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The Impact of *Sphenocentrum jollyanum*, *Baphia natida* and Combined Extract Consumption on Reproductive Indices and Antioxidants Gene of Male Wistar Rats

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Abstract:- This study investigated the impact of ethanol root extract of *Sphenocentrum jollyanum* and *Baphia nitida* on reproductive indices of seventy (70) male Wistar albino rats(b.w 150 to 200g). The rats were randomly grouped into seven of 10 rats per group.Groups 1 and 2 were given 400mg/kg b.w of *S.jollyanum* and *B.nitida*, groups 3 and 4 were given 400mg/kg and 200mg/kg b.w of combined mixture of *S.jollyanum* and *B.nitida*, while groups 5 and 6 were given 200mg/kg b.w of *B.nitida and S.jollyanum* extracts respectively for 60 days and group 7 serves as control.All rats were allowed access to feed and water *ad libitum*.Results were analyzed using GRAPHPAD PRISM in comparing difference among means and level of significance set at p<0.05. There was significant (p<0.05) decrease on serum follicle stimulating hormone (FSH) in group given 400mg/kg of *S.jollyanum* compared with control. Significant (p<0.05) increase was observed in serum testosterone levels in groups two, four and six compared with the control. Furthermore, significant (p<0.05) decline was observed in progressive motility of spermatozoa and total sperm count in experimental groups compared to control. Sperm viability did not show any significant change. However, percentage of irregular spermatozoa was significantly (p<0.05) elevated in experimental groups compared to control. The result also showed down regulation of glutathione reductase, peroxidase, catalase and nitric oxide genes across experimental groups.These results suggesta possible detrimental effect of these extractson the reproductive function of male Wistar rats.

Keywords:- *Sphenocentrum jollyanum, Baphia nitida, Follicle Stimulating Hormone, Testosterone and Antioxidant Enzymes.*

I. INTRODUCTION

Presently in Nigeria, the upholding of sexual strength has been a thing of immense concern among male population. Due to this, variety of sex enhancers including herbal agents have been employed in maintaining sexual strength and management of erectile dysfunction and premature ejaculation. However, it has been documented that the common reason why younger population of men indulge in consumption of sex enhancers is basically to achieve a desired tough and lengthy erection in order to delay ejaculation and boostthe size of the genital (**Bechara et al., 2010**).Studies have shown that some of these sex enhancers including herbal medicinal plants may produce detrimental effects capable of impairing reproductive function and subsequently infertility (**Bliesener et al., 2005; Ahmed &Kurkar, 2014; Mobisson, et al., 2018**).

Moreover, plant material comes with a multiplicity of compounds that may produce physiologic undesired effects. *Sphenocentrum jollyanum* and *Baphia nitida* happen to be some of such medicinal plants utilized for sexual performances. Along west coast African sub region, this perennial plant *S.jollyanum* (*Menispermaceae*) grows naturally. *S.jollyanum* is a green shrub that grows about 1.5 metres high and has been employed intopractice of traditional medicine for ages; of which its roots, leaves and latex have been in use (**Burkill, 1997**).*Baphia nitida* (bar wood or cam wood), and also known as African sandalwood, is aleguminous, shrubby hard-wooded tree from mid African West region. This wood is of an extremely nice colour and is employed in woodturning for manufacturing knife handles and related articles. The tree's bark and heartwood are commonly utilized to craft a bright but non-permanent red dye which is soluble in alkali (**Gill, 1992**).

It has been documented that oral consumption of drugs orcompounds with medicinal may likely exerts either negative or positive effects on the normallevels of blood indices (**Iwu, 2006**). The free radicals generated during metabolism which may likely lead to destruction of important body cell components are eliminated or neutralized by the activities of antioxidants. On the other hand, in view of the fact that reactive oxygen species perform helpful role in cells like redox signalling, the role of antioxidant system is not to entirely get rid of oxidants,rather it maintain their levels optimally. Decrease antioxidants levels or antioxidant enzymes inhibitionresult to oxidative hassle which may likely lead to death of body cells. An oxidative hassle may be an essential component of many humans. The utilization of antioxidants in pharmacology is deeply studied, particularly to alleviate stroke and neurodegenerative ailments (**Lichterman, 2004***).*Therefore, this study for the first time aimed at investigating the impact of *S.jollyanum* and *B.natida* consumption on reproductive indices of male Wistar rats

