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EFFECTS OF ETHANOL EXTRACT OF *MORINGA OLEIFERA* LEAVES ON NIFEDIPINE-INDUCED INFERTILITY IN MALE ADULT WISTAR RATS

Bartholomew Ogbonna[1], Peter Ughachukwu[1], Cornelius Nwozor[2]*, Casmir Ofor[3], Princeton Okam[4], Okechukwu Peter Nwabuokei[5], Henry Enwelum[6] and Kosisochukwu Ifemenam[6]

[1]Department of Pharmacology and Therapeutics, Faculty of Clinical Medicine, Chukwuemeka Odumegwu Ojukwu University, Awka Campus, Anambra State, Nigeria.
[2]Department of Physiology, Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Anambra State, Nigeria.
[3]Department of Pharmacology & Therapeutics, Faculty of Clinical Medicine, Ebonyi State University, Abakiliki, Nigeria.
[4]Department of Pharmacology & Therapeutics, Faculty of Clinical Medicine, Nnamdi Azikiwe University, Nnewi Campus, Nigeria.
[5]Department of Medicine & Surgery, Faculty of Medical Sciences, International University of Health Sciences, St. Kitts and Nevis State, West Indies.
[6]Department of Obstetrics and Gynecology, Faculty of Clinical Medicine, Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Amaku, Awka, Anambra State, Nigeria.

***Corresponding Author: Dr. Cornelius Nwozor**
Department of Physiology, Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli campus, Anambra State, Nigeria.

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ABSTRACT
Infertility has been found to be one of the adverse effects that has hindered the successful management of hypertension in hypertensives using calcium channel blockers especially nifedipine. Suspension of treatment as a result of this adverse effect has contributed to treatment failure, development of resistance to nifedipine, increased morbidity and motality, and increased cost of treatment. Orthodox medical interventions aimed at reducing the prevalence of this infertility present their own challenges. This research aimed to determine the effects of ethanol leaf extracts of *Moringa oleifera* on nifedipine-induced infertility in male adult Wistar rats. Thirty-two male adult Wistar rats (age range of 12-14 weeks and weight range of 110- 115 g) were randomly divided into four groups of 8 rats. Group A (control) was fed rat feed, Group B, was fed rat feed plus 0.5 mg/kg nifedipine in aqueous solution, Group C, was fed rat feed plus 174 mg/kg of ethanol leaf extract of *Moringa oleifera*, and Group D, was fed rat feed, 174 mg/kg of ethanol leaf extract of *Moringa oleifera* plus 0.5 mg/kg body weight nifedipine in aqueous solution. Clean drinking water was freely supplied to all the rats and the whole experimental exercise lasted for 8 weeks, after which the rats in the four groups were sacrificed by hitting their occiputs on the laboratory slab, and their testes and the epididymal sperm cells were collected for analysis in a laboratory. Test for significance was done at P value < 0.05. The results showed that nifedipine has significantly increased the fertility of the male adult Wistar rats in the sperm motility and sperm morphology but not on the sperm total count. (2). The inclusion of the extract triggered significant increase on the sperm motility and sperm morphology but not on the sperm total count. (3). The effect when doses of the extract and nifedipine were administered showed that significant decrease was found only in the sperm morphology where the abnormal morphology significantly increased from 25 to 82.86. This study showed that when 0.5 mg/kg nifedipine in aqueous solution and 174 mg/kg ethanol leaf extracts of *Moringa oleifera* were administered separately to the rats, there were significant increase on the sperm motility and morphology but not on the total count. However, when a combination of nifedipine and the extract were administered to the rats, there was reduction in all the sperm parameters considered, especially on the sperm morphology where there was a significant increase on the abnormal sperm morphology, which was a clear indication that this study led to abnormality in sperm morphology. Therefore, leaf extracts of *Moringa oleifera*, when used in the usual clinical doses and duration of therapy is not likely to serve as protective agent on nifedipine- induced infertility in hypertensive patients using nifedipine.

KEYWORDS: Antihypertensives, *Moringa oleifera*, nifedipine, sperm parameters, Wistar rats.

