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Investigation into the intake of a popular polyherbal drug (Jalin Herbal Mannex Liquid) on selected biochemical indices of male wister rats

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Jalin Herbal Mannex Liquid (JHML) is a polyherbal formulation from honey, Panax ginseng, Liriosma ovate, and Lepidium mehenil, intended for enhanced sexual performance and improve sperm count in men. JHML was studied via its effect on body weight, biochemical indices and histopathology of the testes. Twenty healthy Wistar rats of 120 -150 g weight were allotted into groups A and B., of ten rats each, group A (control), were given 2 mL/kg B.W of tap water, group B were dosed with 2 mL/kg B.W of JHML for four weeks with the animals allowed access to feed and water ad-libitum. Blood were obtained through heart perforation, kidney and testes were excised, washed with normal buffered saline. Results from the investigation indicated that JHML had androgenic properties with marked significant increase ($p<0.05$) in body weight of rats administered with JHML. Significant increase ($p<0.05$) was observed in LH, FSH, testosterone and with a concomitant significant decrease ($p<0.05$) in PRL. The JHML caused non-significant decrease ($p>0.05$) on triglycerides and cholesterol but with positive significant effect ($p<0.05$) on LDL and HDL. No significant changes ($p>0.05$) on creatinine, urea, uric acid and serum electrolytes. Testicular sections of rats treated with JHML exhibited normal features, seminiferous epithelium and interstitial tissues with active spermatogenesis. The significant increase ($p<0.05$) in testosterone, LH, FSH with a concomitant significant decrease ($p<0.05$) in PRL may account for its sex invigorating potential, the non-significant changes observed in some biochemical indices of the rats showed that JHML is relatively safe at the studied dose.

Key words: Jalin Herbal Mannex Liquid, sex hormone, renal function, lipid profile, serum electrolyte.

INTRODUCTION

In recent times, the production, sales, and intake of different brands of packaged polyherbal products in Nigeria had increased astronomically, owing to their general acceptability. They are considered cheap when compared to mainstream medicines, readily available and with little or no stringent pharmacological bottleneck with regards to their prescription and use (Kpomah and Odokwo, 2020). Polyherbal therapy or herbal combination has been in usage in many countries for centuries, however, there is a paucity of scientific evidence to buttress their therapeutic potential (Che et al., 2013). Drug combination therapy often produces a promising effect in the treatment and control of diseases over single-drug therapy. The theory of drug combination has

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been well proven in Western medicine with significant success being accomplished over the decades. In recent times, drug amalgamation recipe in contagious diseases and cancer therapy have presented fresh hopes and frontiers to patients (Risberg et al., 2011; Muhammad et al., 2016). Naturally occurring plants and plant products organized into certain recipes have been shown to have potential interaction effects. According to Kajaria et al. (2011), these effects include mutual potency heightening, mutual assistance, mutual restraint and mutual antagonism. Due to socioeconomic and traditional influences in Africa and most especially the Nigerian system of medicine, polyherbal compounds are used for the treatment of various infections (Chickenpox, *E. coli*, Diphtheria, Common cold, Giardiasis, Infectious mononucleosis, Influenza), and also in the treatment and control of metabolic and neurodegenerative diseases (Akinyemi et al., 2018; Jamshidi-Kia et al., 2018) which may sometimes arise from the use of synthetic drugs (Arhoghro et al., 2012).

