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Evaluation of Sperm and Haematological Parameters Boosting Potential of *Breynia nivosa* (W.Bull) Sm. (Euphorbiaceae) Leaf Extract in Male Albino Rats

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: In Nigeria and almost all countries in Africa, the use of herbal medicines and herbal products had received general recognition by the people owing to their affordable prices as well historical believes associated with the efficacy of these herbs as compared to conventional drugs in the management and treatment of diseases.
Objectives: This study was carried in order to evaluate sperm and haematological parameters boosting potential of leaf methanol extract of *Breynia nivosa* in male Swiss albino rats.
Materials and Methods: Methanol leaf extract of the plant was administered to thirty male Swiss albino rats grouped into six groups of six rats per group orally for four weeks. The rats in group I served as negative control (administered with 10 mL normal saline), group II served as positive control (administered with a standard drug Spermovite 50 mg/kg), while groups III-VI were given 100, 200, 300 and 400 mg/kg body weight (b.w) of leaf extract respectively. Effect of the leaf extract on sexual organ's weight and blood parameters were determined as well as luteinizing hormone (LH) and follicle stimulating hormone(FSH) serum levels, and semen characteristics.
Results: Oral administration of methanol extract at doses of 100, 200, 300 and 400 mg/kg increased the body and sexual organ weights. There was significant increase in serum testosterone, follicle-stimulating hormone and luteinizing hormone in the treated groups (III-VI) compared to the control groups(I-II) at $p \leq 0.05$. The haematological parameters increased in dose-dependent fashion especially the white blood cells (WBC), red blood cells (RBC) and lymphocytes in the experimentally studied rats.
Conclusions: Our study showed that *B. nivosa* crude leaf extract boosts quality of sperm production as well as increases blood parameters in albino rats. Thus, the plant can be useful in the management of male infertility and sexual disorders.
Keywords: *Breynia nivosa*, Sperm boosting, Haematological parameters, Male albino rats

INTRODUCTION

Infertility has become one of the major problems encountered in some marriages in Africa (Bodenner, 2016). Infertility is defined as the inability of becoming pregnant by the females or unable to induce pregnancy by the males. In males, the problem is as a result of defects in sperm in terms of a number of living cells, motility and testicular temperature, and this had created many problems in some marriages (Manhal *et al*., 2014). Haematology on the other hand, is the study of the composition, formation, function and disease in the blood. It is also referred to as the study of all the cellular elements of blood, both in health and disease condition. Haematology includes the study of aetiology,

diagnosis, treatment, prognosis and prevention of blood disease. Plant extracts have been shown to possess blood boosting potential in experimental animals (Okokon *et al*., 2015).

Breynia nivosa is a shrub which is commonly called "ice plant or snow bush" because of its beautiful variegated foliage leaf, especially in winter season. It has round leaves with mottled, multi-colored variegated with white, green and red coloration leaves (Burkill, 1994; Onyebule *et al*., 2014). The plant is a shrub which is about 2m high and primarily used for foliage, and mainly domicile in villages and towns of South-east Nigeria. Leaves are simple, opposite with entire margin and ovate in shape (Evans, 2006). In South-east Nigeria, the plant is locally called "ogwu eze" in Igbo language and the leaf extract is used in traditional medicine in the treatment of headaches, toothaches and tooth infections while the stem is commonly used as chewing sticks (Amadi *et al*., 2008).

Amadi *et al*. (2008), reported that the leaf extract also showed - antibacterial activity against- *Streptococcus mutans* isolated from dental caries patients. The results of their study and other scientists revealed that herbal preparations of *B. nivosa* can be used for cleansing of the oral cavity, maintenance of oral hygiene and prevention of dental caries as well as analgesic and anti-inflammatory agents (Roja and Roa, 2000). The leaf is also used in the treatment of fever and malaria as well ear ache by the Ibibios of Niger Delta region and Igbos in South-east of Nigeria (NNMDA, 2008), and as an antioxidant (Effraim *et al*., 1999). Phytochemical screening of the plant's leaves in increasing order of polarity revealed the presence of alkaloids and various types of glycosides (Onyegbule *et al*., 2014).

The present study evaluated the efficacy of *Breynia nivosa* leaf extract in sperm and haematological parameters boosting in male albino rats.