II. MATERIALSAND METHODS

A. Ethanoic Preparation of Sphenocentrum jollyanum and Baphia nitida root

The fresh root of *Sphenocentrum jollyanum* and *Baphia nitida were* collected and sent to a Botanist in Pharmacology Department Madonna University Nigeria for identification. They were chopped into pieces and air dried (36-39^{o}c). The dried root was ground to a coarse powder with grinder and stored in a closed earthenware pot which was left open when placed on a fire without adding water. The 1000g and 500g of the powered *Sphenocentrum jollyanum* and *Baphia nitida* respectively were extracted with 100% ethanol within 72h. The ethanol filtrate was concentrated to obtain a crude extract and evaporated to dryness in water bath at 60^{0}C for 36hours. The brownish residue were weighed and kept in an air tight bottle in the refrigerator until use. This method was recently used by (**Wopara *et al*, 2019**).

B. Animals Care

Seventy (70) male albino Wistar rats aged 8 weeks and weighing 150–200g were used for this study. The animals were housed in the Department of Biochemistry Animal house, Madonna University, Elele, Rivers State, Nigeria. Standard animal cages with wood dust as bedding were used in keeping the animals. They were allowed access to rat specific feed and clean water*ad libitum*, and exposed to 12/12-hr light/dark cycle. The animals' acclimatization was within 7 days. The animals were kept in line with laid down principles for animal care as approved in Helsinki's 1964 declaration. The animal ethics committee of Madonna University approved our study protocol graciously.

C. Experimental design

Based on body weight, the experimental animals were randomly assigned into 7 groups of 10 rats each. Groups 1 and 2 were fed with 400mg/kg body weight of *Sphenocentrum jollyanum* and *Baphia nitida* respectively, groups 3 and 4 were fed with 400mg/kg and 200mg/kg body weight of diherbal mixture of *S.jollyanum* and *B.nitida* respectively while groups 5 and 6 were fed with 200mg/kg body weight of *B.nitida and S.jollyanum* respectively and group 7 serves as control. Administration of *S.jollyanum* and *B.nitida* crude extract was done via orogastric feeding once daily for 60 days while the control group received normal saline as vehicle after which the animals were sacrificed under chloroform anaesthesia and the epididymis were carefully harvested for semen analysis and blood sample was collected for hormonal assay. Method recently used by, (**Mobisson, *et al.*, 2018, Wopara, et al 2019**).

D. Assessment of sperm motility

The determination of Sperm motility was done by introducing 10µl of sperm suspension extracted from the left epididymis on a clean pre-warmed slide, covered with a cover slip and examined under a light microscope (Leica DM 750, Switzerland) equipped with a heated stage (37°C), at 100× magnification (**Atashfaraz et al., 2013**).

E. Determination of epididymal sperm count

Estimation of epididymal sperm count was done using the procedure illustrated by Freud and Carol (**1964**). The left cauda epididymis from each rat was placed in 2 ml of normal saline, pre-warmed to 37°C. Tinyincision was made in the cauda epididymis and spermatozoa were gotten and introduced in saline solution. Two hundred microlitres of the suspension was introduced to both chambers of a Neubauer haemocytometer using a Pasteur pipette by touching the edge of the cover slip and allowing each chamber to be filled by capillary action. The epididymal sperm count for each animal was subsequently obtained and recorded.

F. Assessment of sperm viability and morphology

Twenty microlitres of 0.05% eosin Y–nigrosin was introduced to the same volume of sperm suspension and incubated for 2 minutes at room temperature. After incubation, all slides were viewed under a light microscope (Leica DM 750) at magnifications of ×100 and ×400. Live spermatozoa were not stained, while dead spermatozoa were stained pink. For each assay, 400 spermatozoa were counted and viability percentages were calculated (**Wyrobek, et al 1983**).