Notwithstanding the medical innovations in treatment and treatment amenities for male sexual malady (Lim et al., 2005), most patients are habitually distrustful of this treatment options and these could be attributed to its sensitivity and social stigma attached to sexual feebleness in African perspective (Lim et al., 2005), these treatment protocols are often very expensive, not easily accessible to the poor and rural dwellers and are often associated with some serious side effects such as headache and heartburn, myalgia and back pain (Ojewole, 2007). Consequently many people self-medicate with unorthodox and unconventional therapies. One common and popular polyherbal used in Nigeria is Jalin Herbal Mannex Liquid (JHML) with Nafdac No A7-2077L. It is a polyherbal formulation used to augment sexual performance in men, combat weak erection, asphyxiate impotence, improvement of sperm count, eliminate waist ache, increase libido and increased sex duration for the satisfaction of both partners. It contains honey, *Lepidium mehenil, Panax ginseng* and *Liriosma ovate. Panax ginseng* commonly called ginseng has functioned as a vital component of many Chinese medicaments for centuries and currently it still fills an enviable and prominent position as the most extensively consumed herbal recipe in the world (Blumenthal, 2001). It is generally believed that ginseng does not only stimulate physical benefits but also has a positive effect on cognitive performance and well-being (Mishra and Verma, 2017). Maca (*Lepidium meyenii Walp*) is widely used as a nutritional supplement and in folk medicine to increase fertility and sexual function (Gonzales et al., 2001; Gonzales, 2012; Del Prete et al., 2018). The desiccated hypocotyls of Maca are abundant in food nutrients, such as lipids, carbohydrates, proteins, free fatty acids, essential amino acids (Canales et al., 2000; Tafuri, et al., 2019). Additionally, Maca comprises several secondary plant metabolites like alkaloids macamides, glucosinolates and macaridine (Tafuri et al., 2019), which are vital in metabolism. *Liriosma ovate* commonly called potency wood, it is a top rated herbs in Amazonian folk medicine, which increases libido and penile stiffness. It functions as a nerve stimulating substance that amplifies the receptivity to sexual stimuli and also the physical perception and sensation of sex (Lim, 2017). It is rich in sterols like sitosterol, campesterol and lupol which activate the body's receptors for hormones like testosterone to heighten libido and enhance performance (Malo et al., 2005). Also present are volatile oils e.g. camphor that help restore sex drive and ease of arousability (Lim, 2017). Honey is a natural product formed from flower nectar by *Apis mellifera.* It is a sugary, piquant vicious liquid. It comprises of sugars, proteins, vitamins, enzymes, minerals, amino acids, scented compounds and polyphones (Alvarez-Suarez et al., 2013; Arawwawala and Hewageegana, 2017; Tafere, 2021). It is generally and extensively used as food and medicaments by many generations, cultures and traditions of the world with great application in religious functions (Tafere, 2021).

Though, herbal drugs are frequently considered safer than orthodox medicines because of their superior lenience. However, there are also documented adverse reactions that are linked with herbal medications (Komlaga et al., 2015; Mensah et al., 2019). The adverse drug reactions linked to herbal medications are largely due to the intrinsic bioactive secondary metabolites inherent in the herbal resources, poor qualities of the plant used which may be due to factors such as contamination with chemicals like pesticides, heavy metals and microorganisms, adulteration with synthetic drug with the principal objective of deceitful improvement in potency and poor quality control methods. Firenzuoli et al. (2005) however, posited that a satisfactory herbal drug must be harmless, unchanging and presented in an appropriate dosage formula and package. In many countries of the world, Nigeria has not been an exception. There are prescribed guidelines for both local and imported herbal products in Nigeria (NAFDAC, 2013), even though herbal medicine practices have not been fully incorporated into the health care delivery system. This investigation is intended at studying the efficacy, potency and biochemical effects of JHML, a popular brand of polyherbal on some vital biochemical indices of male Wistar rats.

MATERIALS AND METHODS

Experimental animals

Twenty (20) healthy adult male Wistar rats weighing 120 to 150 g were used for this study. They were obtained from the animal section of the Pharmacology Department, Niger Delta University, Amasoma, Bayelsa State, Nigeria, and were maintained under standard housing conditions. The animals were adapted for two weeks preceding initiation of experimental regimen, fed with pelletized growers' mash, exposed to clean tap water throughout the period of the study. All animal experimental protocols were

permitted by the Committee of Scientific Ethics at Niger Delta University, Amasoma, and were carried out by its guidelines for animal use.

Experimental design

The animals were allotted into groups 'A' and 'B' of ten (10) rats each in a standard plastic rat cage and were treated as follows according to their body weights for four (4) weeks. Group A: Control rats received 2 mL/kg B.W of distilled water, and group B: The treatment group received 2 mL/kg B.W of JHML.

Sample collection

Upon completion experimental duration, and 24 h after the last oral drug administration, the animals were euthanized under anesthesia in a chloroform chamber and blood were obtained through heart perforation into ordinary sample bottles. The blood samples were made to stand for 20 min for coagulation to occur, and afterwards centrifuged at 2000 rpm for 10 min and the supernatant (serum) collected and kept at 4°C prior to biochemical assay. The testes and kidneys were quickly excised and weighed, after which they were immediately taken and fixed in 10% neutral buffered formalin for histopathological examination.

