Original Article

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DOI: 10.4103/jeca.jeca_6_17

Evaluation of the comparative effects of antihypertensive drugs: Methyldopa and *Moringa oleifera* leaves on the hypothalamic–pituitary–gonadal axis in male Wistar rat

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Abstract:

BACKGROUND: Antihypertensive drugs have been reported to induce reproductive toxicity, and hypothalamic–pituitary–gonadal axis (HPG) is responsible for the control of reproductive functions. Hence, there is a need to compare the effects of taken commonly used synthetic and natural antihypertensive drugs (Methyldopa and *Moringa oleifera*) on HPG-axis.

AIM: This study was aimed to determine the hypothalamic–pituitary–gonadal responses to the administration of methyldopa and aqueous extract of *Moringa oleifera* leaves in male Wistar rats.

METHODOLOGY: Twenty-five male adult Wistar rats weighed between 150 and 200 g were divided into five groups (A–E), with each group comprising fi ve rats. Group E was designated as the control group which received physiological saline while rats of Group A and B received 200 and 400 mg/kg body weight of *M. oleifera*, respectively. Rats of Group C and D received 500 and 1000 mg/kg body weight of methyldopa, respectively, for 30 days.

RESULTS: Andrological parameters (sperm count, sperm morphology, sperm motility, serum testosterone, follicle-stimulating hormone, and luteinizing hormone concentration) in Group B rats showed significant increase when compare with the methyldopa-treated group (C and D) and control group E rats. Group D rats showed slight abnormalities in sperm morphology and slight decrease in sperm motility when compare with the control group E. Histoarchitecture of the testes of Group A, B, and E rats showed normal seminiferous tubules with full maturation of the germinal cell layers and only Group B rats contained more spermatozoa in their lumen. Group C and D rats showed some of their seminiferous tubules with incomplete maturation of germinal cell layers with their lumens contained fat deposit with no spermatozoa.

CONCLUSION: The aqueous extract of *M. oleifera* leaves as an antihypertensive drug showed high beneficiary effects on male fertility over methyldopa.

Keywords:

Hypertension, methyldopa, *Moringa oleifera*, testes

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Introduction

Hypertension is associated with structural changes in the heart and blood vessels which may lead to mortality and morbidity. Hypertension is typically defined as having a systolic blood pressure (SBP) ≥140 mmHg (SBP ≥140 mmHg) and a diastolic blood pressure (DBP) ≥90 mmHg (DBP ≥90 mmHg) (CHEP, 2008) as against 120/80 mmHg which is consider as the normal SBP and DBP, respectively (CHEP, 2008).

How to cite this article: Adeleke OS, Falana BA, Babawale GS, Atere TG, Abayomi TA, Tokunbo OS. Evaluation of the comparative effects of antihypertensive drugs: Methyldopa and *Moringa oleifera* leaves on the hypothalamic–pituitary–gonadal axis in male Wistar rat. J Exp Clin Anat 2017;16:71-6.

Adeloye *et al*. (2015) reported that estimated 1 billion people are affected by hypertension worldwide, of which hypertension is the main risk factor for many other cardiovascular diseases. In Nigeria, the pooled prevalence of hypertension increased from 8.6% within 1970–1979 and this figure increased geometrically to 22.5% as at 2011 (Belleau *et al*., 2012). Ikeoluwapo *et al*. (2016) reported that approximately 33.1% of Nigerian are suffering from hypertension, of which the prevalence in male is higher than female.

In the management of hypertension, different kinds of antihypertensive drugs have been developed over time and methyldopa is one of the first-generation antihypertensive drug and still remains one of the most commonly used drugs in the treatment of hypertension worldwide (Brunton, 2006). Methyldopa (α-methyl-3,4-dihydroxy-L-phenylalanine) is an analog of DOPA-3,4-hydroxyphenylanine. It is white or yellowish white, odorless, and is sparingly soluble in water. The tablets contain colloidal silicon dioxide, Croscarmellose sodium, hypromellose, magnesium stearate, microcrystalline cellulose, polydextrose, and polyethylene glycol as some inactive ingredients (Brunton, 2006). Methyldopa has been implicated in suppressing testicular function and inhibits sperm motility (Brunton, 2006).