INTRODUCTION

Infertility is a disease of the male or female reproductive system defined by the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse.[1] Infertility in males is most commonly caused by problems in the ejection of semen[2], absence or low levels of sperm, or abnormal motility and morphology of the sperm, obstruction of the reproductive tract, hormonal disorder, and testicular failure to produce sperm. Conditions that cause abnormal motility and morphology of the sperm can lead to male infertility. For example, the use of anabolic steroids[3], calcium channel blockers like nifedipine can cause abnormal semen parameters like sperm count, morphology, viability and motility. Nifedipine is a vasodilator used in the management of some medical conditions, like hypertension, migraine headache, angina and arrhythmias.[4] The major side effect of nifedipine is its infertility effects because of its ability to prevent the uptake of calcium ions and hence decrease in spermatogenesis, during the course of its usage.[5] Ethnomedicinal studies are significant for the discovering of new crude drugs from indigenous reported medicinal plants, right from the commencement of ethnobotany, with special emphasis on the documentation of traditional medicinal knowledge of plants discoveries of a number of key modern drugs.[6] In developing countries, traditional medicines are still the main sources of the healthcare system and it is estimated that about 80% of the population in such countries are dependent upon traditional medicine.[7] Awareness in traditional health practice all over the world has attracted the attention of some scientists towards the ethnomedicines, mostly because of the hazards caused by the synthetic drug residues in the environment and the food chain. Moreover, micro-organisms are becoming resistant to modern drugs because of improper uses of these drugs especially among drug abusers.[8]

Moringa oleifera lam (family: *Moringaceae*), a medicinal plant, is a highly valued plant in tropical and subtropical countries where it is mostly cultivated. The leaves are highly nutritious, being a good source of β-carotene, riboflavin, nicotinic acid, folic acid, pyridoxine, protein, vitamin A, B, C and E, amino acid, various phenolic compounds and minerals.[9] Scientific studies have provided information on the use of hydroalcoholic and other organic solvent extraction of *Moringa oleifera* leaves for its therapeutic activities, which include cardio-protective, hepato-protective antioxidant, anti-inflammatory, and other biological activities with a high degree of safety.[10] Extracts of *Moringa oleifera* leaves consist of a variety of alkaloids and sterols, flavanols glycosides, glycosinolate, isothiocyanate, terpene, anthocyanin and polyphenols which are believed to be responsible for its therapeutic effect.[11] *Moringa oleifera* as an edible medicinal plant used to fight malnutrition and health issues was highlighted in Africa, where *Moringa oleifera* flowers, leaves, fruits and seeds from Guinea Bissau were characterized for their nutritional contents. Aqueous and hydroalcoholic extracts of *Moringa oleifera* leaves were investigated for their phenolic profile and bioactivities.[12] Flavonoids are a family of phenolic compounds that have many biological properties including hepatoprotective, antithrombotic, antibacterial, antiviral and anticarcinogenic activities. These physiological benefits are thought to be due to their antioxidant activity and free radical scavenging properties.[13]

MATERIALS AND METHODS

Study Centre

The study centre was at the Department of Pharmacology and Therapeutics, Faculty of Clinical Medicine, Chukwuemeka Odumegwu Ojukwu university, Awka campus, Nigeria.

Extraction of Plant Extract

Moringa oleifera leaves have pharmacological properties because they contain important bioactive compounds that have been commonly extracted from the leaves using the general methanol-based procedures.[14] Dried *Moringa oleifera* leaves were purchased from a Botanist, identified and authenticated by a Taxonomist. The dried *Moringa oleifera* leaves weighing 400 g, were ground into fine coarse powder by hand and mortar. The leaves powder (400 g) was soaked in 95% ethanol (1L) and one liter of water added in a ratio of 1:1 at room temperature for 24 hours. The mixture was subjected to filtration using a Whatman grade 1 filter paper, and then the solvent was evaporated using rotary evaporator until the extract became lyophilized. The weight of the extract was 52 grams. The extract was stored in a dark bottle.[15] Determination of percentage of the extract was calculated thus: weight of extract/ weight of leaves x 100%.

Experimental Animal

Thirty- two (32) male adult Wistar rats with weight range (110 - 115 g) were used for this experiment with an age range of 12-14 weeks. Rat acclimatization was for one week before the actual study started. The rats were housed according to their groups in four cages made from wood, measuring 35 cm × 30 cm × 40 cm. Each of the wooden cages had a square shaped iron base, with a metallic container, having a flat shape placed underneath to collect the droppings of each rat. During this acclimatization stage, the rats were given standard laboratory rat feed and clean drinking water freely.