Purchase of JHML

The JHML with NAFDAC registration number A7-2077L, Batch number 320 and with a production date of August 2020 and expiry date of August 2022 was purchased from Cynflac Pharmacy Limited, Yenegoa, Bayelsa State.

Assay kits/reagents

Luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone and prolactin (PRL) radioimmunoassay test kits are products of BYK-Sangtic Diagnostica. Lipid parameters (Total cholesterol, total triglyceride, low-density lipoprotein) spectrophotometric test kits are products of Randox Laboratories Ltd. United Kingdom, while all other chemicals/reagents used in this investigation were of purest analytical grade.

Changes in body weight

Rats in all groups were weighed on the first day and at the completion of the treatment protocols. The percentage change in body weight was calculated using:

$$\%\ change\ in\ body\ weight = \frac{final\ body\ weight - initial\ body\ weight}{initial\ body\ weight} \times 100\%$$

Testes/kidney as the ratio of body weight

Testes/kidneys were removed and weighed immediately. Testes/ kidney ratio was calculated as a percentage using the expression below:

$$\frac{weight\ of\ organ\ (g)}{body\ weight\ (g)} \times 100\%$$

Hormonal assay

Serum samples were analyzed for the following hormones testosterone, follicle stimulating hormone, and luteinizing hormone by the method described by BYK-Sangtic Diagnostica. This was based on the principle of radioimmunoassay of competitive attachment between the sample serum and the standards for a constant quantity of the antisera (Tietz, 1995).

Lipid parameters

The concentrations of cholesterol and triglycerides were estimated by colourimetric method as described by Ochei and Kolhatkar (2008). High-density lipoprotein (HDL) was determined spectrophotometrically using a commercial assay kit from Biosystems S.A. Costa Brava 30, Barcelona (Spain) by adopting the methods of Grove (1979) and Tietz (1990). Low-density lipoprotein cholesterol (LDL) was also determined spectrophotometrically using a commercial assay kit from Biosystem and adopting the method described by Burstein et al. (1970).

Renal function

Urea was evaluated through the adjusted Berthelot method according to Tobacco et al. (1979). Creatinine was assayed by colourimetric kinetic method of Bartels et al. (1971). Uric acid was assessed using the enzymatic colourimetric method of Duncan et al. (1982).

Serum electrolyte

A clinical chemistry analyzer, ST-200 plus ion selective electrode system (ISE) was used for serum electrolyte ($Na^{+} and\ K^{+}$) estimations.

Histopathological scrutiny of the testes

Histopathological inquiry of the testes for degeneration and derangement was performed using the method described by Krause (2001).

Statistical analysis

The Statistical Package for Social Sciences (SPSS, IBM, USA version 23) Computer software was used for data analysis. The results were stated as mean ± standard deviation (S.D) with the results analyzed using a one-way analysis of variance (ANOVA). $P<0.05$ was regarded as statistically significant.

RESULTS

The effects of administration of the JHML on the body weight and relative organ weight (testes and kidney)

The upsurge in body weight of the rats is expressed in simple percentage (descriptive statistics) as presented in Table 1. After 4 weeks protocols, both experimental groups experienced progressive increase in body weight.

Table 1. The effects of administration of the JHML on the body weight, and relative organ weight (testes, and kidney).

Treatments group	Initial body weight (g)	Final body weight (g)	Changes in body weight (%)	Weight of testes (g)	The relative weight of testes %	Weight of kidney	The relative weight of kidney %
Control (distilled water)	170.00 ± 8.15[a]	199.60 ± 10.03[a]	17.41[a]	1.22 ± 0.15[a]	0.60 ± 0.12[a]	2.55 ± 1.71[a]	1.28 ± 0.36[a]
JHML (2 mL/kg body weight)	162.19 ± 5.04[b]	194.60 ± 8.10[b]	19.98[b]	1.10 ± 0.05[b]	0.57 ± 0.30[a]	2.49 ± 2.40[b]	1.28 ± 0.51[a]

Data are Mean ± SD (n=10). Mean on same column with a different superscript letter(s) are significantly different, (p<0.05). One way analysis of variance (ANOVA) followed by post hoc LSD.
Source: Authors

Table 2. The effects of oral route administration of JHML on the concentrations of LH, FSH testosterone, and prolactin.

