

Comparative Effect of Virgin Coconut Oil (VCO) and Coconut Milk On Fertility Profile (Male Sex Hormonal Analysis) Using Rats as Models.

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Summary. The comparative effects of Virgin Coconut oil (*CocosNucifera*) and Coconut milk on male sex hormone using male rats as model was studied. A total of 72 rats (40 adults male and 32 female) weighing between 200-250g were used in the study. Group 1 served as the control and received distilled water only. Groups 2-4 were the test groups and were fed with the coconut extracts orally. Group 2 was fed with virgin coconut oil (VCO), group 3 was feed with coconut milk (CM) while group 4 was fed with the mixture of coconut oil and coconut milk.(VCO+ CM) respectively. At the end of administration, assay of the reproductive hormones (FSH, LH, Testosterone) were carried out. The results of the study showed that the administration of Virgin Coconut milk (CM) and the mixture of Virgin Coconut oil (VCO) and Virgin Coconut milk (CM) resulted in no significant changes in FSH concentration when compared with the control. The virgin coconut oil (VCO) showed a highly significant decrease in FSH concentration ($P<0.05$) when compared with the control and also with coconut milk. Virgin Coconut milk (CM) administration showed a significant increase whereas Virgin Coconut oil (VCO) a significant decrease in LH concentration when compared with the control respectively. Also the administration of the coconuts extracts all have a decreased effects on the testosterone concentrations, significantly ($P<.05$) decreased by the Virgin Coconut oil (VCO). Administration of Virgin Coconut oil either directly or via our diets should be with caution because of possible impairment in the level of sex hormones, thereby affecting fertility.

Keywords: Virgin coconut oil, coconut milk, testosterone, FSH, LH.

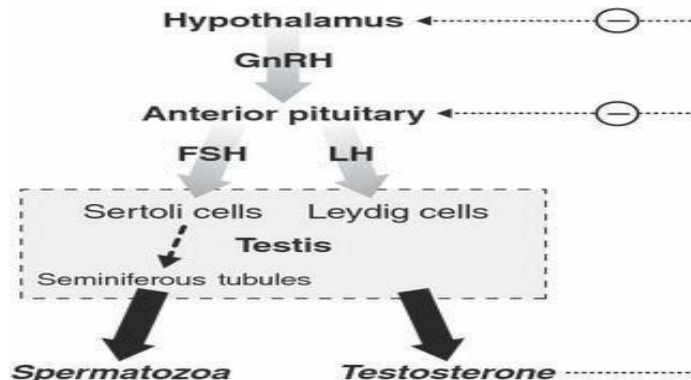
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I. Introduction

Sex hormones such as testosterone, Luteinizing hormone and Follicle stimulating hormone play various physiological roles in the development of male reproductive system during puberty which enhances fertility (David JH *et al.*, 2018).

Regulation of Testosterone secretion



Testosterone is produced in the Leydig cell in the testes (Aumuller G, and Seitz J 1990), the number of Leydig cells in turn is regulated by the luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Paynr AH and O' Shaughnessy P 1996). LH stimulates gene in the sertoli cells which in turn promote differentiation of

spermatogonia (Merita PH *et al.*, 2008). That is low level of testosterone can lead to a decreased in libido or erectile dysfunction that will in turn cause infertility (Ballargeon J *et al.*; 2001).

Testosterone plays a vital role in body hair growth; development of testes and prostate and enhances sexual drive and function (Mooradium AD *et al.*, 1987). Testosterones are secreted in higher concentration in male to produce sperms and enhances fertility (Zhou L *et al.*, 2016).

Fertility is the natural capability to produce children. When a man and woman cannot perform this function or capability to produce children, they are termed 'infertile'. That means fertility problems can happen both in women and men, which account for about 40-50% in WHERE? (Hirsh A, 2003).

Infertility in man is a health issue that lowers the chances of his female partner getting pregnant after 12 months of unprotected and regular sex. It affects about 7% of all men (Loffi F, Maggi M, 2004).

Male infertility is usually caused by testicular damage leading to inability to produce sperm; damaged testicle will not usually regain its sperm-making abilities (Purvisk,E, 1992). Furthermore, dramatic changes in the semen quality have been seen during the past three decades. (Nagiahet al, 2015). High intake of antioxidants, fruits, vegetables, poultry, sea foods, skim milk and shellfish as well as low intake of full-fat dairy, sweet and processed meat, especially with high-saturated fat foods, has favorable association with sperm quality. (Daniel N et al 2019).