Drug Source

1. Nifedipine (20 mg tablet) was one of the drugs made used of in this experiment and was manufactured by Wellona Pharma, India and purchased from Syleon C pharmacy, opposite Nnamdi Azikiwe University teaching hospital, Nnewi, Anambra State.

2. *Moringa oleifera* leaf extract was another drug (herbal) used in this study. It was procured and

authenticated by a botanist, Mrs. Onwunyili Amaka Roseline of the Department of traditional medicine and pharmacognosy Nnamdi Azikiwe University, Agulu campus.

Animal Source

3 .The experimental rats (male adult Wistar rats) were procured and certified by Mr. Obigwe Innocent Mmaduabuchi of the Animal house, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University, Igbariam, Anambra State, Nigeria.

STUDY DESIGN

This was an animal based experimental study. The rats were divided into four experimentation groups (group A, B, C and D) with each group consisting of eight rats as follows

Group A: Feed only.

Group B: Feed + Nifedipine 0.5 mg/kg.

Group C: Feed + 174 mg/kg leaf extract of *Moringa oleifera*.

Group D: Feed + Nifedipine 0.5 mg/kg + Leaf extract of *Moringa oleifera* 174 mg/kg.

All the rats were freely supplied with clean drinking water and the whole experimental exercise lasted for 8 weeks, after which the rats in each treatment group were sacrificed by hitting their occiputs on the laboratory slab.[16] The epididymal sperm cell and testes were taken for laboratory analysis.

LD_{50} of *Moringa Oleifera*

The LD_{50} (Lethal dose -50) for *Moringa oleifera* leaf extract was estimated to be 1585 mg/kg (per oral), in male Wistar albino mice[17,18], found that $LD_{50,}$ using experimental rats ranges from 800-3000 mg/kg.[18] For this study, 174 mg/kg of *Moringa oleifera* leaf extract was used.

Semen Collection

The rats were sacrificed at the end of eight weeks, by hitting their occiputs on the laboratory slab and the testes were dissected out and rinsed in saline solution. Caudal epididymis from each side of the testes of the male adult Wistar rats were dissected out into 10 ml of 0.9% warm normal saline and gently teased to release the sperm cells.[19] The sperm cells were pipetted on grease-free clean slides to determine their morphology and motility using a microscope.

Semen Analysis

Semen analysis involved the determination of some measurable parameters which included sperm count, sperm motility, sperm morphology, sperm viability.

Statistical Analysis and Presentation

The data obtained were computed and analyzed using the Computer Statistical Package for Social Sciences (SPSS) version 29(IBM Company, Chicago, USA 2022). Results were presented as figures. Normality test was conducted on the data. Analysis of variance (ANOVA) was used for test of significance because there were more than two groups of Wistar rats. The post-hoc test (Bonferoni) was used to check for inter-group variations and to detect the group from where the observed difference arose. Descriptive statistics (mean, standard deviation, range, percentage, etc), were determined for continuous variables. P-value < 0.05 at 95% confidence interval was considered statistically significant.

RESULTS

Percentage yield of the extract was 13 %.

Results for Sperm Motility

The results for sperm motility showed relatively closer outcome for rats in group A, on active motile sperms, but differs from results gotten for the sluggish motile and non-motile sperms (figure. 1).The figure also showed that the sperm motility maintained high count in the group B rats (Feed + Nifedipine), and also in the group C rats (Feed + *Moringa*). Similar results were also observed in the group D rats (Feed + Nifedipine + Moringa) where each line plot specifically representing each experimental group is relatively clustered (fig.1).The active motile sperms can be observed to have shown some wider differences from the sluggish and non-motile sperms in both groups.