The search for alternative therapy for the treatment of common diseases has moved human closer to natural products being easy to assess, cheap, and affordable. Use of these natural products cannot be overlooked in the treatment of hypertension and other ailments in Nigeria and other parts of the world.

Moringa oleifera is one of the natural products used traditionally for the treatment of hypertension (Anwar *et al*., 2007). *M. oleifera* is the most widely cultivated species of the genus moringa, which is the only genus in the family moringaceae. *M. oleifera* is a fast growing, deciduous tree. It can reach a height of 10–12 m and the trunk can reach a diameter of 45 cm (Olson *et al*., 2010). The phytochemicals present in this plant include alkaloids, steroids, triterpenoids, tannins, anthracenosides, reducing sugars, flavones, and saponins which are reported to have biological activities on animal tissues (Kasolo *et al*., 2010).

M. oleifera has been discovered to be rich in minerals, antioxidants, high protein density, and vitamins (Anwar and Latif 2007) and Rolf *et al.* (2000) reported that taking certain vitamins may help improve male fertility. Blomhoff *et al*. (2011) evaluated the concentration of zinc and Vitamin C in *M. oleifera* and observed that *M. oleifera* is rich in zinc and Vitamin C. Moreover, Bisong, 2005 also reported that plants rich in both zinc and Vitamin C will cause increase in the testosterone and follicle-stimulating hormone level.

Materials and Methods

Preparation of methyldopa and aqueous extract of *Moringa oleifera*

Methyldopa was purchased from Pharmacy Department, Ladoke Akintola Teaching Hospital, Osogbo, Osun State. The drug was identified and authenticated in the Department of Pharmacology, Osun State University, Osogbo. Ten gram of methyldopa was grinded to powder and dissolved in 30 ml of distilled water.

M. oleifera leaves were obtained from farmland in Osogbo, Osun State, Nigeria. The plant was identified and authenticated at the Department of Plant Biology, Osun State University, Osogbo. *M. oleifera* leaves were air dried and the dried pieces were then pulverized using an electric blender (Blender/Miller III, model MS-223, Taiwan, China). 100 g of *M. oleifera* powder was dissolved in 1 L of ethanol. The solution was allowed to stand for 2 days with constant shaken for proper dissolution. The solution was then filtered with Whatman filter paper so as to separate the filtrate from the residue. The filtrate was concentrated using an electric oven at 40°C so as to evaporate the filtrate into the solid mass (paste) and 4 g of the paste was dissolved in 30 ml of distilled water.

Experimental design

A total number of 25 adult male Wistar rats weighed between 150 and 200 g were used. The animals were divided into five groups (A–E) with each group comprising five rats. Group E was designated as the control group which received physiological saline while rats of Groups A and B received 200 and 400 mg/kg body weight aqueous extracts of *M. oleifera* leaves, respectively. Rats of Groups C and D received 500 and 1000 mg/kg body weight of methyldopa, respectively, for 30 days. Drug administrations were done orally using orogastric cannula. The animals were fed with feed from TopFeeds Ltd. Osogbo, Osun State, Nigeria, with access to drinking water *ad libitum*. All the experimental procedures were done following the experimental guidelines of Health Research and Ethics Committee of the Osun State University, Osogbo Campus, Osogbo, Osun State.

Animal sacrifices and samples collection

The animals were sacrificed on the 30th day of the experiment by anesthetized with 0.5 ml/kg of ketamine hydrochloride and fixed by transcardial perfusion method using 4% paraformaldehyde as fixative. Caudal epididymis was excised from the testes before perfusion fixation for sperm analysis and testes fixed in Bouin's fluid for histological analysis.

