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Full Length Research Article

Methanolic extract of *Citrullus lanatus* Seeds Abates Testicular Degeneration and Dose-Dependently Modulates Testicular Function in Hyperlipidemic Male Wistar Rats

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Summary: Hyperlipidemia is emerging as an important cause of adverse health outcomes including cardiovascular complications, obesity, metabolic disorders, and infertility. A total of twenty-five (25) male albino Wistar rats were divided into five groups (n=5): Normal control, Hyperlipidemic control group which was administered (intra-peritoneal) with 0.2ml/10g body weight of egg yolk and then terminated after twenty-four (24) hours, Hyperlipidemic non-treated group which were administered with 0.2ml/10g body weight of egg yolk and were left throughout the treatment period. Hyperlipidemic low-dose treated group (administered 0.2ml/10g body weight of egg yolk, 800mg/kg body weight of methanolic extract of *Citrullus lanatus* seed-MECLS), and Hyperlipidemic high-dose treated group (administered 0.2ml/10g body weight of egg yolk, and 1600mg/kg body weight of MECLS). No significant change was observed in testosterone levels and sperm count across all groups. However, a statistically significant increase (P<0.05) in luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels was observed in Hyperlipidemic non-treated and Hyperlipidemic low-dose treated group when compared to Normal Control Group. They also showed marked testicular damage and significantly decreased (P<0.05) sperm cell motility and significantly increased sperm cell abnormalities. Hyperlipidemic low and high dose groups exhibited moderate and complete regeneration of testicular histo-architecture respectively. Furthermore, high dose treated group showed a significant decrease in sperm count, motility, LH and FSH levels. This study suggests that MECLS dose dependently ameliorates testicular damage induced by hyperlipidemia but may affect sperm cell characteristics.

Keywords: *Hyperlipidemia, Sperm count, testosterone, LH, FSH, Testicular histology*

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INTRODUCTION

Male infertility accounts for 40–50% of the reasons why couples are unable to conceive (Brugh and Lipshultz, 2004). Various abnormal metabolic and disease conditions increase the risk of male infertility (Ventimiglia *et al.,* 2016). Hyperlipidemia, or high cholesterol, means that one or more fat proteins in the blood is elevated beyond normal levels. It is believed that with rapid socioeconomic development, increasing prevalence of sedentary life styles and dietary changes, hyperlipidaemia is emerging as an important cause of adverse health outcomes globally (Zappalla and Gidding, 2009). An increment in circulatory lipids may induce oxidative stress causing significant production of oxygen free radicals, which may lead to oxidative modification in lipoproteins (Mishra et al., 2011) which predisposes individuals to development of type 2 diabetes (Zhou *et al.,* 2014), atherosclerosis (Snehalatha *et al,* 2014), stroke (Djelilovic-Vranic *et al.,* 2013; Tziomalos *et al.,* 2009) and cardiovascular diseases (Vergani and Lucchi, 2012).

Tanaka *et al.,* (2001) have suggested that hypercholesterolemia is an independent risk factor for testicular dysfunction in animal models. Studies have shown its detrimental effect on testicular histology and functions including spermatogenesis and steroidogenesis, epididymal sperm maturation process, sperm quality parameters, sperm fertilizing capacity and fertility index (Ashrafi *et al.,* 2013; Zhang *et al.,* 2012). The exact forms of Cholesterol and mechanisms by which they potentiate this negative effect remain subject to contemplation. Zmuda *et al.,* 1997 reported a negative correlation between testosterone levels and triglycerides levels, Garcia-Cruz *et al*.,(2012) suggests that hyperlipidemic men have significantly lower testosterone levels compared to non-hyperlipidemic controls.