G. Assessment of Serum Reproductive Hormones

Blood was collected via cardiac puncture, under chloroform anesthesia using a 5 ml syringe and a 21 G needle. The blood samples were introduced into plain capped sample bottles and allowed to stand for 2 hours, after which they were centrifuged at 1,000 rpm for 5 minutes using a bucket centrifuge (B-Bran Scientific and Instrument Company, England). Serum settled on top and was then used for reproductive hormonal assay. Serum

testosterone, luteinizing hormone, follicle stimulating hormone and prolactin concentrations were determined using the ELISA kit for rats, method as used by (**Khaki, et al., 2009**).

H. Antioxidant assessment

➢ *Total RNA Isolation*

The total RNA was isolated from the whole tissue following a method recently used by Omotuyi *etal*.,(2018).

➢ *Procedure*

Tissues were homogenized in cold (4˚C) TRI reagent and the total RNA was partitioned in Chloroform and the mixture centrifuged at 15,000 rpm/15min. Then, the RNA from the clear supernatant was precipitated using equal volume of isopropanol and RNA pellets were rinsed twice in 70% ethanol (70ml absolute ethanol). The pellets were air dried for 5mins and dissolved in buffer (1Mm sodium citrate, p^H 6.4).

❖ *cDNA Conversion*

➢ *Procedure*

Total RNA quantity (concentration (µg/ml) = 40* A_{260}) and quality (≥ 1.8) was assessed using the ratio of A_{260}/ A_{230} (A=absorbance), read using spectrophotometer. The DNA contamination was removed from RNA following DNAse 1 treatment (NEB, Cat; M0253S) as specified by the manufacturer.2µl solution containing 100mg of DNA-free RNA was converted to cDNA using M-MuLV Reverse transcriptase kit (NEB, Cat: M0253S) in 20µ final volume (2 µl, N^9 random primer mix; 2µl, 10X M-MuLV buffer; 1µl, M-MuLV RT (200 U/ µl); 2µl, 10mMdNTP; 0.2µl, RNase Inhibitor (40U/µl) and 10.8µl nuclease-free water).The reaction proceeded at room temperature O/N.Inactivation of M-MulV Reverse transcriptase was performed at 65˚C/20min.

➢ *PCR amplification and agarose gel electrophoresis.*

PCR amplification for the determination of genes whose primers (Primer3 software) are listed below was done using the following protocol:

PCR amplification was performed in total of 25µl volume reaction mixture containing 2µl cDNA (40mg), 2µl primer (100pmol) 12.5µl Ready Mix Taq PCR master mix (One Taq Quick-load 2x, master mix, NEB, Cat: M0486S) and 8.5µl nuclease-free water. Initial denaturation at 95˚C for 5 minutes was followed by 20 cycles of amplification (denaturation at 95˚C for 30 seconds and annealing (see TM values for each primer pair on table 1) for 30 seconds and extension at 72˚C for 60 seconds) and ending with final extension at 72˚C for 10 minutes. In all experiments, negative controls were included where reaction mixture has no cDNA. The amplicons were resolved on 2.0% agarose gel (Cleaver Scientific Limited: Lot: 14170811) in Tris (RGT reagent, china, Lot: 20170605)-Borate (JDH chemicals, China, Lot 20141117)-EDTA buffer (p^H 8.4).

➢ *Amplicon image processing and semi-quantification*

In-gel amplicon bands images captures on camera were processed on Keynote platform. Gel density quantification was done using Image-J software. Each point represent relative expression ((test gene band intensity/ internal control band intensity)* 100) plotted using Numbers software (Mac OSX version).

TARGET GENE	FORWARD 5'-3'	REVERSE 5'-3'
B-actin	**ACACTTTCTACAATGAGCTGCG**	**ACCAGAGGCATACAGGACAAC**
GSR	**TGTAAAACGACGGCCAGT**	**CAGGAAACAGCTATGACC**
GPX-1	**TGTGCCCCTACGGTACA**	**CCAAATGACAATGACACAGG**
CAT	**CCGACCAGGGCATCAAAA**	**GAGGCCATAATCCGGATCTTC**
Inos	**GCTTGGGTCTTGTTAGCCTAGT**	**GTTGTTGGGCTGGGAATAGC**

Table 1:- List of PCR Primer

I. Statistical Analysis

All results are presented as mean ±standard error of mean (SEM). One way analysis of variance (ANOVA) was utilized in comparing the difference within groups. Computer software SPSS (version 20) and graph pad prism were used to analyze the data and level of significance was placed at $p<0.05$.