Sperm count assay

Sperm motility was assessed by the method described by Rouge and Bowen 2002. The spermatozoa were counted

by hemocytometer using the improved Neubauer (Deep 1/10 mm, LABART, Germany) chamber as described by Rouge and Bowen 2002.

Sperm morphology and motility assay

Sperm live/dead ratio and motility was determined using 1% eosin and 5% nigrosin in 3% sodium citrate dehydrate solution according to the method described by Rouge and Bowen 2002.

Testosterone, luteinizing, and follicle-stimulating hormone assay

These were carried out with the use of testosterone, luteinizing (LH), and follicle-stimulating hormone (FSH) ELISA Kit obtained from Monobind Inc. Lake forest, CA, USA. Biotinylated antibody, enzyme-antigen conjugate and a serum native antigen were mixed so that a competitive reaction can set in between the native antigen and enzyme antigen conjugate for a limited number of antibody binding sites. The amount of testosterone, FSH, and LH that were able to bind to the testosterone, FSH, and LH antiserum, respectively, were inversely proportional to the concentration of testosterone, FSH, and LH in the well. The absorbance in each well at 450 nm was read in a microplate reader (using a reference wavelength of 620-630 nm to minimize well imperfections).

Histological examination

Routine histological processing using hematoxylin and eosin staining method was carried out. The testes were fixed in Bouin's fluid, dehydrated in ascending grades of alcohol, cleared in xylene, and infiltrated in molten paraffin wax before finally embedded in molten paraffin wax to form block. The paraffin block containing the tissue was then sectioned by the rotary microtome at 4 µm thickness. The sections were then floated in water bath at 40°C and transferred to a glass slide and stained with hematoxylin and eosin stains. The slides were then viewed under light microscope at × 100 and × 400 magnification and photomicrographs were taken in at both magnifications.

Statistical analysis

Results obtained from the analysis of andrological parameters (sperm count, sperm morphology, sperm motility, testosterone, and FSH and LH concentration) were analyzed statistically, to see the correlation between the results using the Graphpad prism version 5.0.3 (GraphPad Software, Inc., CA 92037 USA). The results were presented as mean ± standard error of mean with significant level at $P < 0.05$ while the histological examination, sperm analysis and serum testosterone, and FSH and LH level were carefully studied and analyzed to establish any correlation between the groups.

Results

Table 1 shows statistical analysis for sperm count, morphology, and motility.

As revealed in Table 1, there was no significant difference in the mean value of sperm count in Groups A, C, and D rats when compare with control group E, but slight significant increase ($P < 0.05$) was observed in Group B rats when compare with the control group E. Moreover, comparison between *M. oleifera* and methyldopa-treated groups showed that Group B rats have slight significant increase ($P < 0.05$) in their sperm count when compare with Group D rats while comparison between Group A and C showed no significant different in the sperm count mean values.

More also, sperm morphology analysis mean value shows that there was no significant difference in Groups A, B, and C rats when compare with control group E, but slight significant decrease ($P < 0.05$) was observed in Group D rats' normal sperm morphology when compare with the control group E. Moreover, comparison between *M. oleifera* and methyldopa-treated groups showed that Group B rats have high significant increase ($P < 0.01$) in their sperm normal morphology when compare with Group D rats while comparison between Groups A and C showed no significant different in the sperm morphology mean values.