Treatment groups	LH (mμ/L)	FSH (mμ/L)	Testosterone (ng/mL)	Prolactin (ng/mL)
Control (distilled water)	19.76 ± 3.21[a]	29.33 ± 3.90[a]	151.60 ± 7.60[a]	6.45 ± 0.32[a]
JHML (2 mL/kg body weight)	25.10 ± 1.89[b]	34.20 ± 2.92[b]	166.70 ± 6.70[b]	4.91 ± 0.21[b]

Data are Mean ± SD (n=10). Mean on same column with a different superscript letter(s) are significantly different, (p<0.05). One way analysis of variance (ANOVA) followed by post hoc LSD.
Source: Authors

However, the findings indicated a significant increase in body and testes weight (p<0.05) of the group administered with the JHML formula, but with no significant difference ($p > 0.05$) in the relative weight of testes, an indication of proportional growth. The result on kidney and relative kidney weight showed no significant difference (p≥0.05) between the control and the JHML treated group.

The effects of oral route administration of JHML on the concentrations of LH, FSH testosterone and prolactin

After 4 weeks of treatment protocols, the oral administration of JHML caused a significant increase (p<0.05) in levels of all hormones investigated in this study namely LH and FSH and testosterone with a concomitant significant decrease (p<0.05) in concentration of prolactin of male albino rats as presented in Table 2.

The effects of JHML on Lipid profile

The oral administration of JHML for 4 weeks as shown in Table 3 caused a non-significant decrease (p≥0.05) in the levels of total cholesterol and triglycerides. JHML also brought about a significant decrease (p<0.05) in the value of LDLc and a significant increase (p<0.05) in HDLc levels.

The effects of JHML on the concentrations of creatinine, urea and uric acid activities following oral administration of JHML

The oral administration of JHML for 4 weeks as presented in Table 4 caused a non-significant change (p≥0.05) in the levels of creatinine, urea and uric acid activities of the male rats.

The effects of JHML on the concentrations of serum electrolyte activities following oral administration of JHML

JHML after 4 weeks of oral administration on the rats as presented in Table 5 caused no significant changes (p≥0.05) in levels of serum electrolytes namely sodium and potassium when compared with the control group that were administered with distilled water.

The effects of JHML on the histology of the testes

The histopathological examination of the testes after 4 weeks of oral dosing of the rats with JHML (Figures 1 and 2) showed no significant changes in histological features between the control group and the JHML treated groups, as both photomicrographs of testicular sections (H & E x

Table 3. The effects of JHML on Lipid profile.

Treatment	Tc (mg/dL)	TG (mg/dL)	LDL-c (mg/dL)	HDLc (mg/dL)
Control (distilled water)	130.90 ± 3.40[a]	95.34 ± 3.30[a]	112.80 ± 4.30[a]	52.60 ± 1.10[a]
JHML (2 mL/kg body weight)	128.40 ± 2.80[a]	94.36 ± 3.23[a]	107.80 ± 4.50[b]	54.70 ± 1.20[b]

Data are Mean ± SD (n=10). Mean on same column with a different superscript letter(s) are significantly different, ($p<0.05$). One way analysis of variance (ANOVA) followed by post hoc LSD.
Source: Authors

Table 4. The effects of JHML on the concentrations of creatinine, urea and uric acid activities following oral administration of JHML.

Treatments	Creatinine (µmol/l)	Urea (mol/l)	Uric Acid (mol/l)
Control (distilled water)	76.9±3.40[a]	6.63±0.74[a]	48.60±3.40[a]
JHML (2 mL/kg body weight)	77.45±2.40[a]	6.60±1.10[a]	49.10±1.30[a]

Data are Mean ± SD (n=10). Mean on same column with a different superscript letter(s) are significantly different, ($p<0.05$). One way analysis of variance (ANOVA) followed by post hoc LSD.
Source: Authors

Table 5. The effects of JHML on the concentrations of serum electrolyte activities following oral administration of JHML.

Treatments	Na^+ (nmol/l)	K^+ (nmol/l)
Control (distilled water)	129.60 ± 2.20[a]	4.79 ± 0.46[a]
JHML (2mL/kg body weight)	130.70 ± 2.43[a]	4.80 ± 0.20[a]

Data are Mean ± SD (n=10). Mean on same column with a different superscript letter(s) are significantly different, ($p<0.05$). One way analysis of variance (ANOVA) followed by post hoc LSD.
Source: Authors

40) showed testes exhibiting normal features of the seminiferous epithelium and interstitial tissues with active spermatogenesis.

