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Modulatory effect of ethanol root extract of *Sarcocephalus latifolius* on Fertility of hypertensive Wistar rats induced by Nw-Nitro-L-Arginine Methyl Ester

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Summary: This study was designed to investigate the modulatory effect of ethanol root extract of *Sarcocephalus latifolius* (SL) on the fertility of hypertensive Wistar rats induced by Nw-Nitro-L-Arginine Methyl Ester. Fifty adult male Wistar rats were randomly divided into 5 groups A-E. The rats in group A (Control) were administered with distilled water while Groups B-E received L-NAME at 40 mg/kg, Groups C, D, were co-administered SL at dosage of 100 and 200 mg/kg body weight, respectively, and group E was co-administered with Captopril 20 mg/kg once daily for 28 days. L-NAME caused a significant increase in blood pressure (mmHg) with Systolic Blood Pressure (SBP) (159.08±2.89), Diastolic Blood Pressure (DBP) (114.67±3.83) and Mean Arterial Pressure (MAP) (120.90±4.65) values when compared with their respective control of (115.00±2.81, 80.91±2.76 and 91.9±2.68) in Group B. The high blood pressure was however lowered im groups co-administered with SL and Captopril. Higher morphological alterations of sperm cells were observed in hypertensive rats and hypertensive rats medicated with captopril in this study, It was noticed that the right testicular weight and right testicular length in group C were affected significantly when compared to the left testicular parameter in groups A and B. Semen characteristics showed a decrease in sperm motility and liveability in hypertensive rats group compared to the control and extract treated groups. This decrease fell below acceptable 60 % minimum sperm motility recommended for breeding animals and percentage of the abnormal sperm cell in group B is higher than 20% maximum acceptable limit in normal breeding animals. Hypertension altered the reproductive indices in rats used for this study and could result in infertility but ethanol extract of *S. latifolius* ameliorated the reproductive organ damage in hypertensive rats.

Keywords*: Hypertension, Infertility, rats, Sarcocephalus latifolius, Nw-Nitro-L-Arginine Methyl Ester*

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INTRODUCTION

Non-communicable diseases such as hypertension, a chronic condition of elevated blood pressure and risk factor for cardiovascular diseases have become a cause for global concern (Adeboye *et al.*, 2015) About a billion people worldwide are affected by hypertension otherwise referred to as the "silent killer" as it has no distinct sign and symptom at the initial stage (Marshall *et al.*, 2012) High blood pressure is defined as a systolic blood pressure at or above 140 mmHg and/or a diastolic blood pressure at or above 90 mmHg. Systolic blood pressure is the maximum pressure in the arteries when the heart contracts (Magder, 2018). The economic burden of hypertension includes cardiovascular complications such as heart failure, hypertrophic cardiomyopathy, others include stroke and renal failure, (Zhou *et al.*, 2021).

Hypertension is associated with an increased incidence of sexual dysfunction (Lou *et al.*, 2023). The aetiology for male factor infertility is multifactorial. Most studies focus on sperm motility (SM), viability (SV), and DNA integrity since these parameters are important characteristics of sperm function (Sati and Huszar, 2015) Infertility in the male is the inability to produce fertile sperm cells or semen for fertilization of fertile ovum, which does not lead to

conception of the female bred (Magalhaes *et al*, 2021). Male infertility may be induced by many factors which include diseases, exposure to environmental conditions, adverse drug effect or plants (Adewoyin *et al*., 2017). One lifelong non-infectious disease with infertility as one of its sequels is hypertension. Several studies examining hypertensive men demonstrated a significant inverse relationship between blood pressure and total serum testosterone, which could be associated with impaired reproductive potential, free testosterone and sex hormone binding globulin (Narinx *et al.,* 2022, Svartberg *et al*., 2004).

Studies have reported that N^{w}-Nitro-L-Arginine Methyl Ester (L-NAME)-induced hypertension has been associated with attenuated endothelium-dependent relaxations, cardiac and aortic tissue damage, renal vascular, and glomerular fibrosis (Francois *et al*., 2004; Nyadjeu *et al*., 2013).