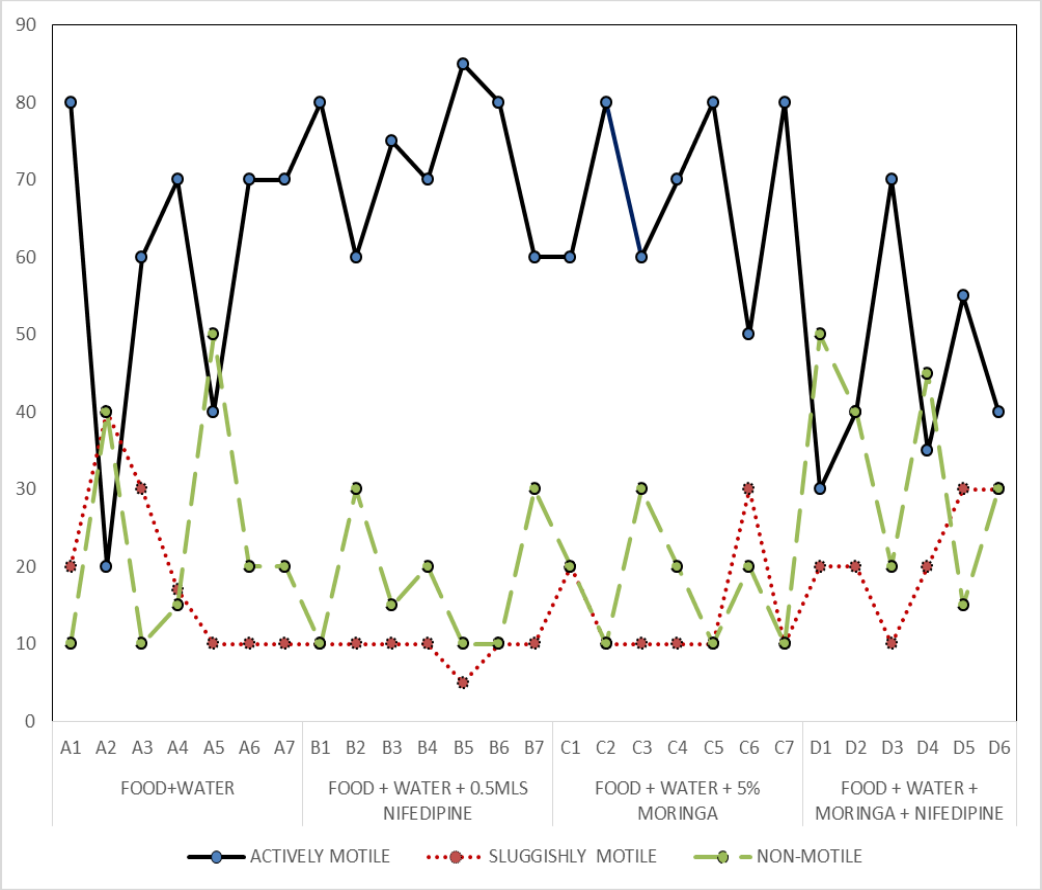

Figure 1: Sperm motility.

Results for Sperm Morphology

The chart for sperm morphology indicated that some differences existed between the normal sperm morphology and the abnormal sperm morphology (Figure 2). During standard laboratory feed with feed and water only, normal sperm morphology and abnormal sperm morphology were equal at one sample point (50), but differ at other samples. They differed continuously at standard laboratory feed plus specific doses of nifedipine (feed+ water + 0.5 mg/kg nifedipine) and also at standard laboratory feed plus a specific quantity of the *Moringa oleifera* leaf extract (feed + water+ 174 mg/kg *Moringa).* They were getting closer at three sample points for the standard laboratory feed plus extract of leaves of *Moringa oleifera* and specific doses of nifedipine rat feed (feed + water + *Moringa* + nifedipine).

