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Co-administration of Extracts of *Lophira lanceolata* and *Cyperus esculentus* Improves Libido and Fertility in Alloxan - Induced Diabetic Wistar Rats

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Abstract

A common complication of diabetes mellitus is the disturbance in the male reproductive system which often leads to impotence. In this study**,** we investigated the effect of co-administration of the aqueous leaf extract of *Lophira lanceolata* (LLAE) and *Cyperus esculentus* (CEAE) on the Fasting Blood Sugar (FBS), sexual behaviour, sperm count and sperm motility of alloxan-induced diabetic wistar rats. Diabetes was induced in rats by intraperitoneal injection of alloxan (150 mg/kg). Twenty (25) adult male diabetic wistar rats were randomized into 5 groups of 5 rats each. Group 1 served as control and received 5ml/kg of 0.9% Normal saline, Groups 2, 3, 4 and 5 received metformin (150mg/kg), LLAE (200mg/kg), CEAE (200mg/kg) and LLAE (200mg/kg)+CEAE (200mg/kg) respectively. Treatment was done orally for 28 days during which FBS was monitored weekly. On the 24th day of the treatment each male rat in each group was put in a cage with two estrous female rats in order to observe mounting and mating frequencies. At the end of the 28- day treatment, the rats were anaesthetized under chloroform and the epididymis was collected for semen analysis. The extracts and the co-administration produced significant ($P < 0.05$) reduction in FBS and significant ($P < 0.05$) increases in the mounting/ mating frequency, mean sperm count, sperm motility, epididymal weight and normal spermatozoa compared to the diabetic control group. The effects of the co-administration were observed to be significantly higher than that of metformin or either of the extracts administered alone. It was concluded that co- administration of the extracts of *Lophira lanceolata* and *Cyperus esculentus* through possible additive interaction possess pro-fertility effects. Hence, could be useful in the management of diabetes- associated sexual dysfunction in men.

Keywords: *Lophira lanceolata*, *Cyperus esculentus*, Libido, Fertility, Alloxan

1.0 Introduction

Diabetes mellitus is a chronic metabolic disorder resulting from deficiency in insulin secretion, insulin action or both (Wadkar *et al.,* 2008). It is characterized by hyperglycemia (Ugochukwu *et al.,* 2003) and abnormalities in lipid and protein metabolism (Scoppla *et al*., 2001). Hyperglycemia is an important factor in the development and progression of the complications of diabetes mellitus such as retinopathy, nephropathy, neuropathy, coronary artery disease, strokes, liver diseases and myocardial infarction (Ghosh and Collier, 2012).

Another common complication of diabetes mellitus is the disturbance in the male reproductive system. Glucose metabolism is an important event in spermatogenesis. Diabetes mellitus induces molecular alterations which negatively affect sperm quality and function as well as fertility (Mallidis *et al.,* 2011). Studies have shown that diabetic patients experience dysfunction in fertility, such as low sexual desire, erectile dysfunction, and difficulties in producing offspring (Kumar *et al.,* 2006). According to La Vignera *et al*. (2012), diabetes-induced rats exhibited low sperm quality and interrupted spermatogenesis in the testes. This statement is supported in the finding of Jangir and Jain, (2014) where diabetes mellitus caused hypospermatogenesis, decreased percentage of sperm motility and normal sperm morphology. Studies show that diabetes mellitus interrupts the production of lactate which is the fuel for sperm development (Jutte *et al.,* 1981). This event caused alteration in seminal parameters, and hence resulted in poor sperm morphology (Rato *et al.,* 2013. According to the meta-data compiled by Vasilios *et al.* (2016) the event of diabetes mellitus affected the total seminal volume and the percentage of motile sperm.

Many plants are used for medicinal purposes. Medicinal herbs and plants extracts are now generally considered as effective medicines that play a major role in modern pharmacy (Galani and Patel, 2010). In 1985, the world health organization estimated that about 80% of the world's population relies on medicinal plant for their primary health care needs. Although herbal medicine has existed since the dawn of time, our knowledge of how plants actually affect physiological function remains largely unexplored (Buckes, 1995). It is believed in some ethno-medicinal practices in Nigeria that the consumption of the decoction of *Cyperus esculentus* and *Lophira lanceolata* leaves can enhance fertility in males. They also believe that this combination can stimulate erection in males and enhance sexual performance of males that are impotent. However, the mechanism by which this combination works remain unknown. Therefore, this study examined the possible mode of action and also the synergistic effect of *Lophira lanceolata* extract co-administered with *Cyperus esculentus* on blood glucose level, libido and fertility, in terms of sperm quality, spermatogenesis, and sexual behaviour in alloxan-induced diabetic male rats.

