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Original Research Article

Hormonal and Semen Production Response to Oral Combination of Tigernut, Soybean, Datefruit Extract and Cod Liver Oil Administration in Male Wistar Rats

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Abstract: ***Aim/objective****:* This study assess the effect of combined oral administration of tigernut, datefruit, soybean extract and liquid codliver oil on semen and hormonal indices such as sperm count, viability, ph., testosterone, FSH, LH, PSA etc using male wistar rats. **Methods**: The Tiet and Layman methods were used to determine the concerned hormones analyzed while the PSA and semen was calculated using calibrated curve method and hemocytometer Method. The animals were grouped into two phases. ***Hormonal (1) phase*****:** This consist of 25 animals with group (1) serving as the control, (2) received 1000mg/kg soybean, (3) received 800mg/kg tigernut and datefruit, (4) 800mg/kg of combined extract of tigernut, datefruit, and soybean daily while group5 received a standard drug of 500mg/kg codliver oil daily. ***Phase (2) Semen analysis*****:** This phase consist of 35 animals with group (1) also serving as control but group (2) receive 1400mg/kg tigernut, (3) receive 800mg/kg datefruit, (4) receive 1000mg/kg soybean, (5) receive 800mg/kg datefruit/tigernut, (6) receive 800mg/kg of tigernut, datefruit, and soybean extract each daily while group7 was administered 500mg/kg codliver oil daily for 35days. ***Results:*** The outcome of this study indicate an increase mean values for insulin, LH and testosterone among the test group compared with control. The FSH level was significantly higher in group2 (3.60m/u/ml) but decreases in group3 - group5 compared with the control (3.00m/u/ml) level. There was a significant positive correlation that exist between FSH and LH while the PSA levels are within normal range. Both active and sperm count were increased in percentage and in millions among group5 and group6 administered the combined extract of the fruits daily in comparison with control group. A significant increase level of testosterone and improved normal/active sperm count among the test group administered soybean extract and codliver oil daily was observed. ***Conclusion*****:** This study is a break through on the physiopharmacological effect of these extract on gonadotropins and pancreatic beta cells of Langerhans in exerting a response on the gonads to secrete testosterone with subsequent improvement in sperm count level among males to reduce infertility and increase insulin secretion that will increase carbohydrate utilization by the tissues to normalize glucose concentration within the blood.

Keywords: Semen, Hormones, Sperm, LH, FSH, PSA, Insulin.

INTRODUCTION

The term soy was initially used by Jordan in 1918 but in 1944 the American soybean association recommend the change to shorter word soy. Soy bean has been the world most cultivated plant with eight fold increase of about 16.5m hectares from 1995- 2020.Most subjects consume the bean on daily basis or even

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weekly(Matild and Lisa, 2023). The soy bean is often called an oil seed because it is usually crushed and processed to yield crude soy. Date fruit are staple food with over 3000 varieties grown in arid part of North America and Middle East that have contributed immensely to the social wellbeing and economy of the regions. Both fresh and dried are produced by its industry with the leaves used as thatches, fodder and basket.

The plant grows well in environment with long and hot temperatures used as wind breakers for other plants. The fruit is rich in glucose, minerals, vitamins, fats, and fructose with low protein content used to feed broilers, pigs, cattle's, layers, tilapias but juts to mention a few (Feedipedia, 2016; Oladzad sepide *et al.*, 2021;).Tiger nut are underutilized fruit belonging to the cyperaceae family producing rhizome from the base and yellow tubers at maturity. The fruit can be roasted, dried and used for baking flour, bait for fish, milk for cows etc. The fruit is rich in carbohydrate, vit C, E, protein, minerals ie iron, calcium and serve as source of food for both humans and animals. Studies have shown its usefulness in reducing bad cholesterol, weight, flatulation, diarrhea, cardiac thrombosis and increase blood cell production (Bamishaiye, 2011; Emily *et al.*, 2024; Solomon *et al.*, 2024).

The percentage motility of sperm velocity in both animals and humans are of great benefit to the determination of semen and sperm quality that can be used to assess the clinical and toxicological physiology of reproductive function (Peter & Mark, 1987). Quality of sperm is determined by its morphology, count and motility using counting chambers such as Hemocytometer and marker chamber. However unusual optical integrated are now available as automated method of determining sperm quality though not available in most labs (Luthfi and Noor, 2021). Lowered sperm count has been reported in certain animals administered medicinal plant extract of ethanolic lagenaria Breviflora roberty but such have not been fully determine among the fruit extract of tigernut, datefruit, and soybean combination (Adebowale *et al.*, 2008).