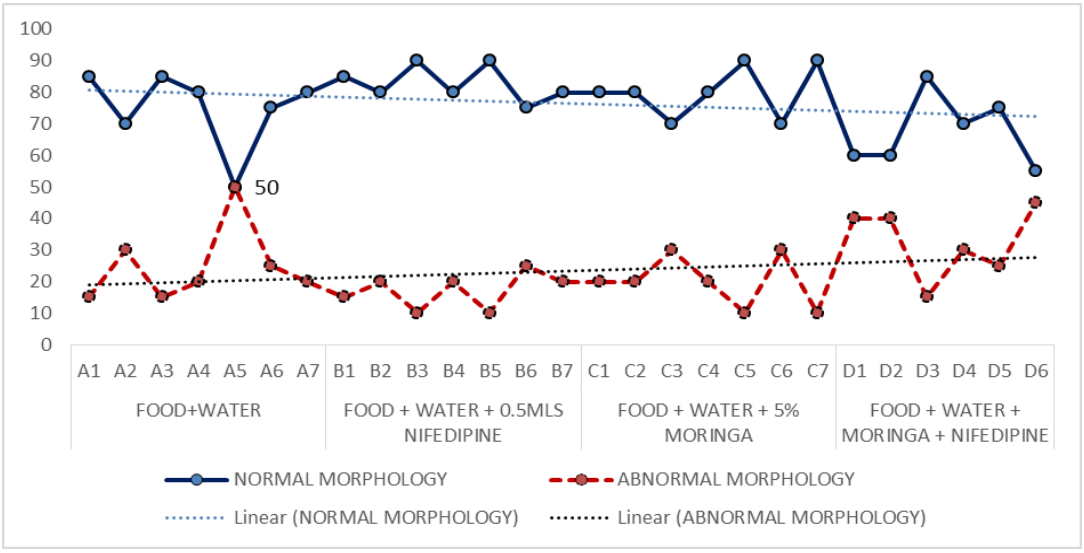

Figure 2: Sperm Morphology.

Results for Sperm Total Count

Figure 3 shows that the movement of the chart from the first group to the last group was close to being the same but with slight differences. At the standard laboratory rat feed (feed + water), the sperm count recorded a lowest value of 30.1 and highest value of 82. Similar sperm count values were obtained in other experimental groups such as in standard laboratory rat feed plus specific doses of nifedipine (feed + water+ 0.5 mg/kg nifedipine: highest value =76, lowest value =35), standard laboratory rat feed plus specific quantity of the extract of *Moringa oleifera* leaves (feed + water + 174 mg/kg *Moringa*; highest value =78, lowest value =35) and, standard laboratory rat feed plus specific quantity of the extract of *Moringa oleifera* leaves and specific doses of nifedipine (feed+ water + *Moringa* + nifedipine: highest value =86, lowest value =21).

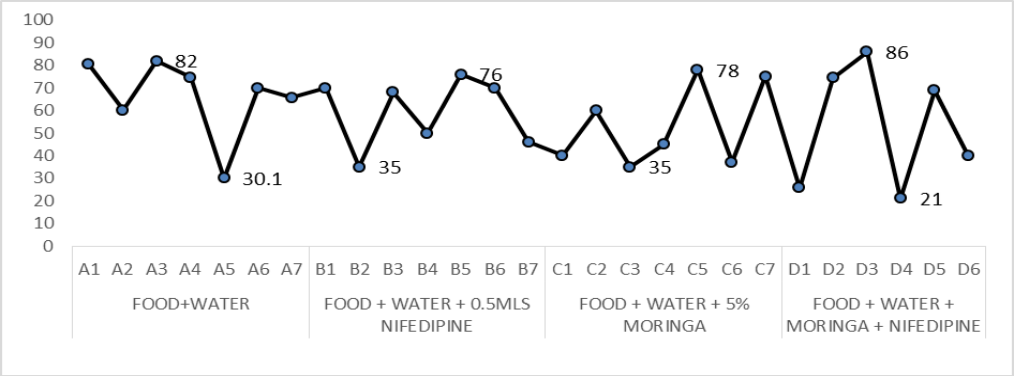

Figure 3: Sperm Total Count.

The Sperm Parameters of Wistar Rats Fed with Normal Feed Plus Nifedipine

The inclusion of nifedipine increased the mean sperm motility for the active motile (from 58.57 to 72.86 µm/s) and reduced the mean for the sluggish sperm motility to 9.29, from 19.57.The mean for the non –motile sperm dropped from 15.47 to 9.06 µm/s.

When fed with the standard laboratory feeds containing feed and water only, the average normal sperm morphology increased to 82.7 (from 75). This led to a decrease in the abnormal morphology (from 25 to17). The mean however got reduced at the total count for nifedipine addition (from 66.14 to 59. 28).

The result showed that nifedipine has significant positive effect on the sperm motility of the rats (P< 0.0001, F = 43.756, df = 2), and sperm morphology (P<0.0001, F=128.647, df=1) but not on total count (P=0.455, T=0.772, df= 12). It showed that significant differences were obtained between active motile and sluggish motile (P<0.0001, 95% Conf. Int: 34.82-67.75) as well as between active motile and non-motile (P<0.0001, 95% Conf. Int: 24.10 - 65.90). No significant difference was found between the sluggish and the non-motile sperms. (P= 0.414, 95% Conf. Int: -17.28 to 4.71). For sperm morphology, the normal morphology significantly differs with the abnormal morphology (P< 0.0001, 95% Conf. Int: 46.73 – 68.97). Both indicated the effects of nifedipine at improvement on active motile sperm and the normal sperm morphology.