The medicinal value of any plant depends on its chemical composition that elicits a specific physiological action on the human body. Over the years, man has explored the potential of plants for a number of other purposes, hence their dependency on plants increased both directly and indirectly (Ali and Qaiser, 2009). *Citrullus lanatus* (commonly known as water melon) is among the variety of fruits and vegetables consumed globally (Erhardt *et al.,* 2003). Water melon seeds have both nutritional and health importance, the seed contain vitamins B2, minerals (such as magnesium, potassium, phosphorous, sodium, iron, zinc,

manganese and copper), riboflavin, fat, carbohydrates, proteins (Lazos, 1986) and phytochemicals (Braide *et al.*, 2012). Seeds of watermelons are also known to be used in the preparation of snacks, flour sauces, cooking Oil and production of cosmetics (Jensen *et al.*, 2011).

This study was aimed at evaluating the potential effect of methanolic extract of *C. lanatus* (watermelon) seeds (MECLS) on testicular function (sperm profile and tissue histology) and some key reproductive hormones (FSH, LH and Testosterone) in hyperlipidemic animal models.

MATERIALS AND METHODS

Experimental animal: A total of twenty-five male albino Wistar rats weighing between 140 -180g was obtained from the animal house of the Faculty of Basic Medical Sciences, Alex Ekwueme Federal University Ndufu Alike Ikwo, (AE-FUNAI) for the experiment. The rats were acclimatized for two weeks before the experiment commenced. The rats were fed normal rat's pellets and water ad libitum and the animals were kept under standard conditions. The animal room was properly ventilated with a temperature range of 27-29^{0}C under 12hours day/light photoperiod regimen.

Plant Material: The seeds were obtained from 30 fruits *of citrullus lanatus* (watermelon) from a local market in Ikwo, Ebonyi State. The plant was authenticated by plant taxonomist from Department of Biology, Faculty of Science, AE-FUNAI. The flesh was removed and the seed collected was washed, sun-dried and milled into fine powder.

Preparation of Extracts: The fruits were thoroughly washed and cut open in order to get the seeds, then seeds were air-dried for three weeks after which it was weighed and the weight was 1kg;. Then pulverized using mortar and pestle under aseptic conditions and ground to powder using a blender (waring commercial blender, model no. HGB2WTS3). The method of extraction employed was percolation. After grinding, the powdered form of the seeds weighed 0.9kg which was soaked into 1500ml of methanol in air tight container kept at room temperature for a period of three days. After three days, it was filtered using cheese cloth in order to remove the chaff after which the filtrate was evaporated using evaporator water bath machine. The extract was constituted using normal saline as a vehicle where by 20g of the extract was dissolved 100ml of the vehicle and 30g of the extract was dissolved in 100ml of the vehicle for low and high dosage respectively.

Experimental Design: Twenty-five (25) male albino Wistar rats was randomly selected and divided into five (5) groups, consisting of five (5) rats per group viz: Control group**.,** Hyperlipidemic control group (Sacrificed 24 hours after inducing hyperlipidemia), hyperlipidemic untreated group (left for the duration of treatment after inducing hyperlipidemia)**,** Hyperlipidemic low-dose treated group: (received 800mg/kg body weight of methanolic extract of *Citrullus lanatus* seed (MECLS) for twenty-one days) and Hyperlipidemic high-dose treated group: received 600mg/kg body weight of (MECLS) for twenty-one days. All rats were allowed free access to drinking water.

Inducing hyperlipidemia: Hyperlipidemia was induced by intra-peritoneal administration of egg yolk (0.2ml/10g body weight) for a day (Song *et al,.* 2013 and Anuwat *et al.*, 2017). After which the HCG were sacrificed after 12 hours fasting period to ascertain the attainment of hyperlipidemia.

Experimental Procedure: The treatment regimen was done through oral gavage. After which blood sample was collected from the animals from all groups via retro-orbital puncture using heparinized capillary tubes.

Laboratory Analysis:

Serum lipid profile: Total cholesterol and triglyceride level was estimated using Enzymatic Method (Allain *et al.*, 1974). Lipoprotein analysis was estimated using the Direct method (Sugiuchi *et al.*, 1995; Rifai *et al.*, 1992).