III. RESULTS

A. The impact of S.jollyanum and B.natidaon serum concentration of reproductive hormones

Serum concentration of follicle stimulating hormone (FSH) was decreased significantly ($p<0.05$) in group fed with 400mg/kg body weight of *S.jollyanum* compared to control, (Table 1).Serum concentration of luteinizing hormone did not show any significant difference with the control. The serum testosterone concentration was significantly ($p<0.05$) elevated in groups fed with 200mg/kg of *S.jollyanum*, 200mg/kg of combined mixture

of *S.jollyanum* and *B.natida* and group fed with 400mg/kg body weight of *S.jollyanum* compared to the control (Table 1).Serum concentration of prolactin did not show any significant difference with the control.

Doses(mg/kg)	Follicle stimulating hormone (FSH) (ng/ml)	Luteinizing hormone (LH) (U/ml)	Testosterone (nmol/ml)	Prolactin (ng/ml)
GRP7(Control)	0.54±0.10	1.16±0.13	0.12±0.00	0.12±0.00
GRP6(200mg/kg of *S.jollyanum*)	0.56±0.00	0.87±0.00	0.32±0.00*	0.10±0.00
GRP5(200mg/kg of *B.nitida*)	0.60±0.00	0.89±0.00	0.09±0.00	0.35±0.00*
GRP4(200mg/kg of *S.jollyanum*+ *B.nitida*)	0.63±0.00*	0.97±0.00	1.14±0.00*	0.40±0.00*
GRP3(400mg/kg of *S.jollyanum*+ *B.nitida*)	0.61±0.00*	0.90±0.00	0.39±0.00*	0.34±0.00*
GRP2(400mg/kg of *S.jollyanum*)	0.51±0.00*	1.05±0.00	0.63±0.00*	0.33±0.00*
GRP1(400mg/kg of *B.nitida*)	0.56±0.00	1.03±0.00	0.50±0.00*	0.21±0.00

Values are expressed in mean ± SEM, (n = 10).
* Statistically significant (p<0.05) compared with the control

Table 2:- The impact of ethanol extract of *Sphenocentrum jollyanum* and *Baphia natida* on serum reproductive hormone concentrations after 60 days of ingestion

B. The impact of S.jollyanum and B.natida on Semen profile

After60 days of extract administration, the sperm count and motility were significantly (p<0.05) reduced in all experimental groups compared to control. Furthermore, the percentage of irregular spermatozoa was significantly (p<0.05) elevated in all experimental groupscompared to control as shown in Table 2.

Dose(mg/kg)	% progressive motility	% viability	Epididymal volume (ml)	Sperm count $\times 10^6$/ml	% irregular sperm
GRP7(Control)	101±1.00	98.6±1.32	5.18±0.03	93.8±2.64	6.8±0.10
GRP6(200mg/kg of *S.jollyanum*)	75±2.55*	58.4±1.90	5.17±0.02	58.8±3.51*	36.9±0.29**
GRP5(200mg/kg of *B.nitida*)	76±5.00*	67.0±4.00	5.14±0.02	52.0±4.11*	39.9±2.64**
GRP4(200mg/kg of *S.jollyanum*+ *B.nitida*)	55±2.44*	64.0±4.63	5.16±0.02	67.8±5.37*	30.3±3.35**
GRP3(400mg/kg of *S.jollyanum*+ *B.nitida*)	56±2.44*	79.0±2.00	5.18±0.02	62.4±3.58*	21.35±2.53**
GRP2(400mg/kg of *S.jollyanum*)	64±6.78*	72.0±2.44	5.18±0.02	58.0±2.69*	23.40±3.32**
GRP1(400mg/kg *B.nitida*)	74±4.00*	82.0±3.39	5.16±0.02	67.6±2.75*	22.85±2.29**

**Reduced significantly (P < 0.05); **p<0.05 increased versus control. Data is represented as mean ± SEM (n=10)*

Table 3:- The impact of *Sphenocentrum jollyanum* and *Baphia nitida* root extract on semen characteristics of rats.