The Sperm Parameters of Wistar Rats Fed With Rat Feed Plus Leaf Extracts *Moringa Oleifera*.

The administration by doses of *Moringa oleifera* leaf extract triggered significant effects on sperm motility (P= 0.0001, F=34.93, df= 2), and sperm morphology (P<0.0001, F=97.71, df=1) but not on total sperm count (P= 0.191, F =1.399, df= 12). The doses of *Moringa oleifera* leaf extract increased the mean value of the active sperm motility from 58.57 to 68.57 µm/s, and consequently the reduction in the sluggish sperm motility (from 19.57 to 14.29 µm/s) and the reduction in the non-motile (from 23.57 to 17.14 µm/s). It also significantly changed the mean ± standard deviations for normal sperm morphology from 75.0 ± 12.25 to 80.0 ± 8.16.

The mean total count decreased from 66.14 to 52.86 on *Moringa oleifera*, but the decline was not found significant in this study.

DISCUSSION

This study, revealed that nifedipine increased the mean sperm motility (from 58.57 to 72.36) and the normal sperm morphology (from 75 to 82.86) but has no significant effect on Wistar rats fertility with regard to the sperm total count (P= 0.455). The sperm total count for nifedipine addition reduced (from 66.14 to 59.28). The result showed that nifedipine has significant effect on the rat's fertility in the sperm motility (P< 0.0001) and sperm morphology (P< 0.0001) but not on the total sperm count, when compared with the control. This was contrary to a study by[20], where there was significant decrease (P< 0.05) in sperm total count and sperm motility. These differences may be as a result of the doses of nifedipine used which was slightly higher (0.57mg/kg) body weight. The age of the rats may have effect on the difference in the result reported. In this experiment, the age of the rats ranges from 12-14 weeks but[20], did not state the age of the rats used in their study. Wistar rats were not sexually matured until 100days. In a study by[21], designed to address the involvement of

CCBs in inducing male infertility, they found out that (after feeding 36 male mice with therapeutic dose of nifedipine for 30 days), CCBs significantly decreased the total count. There was however, slight increase in sperm motility and normal sperm morphology.[21] In another study[22], administered 0.57 mg/kg body weight of nifedipine per oral to male rats (150-200 g), and found that nifedipine significantly decreased ($P < 0.05$) sperm count and motility.[22]