Table 1: Shown values of andrological parameters of the control Group E and treated groups (A-D) after administrations of aqueous extract of *Moringa oleifera* leaves and methyldopa

Parameters	Group E (control)	Group A (200 mg/kg *M. oleifera*)	Group B (400 mg/kg *M. oleifera*)	Group C (500 mg/kg methyldopa)	Group D (1000 mg/kg methyldopa)
Sperm count (×10^6 cell/mL)	42.67±1.20	42.87±0.96	48.53±0.58^{a*,b*}	41.67±1.35	40.90±1.87
Sperm morphology (%)					
Normal	84.34±3.05	84.52±2.79	87.96±0.97^{b*}	72.38±4.24	70.05±1.87^{a*}
Abnormal	15.66±3.05	15.48±2.79	12.02±0.98^{b**}	27.62±4.24	29.95±1.87^{a*}
Sperm motility (%)					
Motile	86.19±0.34	86.60±0.47	87.23±0.60^{b*}	84.44±0.66	83.77±0.56^{a*}
Nonmotile	13.81±0.34	13.40±0.47	12.77±0.60^{b*}	15.56±0.66	16.23±0.56^{a*}

Values presented as mean±SEM, $P<0.05$, $P<0.001$ ($n=5$). ^{a*}Comparison of the treated groups with the control, ^{b*}Comparison between treated groups. *M. oleifera* - *Moringa oleifera*, SEM - Standard error of mean

Furthermore, sperm motility mean value shows no significant difference in Groups A, B, and C rats when compare with control group E, but slight significant decrease ($P < 0.05$) was observed in Group D rats' motile sperm when compare with the control group E. Moreover, comparison between *M. oleifera* and methyldopa-treated groups showed that Group B rats have slight significant increase ($P < 0.05$) in their motile sperm when compare with Group D rats while comparison between Groups A and C showed no significant different in the sperm motility mean values.

Hormonal analysis for testosterone, luteinizing, and follicle-stimulating hormone

Figure 1 shows comparison between the control and treated groups' serum testosterone level after the administration of aqueous extract of *M. oleifera* leaves and methyldopa. It was observed that there was no significant difference in the mean value of serum testosterone in Groups A, C, and D rats when compare with the control Group E rats. Moreover, Group B rats administered with *M. oleifera* extract at 500 mg/kg body weight showed slight significant increase in the mean value of serum testosterone.

Figure 2 shows comparison between the control and treated groups' serum follicle-stimulating hormone level after administration of aqueous extract of *M. oleifera* leaves and methyldopa. Group D rats showed slight decrease in their serum follicle-stimulating hormone level when compare with other groups ($P < 0.05$). However, serum follicle-stimulating hormone level comparison between Groups B and D rats showed high significance ($P < 0.01$). Furthermore, comparison between the control and treated groups' serum luteinizing hormone level after the administration of aqueous extract of *M. oleifera* leaves and methyldopa as seen in Figure 3. Group B rats (400 mg/kg/body weight) *M. oleifera* showed slight increase in serum luteinizing hormone level when compare with other groups ($P < 0.05$).

Photomicrograph of the testes

Groups E (Control), A (200 mg/kg/body weight) M. oleifera and B (400 mg/kg/body weight) M. oleifera revealed normal histological sections of the testes, the germinal cell layers show normal maturation stages, the lumen appears normal with the presence of spermatozoa while the interstitial spaces and Leydig cells also appear normal (Figures 4a and b, 5a and b and 6a and b respectively). Group C (500 mg/kg body weight) methyldopa shows normal germinal cell layers with very few spermatogonia cells that showed maturation arrest at secondary level of maturation. The interstitial spaces and Leydig cells also appear normal [Figure 7a and b]. Group D (1000 mg/kg body weight) methyldopa shows germ cell maturation arrest in several seminiferous tubules while the lumen appears widen with absence of spermatozoa [Figure 8a and b].