Lophira lanceolata is a tree of the tropical and sub-tropical regions. It is a common tree in Cameroun, Nigeria and Sudan. It often grows gregariously on fallow land at the edge of forests. It is a tree of 8 to 10 m tall, straight or twisted, with leaves alternate, clustered at the end of short straight branches, glabrous, bright and blade oblong-lanceolate. The bark surface is corky grey (Arbonier, 2000). *Lophira lanceolata* is used in traditional medicine to treat several illnesses. The decoction of the fresh leaves is administered orally against headaches, dysentery, diarrhoea, cough, abdominal pains and cardiovascular diseases. It is also used on skin to cure wounds (Arbonier, 2000). *Cyperus esculentus* is an underutilized tuber of family *Cyperaceae*, which produces rhizomes from the base of the tuber that is somewhat spherical (Devries *et al.,* 1999). It is a tuber that grow freely and is consumed widely in Nigeria, other parts of west Africa, east Africa, parts of Europe particularly Spain as well as in the Arabian Peninsula (Abaejoh *et al.,* 2006). Tiger nut has excellent nutritional qualities with a fat composition similar to olives (Coskunerm *et al.,* 2002). It is also gluten and cholesterol free (Wills, 1980). It is the richest food source of flavonoids (El-Habshy, 1988) and also rich in water, fibers, alkaloids, digestible carbohydrates, saponins and fatty oils (glycerides). In addition, it also contains some elements, like phosphorus, potassium, calcium, iron, zinc, magnesium and manganese (Addy & Eteshola, 1984; Jeong *et al.,* 2000). Among the Egyptian herbal remedies, consumption of *cyperus esculentus* is relatively popular in some societies as an anti-diabetic agent (Gupta *et al.,* 1971: Ghazanfar, 1994). It is said to be suitable for diabetic persons (Borges et al., 2008) as well as being a powerful aphrodisiac (Caius, 1998). A study carried out by Hassan (2007) reported an appreciable hypoglycemia and hypolipidemia on streptozotocin-induced diabetic rats fed with tiger nut tubers.

2.0 Materials and Methods

2.1 Chemicals and drugs

Ethinyl estradiol, progesterone and all the chemicals used were of analytical grade and were purchased from Sigma Chemical Co. Ltd (USA) through a local vendor.

2.2 Animals

Adult Wistar rats of either sex weighing 150–200g were used for this study. They were kept in stainless steel cages under standard laboratory conditions. They were maintained on clean water and standard rodent feed.

2.3 Plant Collection and Identification

The leaves of *Lophira lanceolata* and nuts of *Cyperus esculentus* were bought from a market in Lokoja, Kogi State, Nigeria and were identified at the Herbarium Unit of the Department of Biological Sciences, Federal University Lokoja.

2.4 Preparation of Extracts

The plant materials were shade- dried for fifteen (15) days and pulverized separately using an electric blender. One thousand (1000) gram of each of the pulverized plant materials was soaked in distilled water separately for 72- hours. The resulting mixtures were filtered using Whatmann filter paper (Size No1) and the extracts were concentrated using free- dryer. The extracts of *Ficus Lophira lanceolata* and *Cyperus esculentus* shall henceforth be referred to as LLAE and CEAE respectively.

2.5 Experimental Design

2.5.1 Induction of diabetes

Diabetes was induced in adult male albino rats according to the method of Dunn and Mc Letchie (1943). The animals were fasted overnight and administered intraperitoneally 150 mg/ kg Alloxan monohydrate**.** After 72 h of administeration, rats having Fasting Blood Sugar (FBS) >200 mg/dl were considered hyperglycaemic and hence diabetic and used for the study.

2.5.2 Grouping of animals/ Treatment

Twenty-five (25) adult male diabetic albino rats were divided into 5 groups of 5 animals each and treated as follows:

Group 1: Diabetic control and received 5ml/kg 0.9 %Normal saline
Group 2: Diabetic and received 150mg/ kg Metformin
Group 3: Diabetic and received 200 mg/ kg LLAE
Group 4: Diabetic and received 200 mg/ kg CEAE
Group 5: Diabetic and received 200 mg/ FEAE + 200 mg/ kg CEAE

All treatments were carried out orally for 28 days. Fasting Blood Sugar (FBS) of the rats was monitored weekly during the period of treatment using Fine Test® glucometer and its corresponding strips.