METHODOLOGY

Plant collection and identification

Fresh leaves of *Breynia nivosa* were collected from by 6 am from a forest in Takum, Taraba State and authenticated by a biologist at the Department of Science Laboratory Technology, Federal Polytechnic Bali, Taraba State with a voucher specimen number of *EUP002* .

Preparation and extraction of plant materials

Leaves of *B. nivosa* were dried in the shade for two weeks, and powdered and defatted with petroleum-ether before extracting with methanol in a Soxhlet extractor for 48 h at 60^{o}C. After extraction, the mixture was filtered and evaporated to dryness at 60 ^{o}C using a rotary evaporator. The percentage yield was 14.8 % and stored at 4 ^{o}C in the refrigerator for future usage. The extract was dissolved in distilled water to give the final concentration of 100 mg /kg, 200 mg /kg, 300 mg /kg and 400 mg/kg b.w.

Plant extract administration via oral route

Thirty male Swiss albino rats weighing between 150-200 g were maintained under standard environment conditions and fed with standard pellet diet and water *ad libitum*, and allowed to acclimatized in the laboratory for one week. After a week of acclimatization, they were grouped into six groups of six rats per group as follows:

Group I (negative control), was administered 2 mL normal saline once daily,

Group II (positive control) was administered with 50 mg Spermovite once daily,

Group III was administered with 100 mg/kg b.w methanol extract of *B. nivosa (*MEBN) once daily.

Group IV was administered with 200 mg/kg b.w MEBN once daily

Group V was administered with 300 mg/kg b.w MEBN once daily, and

Group VI was administered with 400 mg/kg b.w MEBN; orally via gavaged once daily for 30 days.

Body weights of rats were recorded at the termination of the experiment. After the administration of the last treatment, the animals were fasted overnight and then on the next day, they were sacrificed under light ether anaesthesia. Blood samples were collected by heart puncture for determination of testosterone, luteinizing hormone and follicle-stimulating hormone serum levels as well as haematological parameters. The reproductive organs; testes, Epididymis, seminal vesicles and ventral prostate were removed and weighed. The testes were immediately fixed in Bolin's solution for morphometrical study.

Analysis of haematological parameters

The following parameters were analysed: PCV, MCV, WBC, HB, Lymphocytes, MCHC, MCH, and Platelets at Sancta Maria Integrated Laboratory (USAID/Fhi360 Affiliate), Bali, Nigeria.

Determination of body weights for percentage gain

Body weights of experimental animals before and after experiments were measured using

electronic balance, following an overnight fasting. The body weights were used to calculate the daily weight gained by the animals.

Determination of sex organ weights

The animals were completely anaesthetized with anaesthetic ether (Swipha Pharm Ltd), sacrificed by cervical decapacitation and then testes were carefully removed through the lower abdominal incision and separated from the epididymis, and then weighed using an electronic balance. The organ weight of each sex organs was determined using standard protocol (Brown, 1990; Mehran *et al*., 2013).

Semen collection from the male rats

The testicles were removed through lower abdominal incision and testes were then separated from the epididymis. The right and left epididymis were trimmed off the testes and semen were collected from the tail of the epididymis through an incision made with a razor blade. Sperm cells were sucked into Pasteur pipette from the caudal epididymis. Incisions were also flushed with 2 drops of 1% buffered sodium citrate kept at 37 ^{o}C .

Morphometrical analysis of testes

Tissue samples from right testes were excised and processed for paraffin embedding sections. Serial sections with 5µm thickness were stained with haematoxylin and eosin and used for morphometrical studies under a light microscope. For measuring of seminiferous tubules diameter and germinal epithelium height, 90 round cross-sections of seminiferous tubules were randomly chosen in each rat. Then, using an ocular micrometer of light microscopy (Olympus BH, Japan, Tokyo), at a magnification of 40x, two perpendicular diameters of each cross-section of seminiferous tubules were measured and the mean of these was calculated. Also, germinal epithelium height in four equal distance of each cross-section of seminiferous tubules measured and the mean of these was calculated.

Determination of sperm morphology

Sperm cell morphology was also determined using eosin staining method. For this purpose, 10 µL of 1% eosin was added to a test tube containing 40 µL of sperm suspension and were mixed by mild agitation. Then, sperm was incubated at room temperature for 30 min for staining and then re-suspended with a Pasteur pipette. 200 sperm cell per rat were examined microscopically at 40x magnifications and the number of morphologically abnormal sperm was recorded to give the percent abnormal sperm (Raji *et al.*, 2003; Kisa *et al.*, 2004).