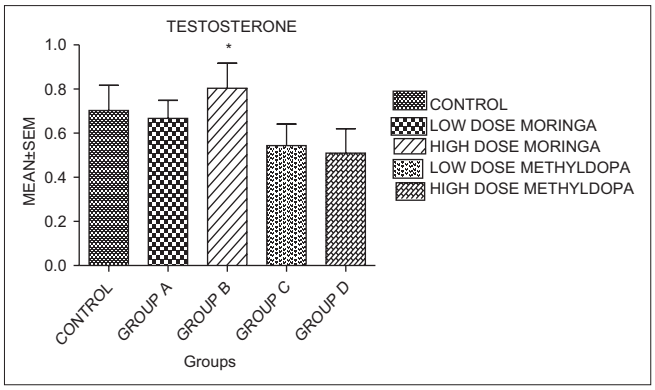

Figure 1: Comparison between the control and treated groups' serum testosterone level after administration of aqueous extract of *Moringa Oleifera* leaves and methyldopa. Group B rats showed slight increase in serum testosterone level when compare with other groups (*$P < 0.05$)

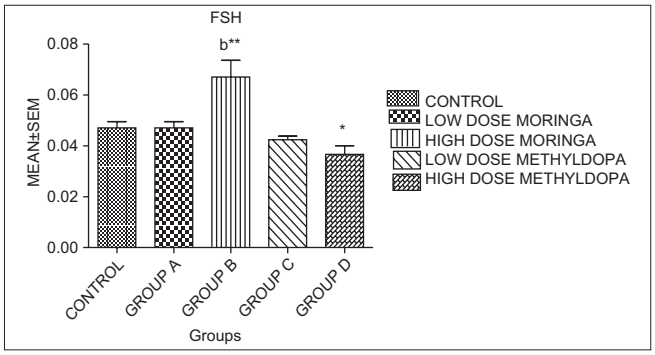

Figure 2: Comparison between the control and treated groups serum follicle-stimulating hormone level after administration of aqueous extract of *Moringa oleifera* leaves and methyldopa. Group D rats showed slight decrease in their serum follicle-stimulating hormone level when compare with other groups (*$P < 0.05$). However, serum follicle-stimulating hormone level comparison between Group B rats and Group D rats showed high significance (b**$P < 0.01$)

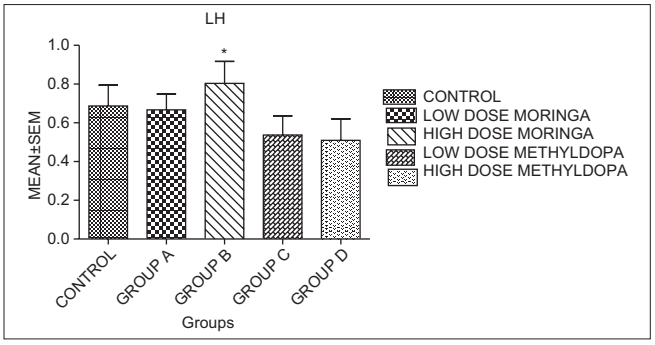

Figure 3: Comparison between the control and treated groups serum-luteinizing hormone level after administration of aqueous extract of *Moringa oleifera* leaves and methyldopa. Group B rats (400 mg/kg/body weight) *Moringa oleifera* showed slight increase in serum-luteinizing hormone level when compare with other groups (*$P < 0.05$)

Discussion

In this present study, it was observed that the aqueous extract of *M. oleifera* leaves promotes fertility in dose-dependent manner in male Wistar rats as a result of

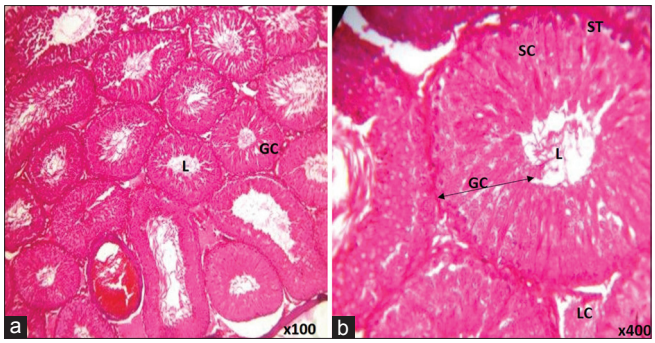

Figure 4: (a) Histoarchitecture of the control group E rat testes "stain with H and E" (×100). Shown normal spermatogonia cell, sertoli cells, germ cell layer with normal maturation stages, the lumen (L) appears normal with the presence of spermatozoa. The interstitial spaces and Leydig cells also appear normal. (b) Histoarchitecture of the control group E rat testes "stain with H and E" (×400). Shown normal spermatogonia cell, sertoli cells, germ cell layer with normal maturation stages, the lumen (L) appears normal with the presence of spermatozoa. The interstitial spaces and Leydig cells also appear normal