2.5.3 Mounting frequency test

On the 24th day of the treatment each male rat in each group was put in a cage with two estrous female rats. Oestrous was induced in the female rats using 1mg progesterone and 100µg ethinylestradiol 6 and 48 hours respectively before the pairing (Varsha *et al.*, 2013). The rats were observed for mounting behavior. The number of times the male rat mounts the female within five minutes time frame was counted and recorded.

2.5.4 Mating frequency test

On the 26th day of treatment the sexual episode/intromission is usually established when a male rat mount a female rat and lick its penis. The number of times each male rat in all the groups mounted a female and licked its penis was recorded for a period of five minutes (Varsha *et al.*, 2013).

2.5.5 Sperm analysis

At the end of the 28- day treatment, the animals were anaesthetized under chloroform vapour and sacrificed. The epididymis was exposed by scrotal incisions and transferred into petri-dish. The weight of the epididymis was recorded for each rat. The epididymis was crushed using a blunt forceps in a petri-dish and 1 ml of normal saline was added to semen and mixed thoroughly using a syringe to draw and release the mixture continuously (Verma *et al.*, 2002). The semen mixture was then sucked into a red blood cell pipette to the 0.5 mark, then normal saline was sucked up to

the 101 mark. The normal saline in the stem of the pipette was discarded and the content of the bulb of the pipette was mixed thoroughly. A drop of the mixture was placed on the counting chamber which then spreads under the cover slip by capillary action. The counting chamber was then mounted on the slide stage of the microscope and viewed under x40 magnification. A grid system divides the counting chamber into five major squares each containing 16 smaller boxes. The count included all the sperm cells within the five major squares using the top and right or left and bottom system of counting as described by Verma *et al*. (2002) and Zaveneid and Polakoski (1977). The sperm count for a rat was calculated as = n x $1x10^{-6}$/ml of semen.

2.5.6 Sperm motility

A drop of the semen mixture was placed on a glass slide using 2 ml syringe, the preparation was placed on a microscope. Sperm motility was assessed as described by Sonmez *et al*. (2007). The motility of epididymal sperm was evaluated microscopically within 2–4 min of their isolation from the caudal epididymis and data were expressed as percentages of fast motile, slow motile and non-motile spermatozoa. The percentage of motility was evaluated visually at x40 magnification.

2.6 Statistical Analysis

Data were expressed as mean standard error of mean (SEM). Statistical comparisons were performed by one-way ANOVA, followed by Duncan's multiple comparisons test. Mean values were considered statistically significant when p-value is less than 0.05.

3.0 Results

The effect of the extracts co- administerations on the FBS of diabetic Wistar rats is presented in **Table 1**. Following alloxan administeration, there was an elevation in the FBS of rats compared to non- diabetic rats. Treatment with the standard drug- metformin and the extracts produced no significant (P>0.05) changes in FBS on Days 7 and 14 compared to diabetic control. However, on days 14, 21 and 28, Metformin (150mg/kg), LLAE (200 mg/kg), CEAE (200 mg/kg) and LLAE (200 mg/kg) + CEAE (200 mg/kg) produced significant (P< 0.05) reduction compared to diabetic control. The reduction in FBS produced by the co- administeration LLAE (200 mg/kg) + CEAE (200 mg/kg) was significantly (P< 0.05) higher compared to those produced by metformin, LLAE or CEAE alone. **Table 2** shows the effect of the extracts and their co-administeration on the mean sperm count and epididymal weight of the rats. The mean sperm counts of all the treatment groups were significantly (P < 0.05) increased compared to the control group. The mean epididymal weight of all the treated groups except the metformin- treated group were also significantly (P < 0.05) increased compared to control group. The increases in sperm count and epididymal weight produced by the co- administeration LLAE (200 mg/kg) + CEAE (200 mg/kg) were also significantly (P < 0.05) higher compared to those produced by metformin, LLAE or CEAE alone. **Figure 1** shows the effect of the treatments on mounting frequency of rats. There was a significant (P < 0.05) increase in mounting frequency in the treatment groups compared to the control group. The most significant (P < 0.05) increase in mounting frequency was observed in the group that received the co- administeration**.** Similarly, there was significant (P < 0.05) increase in mating frequency in the treatment groups compared to control with the co-administeration producing a significantly (P < 0.05) higher increase compared to other treatments **(Figure 2).** The effect of treatments on the motility of sperm cells of rats is presented in **Figure 3.** All the treatments produced significant (P < 0.05) increase in actively motile sperm compared to control. **Figure 4** shows the effect of treatments on the morphology of sperm of rats. All the treatments also produced significant (P < 0.05) increase in normal sperm with a corresponding decrease in abnormal sperm compared to control. The increase in normal sperm and decrease in abnormal sperm produced by the co-administeration LLAE (200 mg/kg) + CEAE (200 mg/kg) were also significantly (P < 0.05) higher compared to those produced by metformin, LLAE or CEAE alone.