Study also has shown that sperm motility can be change in a short period and seems to be closely coupled to diet. (Daniel N et al 2019).

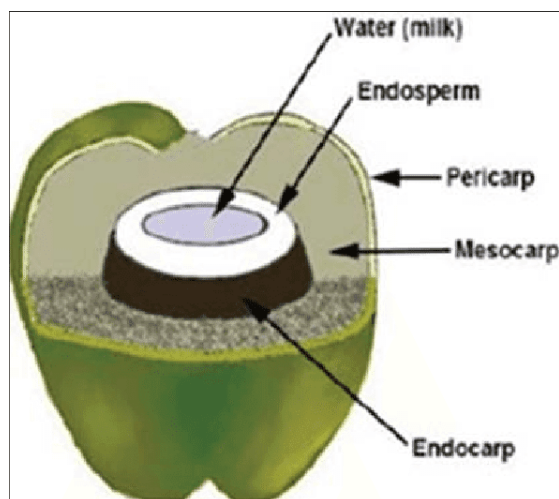
Medicinal herbs gotten from plants and plants extracts have been shown to improve infertility problems in men (Gonzales GF 2002).

Infertility is a growing problem worldwide(Okanufa FE, Odunsi OA, 2003).Epidemiologic reports indicated that prevalence of infertility ranges from 3.5% to 16.7% in developed countries and from 6.9-9.3% in developing countries (Boivin J et al 2007).

About 8-12% of couples globally are experiencing infertility during their reproductive lives estimated by world health organization in 1991, thus affecting 50-80 million couples with 20-35 million in Africa (WHO, 1991).

It affects about one in every six couples and researchers estimate about one in every three cases is due to fertility problems in the male partner alone (Brugo-Olmedos, Chilik C, etal, 2001).Also despite the numerous advance methods of treatment, there have been increased number of infertile couples at the past decade. (Heitman E, 1995).

Assisted reproduction methods have been developed to overcome infertility. However, due to high cost most couples cannot afford it. (Heitman E, 1995).Fertility incidence has remained very high, despite the increased use of assisted reproductive technologies (ART) in recent years. (Salas-Huktos A, et al 2017).In Nigeria, it is estimated that 3-4 million couples were affected by infertility issues (Thomas K D et al 1995).Infertility comes with a high financial and physical price for many couples, this has made a lot of couples to discontinue from their treatments (Akinoye O, and Truter EJ, 2011), which has led to significant increase in psychological trauma among couples (Umezulike A C et al 2004).Despite its high prevalence, not much efforts has been made in tackling this problem. The impact of male infertility is likely to increase if adequate measures are not taken.



Although researchers have suggested that the kind of food we eat can play an important role in alleviating fertility related outcomes in both men and women, the current research examining the effect of dairy on fertility is limited in scope. (Cousineau TM et al 2018).

Plants like coconuts, have been used for many studies arising from heart diseases, stress reduction, antioxidant, and source of hydration, kidney stone prevention and reduction of blood pressure but only very few research work has been done on the use of coconut with a view to reducing infertility. Most recently, the water of immature coconut was associated with increased fertility by increasing sperm motility and count (Augustine A. et al 2019), no substantial work has been done in consumption of coconut meat which contains coconut oil and milk.

Sample collection and identification.

Solid dry mature coconut was purchased from Swali market, Yenagoa, Bayelsa state, Nigeria. The coconuts were identified by the department of crop and pest management, faculty of agriculture, university of Africa.

Procurement of experimental animals.

Healthy Wister rats, of two months old and weighing between 160-200g were procured from Human Physiology Department, University of Port Harcourt.

Experimental design

The animals after acclimatization were randomized and grouped for two(2) weeks in accordance to the method of Morton and Han (Morton D.B, Han J, 2010) and placed in a wooden netted cages and maintained under environmentally controlled room provided with 12:12 hours light and dark cycle approximately at 25C. They were grouped into 4 (n=10 per group)

- Group 1 (control)
- Group 2 (VCO)
- Group 3 (CM)
- Group 4 (VCO+CM)

The test group (2-4) were fed orally with the extracts while the control were fed distilled water and normal feed. Furthermore, the procedures involving the animal models conformed to the guiding principles in the care and the use of animals by the American Physiological society (American Physiological Society .,2002).

Preparation of plant extracts.