Major health challenges among male humans today include the loss of libido and decrease sexual performance in most region of the world. Pollutant from the surrounding and consumption of some certain chemicals and herbal drugs are among the determinant factors responsible for male reproductive disorder. Hence due to high cost of drugs coupled with low minimum wage income in Nigeria most citizens of the country have align to the use of alternative (Medicinal plants) medicine which have proven beneficial in treating most illness such as low sperm count, and motility (Joseph *et al.*, 2019) Studies have shown that about 50% of infertility among couples is partly from the male counterpart making the evaluation of treatment to be more important in males.

The development of male germ cell are regulated by the gonadotropin that depend on the hypothalamus, testes and the pituitary gland. The LH, FSH act on the cells of leydig to stimulate the production of testosterone while FSH act on the sertoli cell to stimulate spermatogenesis. An increase level of FSH in men with azoospermia (<5 million/ml) is a biomarker indicating seminiferous tubule damage and the inability of the pituitary to produce FSH and LH will lead to testicular dysfunction and infertility (Lubna *et al*., 2022; Nelma *et al.*, 2015). Various studies have been conducted using the extract of tigernut, soybean and date fruit separately but the concomitant oral administration of these extract in a combined fashion daily to ascertain their effect on hormonal and semen response profile have not been fully researched and that form the basis for this study.

MATERIALS AND METHODS

Animal Purchase: The animals were purchased from the departmental animal house used purposely for research studies in Uniport, Nigeria and were acclimatized for two weeks before the administration of the various extract.

Fruit Purchase: The net fruits were bought at the popular swali market from malam Ibrahim Yusuf and the soybean at mile1 market portharcout while the liquid codliver oil was purchased at Denson pharmaceutical shop in Bayelsa state.

Extract Preparation: The fruits were gathered separately and washed with clean running tap water to clear off extraneous particles and dried for one week at room temperature and pulverized mechanically. The concentrated crude extract was obtained using a rotary evaporator and the yield was kept in a refrigerator at 4℃.

Statistical Analysis: The analysis was done using spss version 23.0 and a one way ANOVA mean values were represented as mean ±S.E.M.

EXPERIMENTAL DESIGN

This research study was carried out in two phase as indicated below:

- **Hormonal phase:** This comprises of five animal each in five groups making a total of twenty five animals.
  Group1 serve as the normal control fed with local feeds and water ad libitum
  Group2 received 1000mg/kg of soybean extract daily
  Group3 was administered 800mg/k Tigernut Datefruit extract daily
  Group4 received 800mg/kg combination extract of Tigernut, Datefruit and soybean differently daily.
  Group5 was administered a standard drug (codliver oil) at 500mg/kg daily.

- **Semen Analysis Phase:** This phase consist of thirty five male animals of five per group divided into 7 groups.
  Group1 – control
  Group2 received 1400mg/kg Tigernut extract daily
  Group3 received 800mg/kg Datefruit
  Group4 received 1000mg/kg soybean extract daily
  Group5 received 800mg/kg Datefruit and Tigernut extract daily
  Group6 received 800mg/kg Datefruit, soybean and tiger nut extract separately daily
  Group7 was administered 500mg/kg liquid codliver oil daily. NOTE; the administration of the various extract was done in thirty five days.

Blood Sample collection: The experimental animals blood was collected through cardiac puncture (5ml) each and introduced into plain bottles for the concern parameters analysis after anesthetizing the animals using a general anesthesia –ketamin.The serum were suctioned from the cells for hormonal indices analysis. The experimental animals were only males to prevent sperm release during mating that may have increase PSA level during blood /semen sample collection.

Methods for Hormonal Analysis: The Tiet method was used to determine the level of testosterone, FSH and LH was determine using Layman method while the PSA levels were calculated using a calibrated curve method.

SEMEN ANLYSIS

Hemocytometer Method

1. The epididymis was lacerated to press out the semen.
2. Emulsify with 0.5% Eosin and examine using x10 & x 40 objective lens
3. 10-12 fields were examine to check for viable cells which is the % of stain cells as against the un-stained.
4. 10 -12 field were also examine to check for nominal cells as against the abnormal ones in %. The same for actively motile cells, sluggishly cells and the death cells.
5. The sperm count was done using counting chamber 1:20 while dilution of the semen was done using normal saline.
6. The chamber was assembled and filled with the diluents while 4 x 16 squares were counted and multiply by 100,000.
7. Example no of cells counted x 100,000 = sperm count.