Hormonal Assay: An enzyme-based immunoassay system was used to measure testosterone, FSH and LH levels in serum samples collected. Blood serum was introduced into micro–plate well for each sample to be measured. Thereafter, an enzyme antigen linked conjugate for each hormone was added respectively and then incubated for 1 h at room temperature. The plate was then washed with micro plate washer to remove all unbound material. After washing, excess fluids were taped off. Then color was developed by adding color reagent to determine the bound hormone. Quantitative test result was obtained by measuring the absorbance. The color intensity was checked by tasking the ELISA plate to an ELISA reader which is attached to spectrophotometer that read the absorbance.

Testicular histology: The testes were fixed in 10% Formalin, after complete fixation the blocks was embedded in paraffin and sections cut at 5mm (micron) using a microtome and then stained with hematoxylin and eosin and mounted. Microscopic examination of the sections was then carried out under a light microscope and later the microscopic slides of the testes were photographed at magnification ×400.

Sperm profile: Sperm morphology was investigated using a microscope, Sperm motility was evaluated by an earlier method by saalu *et al.*, 2010. Sperm count was done using methods by Neubauer's counting chamber method.

RESULTS

Lipid profile: High cholesterol intake is known to induce hyperlipidemia. As shown in Table 1, we recorded a significant increase of total cholesterol, triglyceride, low density lipoprotein, and a significant decrease (P<0.05) in HDL in hyperlipidemic control group when compared to other groups. This finding demonstrates that intra-peritoneal administration of egg yolk induced hyperlipidemia. This is consistent with other studies by Pashaie *et al.*, 2017 and Sumbul and Ahmed, 2012. Results from Table 1 although shows that over time, there was a significant recovery. With cholesterol levels returning back to normal in hyperlipidemic non-treated group. Subsequent findings however convey the fact that despite this recovery, significant alterations in reproductive parameters were still persistent

Table 1
Lipid profile of the various groups.

Lipid profile	Normal control group (*n*=5)	Hyperlipidemic control group (*n*=5)	Hyperlipidemic non-treated group (*n*=5)	Hyperlipidemic low-dose treated group (*n*=5)	Hyperlipidemic high-dose treated group (*n*=5)	*P*- value
Total cholesterol (mmol/l)	1.20±0.01	4.44 ± 0.21*	1.44 ± 0.05	1.08± 0.03	1.45± 0.02	<0.001*
Triglyceride (mmol/l)	0.45± 0.01	4.03± 0.11*	0.53±0.05	0.36± 0.01	0.53± 0.08	<0.001*
HDL (mmol/l)	1.13± 0.02	0.43± 0.12*	0.86± 0.02	1.00± 0.11	1.05± 0.01	<0.001*
LDL (mmol/l)	0.31± 0.01	1.58± 0.58*	0.32± 0.04	0.19± 0.01	0.29± 0.02	<0.006*

mmol/l = milimole per litre. HDL= High density lipoproteins, LDL = low density lipoproteins. Values are mean ± SEM, (n=5). One way ANOVA followed by Newman-Keuls Multiple Comparison Test was used for statistical significance. "" denotes statistically significant compared to other groups (P < 0.05).*