DISCUSSION

JHML is a well-known herbal drug consumed by many for many health reasons, but mostly for aphrodisiac purposes. Kpomah et al. (2012) and Nwafor et al. (2020) described aphrodisiac as any drug, food or food products (e.g. chocolate), topical rubefacient, etc. that stimulates sexual desire, sex drive or sexual pleasure. The incidence of male sexual dysfunction is on the increase globally as it is reported by Chen et al. (2019) that 52% of men aged amid 40 and 70 years suffer from differing levels and forms of sexual dysfunction (erectile dysfunction, low libido, premature ejaculation etc.). In Nigeria, Abu et al. (2019) reported that 66.4% of men of the ages of 18 and 76 years suffer from erectile dysfunction but only 39.4% are aware of treatment and surprisingly only 26.5% of this population seek treatment.

One major reason for the low treatment-seeking behaviour could be attributed to its sensitivity and the social stigma attached to sexual dysfunction in the African context (Lim et al., 2005), and this makes many patients to self-medicate by resorting to local herbs and sex tonics.

Body weight fluctuations serve as a sensitive indicator of the general health status of animals (Arthur et al., 2011). The results obtained showed that all rats in the two groups experienced a sizeable surge in body weight following the treatment regimen. The amplification in body weight were more significant and pronounced in the group administered with the JHML. The surge in body weight might occur owing to the androgenic properties of the plant extracts in the JHML (honey, *Panax ginseng*, *Lepidium mehenil* and *Liriosma ovate*). Androgen possesses anabolic activity (Mbongue et al, 2005; Thakur and Dixt, 2007; Yakubu et al, 2007). The steroids also block the action of other bodily chemicals that signal muscle fatigue, thereby allowing the individual to perform longer with more intensity and endurance (Singh et al., 2003). Vicissitudes in organ weight are often regarded as a positive indicator of chemically induced organ damage also had no significant change ($p > 0.05$) in the relative

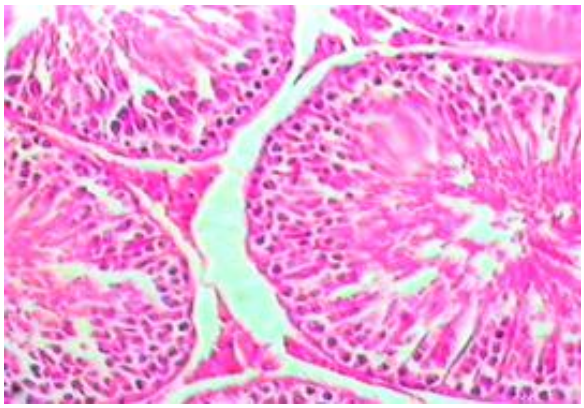

Figure 1. Photomicrographs of testicular sections of control rats (H & E x 40). Atrophed seminiferous tubules with widened interstitial space, spermatogonia spermatids and spermatocytes.
Source: Authors

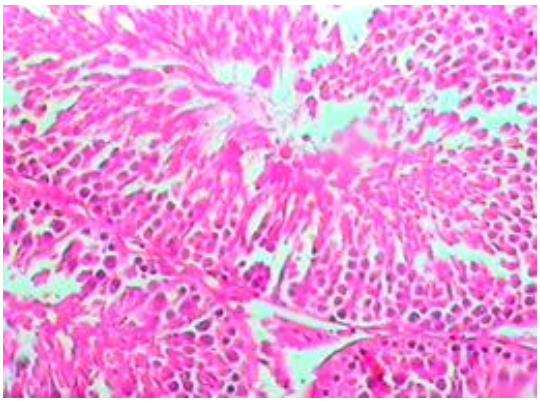

Figure 2. Photomicrographs of testicular sections of rats treated with JHML (H & E x 40). Testes exhibiting a normal feature of the seminiferous epithelium and interstitial tissue with active spermatogenesis.
Source: Authors

weight of testes, and kidneys when matched with the control group, an indication of proportional growth with no adverse or toxicity effect.