Table 1: Mean Values for Hormonal Indices

GROUPS	INSULIN (u/U/ml	TES (ng/ml)	FSH (m/u/ml)	LH (m/u/ml)	PSA (ng/ml)
1	0.366±0.12	1.73±0.25	3.00±0.14	1.16±1.18	0.55±0.06
2	1.17±0.26	9.05±2.19	3.60±0.14	2.09±0.16	0.31±0.07
3	0.83±0.75	2.95±0.21	1.62±0.09	2.45±0.49	1.02±0.06
4	1.38±1.07	1.27±0.49	0.49±0.06	1.66±0.08	0.68±0.06
5	0.48±0.16	4.25±1.63	1.26±0.59	1.91±1.26	0.74±0.34
Sig	0.47	0.00	0.00	0.40	0.05

Table 2: Correlation between Insulin versus Other Hormonal Parameters

Variables	r-values	p-value
Insulin versus Testosterone	**0.16**	**0.67**
Insulin versus FSH	**-0.143**	**0.69**
Insulin versus LH	**0.15**	**0.68**
Insulin versus PSA	**-0.01**	**0.98**

NB: PSA –prostate specific antigen

Table 3: Correlation between FSH, PSA versus other parameters

Variables	r-values	p-values
FSH versus TES	**0.56**	**0.09**
FSH versus LH	**0.95**	**0.02sig.**
PSA versus TES	**-0.59**	**0.06**
PSA versus FSH	**-0.53**	**0.11**
PSA versus LH	**0.52**	**0.11**

Pearson correlation significant (2-tailed) only exist between FSH and LH while no further significant correlation exist among other variables investigated.

Table 4: Semen Variables Compared with Combined Fruits Extract Administration and Codliver Oil

Groups	Appearance(color)	Volume (ml)	PH	Viability (%)	Normal (%)
1(control)	Milky	0.25±0.07	8.00±0.00	85.00±7.07	85.00±7.07
2	Milky	0.10±0.00	8.00±0.00	57.50±3.53	57.50±3.53
Mean Diff.	-	0.15	0	27.50	27.50
3	Milky	0.20±0.00	8.00±0.00	77.50±3.53	77.50±3.53
Mean Diff.	-	0.05	0	7.50	7.50
4	Milky	0.25±0.07	8.00±0.00	75.00±7.07	72.50±3.53
Mean Diff.	-	0.00	0	10.00	12.50
5	Milky	0.25±0.07	8.00±0.00	80.00±5.00	82.50±3.53
Mean Diff.	-	0.00	0		2.50
6	Milky	0.35±0.07	8.00±0.05	85.00±7.07	85.00±7.07
Mean Diff.	-	-0.10	0.00	0.00	0.00
7	Milky	0.25±0.07	8.00±0.00	77.50±3.53	72.50±3.53
Mean Diff.	-	0.00	0	7.50	12.50
P-value	-	0.09	0.03	0.03	0.03

Table 5: Effect of the Fruits Extract and Cod Liver Oil on Sperm Motility

Groups	Abnormal (%)	Active (%)	Sluggish (%)	Dead (%)	Sperm count (millions)
1(control)	15.00±7.07	80.00±7.07	7.50±3.53	12.50±3.53	575.00±176.77
2	42.50±7.07	50.00±4.07	10.00±0.00	10.00±0.00	125.00±35.35
Mean Diff.	-27.50	30.00	-2.50	-27.50	450.00
3	22.50±3.53	77.50±73.53	10.00±0.00	12.50±3.53	350.00±70.71
Mean Diff.	-7.50	2.50	-2.50	0.00	225.00
4	27.50±3.53	70.00±0.00	10.00±0.00	20.00±0.00	250.00±70.71
Mean Diff.	-12.50	10.00	-2.50	-7.50	325.00±
5	17.50±3.53	80.00±0.00	10.00±0.00	10.00±0.00	650.00±70.71
Mean Diff.	-2.50	0.00	-2.50	2.50	-75.00
6	15.00±7.07	85.00±7.07	7.50±3.53	7.50±3.53	600±111.42
Mean Diff.	0.00	-5.00	0.00	5.00	-25.00
7	27.50±3.53	72.50±3.53	3.53±10.00	17.50±3.53	550.00±70.71
Mean Diff.	-12.50	7.50	-5.00	-5.00	25.00
P-value	0.15	0.31	0.29	0.29	0.87