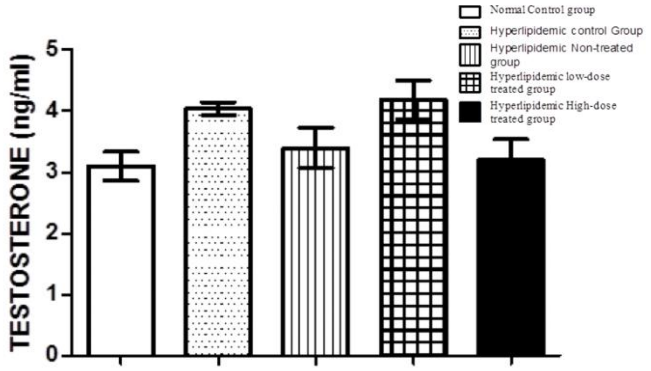
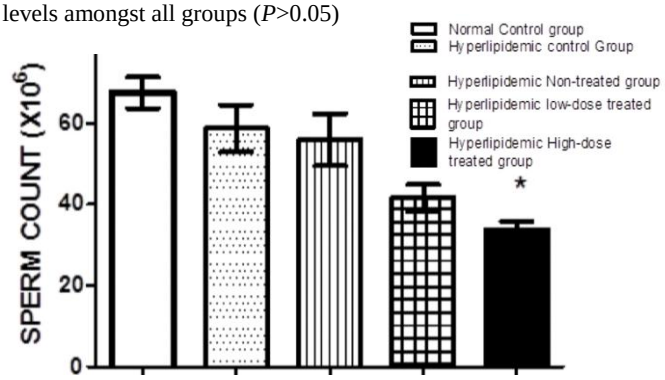


Figure 1:
A comparison of Testosterone levels among the various groups. There was no statistical significance difference in testosterone levels amongst all groups (*P*>0.05)



Testosterone Levels: Figure 1 shows that there was no significant change in testosterone levels among the various group. It remained steady both in hyperlipidemic rats and hyperlipidemic treated rats.

Sperm Count: We observed a significant decrease (*P*>0.05) in sperm cell count in hyperlipidemic high-dose treated group when compared to hyperlipidemic control and hyperlipidemic non-treated groups (Figure 2).

Mid-piece Abnormality: We observed statistically significant increase (*P*<0.05) in sperm cell with mid-piece abnormality in hyperlipidemic non-treated group and hyperlipidemic low-dose treated group when compared to normal control group and hyperlipidemic control group. (Figure 3).

Active Motility: Figure 4 shows a statistically significant decrease (*P*<0.05) in sperm cell with active motility in hyperlipidemic control group when compared to normal control and hyperlipidemic control group. There was also a significant decrease (*P*<0.05) in hyperlipidemic high-dose treated group when compared to other groups.

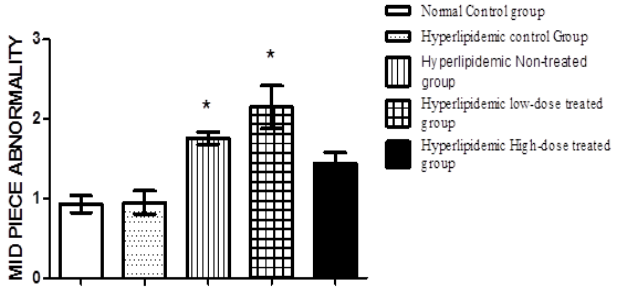


Figure 3:
Comparison of sperm cell with mid-piece abnormalities. *= statistically significant difference amongst the various groups (*P*<0.05).

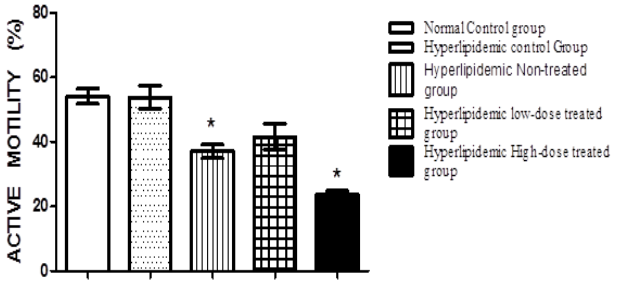


Figure 4:
Comparison of sperm cells with active motility. * = Significant decrease (*P*<0.05).

Luteinizing Hormone: Results from this study (Figure 5) shows that there was a significant increase in LH levels in Hyperlipidemic non-treated group and Hyperlipidemic low-dose treatment group when compared to another group.

Follicle Stimulating Hormone: A statistically significant increase (*P*<0.05) in FSH levels in Hyperlipidemic control group, Hyperlipidemic non-treated group and Hyperlipidemic low-dose treated group when compared to normal control group. A statistically significant decrease (*P*<0.05) in FSH levels in hyperlipidemic high-dose treated group when compared to Hyperlipidemic control group, Hyperlipidemic non-treated group and Hyperlipidemic low-dose treated group.