Coconut milk: the coconuts were broken, the meat scrapped from the shell and cut into small piece using a sharp knife. The cut pieces were grinded in a grinding machine into viscous slurry and therefore squeezed through cheese cloth (filter) to obtain coconut milk which was put into a glass jar^[22]. The glass jar containing coconut milk were kept in a refrigerator for preservation.

The Virgin coconut oil prepared by the method as described by Nevin KG and RajamohanT. (NevinkG, Rajmohan T. 2006).

The solid matured coconut were crushed manually and the meat was removed and cut into pieces using a sharp knife, the pieces was grinded in a grinding machine into viscous and slurry and therefore squeezed through the cheese cloth to obtain coconut milk. The coconut milk produced was left for 24 hours to aid the gravitational separation of the milk, which was in accordance with (NevinkG, Rajmohan T. 2006).

Three phases resulted: The lower aqueous phase, a middle emulsion phase and an upper oily phase. The upper oily phase was then decanled and heated for 10 minutes to remove moisture.

The resultant virgin coconut oil (V.C.O) was then filtered with a fine sieve, stored in bottles at room temperature and used for experiment.

Coconut milk was obtained by the method as described by Nevin KG and Rajamohan T (NevinkG, Rajmohan T. 2006).The solid matured coconut were crushed manually and the meat was removed and cut into pieces using a sharp knife, the pieces was grinded in a grinding machine into viscous and slurry and therefore squeezed through the cheese cloth to obtain coconut milk. The coconut milk produced was left for 24 hours to aid the gravitational separation of the milk, which was in accordance with (Nour 2009).

Three phases resulted: The lower aqueous phase, a middle emulsion phase and an upper oily phase. The upper oily phase was then decanled and heated for 10 minutes to remove moisture.

The resultant virgin coconut milk (MC) was then filtered with a fine sieve, stored in bottles at room temperature and used for experiment.

Acute Toxicity test

This is the minimum dose that is required to cause 50% death of the rat. It was determined using arithmetic method of Lorke's method (Dietrich L., 1983)

Following a 24-hour period of Acute toxicity study, no deaths were recorded in the animal groups 1, 2, 3, 4, 5, 6 and 7 treated with 500, 1000, 2000, 3000, 4000, 5000, 6000, mg/kg body weight of *CocosNucifera* extract. The animals spontaneously regained their activity within one hour of treatment and all survived the acute test.

In contrast, one death was recorded in group 8 which was treated with 7000mg/kg. Hence the acute toxicity value obtained by the application of looke's after shown in formula was 4.582.56 mg/kg

Sex hormone assay

Blood samples were taken on the first day of administration of extracts on the 19th day before the first day of mating with the female rats and continued until the final day of mating.

To draw the blood, the animals were placed in a plastic restrainer and anestheticcetacaine was applied to the tail.

Approximately 1mm of the tail was then cut using a sterile blade and 0.3ml of blood was collected and put into a micro centrifuge tube within 6minutes.

Following each blood collection, the tail tip was pinched to induce clothing.

For rat in the sex group, blood was collected within 15 minutes of the end of mating, samples were stored overnight at 4^oC to coagulate. The following day, samples were centrifuged for 15 minutes at 9000g and serum was extracted and stored at 20o^oC.

Serum testosterone, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) was assayed using coated tube radio immunoassay (Sun et al., 1989).

Statistical analysis

Data were expressed as MEAN± SEM and their group will be evaluated by one way analysis of variance (ANOVA)

II. Results

Table 1. COMPARING THE EFFECTS OF COCOS NUCIFERA EXTRACTS ON FSH LEVEL

GROUP	TREATMENTS	INITIAL CONC OF FSH (MUL/ML)	FINAL CONC. BEFORE MATTING (MEAN±SEM)FSH (MU/ML)
1	Control	2.50±1.60	2.52±0.27
2	Coconut Milk (CM)	2.40±1.50	2.40±0.8**
3	Virgin Coconut Oil (VCO)	2.40±1.60	1.42±0.41**
4	Mixture of CM + VCO	2.65±0.80	2.42±0.414**

*Mean±SEM. marked * is significantly different from control while means marked ** is significantly different from other test*