DISCUSSION

Insulin is anabolic hormone produced by the beta cell of Langerhans responsible for carbohydrate metabolism. Deficiency of insulin result in the so called type 1 diabetes mellitus (Adrogue 2000; Banerjee 2004; Kulkarni *et al.,* 2004; Yanovski, 2002) The results from phase 1 (Hormonal) of this study indicate an improved insulin level among group4 that received the combined extract of the three fruits at 800mg/kg concomitantly and in group2 (1.17m/u/ml) compared with control (0.36m/u/ml).More so other group levels of insulin was also observed to be slightly higher than the control group. Furthermore the testosterone level was significantly increased in group2, 3 and group4 above that of the control. Low testosterone level may lead to reduced sexual function. Testosterone lead to the development testicular cells and sperm development (Andrea *et al*., 2020; Lubna *et al.,* 2022; Solomon *et al.,* 2024).

There was a surge in LH level among all the test group compared with the control but most especially in group3 administered 800mg/kg tigernut and Datefruit extract daily.LH is a hormone synthesize from the adenohypophysis gonadotrope cells whose release depend on hypothalamic releasing GnRH hormone. The main physiologic role include the stimulation of Graafian follicle rupturization and the release of the ovum to form corpus luteum and progesterone / estrogen secretion in the females. However in the males it stimulate the secretion of testosterone needed for the growing and maturation testicle and sperm production from leydig cells (Willett *et al.,* 1999; Daniel and Gurdeep, 2022; Serge, 2023). The FSH whose role is to promote the development of ovarian follicular cells and the development of ova within the follicles was significantly elevated in group2 above other test and control group. The normal FSH level in males falls between 1.5 to 12.4 m/U/ml while LH varies between 1.7 to 8.6m/U/ml (https://www.andrologycenter.in; Anthony *et al.,* 1983).

PSA:

This is a plasma test concern with protein secreted by cancerous and normal prostate cells. Though the level of PSA can varies between individuals but are usually higher among men with prostate cancer and has

been a useful tool in the detection of enlarged prostate gland in patients. Levels of 0 - 2.5ng/ml at age 40-50 indicate an unlikely presence of prostate cancer, hence normal psa ranges between 2.5- 4.0ng/ml. However infection of the urinary tract, BPH, prostatitis can increase PSA level but levels above 10ng/ml are usually cause for concern (Yen, 1999; Primakoff, 2002).

Phase (2) Semen Analysis:

The percentage of normal sperm and viability was the same (85.00%) with the control in group6 and 7 that received 800mg/kg combined extract of the fruits compared with group5 (80.00%) and other test group. However we observed that the volume of semen extracted for the analysis of this study has a bearing as a determinant factor in the percentage variation among the test group compared with control. The combination of the three fruit extract administered to group6 result in an increase production of sperm count (600 in million) and percentage number of active sperm (85%) than the control and other test group. However in group5 administered 800mg/kg datefruit and tiger nut extract daily has the highest number of sperm count 650 (in million) level in this research study.

Infertility is the inability of a man to pregnant his wife after a regular unprotected sex for a period of twelve months and above. The fertility of a man and the continuous existence of the human race depend on the normal functioning of the sexual organs responsible for normal sperm production that are active and viable. Low sperm count associated with low testosterone and high LH and FSH are sign of hypogonadotropic hypogonadism affecting the leydig cell function and sperm production (Meera *et al.*, 2022; Vander and Wyns, 2018; Leslie *et al.*, 2023).

CONCLUSION

Oral administration of soybean extract result in the stimulation of pancreatic islet of Langerhans beta cells to secrete more insulin needed for the body to use carbohydrate effectively as the source of energy by regulating its level in the blood. The test group with lower semen volume (ml) collected had lower sperm indices compared with higher volumes. Additional observation from this study also indicate an increase normal sperm (%), active (%) and sperm count (millions) among test groups administered the combined extract orally on daily basis. While this study will help to reduce the menace of infertility among men, semen volume (ml) collected for fertility screening should also be considered a determinant factor in sperm count and viability.

Conflict of Interest: None declared

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