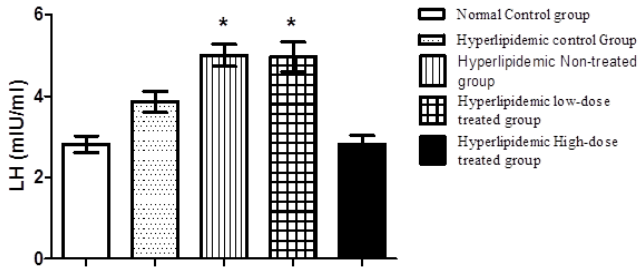


Figure 5:
Effect of *Citrullus lanatus* seed on the luteinizing hormone (LH) level of the groups. * = significant difference, which means that there is a significant increase (P<0.05).

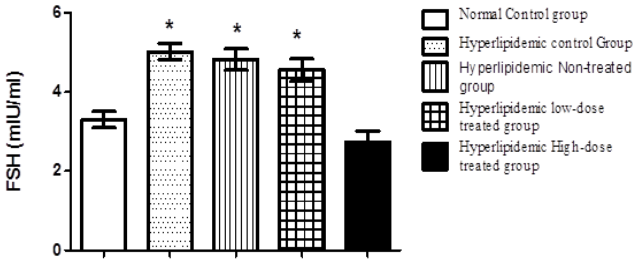


Figure 6:
Comparison of the FSH levels amongst the various groups *= statistically significant difference in that group (P<0.05).

Histological Findings:

Normal control group: Light microscopic examination of the testis showed normal testicular architecture with seminiferous tubule (ST) that are lined with interstitial cells of leydig (ICL), sertoli cell (SC) and well enhanced spermatogenesis (WES). The overall feature appears normal.

Hyperlipidemic control group: Light microscopic examination of the testis showed moderate to severe degeneration with moderate arrest of the spermatogenesis (MAS), focal area of hemorrhage (FAH)within the lumen and moderate apoptosis of the interstitial cell of leydig (MAICL).

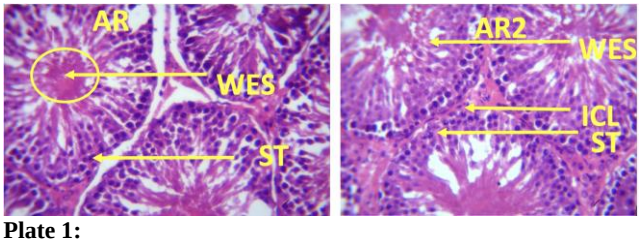


Plate 1:
Photomicrograph of section of testis (x400)(H/E) for normal control group.

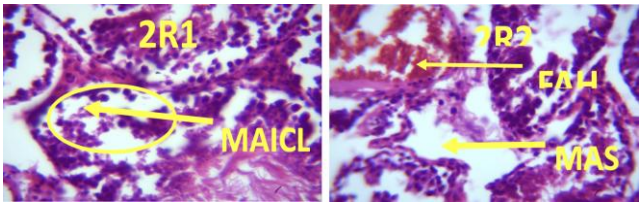


Plate 2:
Photomicrograph of section of testis of hyperlipidemic control groups (x400) (H/E).

Hyperlipidemic non-treated group: Light microscopic examination of the testis showed severe degeneration with severe arrest of the spermatogenesis (SAS), focal area of necrosis (FAN), moderate aggregate of inflammatory cell in the background of the necrotic area coupled with moderate apoptosis of the interstitial cell of leydig (MAICL).

Hyperlipidemic non-treated group: Light microscopic examination of the testis showed severe degeneration with severe arrest of the spermatogenesis (SAS), focal area of necrosis (FAN), moderate aggregate of inflammatory cell in the background of the necrotic area coupled with moderate apoptosis of the interstitial cell of leydig (MAICL).