Serum hormonal assay

Blood samples were left for 30 min to clot and then centrifuged for 10 min at 1000×g. The obtained clear sera were stored at -80 ^{o}C until testosterone, luteinizing hormone and follicle-stimulating hormone levels were measured by radioimmunoassay (Sabu and Subburajub, 2002; Sumalatha *et al*., 2010; Yakubu *et al*., 2007; D'cruz and Mathur, 2005).

STATISTICAL ANALYSIS

Data were analyzed using the Statistical Package for Social Science program version 10 (SPSS 22). Statistical analysis was done using analysis of variance (one-way ANOVA) followed by Pearson's correlation test. Data are expressed as the mean ± S.E.M at a significance level of $p \leq 0.05$.

RESULTS

Evaluation of the effect of *Breynia nivosa* leaf extract on blood parameters

Results in Table 1 showed that the plant extract increased most blood parameters in dose-dependent fashion. The white blood cell(WBC) was 40.4 % while mean corpuscular haemoglobin concentration (MCHC) was 44.1% at dosage of 400 mg/kg in Group VI compared to the standard drug chemiron. However, the percentage red blood cells (RBC) reduced as with increase dosage of extract orally. There was statistical difference between the standard drug Chemiron (10 mg/kg) and the extract at $p \leq 0.05$.

using one-way ANOVA. We showed that the leaf methanol extract of *Breynia nivosa* had increased effects on these important haematological parameters viz: RBC, WBC, PCV and MCHC. For instance, reduction in PCV showed how anaemic a person is. This observed effect was comparable to that of the standard drug Chemiron . The result of our study further showed that rats in group with dosage 400 mg/kg had the best blood boosting parameters which implied that better results are obtained at high dosages

Table 1: Effect of *B. nivosa* leaf extract on haematological parameters of treated albino rats

Animal	Hb(gL/)	RBC(%)	WBC(%)	PCV(%)	MCV(f/L)	PLT(%)	MCH(%)	MCHC(g/L)
GPI(n/saline)	14.88	8.62	15.82	23.1	8.2	12.4	25.4	32.12
GPII(10mg/kg)	9.82	3.81	20.07	10.12	12.1	14.2	26.2	36.1
GPIII(100mg/kg)	10.60	4.75	26.92	22.4	18.5	20.1	28.1	38.3
GPIV(200mg/kg)	11.45	5.42	37.85	26.3	24.1	22.4	32.1	40.1
GPV(300mg/kg)	11.70	5.81	38.2	27.3	26.1	24.1	34.1	42.2
GPVI (400 mg/kg)	12.3	6.21	40.4	27.8	28.2	26.1	35.2	44.1

* All values are mean of original values at n= 5 and p≤0.05 (one-way ANOVA), GP (group)

Effect of *Breynia nivosa* leaf extract on organ weights

Weight of epididymis (WOE), weight of testis (WOT) and final body weight, increased from Group I (GP I) to group VI (GP II) after treatment with the leaf extract orally for 15 days. However, significant increased in the weight of testis was witnessed in group VI while the control group I was the least as can been seen in the Table 2.

Table 2: Body and sexual organs weights of *B. nivosa* leaf methanol extract (BNME)

Animal	Initial body weight(g)	Final body weight (g)	WOE	WOT
GP I	150	155	6.5±0.01c	12.1±0.11a
GP II	165	169	7.2±0.01c	12.5±011a
GP III	168	171	10.5±0.20	12.8±0.10b
GP IV	170.2	178.4	12.2±0.10a	14.1±0.10b
GP V	175.1	179.3	12.6±0.80d	14.8±0.90
GP VI	200	205.1	14.2±0.10a	16.2±0.80d

Results are mean ± SEM, n =6, numbers followed by the same alphabet are statistically significant at p≤0.05 Duncan's multiple range test (DMRT), WOT (weight of testes), WOE (weight of epididymis).

Semen analysis of rats treated with the leaf of *B. nivosa* extract orally

Most of the parameters such as sperm motility, normal morphology, and viability showed significant increase as the dosages increased from 10, 100, 200, 300 and 400 mg/kg body weight(b.w). There was however reductions in the number of abnormal morphology as the doses increased as seen in Table 3 below. In Table 4, semen hormones also increased with increased dosages. Follicle stimulating hormone (FSH) and Luteinizing hormone (LH) showed remarkable increase in all the groups after treated as compared to the control group of rats at p≤0.05 (one-way analysis of variance; ANOVA).