Sarcocephalus latifolius belongs to the family of Rubiaceae, the common names include pin cushion tree or African peach. It is locally called ‘’Egbesi’’ in Yoruba, ‘’Ubuluino’’ in Igbo, ‘’Marga’’ in Hausa and ‘’Mbom-ibon’’ in Ibibio. *Sarcocephalus latifolius* is a small evergreen tree or straggling shrub with leaves rounded ovate, apex shortly acuminate, rounded or luneate base and stipulates ovates (Me *et al*., 2016). Parts used include leaves, roots, stem and fruits. It has been found useful in folk medicine for treatment of malaria, hypertension, diarrhoea, tuberculosis, dysentery and as a laxative (Okiemy-Andissa *et al*., 2004). *S. latifolia* has been reported to be effective at lowering blood pressure at a dose of 2.5 – 20 mg/kg in normotensive rats and 2.5 – 10 mg/kg in hypertensive rats. The fact that *S. latifolia* had blood pressure lowering effect seems to justify its use as antihypertensive agent by the traditional medicine practitioner. The extract also decreased the heart rate dose dependently. Magnitude of response produced by the extract of *S. latifolius* was higher in hypertensive rats than in normotensive rats (Nworgu, 2008).

The antioxidant activity of the ethanol and aqueous extracts of the *S. latifolius* fruit has been investigated by Osama *et al*. (2017) using the DPPH scavenging assay, which determines the reducing power of the extract compared with the reference standard antioxidant ascorbic acid. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron that is responsible for the absorbance at 540 nm and also for the visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance (Mensor *et al*., 2001). The ethanol and aqueous extracts of *S. latifolius* exhibited a significant dose-dependent inhibition of DPPH activity. However, the aqueous extract seems to have shown better DPPH scavenging activity across the different concentrations except at the final dose where both extracts showed comparably significant inhibition when compared to the standard (ascorbic acid).

It was also reported that the leaf extract of Sarcocephalus latifolius possesses hypoglycaemic effect (Gidado et al., 2008). Diabetes mellitus is the commonest non-communicable endocrine disease and is considered one of the leading causes of death all over the world. The presence of phytochemicals in high concentration accounts for the significant hypoglycemic effect of Sarcocephalus latifolius (Ndukwe et al., 2017). Despite the vast medicinal actions of Sarcocephalus latifolius documented in literature, little is known about its anti-hypertensive property in the male animals exposed to Nw-Nitro-L-Arginine Methyl Ester. Therefore, the current study was designed to examine the modulatory effect of ethanol root extract of Sarcocephalus latifolius on reproductive parameters of hypertensive Wistar rats induced by Nw-Nitro-L-Arginine Methyl Este.

MATERIALS AND METHODS

Experimental Animals: Fifty male albino rats were obtained from a reputable breeder. Their weights range from 95 to 230 g and were kept in the animal house throughout the period of the experiment. The rats were kept in a well-ventilated cage at optimum temperature and 12 hours light/dark cycle. They were fed with commercial growers mash and water. The experiment was carried out following current guidelines established for the care of laboratory animals.

Experimental Design: Fifty adult male rats were randomly assigned into five different groups of ten animals each as follows: Group A serves as the control and was provided daily with feed and distilled water only. Group B was given 40 mg/kg of Nω-nitro-L-arginine-methyl ester (L-NAME) for 28 days via oral gavage while groups C was given 40 mg/kg of Nω-nitro-L-arginine-methyl ester and 100 mg/kg of *Sarcocephalus latifolius* root extract orally for 28 days, Group D: 40 mg/kg of Nω-nitro-L-arginine-methyl ester and 200 mg/kg of *Sarcocephalus latifolius* root extract orally for 28 days. Group E: was administered 40 mg/kg of Nω-nitro-L-arginine-methyl ester and 20 mg/kg of Captopril orally for 28 days.

Blood Pressure Measurement and Electrocardiography
The systolic, diastolic, and mean arterial blood pressures can be determined non-invasively in conscious animals by tail plethysinography using an automated blood pressure monitor, CODA SI, Kent Scientific Corporation, Connecticut, USA).