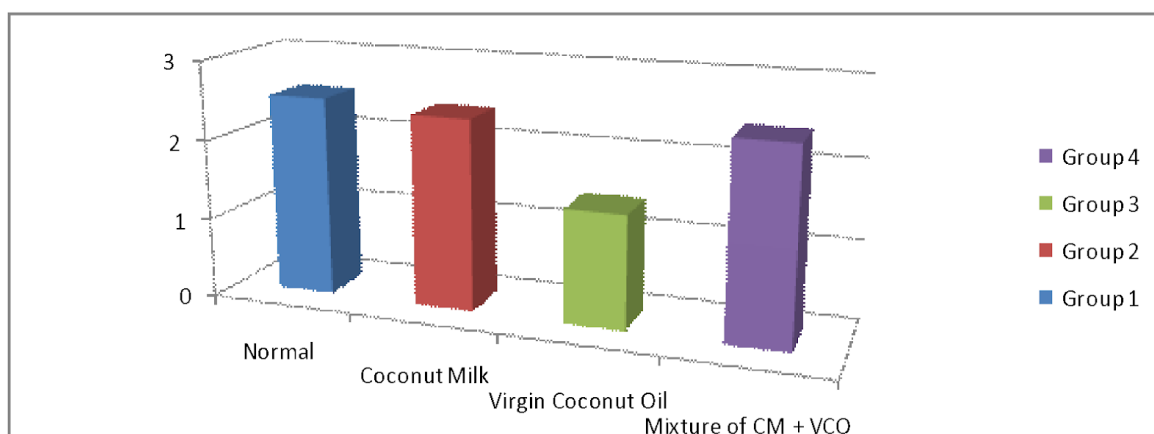


Figure 2: Comparing the Cocosnucifera extracts on FSH Level. The results were graphically represented as mean/ standard deviation in each group. One-way ANOVA analysis showed a significant decrease between the treated and control rats

Table 2: COMPARING THE EFFECTS OF COCOS NUCIFERA ON LH LEVEL

GROUP	TREATMENTS	INITIAL CONC OF LH (IU/L)	FINAL CONC.(AFTER MATTING) OF LH (IU/L)
1	Control	4.5±1.58	4.65±0.11
2	Coconut Milk (CM)	4.4±2.58	4.80±0.15**
3	Virgin Coconut Oil (VCO)	4.09±2.05	3.25±0.18**
4	Mixture of CM + VCO	4.04±1.00	4.60±0.10*

Mean±SEM. marked * is significantly different from control while means marked ** is significantly different from other test.

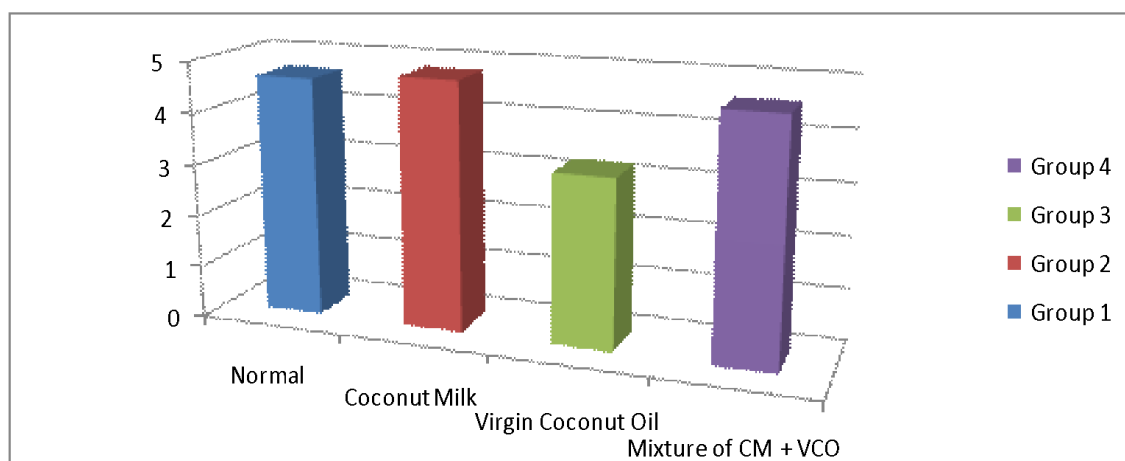


Figure 3: Comparing the Cocosnucifera extracts on LH Level. The results were graphically represented as mean/ standard deviation in each group. One-way ANOVA analysis showed a significant decrease between the treated and control rats

Table.3: COMPARING THE EFFECTS OF COCOS NUCIFERA ON TESTOSTERONE LEVEL

GROUP	TREATMENTS	BASE LINE (INITIAL) CONC. OF TEST. (NMOL/L)	FINAL CONC. (AFTER MATTING) Of TESTOSTERONE (NMOL/L)
1	Control (a)	2.5±1.0.63	2.6±1.8
2	Virgin Coconut Milk (CM) (b)	2.55±0.22	2.0±1.2**
3	Virgin Coconut Oil (VCO) (c)	2.40±0.55	0.7±0.6**
4	Mixture of CM + VCO (d)	2.5±0.53	2.4±1.2*

Mean±SEM marked * is significantly different from control while means marked ** is significantly different from other test.