Table 1: Effect of Co-administeration of the Extracts of *Lophira lanceolata* and *Cyperus esculentus* on Fasting Blood Sugar (FBS) of Alloxan- induced Diabetic Wistar Rats

Groups	Post- Treatment Time in days (d) 0	7	14	21	28
Control	334.3±31.24^{b}	350.5±35.41^{c}	356.4±51.26^{c}	350.5±58.42^{c}	306.8±44.82^{c}
Met 150mg/kg)	341.2±32.11^{b}	340.1±38.35^{c}	300.2±43.23^{bc}	285.6±45.31^{bc}	255.2±45.56^{bc}
LLAE (200mg/kg)	338.5±34.21^{b}	321.5±33.98^{bc}	299.4±27.65^{bc}	269.3±28.73^{bc}	249.5±28.53^{bc}
CEAE (200mg/kg)	330.2±23.16^{b}	305.4±29.61^{bc}	300.6±31.71^{bc}	273.1±30.34^{bc}	205.4±21.43^{b}
LLAE (200mg/kg)+ CEAE (200mg/kg	346.2±44.28^{b}	266.4±28.34^{b}	255.1±30.59^{b}	160.5±22.66^{ab}	103.5±21.23^{a}

Data are presented as mean ± SD. Data were analysed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=5). Mean values having different lower case alphabets as superscripts are considered significant ($p< 0.05$) down the columns.

Table 2: Effect of Co-administeration of the Extracts of *Lophira lanceolata* and *Cyperus esculentus* on Sperm Count and Epididymal Weight of Alloxan- induced Diabetic Wistar Rats

Groups	Sperm Count (million cells/ml)	Epididymal Weight (g)
Control	28.23 ± 1.55^{a}	0.18 ± 0.01^{a}
Met 150mg/kg)	40.21 ± 2.89^{b}	0.19 ± 0.05^{a}
LLAE (200mg/kg)	48.29 ± 2.43^{b}	0.27 ± 0.05^{b}
CEAE (200mg/kg)	45.26 ± 3.26^{b}	0.29 ± 0.07^{b}
LLAE (200mg/kg)+ CEAE (200mg/kg	48.39 ± 4.41^{b}	0.28 ± 0.09^{b}

Data are presented as mean ± SD. (n=5). Mean values with different alphabets as superscript are significantly ($P < 0.05$) different.