Table 3: Effects of *Breynia nivosa* leaf extract on semen characteristics

Animal (g)	Sperm motility	Normal morphology	Viability	Sperm count	Abnormal morphology
GP I (n/saline)	65.20±0.18	70.2±0.10a	80.1±0.30c	53.1±0.10a	8.2±0.01
GP II (10 mg/kg)	68.1±0.10	75.4±0.20b	83.3±0.10a	55.1±0.10a	6.1±0.01
GP III (100 mg/kg)	69.2±0.18	80.2±0.10a	85.1±0.10a	58.6±0.20b	5.8±0.01
GP IV (200 mg/kg)	70.9±0.90	82.4±0.10a	88.4±0.30c	62.1±0.20b	5.2±0.02
GP V (300 mg/kg)	74.2±010	86.1±0.30c	91.2±0.30c	66.3±0.30c	4.4±0.02
GP VI (400 mg/kg)	78.1±0.20	90.1±0.30c	94.2±0.10a	69.2±0.20b	4.0±0.20

* All values are mean ± S.E.M of original values at n= 5 and p≤0.05 (one-way ANOVA), GP (group), sperm count(million/mL), other parameters (%), numbers having the same letters in a row are significantly different (p≤0.05) following DMRT, morph(morphology).

In Table 4 also, our study showed there were no much increase in the quantity of testosterone hormone between the control and the experimental groups at p≤0.05 (one-way ANOVA). However, there was a progressive increase in the amount of follicle stimulating and luteinizing hormones in the rats after three weeks of administration of the extract when compared with the standard drug Spermovite capsule (10 mg/kg).

Our finding further suggests that increase in these hormones was a clear ability of the plant extract to correct male infertility problems, and the plant can then be used for this mentioned purpose in ethno-pharmacology.

Table 4: Fertility hormonal assay of rats treated with *B. nivosa* leaf methanol extract (BNME)

Animal (g)	Testosterone(µg/mL)	FSH (mIU/mL)	LH(mIU/mL)
GP I(n/saline)	1.5±0.1a	9.1±0.04	12.1±0.1a
GP II(p/control)	4.2±0.2b	11.2±0.01c	14.5±0.1a
GP III (100mg/kg)	4.5±0.2b	11.5±0.01	16.2±0.2b
GP IV(200mg/kg)	6.3±0.1a	12.2±0.10a	16.8±0.2b
GP V(300mg/kg)	6.8±1.8c	14.6±0.80c	17.2±1.8c
GP VI (400mg/kg)	7.2±1.8c	16.2±0.10a	17.6±1.8c

*Results are mean ± SD, n =6, numbers followed by the same alphabet are statistically significant at p≤0.05 Duncan's multiple range test (DMRT), FSH (follicle stimulating hormone), LH (luteinizing hormone), p/control (positive control ; Spermovite tablets by Leading Edge , Uk).

DISCUSSION

In this present study, oral administration of methanol extract of *Breynia nivosa* at doses of 100, 200, 300 and 400 mg/kg b.w in Swiss male albino rats for three weeks caused a significant increase in sperm boosting and haematological parameters in dose-dependent fashion. There was exponential increase in body weights of the animals three weeks of drug administration (Table 2). Our results showed that WOE and WOT were high due to an increased in excess sperm production, and this indicated the potential of *B. nivosa* leaf extract to boost sperm production by increasing the WOE and WOT in male albino rats. We compared our results with animals in

group I administered with Spermovite (Leading edge Health Pharmacy) standard drug.

The result further revealed that the presence of certain secondary metabolites like phytosterols reported to be present in the leaf extract was responsible for the increased in the WOE and WOT as well as rapid sperm cell production within three weeks of administration. The ability of plant extracts to affect blood building cells in the body has been attributed to the presence some secondary metabolites they contained. For example, flavonoids and cardiac glycosides had been reported to contribute very immensely in blood formation and improving the rate of blood, thereby affecting positively other blood cell formation (Ashafa *et al.,* 2010; Amini and Kamkar, 2005; Gauthaman and Adaikan, 2008).