Hyperlipidemic low-dose treated group: Shows moderate regeneration with moderate enhanced spermatogenesis (MES) in R2. Mild distortion of the testicular architecture (MDTA) and moderate apoptosis of the interstitial cell of leydig (MAICL) in R1.

Hyperlipidemic high-dose treated group: Shows a well regenerated testicular tissue coupled with well-established spermatogenesis (WES) and mild focal area of inflammation (MFAI) otherwise normal.

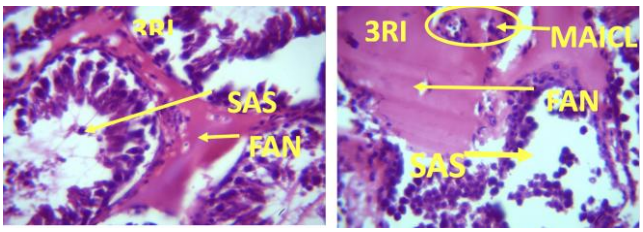


Plate 3
Photomicrograph of section of testis hyperlipidemic non-treated group. (x400) (H/E).

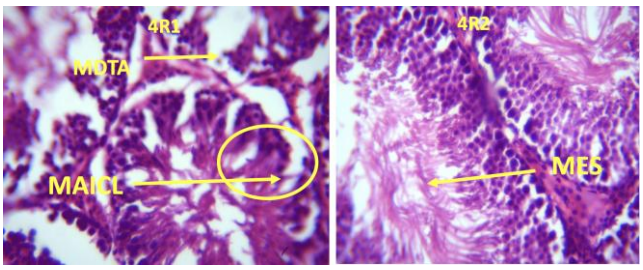


Plate 4
Photomicrograph of section of testis of hyperlipidemic low-dose treated group (x40o)(H/E).

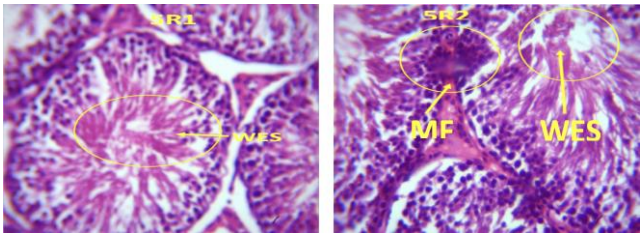


Plate 5
Photomicrograph of section of testis of hyperlipidemic group treated with a high dose of MECLS).(x400)(H/E).

DISCUSSION

It is well established that hypercholesterolemia induces reproductive and testicular damage by increasing lipid peroxidation and excessive generation of free radicals and increased oxidative stress, which are cytotoxic to spermatozoa (Bashhandy, 2007). Testicular histo-pathological reports from this study reveals that there was a severe degeneration with marked cell inflammation, necrosis, moderate apoptosis of interstitial cell of leydig and severe arrest of the spermatogenesis in Hyperlipidemic control group and Hyperlipidemic non-treated group when compared to Normal control group These findings are similar to those of Zhang *et al.,* 2012 who reported degenerative changes in testicular tissues in hyperlipidemic rats.

Sperm parameters such as count, motility and morphology are reportedly key indices of male fertility; being fair markers of spermatogenesis and epididymal maturation of spermatocytes (Morakinyo, 2010). There are valid evidences that hyperlipidemia negatively affects sperm cell structure and motility (Yamamoto *et al.,* 1999; Bashandy, 2007; Ghanayem *et al.,* 2010). Comparably, in this study we observed that over time, hyperlipidemia seem to induce morphological abnormalities in spermatocyte as a significant increase in sperm cells with mid-piece abnormalities in Hyperlipidemic non-treated group when compared to Normal control group and Hyperlipidemic control group. The mid-piece regions of sperm cells are known to contain numerous amounts of mitochondria which provide energy for motility (Mukai and Travis, 2012). Furthermore, a proportionate significant decrease in sperm cell motility was seen in Hyperlipidemic non-treated group. This decrease could thus be a complementary effect of the structural abnormality in mid piece section.