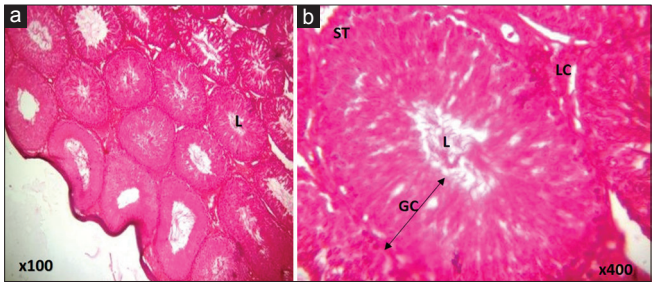

Figure 6: (a) Histoarchitecture of the Group B rat testes "stain with H and E" (×100). Shown normal spermatogonia cell, sertoli cells, germ cell layer with normal maturation stages, the lumen (L) appears normal with the presence of spermatozoa. The interstitial spaces and Leydig cells also appear normal. (b) Histoarchitecture of the Group B rat testes "stain with H and E" (×400). Shown normal spermatogonia cell, sertoli cells, germ cell layer with normal maturation stages, the lumen (L) appears normal with the presence of spermatozoa. The interstitial spaces and Leydig cells also appear normal

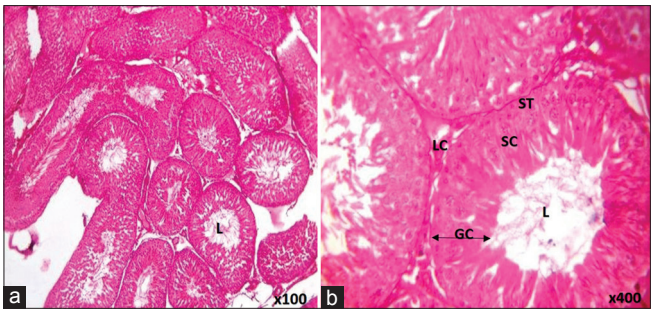

Figure 5: (a) Histoarchitecture of the Group A rat testes "stain with H and E" (×100). Shown normal spermatogonia cell, sertoli cells, germ cell layer with normal maturation stages, the lumen (L) appears normal with the presence of spermatozoa. The interstitial spaces and Leydig cells also appear normal. (b) Histoarchitecture of the Group A rat testes "stain with H and E" (×400). Shown normal spermatogonia cell, sertoli cells, germ cell layer with normal maturation stages, the lumen (L) appears normal with the presence of spermatozoa. The interstitial spaces and Leydig cells also appear normal

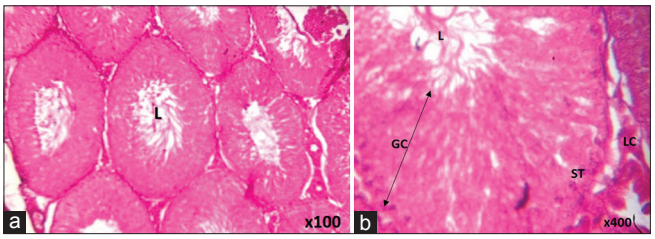

Figure 7: (a) Histoarchitecture of the Group C rat testes "stain with H and E" (×100). Shown normal spermatogonia cell, sertoli cells, germ cell layer with very few spermatogonia cells that showed maturation arrest at secondary level of maturation. The interstitial spaces and Leydig cells also appear normal. (b) Histoarchitecture of the Group C rat testes "stain with H and E" (×400). Shown normal spermatogonia cell, sertoli cells, germ cell layer with very few spermatogonia cells that showed maturation arrest at secondary level of maturation. The interstitial spaces and Leydig cells also appear normal