Sample Collections and Analysis

Blood collection and Haematology: Blood was taken from the retro-orbital plexus into heparinised vials; transferred to heparinised bottles and plain bottles for the collection of serum and plasma, respectively. The blood taken was analysed for blood parameters including, haemoglobin concentration, full blood count, total leukocyte and differential leukocyte counts, packed cell volume (PCV) as well as serum protein analysis and Albumin: Globulin ratio in serum. The animals were then humanised by cervical dislocation while the final weight, relative organ weight (organ weight/100g of body weight) of thymus, kidney, liver and spleen was determined for each animal.

Experimental Animals Sacrifice: The rats were sacrificed by cervical dislocation, the animals were quickly dissected, and the testes were immediately harvested, rinsed with distilled water, blotted with tissue paper, and weighed.

Gonadometrics: The testes, after being separated from the epididymis and weighed. The length and diameter of the

testes were measured with the use of Electronic Veiner Caliper as described by Ajani *et al*. (2021).

Semen Analysis: Semen sample was collected from the caudal epididymis through an incision made with a scalpel blade. The sperm motility was assayed by dropping semen sample and 2.9 % buffered Sodium Citrate on a warm glass slide and then covered with a glass slip to view under a light microscope as described by (Zemjanis 1970, Ajani *et al.*, 2021). The sperm liveability was evaluated using a drop of semen on a warm glass slide, then stained with one drop of warm Eosin-Nigrosin stain. A thin smear was thereafter made of the mixture of semen and was stained on another glass slide. The smear was air dried and viewed under the microscope and assayed according to Zemjanis (1970).

Histopathological Examination: The histopathology of the testes was done using the method described by Drury *et al*, 1976.). Testes were fixed in formalin and later embedded in paraffin wax. To perform histology of tissues 4 µm sections were prepared with the help of Microtome (Leica, RM 2145). These sections then were deparaffinized in xylene. Xylene was removed using 100 % ethanol and then hydrated through reducing concentrations of ethanol, the ethanol was later rinsed from the tissue section with running water and dried. They were stained with Haematoxylin and Eosin and viewed under light microscope.

Statistical analysis: The data obtained were presented as mean ± SD (standard deviation). All mean differences were considered significant at 5% level, therefore *P*-values less than 0.05 ($P<0.05$) were considered statistically significant at $p<0.05$ using version 2.3 statistical software for Microsoft Excel. Significant elemental concentration differences in plants samples were determined by analysis of variance (ANOVA).

RESULTS

The body weight changes in animals before and after treatments with extracts of *Sarcocephalus latifolius* are shown in Table 1.

Effect of *Sarcocephalus latifolius* root extract on Haematological parameters: There was an increase in the PCV (%) of rats in all the treatment groups (35.2±4.86, 36.2±6.14 and 33.6±3.91) when compared to the control group (32±1.87). The mean total red blood cell counts, and haemoglobin concentration (Hb) also increased, while MCV, MCH and MCHC were significantly unchanged (Table 2)

Effect of *Sarcocephalus latifolius* root extract on Blood pressure profile of rats: There were significant ($P<0.05$) increases in the systolic, diastolic and mean arterial blood pressure of the rats given L-NAME alone (Hypertensive group) when compared to the control, extract treated and captopril treated groups. Also, a significant ($P<0.05$) decrease was observed in the blood pressure of rats treated with the *S. latifolius* extracts at 100 mg/kg and 200 mg/kg body weight. Likewise, there was a significant ($P<0.05$) decrease in the systolic and diastolic blood of the hypertensive rats treated with captopril when compared with those of hypertensive group (B) and hypertensive extract treated groups (C and D) at the respective doses of 100 mg/kg and 200 mg/kg (Table 3)

Table 1:
Percentage increase in body weight before dozing till time of sacrifice

Treatment	Initial weight (g)	Final weight (g)	% Increase /Decease
Control (-ve)	188.29	258.5	37.28
L-NAME	166.61	170.61	2.4
L-NAME + (100 mg/Kg extract)	138.41	173.89	25.64
L-NAME + (200 mg/Kg extract)	150.73	158.21	4.96
L-NAME + Captopril	178.48	165.88	-7.0[a]

Effect of *Sarcocephalus latifolius* root extract on sperm motility and liveability: A significant ($P<0.05$) reduction was observed in the motility in motility and liveability of the spermatozoa of hypertensive rats compared to the control. On the other hand, a significant ($P<0.05$) increase was observed in the sperm motility and liveability of hypertensive rats treated with *S. latifolius* extract and captopril when compared with those of the hypertensive group (B) (Table 4).