C. The impact of S.jollyanum and B.natida on the expression of glutathione reductase (GSR) gene

Figure 1 show theimpact of 200 and 400mg/Kg Body Weight of *S. Jollyanum* and *B. Natida* on the expression of glutathione reductase (GSR) gene in Pg/Ml of male rats after 60days.The expression glutathione reductase (GSR) gene revealed that, there were down regulation of GSR gene across group than the control.

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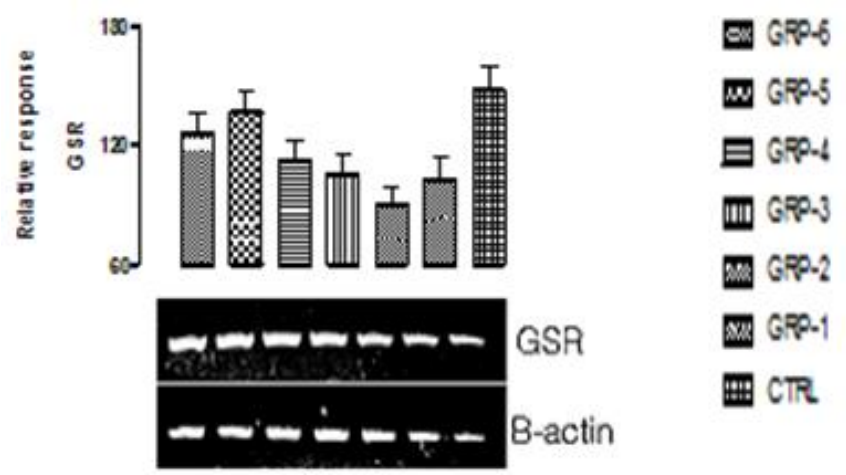

Fig 1:-The Impact of 200 and 400mg/Kg body weight of *S. Jollyanum* and *B. natida* on the expression of glutathione reductase (GSR) gene in pg/ml of male rats after 60 days.

D. The impact of S. jollyanum and B. natida on the expression of glutathione peroxidase (GPX-1) gene

Figure2 show the impact of 200 and 400mg/kg body weight of *S. Jollyanum* and *B. natida* on the expression of glutathione peroxidase (GPX-1) gene in pg/ml of male rats after 60 days.The result from probing the expression of GPX-1 gene in this experiment showed that there was upregulation of the gene in group 5 whereas the gene was downregulated in the other treated groups compared to the control.

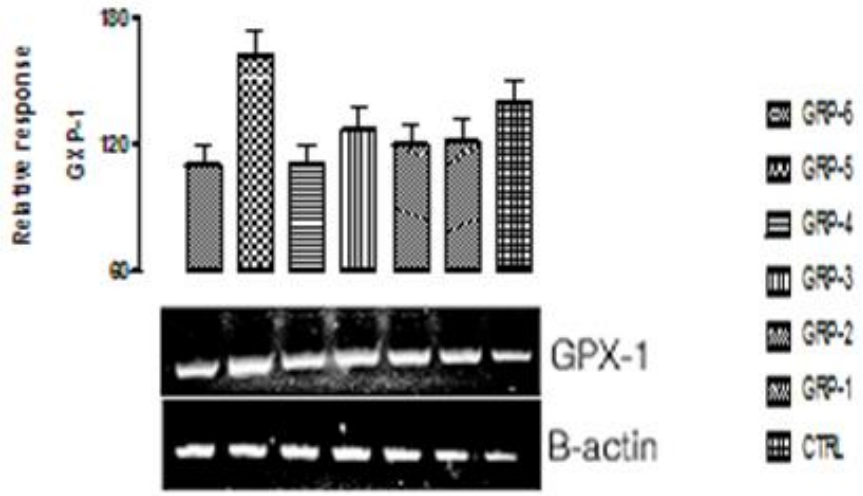

Fig 2:- The Impact of 200 and 400mg/Kg body weight of *S. Jollyanum*and *B. natida* on the expression of glutathione peroxidase (GPX-1) gene in pg/ml of male rats after 60 days.