Clearly, sperm count and motility were affected by the treatment with nifedipine. The difference in the result gotten may be due to the strain of the rats (sexually matured albino rats of Rattus Novergicus). The administration of doses of leaf extract of *Moringa oleifera* triggered significant effect on sperm motility ($P< 0.001$). The sperm motility showed relatively closer results when compared with control. The doses of *Moringa oleifera* leaf extracts increased the mean value of the active sperm motility (from 58.57 to 68.58µm/s). Hence leaf extracts of *Moringa oleifera* enhanced sperm motility. This result was supported by a study by.[23] They found that leaf extracts of *Moringa oleifera* increased sperm motility and morphology when fed to rats.[23] This study was also supported by[24], when they fed two groups of rabbits respectively with 200 and 400 mg/kg body weight of *Moringa oleifera* leaf extracts in their diet. They observed that there was an increase in sperm motility and viability.[24] Also in this study, the administration of doses of *Moringa oleifera* leaf extracts triggered significant effect on sperm morphology ($P<0.0001$). The dose of leaf extract of *Moringa oleifera* significantly changed the mean and standard deviation for normal sperm morphology (from 75.0 ± 12.25 to 80.0 ± 8.16). Therefore leaf extract of *Moringa oleifera* increased normal sperm morphology and hence enhanced fertility in the Wistar rats. *Moringa oleifera* was found to reduce abnormal sperm morphology (thereby increasing normal sperm morphology) in male Swiss mice fed with 4% and 8% *Moringa oleifera* in their diet.[25] In this study, the administration of dose of *Moringa oleifera* leaf extracts did not trigger significant effect on total sperm count ($P=0.191$). The mean total sperm count decreased (from 66.14 to 52.86) when compared with the control but the decline was not found significant in this study. In a closely related study[26] found that there was also a rise in the sperm count of male rats fed with *Moringa oleifera* leaf extracts.[26] In a study by[27], they fed male rats with leaf extracts of *Moringa oleifera* in their diet and observed that there was increase in the percentage of sperm count, motility, normal sperm morphology and viability.[27] In this study, when doses of *Moringa oleifera* and nifedipine were administered to the group D rats, there was no significant effect found in sperm motility ($P=0.540$). There was a decrease in the active sperm motility (from 58.57 to 45). There was slight increase for the sluggish motile (from 19.57 to 21.67), and the non-motile (from 23.57 to 33.33). This indicated some negative effect on the combination, though not found significant. There was also a mean reduction in normal sperm morphology (from 75 to 67.5). Abnormal sperm morphology significantly increased (from 25 to 82.86). This showed that this experiment led to abnormality in sperm morphology. There was also a decrease in the sperm total count (from 66.14 to 52.7). Significant effects were found only in the sperm morphology ($P=0.010$) No significant effects were found in the sperm motility ($P=0.540$) and the total sperm count ($P=0.408$). The effect of administering *Moringa oleifera* and nifedipine to the group D rats was different from the effect gotten when nifedipine and *Moringa oleifera* were administered to the rats of group B and C respectively. The effect gotten in group D may be as a result of herb-drug interaction between leaf extract of *Moringa oleifera* and nifedipine on the semen parameters of the Wister rats used in this study.

In a study by[28], Nifedipine is metabolized by cytochrome P450 (CYP3A sub-family of the enzyme).[28] Also a study by[29], showed that leaf extract *Moringa oleifera* has a weak inhibition on CYP1A2 and CYP2C9 (isoenzymes of Cytochrome P450).[29] A similar study by[30] showed that *Moringa oleifera* leaf ethanolic and aqueous extracts inhibited CYP1A2.[30] In another study by[31] using mice with a targeted cytochrome P450 isoenzyme (CYP 17) deletion concluded that CYP17 plays a critical role in the organization and structure of sperm mitochoridria. There were mitochondrial defects in the CYP17 gene deleted mice. As a result, cellular power generation was impaired (because ATP (adenosine triphosphate) production was also altered). The mitochondrial defects were likely the cause of defects in sperm morphology, altered fertilization and sperm motility in the mice. Hence CYP17 is necessary for proper sperm development and function.[32] Nifedipine is metabolized by Cytoctrome P450 (CYP3A isoenzyme). *Moringa oleifera* leaf extract is a weak inhibitor of cytochrome P450.[33] The result in this present study with the Wistar rats in group D (fed with 0.5mg/kg nifedipine plus 20mg of *Moringa oleifera* leaf extract in their diet) could be as a result of the inhibitory effect of *Moringa oleifera* leaf extract on the enzyme, cytochrome P450, which is the enzyme involved in Nifedipine metabolism. The inhibition of this enzyme by *Moringa oleifera* leaves, led to nifedipine accumulation in the sperm cells especially.[20] in a study showed that high doses of nifedipine has a deleterious effect on the semen of male rats (reduced sperm motility, morphology and count).[20] This was supported by a closely related study by[21] where significant decreases were found in the sperm count and motility of mice after administering therapeutic doses of nifedipine for 30 days.[21] This was also supported by a study by.[22] They found that significant decrease was obtained on sperm count and motility when 0.57 mg/kg of nifedipine was given to male rats.

CONCLUSION

Finally, this study showed that when specified doses of nifedipine and *Moringa oleifera* leaf extract were

administered separately to the rats, there were significant positive effects on the sperm motility and morphology and not on the total count. However, when a combination of specified doses of nifedipine and leaf extracts of *Moringa oleifera* was administered to the rats, there was reduction in all the sperm parameters considered especially on the sperm morphology, where a significant increase was found in the abnormal sperm morphology, which was a clear indication that this study led to abnormality in sperm morphology.

Therefore, leaf extracts of *Moringa oleifera*, when used in the usual clinical doses and duration of therapy, is not likely to serve as protective agent on nifedipine induced infertility in hypertensive patients using nifedipine.

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