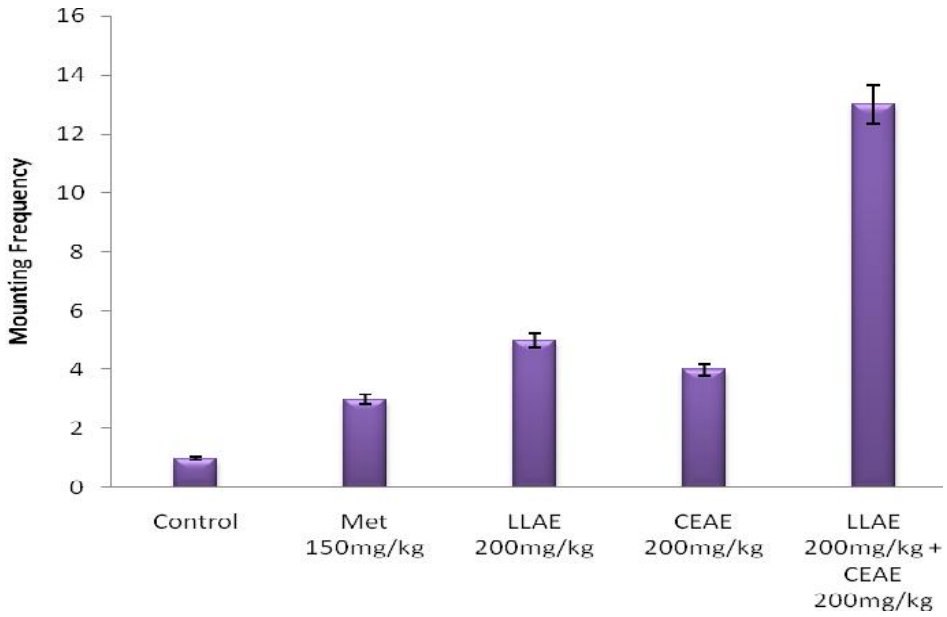

Figure 1: Effect of Co-administeration of the Extracts of *Lophira lanceolata* and *Cyperus esculentus* on mounting Frequency in Alloxan- induced Diabetic Wistar Rats

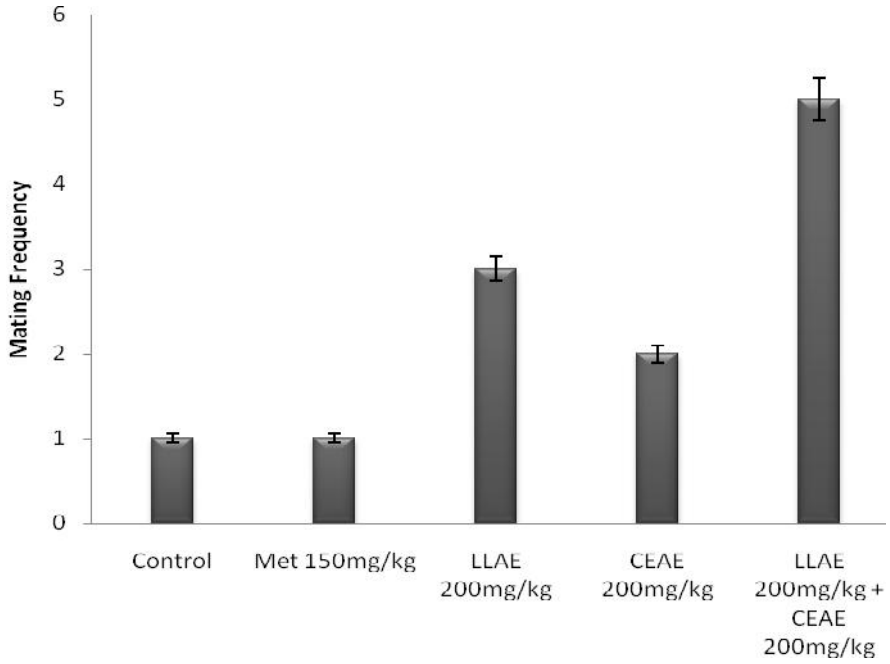

Figure 2: Effect of Co-administeration of the Extracts of *Lophira lanceolata* and *Cyperus esculentus* on Mating Frequency in Alloxan- induced Diabetic Wistar Rats