Lipid peroxidation is an important factor that may induce morphological changes in the spermatozoa (Sanchez *et al.,* 2006), however, Various testicular mechanisms involved in lipid homeostasis in male reproductive tract (Maqdas *et al.,* 2013; Lobaccaro *et al.,* 2012). From this study, hyperlipidemia had no significant effect on testosterone levels and sperm count. These findings with those of Zarei *et al.,* 2014 and Saez *et al.,* 2010.

The production of male gametes depends on the concerted action of the two gonadotropins FSH and LH on the testis (Simon *et al*., 1999). LH plays an important role in regulating testosterone secretion by the interstitial cells of the testes and FSH helps in increases germ cell proliferation (Vihko *et al.,* 1991; McLachlan *et al.,* 1995). There was a significant increase in LH and FSH levels in hyperlipidemic non-treated group and low dose treatment group when compared to normal control group. This increase may perhaps be due to the endocrine systems response to testicular damage induced by hyperlipidemia, which successively may have bolstered testosterone levels and sperm count from declining. A surge in LH elicits a proportionate increase in testosterone (O'Donnell *et al.,* 2006). However, the surge in LH observed in the hyperlipidemic non-treated group didn't potentiate a corresponding increase in testosterone levels. There is a possibility that hyperlipidemia may have activated some regulatory mechanisms such as the unique rennin-angiotensin system localized in the testis which stabilizes steroidogenesis and consequently limiting the increase in testosterone production (Martinez-mortos *et al.,* 2011).

Methanolic extract of *C. Lanatus* seed (MECLS) is known to improve testicular function and morphology (Kolawole *et al.,* 2014). Histo-pathological studies as shown in Figure 10, are affirmative that mild dose (800mg/kg bw) of MECLS to an extent, ameliorated structural damages on testicular tissues induced by hyperlipidemia. This was reflective in hyperlipidemic low-dose treated group as moderate regeneration with moderate enhanced spermatogenesis, mild distortion of the testicular architecture and moderate apoptosis of the interstitial cell of the testis. However, these ameliorating effects wasn't reflected in testicular function because there was no significant change in sperm count. Also, the increase in sperm cells with mid-piece abnormality in hyperlipidemic non-treated group was persistent in hyperlipidemic low-dose treated group when compared to Normal Control Group (NCG) and Hyperlipidemic Control Group (HCG),

Methanolic extract of *C.Lanatus* seed had earlier been reported to increase LH and FSH levels (Kolawole *et al*., 2014), results from this study is in tandem with prior reports as a sustained significant increase in LH and FSH levels was observed in hyperlipidemic low-dose treatment group when compared to NCG and HCG. This sustenance could be as a result of the physiological compensatory feedback mechanism ensuring steady levels of testosterone and sperm count.

Furthermore, Amedu and Idoko (2016) had earlier reported that Methanolic extract of C. Lanatus improves sperm count and increases FSH and LH secretion. From this study, in the hyperlipidemic, high-dose treated group, there was regeneration of testicular tissue coupled with well-established spermatogenesis. However, there was an observable significant decrease for FSH levels, LH levels and sperm count in hyperlipidemic high-dose treated group when compared to Hyperlipidemic low-dose, Hyperlipidemic non-treated and hyperlipidemic control groups. This decrease suggests a possibility of a negative (inhibitory) effect of a high dose of methanolic extract of *C. Lanauis* seed on the anterior pituitary which inhibits the release of both FSH and LH.

In conclusion, hyperlipidemia induced an increase in FSH and LH levels without affecting sperm count and testosterone levels respectively, but decreased sperm quality via its cytotoxic effect on testicular tissues. Mild dose MECLS seems beneficial in repairing damages induced by hyperlipidemia on testicular tissues. However, a high dose of MECLS (despite provoking testicular rejuvenation), has an inhibitory effect on gonadotropic hormones and testicular function.

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