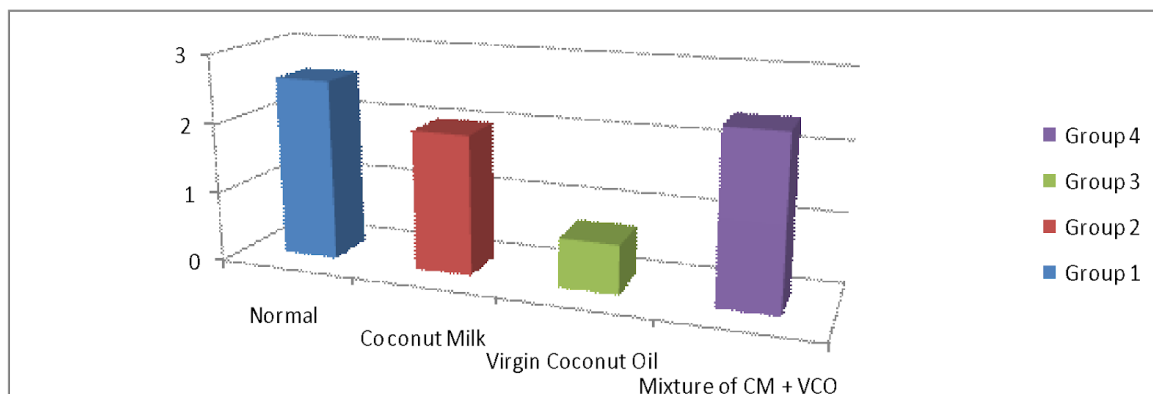
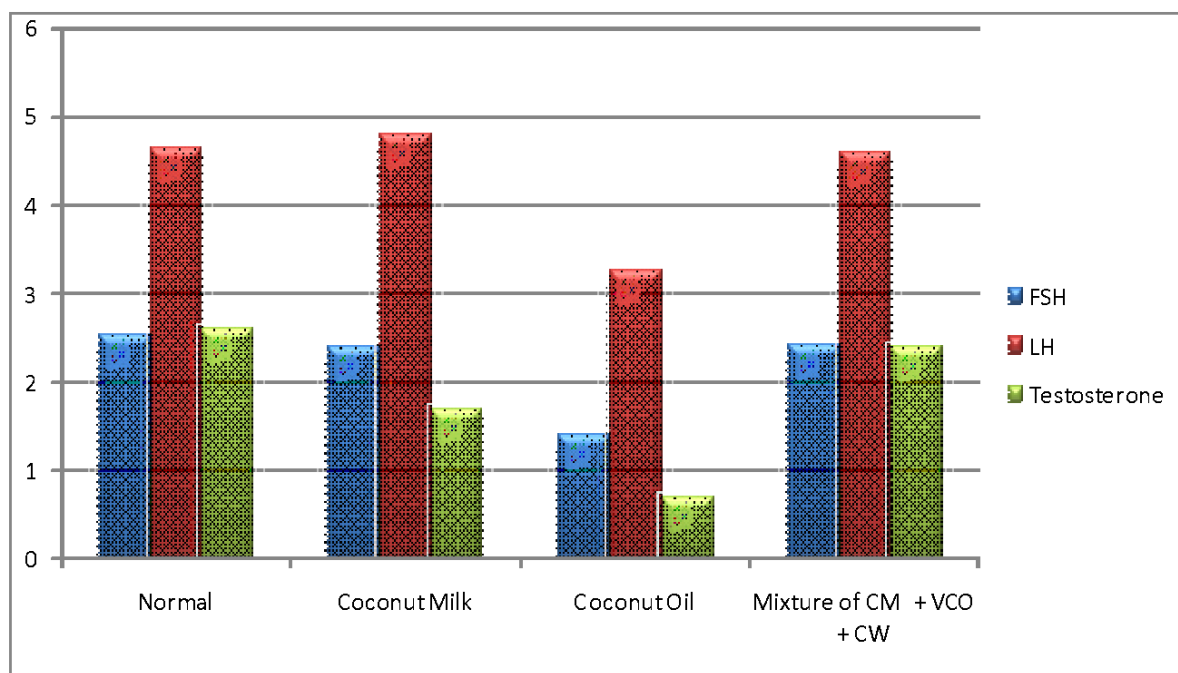