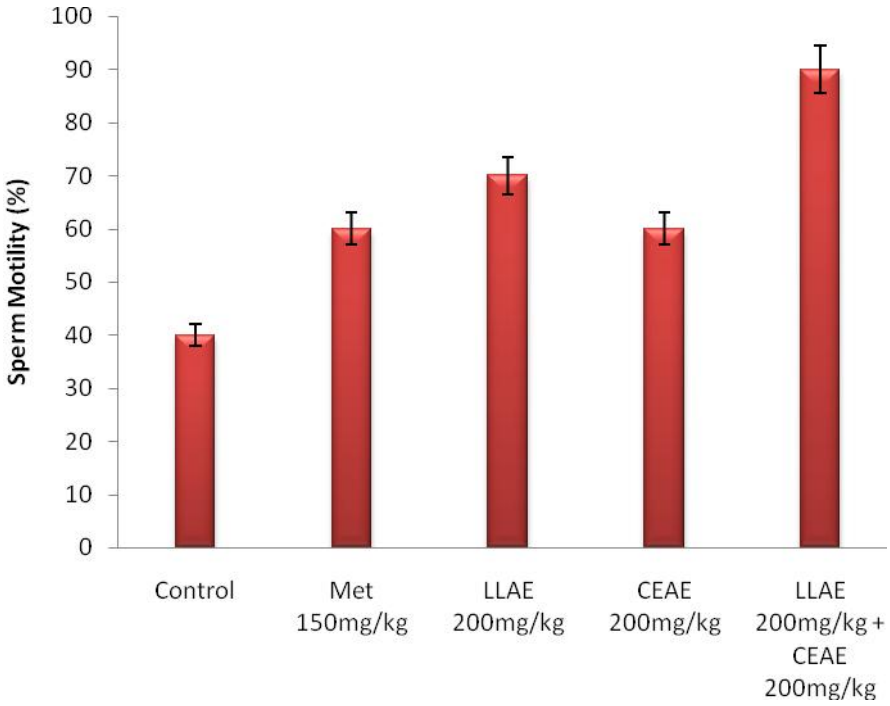

Figure 3: Effect of Co-administeration of the Extracts of *Lophira lanceolata* and *Cyperus esculentus* on Sperm Motility of Alloxan- induced Diabetic Wistar Rats

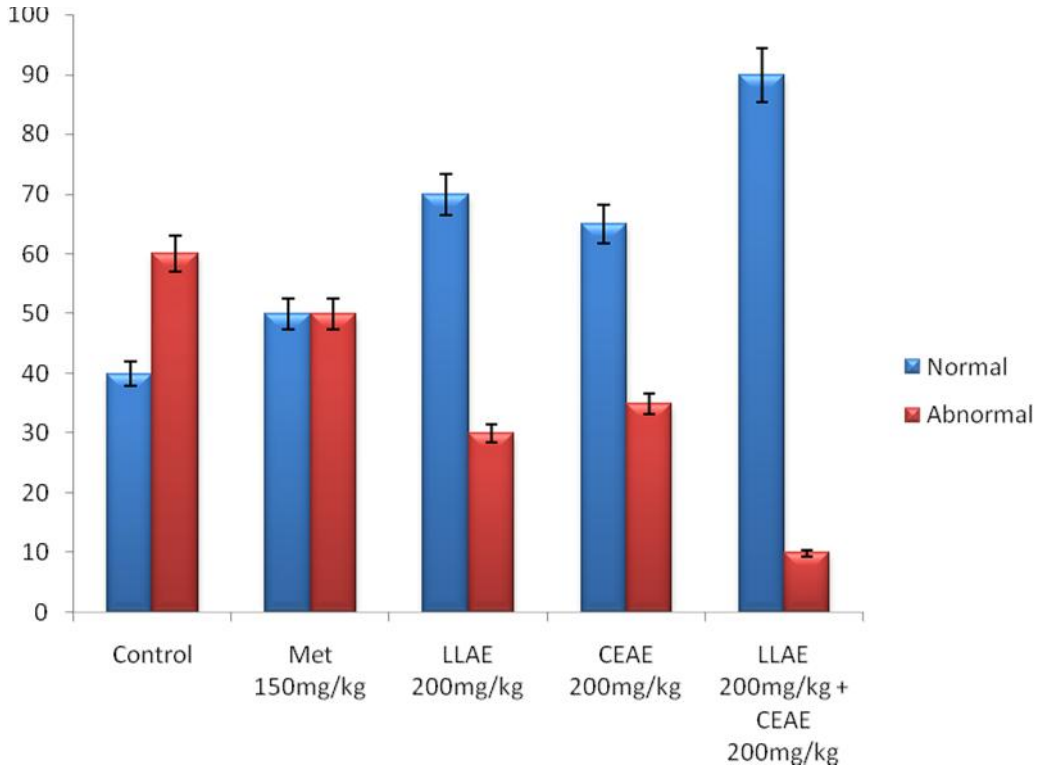

Figure 4: Effect of Co-administeration of the Extracts of *Lophira lanceolata* and *Cyperus esculentus* on Sperm Morphology of Alloxan- induced Diabetic Wistar Rats

4.0 Discussion

Alloxan, used to induce diabetes acts by selectively destroying the insulin- producing beta cells of the Islet of Langerhans producing hypoinsulinaemia (Szkudelski, 2001). This resultant insulin deficiency, leads to various metabolic alterations in the animals such as, increase in blood glucose, dyslipidemia and alterations in serum protein profile (Vivek *et al.*, 2010). Diabetes is associated with cardiovascular, obesity, neurological and reproduction disorders (Argiolas, 1999; Maiorino *et al.*, 2014; Tamas, 2014). Reproductive disorders in diabetic patient including: depression, anxiety, ejaculation dysfunction, decrease in levels of testosterone, progressive of endothelium tissue and smooth muscle tissue, damage to regulation of smooth muscle tone, decrease in number of Leydig cells (Argiolas, 1999; Tamas, 2014; Wankeu- Nya *et al.*, 2014). This study evaluated the effect of the co-administeration of the extracts of *Lophira lanceolata* and *Cyperus esculentus* on sexual behavior dysfunction and infertility in alloxan-induced diabetic rats.