All doses of the JHML significantly increased ($P < 0.05$) testosterone, LH and FSH and significantly reduced ($P < 0.05$) prolactin levels. Testosterone is an essential steroid hormone and the most vital androgen for libido and spermatogenesis in males (Fugl-Meyer et al., 2017) A normal level of testosterone sustains the male secondary sex characteristics and normal sexual desire, which stimulates sperm maturation and protein synthesis, specifically in reproductive organs and muscles (Chen et al., 2019). Androgen insufficiency is a primary causative factor of many common clinical diseases and may lead to one form of sexual dysfunction or another and a decline in reproductive capacity in men (Chen et al., 2019). FSH is secreted by the Sertoli cells it plays a vital part in testicular development (Algeffari et al., 2018). FSH also sustains the testosterone level in spermatogenic cells, promotes the binding of androgen binding protein (ABP) to testosterone, and regulates the number of spermatogenic cells (Chen et al., 2019). LH are also needed for upholding testosterone levels, hence an increase in LH and FSH automatically causes an increase in testosterone levels (Yakubu et al., 2007). Studies have shown that testosterone supplementation helps to improve sexual function and libido (Grahl et al., 2007; Fugl-Meyer et al., 2017), in addition to the magnification of orgasm intensity (Morales, 1996). The concentrations of testosterone, FSH and LH are often used as an indirect indices for evaluating male sexual function. The likely inability of the pituitary gland to sustain the ratio of these hormones may possibly upset several processes involved in sexual function to different levels. High concentration of prolactin in men (hyperprolactinemia) has been linked with hypogonadism, decreased sperm count and motility, erectile dysfunction and decreased libido (Paick et al., 2006; Chen et al., 2019). The positive stimulatory capability of the JHML on these sexual and reproductive hormones can be attributed to its positive stimulatory effect on sexual function.

Serum levels of lipoproteins, such as LDL-c, total cholesterol, and HDL-c are often used as a screening guide for disease diagnosis. The oral route administration of JHML caused no significant change ($p \geq 0.05$) in values of triglycerides and total cholesterol but with a significant reduction *($p<0.05$)* in LDLc, and a significant increase *($p<0.05$)* in HDLc levels. The significant increase in HDL-cholesterol levels and a reduction in LDL-cholesterol levels observed in rats administered with JHML is an indication that the polyherbal can reduce the cardiovascular risk (Chu et al., 2016; Hedayatnia et al., 2020)

Renal function is vital for homeostasis, as the kidneys play vital pleiotropic functions comprising the elimination of metabolic waste and the sustainability of water-electrolyte equilibrium as well as blood pressure (Arthur et al., 2011), thus measuring renal function is vital to ensuring safety of drug dispensing and the detection of acute kidney damages at early onset. The non-significant changes ($p \geq 0.05$) in renal function parameters (creatinine, urea, and uric acid) of the rats administered with JHML when compared with the control group is an indication of the non-adverse effect of the JHML on the kidney (Nwankpa et al., 2018; Tejchman et al., 2021).

Electrolytes are essential in maintaining homeostasis by regulating fluid equilibrium, oxygen transport, acid-base equilibrium, heart and neurological functions (Woyesa et al., 2022). However, an electrolyte imbalance may occur causing *hyper* or *hypo* level of this electrolyte in the body. Abnormalities in electrolyte balance are seen in certain metabolic diseases like diabetes mellitus

(Hasona and Elasbali, 2016; Ashraf et al., 2018; Woyesa et al., 2022). Sodium and chloride ions are the chief electrolytes in the extracellular fluid whereas potassium, magnesium and phosphate are the chief electrolytes in the intracellular fluid. Diffusion of cellular K^+ out of the cells and Na^+ into the cells is caused by trans-membrane electrical gradients. Sodium-potassium ion (Na^+-K^+) pump, which is stimulated by insulin and catecholamine hormones, reverses the movement of these electrolytes to maintain their extracellular and intracellular homeostasis (Woyesa et al., 2022). The non-significant changes (p≥0.05) in concentration of serum electrolytes namely sodium and potassium investigated in this study is an indication that the polyherbal had no adverse effect on electrolyte balance.

Histomorphological check of the control group testes (Figure 1) and the JHML administered groups (Figure 2) showed cells with seminiferous tubules containing orderly maturation of germ cells with normal spermatogenesis, an indication of no adverse or negative effect on gametogenesis and sexual function.

Conclusion

The outcome from this present investigation has shown that JHML has a positive stimulatory effect by significantly increasing (P<0.05) the levels of testosterone, follicle stimulating hormone, and luteinizing hormone, while significantly decreasing (P<0.05) the value of prolactin. The use of this polyherbal drug at the dose used for this study showed no statistically significant change (p≥0.05) in body weight and relative organ weight, lipid profile, renal function and serum electrolyte balance.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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