The role of the plant extract in this case was not different from this report as seen in Table 1. This is because all haematological parameters were increased three weeks of treatment, examples WBC, PCV, MCV, MCH, MCHC, Hb, among others. Rats given methanol leaf extract of *Breynia nivosa* at the doses 100, 200, 300 and 400 mg/kg for three weeks increased the body weight of rats, at $p \leq 0.05$ when difference between initial weight and final body weight were compared (Table 2). This observation showed that the formation of sexual hormones and gametes must be correlated with blood formation (Ogbuewu *et al*., 2011). Similarly, weight of vital sexual reproductive organs such as testes and epididymis, increased significantly when compared with that of control animal in groups I and II (Table 2).

Our result showed that *Breynia nivosa* leaf extract had improves sperm motility, normal morphology, sperm viability, high number of sperm count with reduced abnormal morphology as seen in Table 3. These improvements in the parameters are clear remedies for impotency and low sperm count in males. These results were comparable to that of the animals in Group II (positive control) and the experimental Groups III, IV, V and VI at $p \leq 0.05$(one-way ANOVA).

Sex hormones such as testosterone and luteinizing hormone are class of steroids which caused an increase in body and sexual organ weight (Table 4). This increase in these parameters could be regarded as biological indicator for the efficacy of the plant extract in improving the biosynthesis of steroidal hormones (Ukoha *et al*., 2014). Androgenic effect has been linked to testosterone levels in blood (Mohammadi *et al*., 2013), it is possible that *B. nivosa* leaf methanol extract have a role in testosterone secretion allowing better availability of hormone to the gonads. The testes, epididymis and other reproductive organs are structurally and physiologically dependent upon the testosterone and other androgens. Testosterone stimulates growth and secretary activity of the reproductive organs (Chanda *et al*., 2009), thus, an increase of these hormones as seen in this study may increase the number and function of body and germinal cells of testis. Sperm motility, sperm count, normal morphology, and viability increased at dose of 100, 200, 300 and 400 mg/kg a result which was comparable to the control groups (Table 3).

Isoflavones, a type of flavonoid glycosides has been reported to increase in sperm count as well as antioxidant activity in male rabbit (Takihara *et al*., 1983). It is therefore, possible that increases seen in sperm cell count and viability in this study is due to the presence of this glycoside. Flavonoids also stimulate testicular androgenesis and it is essential for testicular differentiation, integrity and steroidal functions (Henderson *et al*., 2006). These findings from our study were also corroborated with the finding of Pundir and Jain (2010), who studied the effect of *Nigella sativa* on spermatogenesis and fertility of male albino rats. LH and FSH are two gonadotropins which regulate androgenesis and steroidogenic in animals. LH stimulates the production of testosterone in Leydig cells, which in turn may act on the sertoli and peritubular cells of the seminiferous tubules and indirectly stimulates spermatogenesis via testosterone (Tahir and Moeen, 2011). It is a well confirmed that, these sperm parameters in mammals are regulated by LH and FSH which are gonadotropine. For instance, FSH binds with receptors in the sertoli cells and directly stimulates spermatogenesis in the animals (Dafaalla *et al*., 2015). This is likely to be the exact mechanism by which this steroid had increased sperm cell production and triggered haematological parameters values in the rats.

From our study, LH hormone concentration in treated rats could result in increased testosterone secretion from Leydig cells which are crucial in reproduction.

CONCLUSION

The study showed that *Breynia nivosa* leaf methanol extract produced increased effects on male sperm production, blood parameters, hormone and sperm indices in male albino rats. Our findings further showed that the plant extract had the potential to remedy anaemic condition and well as boost immunity, since WBC is crucial in immunoglobulin regulation in human. The study also revealed that leaf extract of *B. nivosa* could be used as an alternative blood boasting medication in anaemic situation. The study therefore supports the claims for traditional use of *B. nivosa* as sperm boosting ethnomedicinal

prescription and blood-building medication in traditional medicine. It therefore represents a vital source towards infertility and anaemia drug development in health care systems.

LIST OF ABBREVIATIONS

LH: luteinizing hormone,
FSH: follicle stimulating hormone,
Hb: haemoglobin,
RBC: red blood cell,
WBC: white blood cell,
PCV: packed cell volume,
MVC: mean corpuscular volume, PLT : platelets, MCH : mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, GP: groups.

DECLARATIONS

Ethical approval

The guidelines for animal in research act of the National Institutes of Health (1985) were followed.

COMPETING INTEREST

There was no competing interest.

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