E. The impact of S. jollyanum and B. natida on the expression of catalase (CAT) gene

Figure 3 show the impact of 200 and 400mg/kg body weight of *S. Jollyanum* and *B. natida* on the expression of catalase (cat) gene in u/l of male rats after 60 days. The result from probing the expression of CAT gene in this experiment revealed that there was downregulation of the gene in the treated group than the control, although statistically, there was no significant difference.

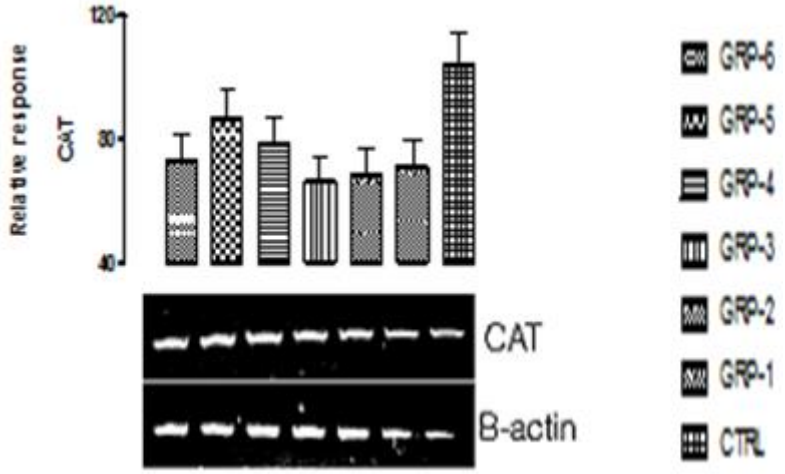

Fig 3:- The Impact of 200 and 400mg/Kg body weight of *S. Jollyanum* and *B. natida* on the expression of catalase (CAT) gene in pg/ml of male rats after 60 days.

F. The impact of S. jollyanum and B. natida on the expression ofnitric oxide (INOS) gene

Figure 4 show the impact of 200 and 400mg/kg body weight of *S. Jollyanum* and *B. natida* on the expression of nitric oxide (inos) gene in pg/ml of male rats after 60 days**.**The result from probing the expression of INOS gene in this experiment revealed that there was downregulation of the gene in the treated group than the control.

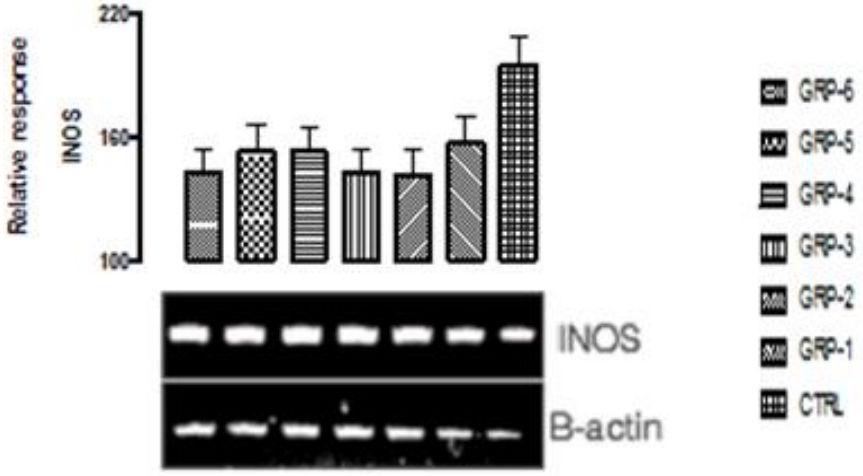

Fig 4:- The Impact of 200 and 400mg/Kg body weight of *S. Jollyanum*and *B. natida* on the expression of nitric oxide (iNOS) gene in pg/ml of male rats after 60 days.