Figure 1 Comparing the *Cocos nucifera* extracts on Testosterone Level. The results were graphically represented as mean/ standard deviation in each group. One-way ANOVA analysis showed a significant decrease between the treated and control rats

Table 4. COMPARING THE EFFECTS OF COCOS NUCIFERA ON THE SEX HORMONE (FSH, LH AND TESTOSTORONE)

GROUP	TREATMENTS	FSH (MU/L)	LH (IU/L)	TESTOSTERONE (NMOL/L)
1	Control (a)	2.52±0.27	4.65±0.11	2.6±1.8
2	Coconut Milk (b)	2.408±0.8	4.80±0.15	1.7±1.2*
3	Virgin Coconut Oil (c)	1.42±0.41*	3.25±0.18*	0.7±0.16*
4	Mixture of VCM + VCO (d)	2.42±0.44	4.60±0.10	2.4±1.2

Results = Mean±SEM marked * is significantly different from control.



III. Discussion

Sex hormones are hormones produced by testes or ovaries (Frick *et al.*, 2015)

Male sex hormones have been known to play an important role in male fertility as they show an effect in sperm's health and production (Moradian AD *et al.*, 1987), thereby deficiency in male sex hormones could be a possible cause of male infertility (Vineet T *et al.*, 2017).

Testosterone is the major sex hormone in male and plays a vital function in sexual performance and reproductive functions (Allen N E, Key J K; 2007). In this research study, administration of 450mg/kg of *Cocos nucifera* extracts showed that the extracts (VCO) had a decreasing effect on testosterone, LH and FSH production when compared with the control and other extracts (CM and CM + VCO). This decrease in sex hormones level could be connected to a decrease in the serum level of sex hormones (LH, FSH and Testosterone) due to the effect of flavonoid (Puranik NV *et al.*, 2019). Coconut extracts contains flavonoid, which might cause low level of testosterone in the body (Puranik NV *et al.*, 2019).

Similarly, research study reported that flavonoid are estrogenic compounds that may occupy estrogen receptors in the body (Benghuzzi *et al.*, 2018) and could cause a decrease in follicle stimulation hormone secretion and inhibit luteinizing hormone (LH) secretion.

LH secretion is important for growth and control the number of leydig cells (Castro ACS; 2002) and its inhibition causes a decrease of the secretion of testosterone and a decrease in the testosterone will reduce sperm production (Olesen IA *et al.*, 2018) as well as cause the cells of the seminal vesicles organs become atrophied, the entire gland would become small and fructose source will decrease. (Arafa M *et al.*, 2019). The concentration of testosterone in the seminal plasma correlates to sperm concentration, motility and percentage and other sperm characteristics (Laudat *et al.*, 1998).

Furthermore, the decrease in testosterone could be associated with the high percentage content of medium chain triglycerides (MCTs) as reported in a test tube studies, which showed that MCTs could block 5-alpha reductase, an enzyme capable of stopping the production of dihydrotestosterone (DHT) from testosterone (Jean P.R *et al.*, 2012). Dihydrotestosterone (DHT), a hormone similar to testosterone (Taylor CW., 2019) helps in the development of penis and scrotum during puberty and prostate gland and seminal vesicles maintenance and development (Mark LS.; 2004).

In addition, a decrease in the sex hormone may be associated with the presence of alkaloids as seen in the phytochemical analysis of *Cocos nucifera* extracts, this is in agreement with a research work done, which showed that alkaloids have a decreasing effect on LH, FSH and testis and serum testosterone level, which also could lead to a decrease in sperm count, motility and density as well as morphological changes in sperm (Musa TV; 2012).

A similar study showed that alkaloids extracts from carica papaya seeds inhibited serum level of androgen and testosterone which might result in male infertility (Udoh PB *et al.*, 2019).

The high saturated fat content found in the extract VCO might have also contributed to the decrease in testosterone level in the rats, which are in agreement with Volek J S *et al.*, (2001) which demonstrated that the effects of diets with high saturated fat decreases testosterone level.

In the light of the aforementioned, we might conclude that the decrease in the level of male sex hormones may be as a result of the presence of high amount of saturated fat found in the Virgin Coconut oil extracts. Therefore coconut milk is preferred to enhance fertility in men.

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