared to the normal control group. However, a significant ( $P < 0.05$ ) reduction was observed in both the extract-treated non diabetic groups (B and C) and the extract-treated diabetic groups (D and C) respectively, when compared to the diabetic control group.

Figure 1 shows the effect of *Englerina drummondii* leaves on serum testosterone level. There was a significant ( $P < 0.05$ ) increase in testosterone level in the rats treated with 500mg/kg and 250mg/kg bw of extract respectively, when compared with the normal control rats. Also, rats in the untreated diabetic group had significant ( $P < 0.05$ ) decrease in testosterone level, when compared with the normal control group; while the testosterone level increased significantly ( $P < 0.05$ ) in the groups treated with extract only, as well as in the extract-treated diabetic groups (D and E), when compared with the diabetic control group.

**Table 2: Effect of *Englerina drummondii* leaf extract on sperm parameters in diabetic male rats**

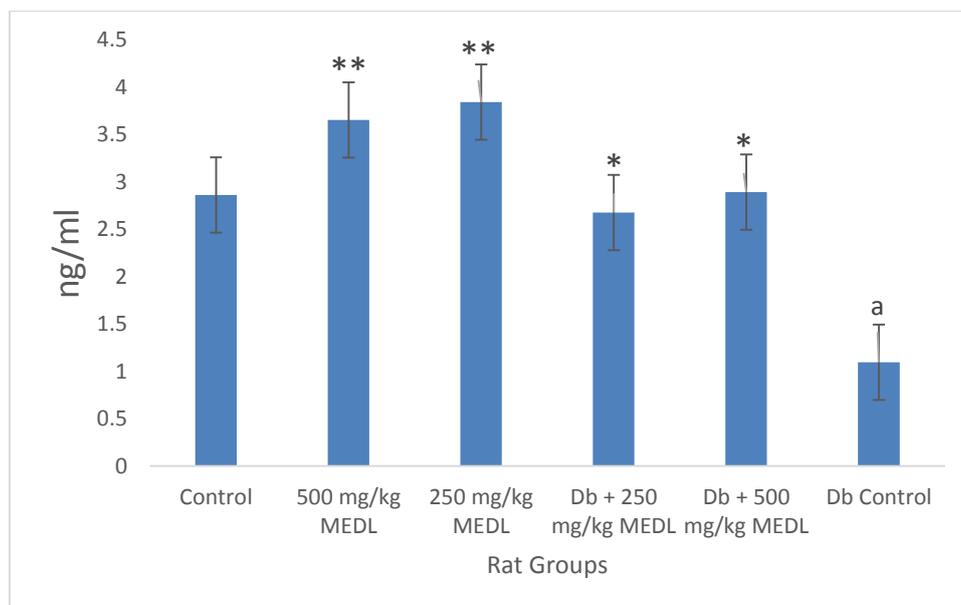
Groups (n = 5)	Sperm Motility (%)	Sperm Viability (%)	Sperm Count (million cell/ml)	Sperm Abnormality (%)
Group A (Control)	89.00 $\pm$ 1.87	85.60 $\pm$ 2.62	152.00 $\pm$ 6.26	9.08 $\pm$ 0.65
Group B (500 mg/kg MEDL)	95.20 $\pm$ 1.46 <sup>a</sup>	96.20 $\pm$ 1.20 <sup>**a</sup>	154.00 $\pm$ 6.22 <sup>a</sup>	8.78 $\pm$ 0.78 <sup>a</sup>
Group C (250 mg/kg MEDL)	84.20 $\pm$ 6.68 <sup>a</sup>	97.80 $\pm$ 0.86 <sup>**a</sup>	160.00 $\pm$ 3.37 <sup>a</sup>	7.35 $\pm$ 0.35 <sup>a</sup>
Group D (Db + 250 mg/kg MEDL)	70.00 $\pm$ 2.74 <sup>*</sup>	73.40 $\pm$ 2.66 <sup>*a</sup>	111.60 $\pm$ 5.01 <sup>*a</sup>	18.71 $\pm$ 1.11 <sup>*a</sup>
Group E (Db + 500 mg/kg MEDL)	77.00 $\pm$ 3.39 <sup>*</sup>	67.00 $\pm$ 3.74 <sup>*</sup>	103.60 $\pm$ 3.80 <sup>*</sup>	22.29 $\pm$ 2.10 <sup>*a</sup>
Group F (Db Control)	71.00 $\pm$ 4.00 <sup>*</sup>	65.00 $\pm$ 3.54 <sup>*</sup>	95.40 $\pm$ 2.73 <sup>*</sup>	27.49 $\pm$ 2.22 <sup>*</sup>

Values are presented as Mean  $\pm$  S.E.M.

\* Indicates significant decrease ( $p < 0.05$ ) compared to normal Control.

\*\* Indicates significant increase ( $p < 0.05$ ) compared to normal Control.

<sup>a</sup> Indicates significant difference ( $p < 0.05$ ) compared to Diabetic Control.



**Figure 1: Bar chart showing effect of methanol extract of *Englerina drummondii* leaves on Testosterone Hormone in male *Wistar* rats after 8 weeks of administration.**

\*\* Indicates significant increase ( $P < 0.05$ ) compared to Control group.

\* Indicates significant increase ( $P < 0.05$ ) compared to Diabetic Control group.

<sup>a</sup> Indicates significant decrease ( $P < 0.05$ ) compared to Control group and other groups.

From the results, the administration of the methanol extract of mistletoe (*Englerina drummondii*) leaf extract to the diabetes mellitus-induced rats significantly lower the FBG level when compared to the untreated mellitus-induced (DM) control group. This antihyperglycemic effect of the methanol extract of *Englerina drummondii* leaves (MEDL) as seen in this study, is similar to the effect of *Viscum album*; another species of mistletoe, on diabetic rats (Adaramoye, 2012)<sup>16</sup>.

The sperm count, motility and viability of rats in the non-diabetic rat groups administered with 500mg/kg and 250 mg/kg MEDL increased significantly when compared to the rats in the DM control group and the diabetes mellitus-induced rat groups treated with varied doses of MEDL. Treatment with MEDL to the diabetes mellitus-induced rat groups significantly improved sperm count and viability, especially at extract dose of 250mg/kg. These ameliorative effects of *Englerina drummondii* leaf extract may not be unrelated to the significantly increased level of testosterone seen in the non-diabetic and diabetic rats treated with the extract. Similarly, MEDL administration to the diabetes-induced rat groups significantly increased the level of testosterone to similar values found in the normal control rats. These enhancing effects of the methanol extract of *Englerina drummondii* leaves on sperm parameters agrees with earlier reports by Ojezele *et.al.* (2016)<sup>17</sup>, who investigated the effects of *Viscum album*, a different variety of mistletoe, on semen quality of *Wistar* albino rats.

## CONCLUSION

From the study, administration of mistletoe (*Englerina drummondii*) leaf extract has antihyperglycaemic effect in diabetic rats. It also improves diabetes-induced reductions in sperm count and sperm viability, as well as enhancement of testosterone level. Therefore, we conclude that the methanol extract of *Englerina drummondii* leaves possess ameliorative effects on diabetes-induced hyperglycaemia and testicular dysfunction in male *wistar* rats.

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