IV. DISCUSSION

The reproductive function parameters assessed in this study were serum concentration of luteinizing hormone, follicle stimulating hormone, and testosterone, prolactin and sperm analysis. Gene expression of some antioxidant parameters such as glutathione reductase and peroxidase, catalase and nitric oxide were assessed. The aforementioned parameters were assessed in both the control and extracts fed rats.

After 60 days of *S. jollyanum and B.nitida* ethanolic root extract ingestion, there was an elevated level of testosteronebase on different doses of extract fed rats. Rats fed with 200mg/kg and 400mg/kg of *S. jollyanum* and 200mg/kg combined extract had significantly ($p<0.05$) elevated testosterone levels. It is documented that an enhanced testosterone levels aidsincrease in sexual activities and libido (**Aversa &Fabbri, 2001; Guyton & Hall, 2012**), and improves the intensity of orgasm and ejaculation in males (**Morales, 1996).** The significant increase in serum testosterone in these groups may likely be attributed to the aphrodisiac effect of *S. jollyanum*, as reported by Olubukola, Olumide, and Peter, (**2017**). Furthermore, the significant increase in testosterone concentration corresponds to the study done by Owiredu et al., (**2007**).The significant reduction in FSH in rats fed with 400mg/kg body weight of *S. Jollyanum* may likely be attributed to significant increase in testosterone and prolactin in this group which may have sent ashort loop negative feedback effectto pituitary gland to inhibit FSH production, (**Guyton & Hall, 2012**). Thesignificant ($p<0.05$) increase in mean serum concentration of prolactinin groups 2, 3, 4 & 5, may likely be attributed to reduction in LH levels in this groups. High level of prolactin in male has been connected with hypogonadism,

reductionin sperm count and motility (**Jeremy & Heaton, 2006;Johril, et al, 2001**), hence the reduction in sperm count and increase in irregular morphology of the sperm in this study.

The sperm analysis is the key marker in assessing male reproductive indices (**Hargrave, et al., 1986**). The progressive motility, viability, sperm count had significant ($p<0.05$) reduction in all extract fed groups compared to the control.However, the percentage of irregular sperm increasedsignificantly ($P<0.05$) across the extract fed groups when compared to the control. The decline in sperm count and elevated level of morphologically deformed sperm may indicate the negative effects of the extract on the gonads which may probably affect spermatogenesis. This result corresponds to the study done by Raji et al., (**2006**), who reported reduction in sperm count in ethanol extract of *S. jollyanum* fed rats. The significant reduction in sperm motility on extract fed rats may likely be due to decreased sperm count.

In this study, the administration of *S. jollyanum* decreased the gene expression of antioxidant enzymes (GSR, CAT, GPx, INOS) compared with control rats. Although,GPx levels in rats fed with 200mg/kg body weight of *B. nitida* was up regulated compared to the control. It has been ascertained that CAT aids to scavenge H_2O_2, while GPx metabolises hydroperoxides. The down regulation of the concentrations of these antioxidant enzyme genes in the testis of the extract rats compared to control may be attributed to the marked reduction in sperm characteristics.These results correspond with the report of Olorunnisola et al. (**2011**); Olorunnisola and Afolayan, (**2013**), who reported reduction in antioxidant enzymes in *s. jollyanum* treated rodents. Hence, it is of our view that the reproductive toxicities seen in this study may likely be due to oxidative stress and may be dose dependent and may be linked to decreased antioxidant enzyme genes in the testicular tissue and production of huge quantities of free radicals relative to the little quantity of antioxidant enzymes present to get rid of them. It is possible that high doses of these extracts may reduce the gene expression of antioxidant enzymes in the testis of treated rats, which may alter the process of spermatogenesis.

V. CONCLUSION

The rate at which male population seek for sexual strength today in Nigeria with the use of different kinds of sex enhancers and resultant psychological dependence prompted this study, hence we conclude that, abusive use of *S. jollyanum and B. nitida*or their combination at high dosesleads to oxidative stress-mediated degenerative changes in testis and epididymis of rats with detrimental effects on sperm characteristics. Therefore, it is necessary that people who indulge in the use of these *S. jollyanum and B. nitida* should be cautious of their reproductive detrimental effects.

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