Table 2:
Hematological parameters of rats treated with or without *Sarcocephalus latifolius* root extract and L-NAME

Parameters	A	B	C	D	E
	Control	**L-NAME (40 mg/kg)**	**L-NAME (40 mg/kg) + SL (100 mg/kg)**	**L-NAME (40 mg/kg) + SL (200 mg/kg)**	**L-NAME (40 mg/kg) + Captopril (20 mg/kg)**
PCV (%)	32±1.87	32.4±3.64	35.2±4.86	36.2±6.14	33.6±3.91
Hb (dL)	10.4±0.30	10.76±1.24	11.66±1.64	12.02±2.07	11.14±1.32
RBC ($X10^6$/µL)	10.07±1.64[c]	12.31±2.65	15.16±1.31	13.3±1.09	13.38±0.84
WBC ($X10^6$/µL)	10.84±1.86	10.92±1.67	9.96±1.62	9.8±2.48	11.84±1.69
Platelet (10^3/ µL)	10±0.0	9.6±0.89	10.4±0.89	10.4±1.67	10.4±0.89
MCV (fL)	31.6±4.27	24.4±2.88	23.6±5.98	26.8±3.27	24.8±2.58
MCH (pg)	10±1.41	7.8±0.83	7.6±1.94	8.4±1.14	8±1
MCHC (%)	33±0	33±0	33±0	33±0	33±0
LYMPH (%)	41.4±1.81	39.6±2.07	37.6±1.51	37.6±2.40	39±3.08
NEUT (%)	58.4±2.19	60.2±1.78	61.6±1.51	62.2±2.58	60.6±3.20
MONO (%)	0.2±0.44	0.2±0.44	0.8±1.09	0.2±0.44	0.4±0.54

Significance was at P<0.05, Means with superscript a, b showed significant differences when compared to the control and hypertensive groups, respectively

Table 3:
Blood Pressure (mmHg) profile of rats treated with or without *Sarcocephalus latifolius* root extract and L-NAME

Parameters	A	B	C	D	E
	Control	L-NAME (40 mg/kg)	L-NAME (40 mg/kg) + SL (100 mg/kg)	L-NAME (40 mg/kg) + SL (200 mg/kg)	L-NAME (40 mg/kg) + Captopril (20 mg/kg)
Systolic	115±2.81 bcd	159.08±2.89 cde	127.67±1.70 abe	127±0.98 abe	118.5±2.44 bcd
Diastolic	80.91±2.76 b	114.67±3.83 acde	80.17±7.62 b	88.58±4.69 b	83.08±2.17 b
MAP	91.9±2.68 b	120.9±4.65 cde	96.33±5.35 b	100.75±3.04 b	95.33±2.22 b

Significance was at P<0.05, Means with superscript a, b showed significant differences when compared to the control and hypertensive groups, respectively.

Table 4
Sperm Motility and Liveability of Experimental Groups

Parameters	A	B	C	D	E
	Control	L-NAME (40 mg/kg)	L-NAME (40 mg/kg) + SL (100 mg/kg)	L-NAME (40 mg/kg) + SL (200 mg/kg)	L-NAME (40 mg/kg) + Captopril (20 mg/kg)
Sperm motility	66±27.93 a	46±26.07 a	82.8±25.16 a	85.2±20.08 a	90.8±12.13 a
Sperm liveability	88.8±13.08 a	64±19.49 a	81.2±20.80 a	85.2±20.08 a	91.6±3.58 a

Significance was at P<0.05. Means with the same superscript are not statistically different at P < 0.05