Treatment with the standard drug- metformin and the extracts produced significant reduction in fasting blood sugar compared to diabetic control. The reduction in FBS produced by the co- administeration of *Lophira lanceolata* and *Cyperus esculentus* extracts was significantly higher compared to those produced by metformin and each of the extracts administered alone. The therapeutic actions of these plants might be attributed to their relatively high antioxidant potentials. Hypoglycaemic activity of phenolic compounds such as flavonoids and tannins from many medicinal plants has been reported by several studies. These antioxidants might have played a role in scavenging the free radicals generated by alloxan leading to the regeneration of the beta-cells destroyed by alloxan, hence an increase in release of insulin and reduction in glycaemia.

In this study, treatment with the extracts and their co-administeration significantly increased the mean sperm counts of the rats compared to the control group. The mean epididymal weight of all the treated groups except the metformin- treated group were also significantly increased compared to control group. The increases in sperm count and epididymal weight

produced by the co- administeration of the extracts were significantly higher compared to those produced by metformin or each of the extract administered alone. The observed increase in the sperm functions in the treated rats could be attributed to favourable and increased spermatogenic activities as results of high testosterone levels. Testosterone is known to be critically involved in the development of sperm cells and derangement results widely in leydig cell dysfunction and testicular steroidogenic disorder (Zhang *et al.,* 2001). It is known that a major function of the epididymis is sperm maturation which leads to the acquisition of fertilizing ability and viability of spermatozoa. Therefore, improvement in the activities of the epididymis could have led to an increase in progressive motility of sperm in the experimental rats.
This study also revealed a profound effect on the sexual behaviour of rats as it is evident in the mounting and mating frequencies of the rats. There was a significant increase in mounting frequency in the treatment groups compared to the control group. The most significant increase in mounting frequency was observed in the group that received the co-administeration**.** Similarly, there was significant increase in mating frequency in the treatment groups compared to control with the co- administeration producing a significantly higher increase compared to other treatments. An increased mounting frequency is considered an indication of sexual arousal and desire (Neil *et al.,* 1990). Disorder of sexual desire (libido) can involve either a deficient or compulsive desire for sexual activity and may include hypoactive sexual desire, a persistent or recurrent deficient or absence of sexual fantasy and desire for sexual activity (APA, 1994). The significant increase in mounting frequency observed with the extract- treated rats compared to the control group indicates an increase in sexual desire thus aphrodisiac activity. The significant increase in mating frequency produced by the extracts and their co-administeration implies aphrodisiac properties specifically, arousal, motivation and vigor which enable penetration and consequently sexual intercourse (Yakubu *et al.,* 2007).

This study also reveals that the extracts and the co-administeration produced significant increase in actively motile sperm compared to control. All the treatments also produced a significant increase in normal sperm with a corresponding decrease in abnormal sperm compared to control with the increase in normal sperm and decrease in abnormal sperm produced by the co- administeration significantly higher compared to those produced by metformin, or either extract administered alone. Fertility abnormalities are associated with low sperm count and abnormal sperm cells (Jia-Huhua and Ying-Hua, 2013) Fertilization therefore, requires adequate and normal sperm count, morphology and motility to occur. The observed effects produced by the extracts and the co-administeration imply fertility - enhancing potentials.

5.0 Conclusion

It can be concluded from the results of this study that *Cyperus esculentus* and *Lophira lanceolata* extracts appeared to be more potent in increasing libido and enhancing fertility of diabetic rats when co-administered possibly through additive interaction. The possible mechanisms of aphrodisiac and fertility-enhancing properties of the co- administered extracts include the ability to manage hyperglycaemia, increase libido and increase sperm count and motility. The co-administration of the extracts of *Cyperus esculentus* and *Lophira lanceolata* may therefore, play an important role as an alternative remedy in the treatment of diabetes associated- sexual dysfunction.

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