increase in andrological parameters seen in the Group B rats treated with 400 mg/kg aqueous extract of *M. oleifera* leaves and this observation is in agreement with the study of Blomhoff *et al*., 2011 who evaluated the concentration of zinc and Vitamin C in *M. oleifera* and concluded that *M. oleifera* is rich in zinc and Vitamin C, the element which is essential to promote male fertility (Bisong, 2005). The increase in sperm parameters as well as normal testicular histoarchitecture observed in Group A and B may be as a result of antioxidant effect of zinc and Vitamin C present in the *M. oleifera* plant. It is believed that taking certain vitamins may help improve male fertility (Rolf *et al*., 2000). The mechanism of action is believed to be as a result of antioxidant present in the *M. oleifera* leaves which protect the STs against destruction from free radicals' damage.

Methyldopa at dose 500 mg/kg per body weight (Group C) showed no significant difference when compare with the control group E and Group A rats (200 mg/kg/body weight) of *M. oleifera*. This observation showed that methyldopa at low dose 500 mg/kg/body weight did not show any significant effect on andrological parameters, but mild abnormalities were observed in the testicular histoarchitecture.

Methyldopa at high dose (1000 mg/kg) was revealed to cause decrease in the andrological parameters while some structural alterations were observed in the histoarchitecture of the testes. This finding is in line with the work of Dunnick *et al*., 1986 & Brunton, 2006 in which they reported that chronic administration of methyldopa suppresses testicular functions in albino rats. However, decrease in sperm motility observed in Group D (1000 mg/kg of methyldopa) is also in agreement with the work of Brunton, 2006 who studied the effects of methyldopa in human and reported that methyldopa inhibits human sperm motility. Reproductive toxicity induced by methyldopa at high dose may be as a result of increase in oxidative stress.

Recommendation

It is, therefore, recommended that further research should be done to evaluate molecular mechanism behind methyldopa reproductive toxicity and molecular

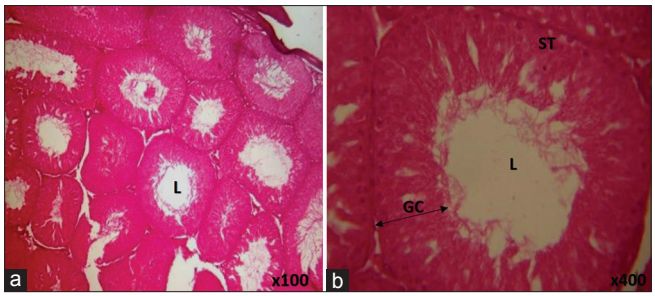

Figure 8: (a) Histoarchitecture of the Group D rat testes "stain with H and E" (×100). Shown several seminiferous tubules with maturation arrest, the lumen is widened and lack spermatozoa with few normal tubules show germ cell layer with normal maturation stages. (b) Histoarchitecture of the Group D rat testes "stain with H and E" (×400). Shown several seminiferous tubules with maturation arrest, the lumen is widened and lack spermatozoa with few normal tubules show germ cell layer with normal maturation stages

mechanism involve in the use of aqueous extract of *M. oleifera* leaves in the treatment of hypertension and reproductive-related diseases. Furthermore, combination of active antihypertensive phytochemicals and antioxidants as found in *M. oleifera* can be process to synthetic drugs that will be more beneficiary to hypertensive patients having fertility problem.

Conclusion

The aqueous extract *M. oleifera* at a moderate dose is a very good antihypertensive drug when compared with methyldopa and this present study also established its high beneficiary effect on male fertility over methyldopa.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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