Table 5:
Morphological abnormalities of Sperm cells of experimental groups rats

Parameters	A	B	C	D	E
	Control	L-NAME (40 mg/kg)	L-NAME (40 mg/kg) + SL (100 mg/kg)	L-NAME (40 mg/kg) + SL (200 mg/kg)	L-NAME (40 mg/kg) + Captopril (20 mg/kg)
HWT	4 (0.8%) a	6 (1.2%) a	5 (1%) a	15 (3%) a	33 (6.6%) a
TWH	12 (2.4%) a	31 (6.2%)	30 (6%) a	18 (3.6%) a	42 (8.4%) a
CT	42 (8.4%) a	79 (15.2%) a	38 (7.6%) a	16 (3.2%) a	72 (14.4%) a
LT &MP	3 (0.6%) a	12 (2.4%) a	1 (0.2%) a	5 (1%) a	51 (10.2%) a
BENT MP	0 (0%) a	4 (0.8%) a	0 (0%) ab	1 (0.2%) a	0 (0%) b
BENT TAIL	0 (0%) a	2 (0.4%) a	1 (0.2%) a	1 (0.2%) b	4 (0.8%) a
TOTAL	61 (12.2%) a	134 (26.8%) a	75 (15%) a	56 (11.2%) a	202 (40.4%) a

Significance was at P<0.05. Means with the same superscript are not statistically different at P < 0.05.
***HWT** = Head without Tail, **TWH** = Tail without Head, **CT** = Curved Tail, **LT&MP** = looped Tail and Mid-piec*

Table 6:
Gonadometric indices of rats treated with or without *Sarcocephalus latifolius* root extract and L-NAME

Parameters	A	B	C	D	E
	Control	L-NAME (40 mg/kg)	L-NAME (40 mg/kg) + SL (100 mg/kg)	L-NAME (40 mg/kg) + SL (200 mg/kg)	L-NAME (40 mg/kg) + Captopril (20 mg/kg)
Left testis weight	0.9±0.50 a	1.16±0.35 a	1.04±0.15 a	1.04±0.17 b	1.18±0.08 a
Right testis weight	1.14±0.05 a	1.2±0.25 a	1.14±0.08 b	1.06±0.15 a	1.16±0.05 a
Left testis length	11.55±9.21 a	20.08±2.13 a	19.15±0.97 a	18.78±1.06 a	19.84±0.18 b
Right testis length	15.11±6.42 a	19.27±1.88 a	18.18±0.75 b	18.88±1.18 ab	18.88±1.25 a
Left testis diameter	7.23±4.28 a	9.85±0.57 a	9.11±1.27 a	9.56±0.36 b	9.47±0.54 ab
Right testis diameter	9.72±1.06 a	9.73±0.68 ab	9.07±0.60 a	8.96±0.57 a	9.44±0.41 a

Significance was at P<0.05. Means with the same superscript are not statistically different at P < 0.05

Effect of *Sarcocephalus latifolius* root extract on Sperm morphology: The percentage of sperm morphological abnormalities were significantly higher (P<0.05) in hypertensive rats when compared with the control, normotensive rats and extract treated rats. The percentage sperm morphological abnormalities of the rats treated with 200 mg/kg extract was observe to have the least abnormalities when compared with the control, captopril treated and the other extract treated groups while that of captopril medicated group had higher abnormality percentage when compare to the hypertensive and extract treated groups. (Table 5).

Effect of *Sarcocephalus latifolius* root extract on Gonadometric indices of rats with or without L-NAME The gonadometric indices in hypertensive rats were slightly higher in hypertensive rats than the control group. However, there were no significant differences (P>0.05) in the indices of extract and captopril treated groups when compared to the hypertensive group (Table 6).

Effect of *Sarcocephalus latifolius* root extract on the histopathology of the Testicles of Rats: The results of the histopathological investigations of the testis of the animals are shown in Plate 1.

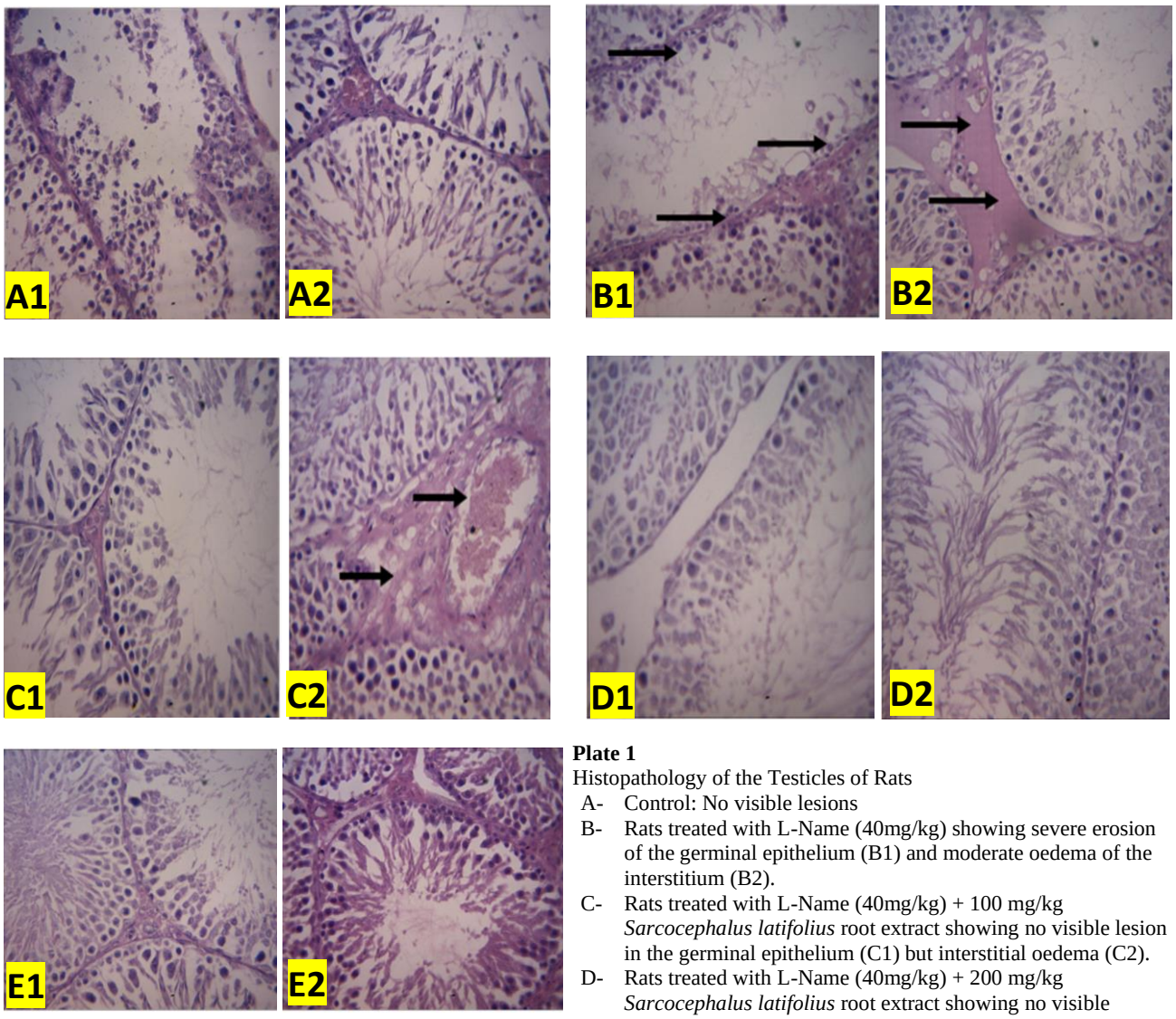

Plate 1
Histopathology of the Testicles of Rats

A- Control: No visible lesions
B- Rats treated with L-Name (40mg/kg) showing severe erosion of the germinal epithelium (B1) and moderate oedema of the interstitium (B2).
C- Rats treated with L-Name (40mg/kg) + 100 mg/kg *Sarcocephalus latifolius* root extract showing no visible lesion in the germinal epithelium (C1) but interstitial oedema (C2).
D- Rats treated with L-Name (40mg/kg) + 200 mg/kg *Sarcocephalus latifolius* root extract showing no visible lesions.
E- Rats treated with L-Name (40mg/kg) + 20 mg/kg Captopril showing no visible lesions.

DISCUSSION

This study was carried out to investigate the modulatory effects of ethanol root extract of *Sarcocephalus latifolius* on the fertility potential of hypertensive male Wister rat induced by L-NAME. Our findings in this study showed that the L-NAME treated group became hypertensive while the groups co-treated with *S. latifolius* and captopril had their blood pressure reduced to normal values comparable to that of the control group. This finding is supported by the report of Nworgu *et al.* (2008) that S. latifolius has blood pressure lowering effect in rats.

Findings from this study showed that packed cell volume and red cell indices of all hypertensive rats treated with *S. latifolius* increased significantly ($p<0.05$). This shows that *S. latifolius* has haemopoietic potential. This finding agreed with Saba *et al.* (2009) study on the fruit extract, where the treated groups demonstrated to possess heamotopoietic effect of the extract. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were not significantly different, which indicate that the red cells were matured cells. This homogeneity in size may be due to the period of the study which was long enough for hematopoiesis to occur and maturation of the cells.

It was noticed that the left testicular weight, length and diameters in groups B, C, D, and E were significantly different (<0.05) when compared to those of their respective control group. The right testicular diameter also increased on groups B, C, D, and E when compared to the control. However, there were no significant differences in the right testicular weights and diameters across all the treatment groups and control. This indicates that left testes were more affected by hypertension and treatments with *S. latifolius* extract and captopril. This agrees with a study that reported that hypertension is associated with an increased incidence of sexual dysfunction (Lou *et al.*, 2023). Some researchers also found out that lower semen volume, sperm mobility, sperm count and total motile sperm count were associated with hypertension (Guo *et al.*, 2017). Anti-hypertensives were also shown to have adverse effects on fertility (Guo *et al.*, 2015). Angiotensin Converting Enzyme Inhibitors were reported to cause relatively decreased semen volume and decreased sperm motility (Guo *et al.*, 2015)

Semen characteristics showed a decrease in sperm motility in group B. This decrease falls below acceptable 60 % minimum for sperm motility for a breeding animal (Palmer *et al*., 2013). Also the liveability value showed a decrease in group B when compared to other group. Since motility and liveability determine fertility, findings from this study therefore showed that hypertension may cause infertility problem in male rats if left untreated (Eisenberg *et al.,* 2016). The extract of *S. latifolius* however improved the sperm motility and liveability in hypertensive rats. It shows that *S. latifolius* has a great reproductive modulatory potential. This agrees with the report of Balogun *et al*. (2016) that *S. latifolius* has diseases prevention and curative effects through antioxidant, anti-inflammatory, antihypertensive, anti-secretory and anti-ulcerative mechanisms.

The percentage of the abnormal sperm cell in group B and E were higher than 20% maximum acceptable in normal sperm breeding animal. Therefore, group B and E having 26.8% and 40.4 % respectively are too high. This findings is similar to Saba *et al*. (2009) who observed sperm abnormality of rats in treatment group were significantly ($P<0.05$) higher in relative to the control rats. This observation is supported by histological findings of the testes of the hypertensive rats which exhibit erosion of the germinal epithelium and interstitial oedema. The histology of the group treated with captopril also revealed severe congestion and interstitial eodema in the section. This findings is supported by the fact that hypertension cause organ damage and some antihypertensive may fail to reverse the damage caused by hypertension or they have adverse effects on the reproductive organs (Eisenberg *et al.,* 2016; Guo *et al*., 2015). The extract treated groups however did not show any abnormality on the histology of the testes. This showed the ameliorative effect of the extract on the testicular damage caused by hypertension.

In conclusion, this study showed that treatment with *S. latifolius* extract reduced the blood pressure and ameliorated the testicular damage and sperm abnormality caused by hypertension.

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