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*Full-Length Research Article*

# Comparative Study on the Effects of *Cymbopogon citratus* (DC.) Stapf and *Mangifera Indica* (L.) on Sperm Indices of Male Rats

***Beshel S.B.[1], Ekpenyong, J.F.[2], Daben, M.R.[3], Chinedu-Ndukwe P.A.[4], Adie, P.I.[5], Asuquo, G. O.[6], Anoyom O.E.[7]**

*[1, 2, 5, 6 & 7]Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Calabar, Calabar – Nigeria*

*[3]Department of Science Laboratory Technology (Biological Sciences Techniques), University of Jos, Jos, Plateau State, Nigeria*

*[4]Department of Zoology and Environmental Biology, College of Natural and Applied Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria*

*[6]Department of Histopathology and Cytology, Faculty of Medical Laboratory Science, University of Calabar, Calabar, Nigeria.*

**Summary:** This study investigated the effects of varying doses of Cymbopogon citratus (DC.). Stapf and *Mangifera indica* (L.) ethanolic leaf extract on the fertility of male *Rattus norvegicus*. Twenty-five (25) adult male rats were randomly assigned to five (5) cages, with five rats per cage. *C. citratus* extract significantly increased the mean (total number of sperm counts obtained from all the rats in a given group divided by the number of rats present in that group) sperm count of the treated rats at both 150 and 300 mg/kg body weight groups, compared to those of *M. indica* extract, respectively. Also, *C. citratus* produced a reduction in abnormal sperm morphology for 150 and 300 mg/kg body weight, respectively, compared to *M. indica* extract. Our findings indicated that *C. citratus* may influence fertility by stimulating sperm count production, and this effect is dose-dependent. The reverse is true for *M. indica*.

**Keywords***: Cymbopogon citratus*, *Mangifera indica*, fertility, abnormal sperm, sperm production, sperm indices,

**Authors for correspondence: beshelsolo@gmail.com, Tel: +234-7033381424*

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## INTRODUCTION

Sperm indices are good markers of animal fertility. The use of plant parts such as acacia leaves, cotton lint, and honey was used as contraceptives (Cuomo, 2010), meaning that they possess the potential to affect sperm indices. Sperm indices such as sperm count, motility and morphology play significant roles in fertility of male animals, thereby indirectly affecting animal population. Acharya and Gowda (2017) posited that fertility in man declines more gradually with age due to factors such as sperm motility and morphology. Certain factors such as chemical consumption from food additives and other environmental factors could play a role in this respect. Reductive effect on sperm count of rats have been reported in some herbal plants (Beshel, 2023; Beshel, 2018; Ogedengbe *et al.,* 2016; Ahmed *et al.,* 2013; Saba *et al.,* 2009). These herbal materials are capable of disrupting spermatogenesis thereby affecting sperm count, motility and morphology (Cavalleri *et al.,* 2018; Nirmal *et al.,* 2017; Hasim *et al.,* 2015; Ahmed *et al.,* 2013). Kumar *et al.* (2012) identified potential antifertility agents from plants which in different ways affect sperm indices of male animals. Kamath and Rena (2001) reported antifertility effect of *Calotropis procera* roots.

Daniyal and Akram (2015) provided a comprehensive summary of medicinal plants used as antifertility agents in females; and Saalu (2016), documented a scientific appraisal of Nigerian folklore medicinal plants with potential antifertility activity in males. Extracts from grapefruit seeds have profertility effects in male rats (Saalu *et al.,* 2008, 2010); *Sesame radiatum* extract facilitates fertility in male (Ukwenya *et al.,* 2008); leaf extract of *Moringa oleifera* modulates testicular toxicity in rats (Saalu *et al.,* 2011). The modulating role of bitter leaf on spermatogenic and steroidogenesis functions of the rat testis has also been reported (Saalu *et al.,* 2013). Further, *Jatropha curcas* extract has shown ameliorating effect on the rat testis (Oyewopo *et al.,* 2014), while aqueous extract of date (*Phoenix dactylifera*) has demonstrated protective effect on the testis (Akunna *et al.,* 2012). Ethanolic extract (250

mg/kg) from *Hibiscus rosasinensis* flowers administered to albino rats revealed strong anti-implantation property (Kamath & Rena, 2001).

Medicinal plants with antispermatogenic effect either interfere with any phase of sperm production or the entire process of spermatogenesis (Meymand, 2002; Meymand, 2002). For instance, Meymand (2002) reported induced abnormality in spermatogenesis and sperm production in some of the seminiferous tubules of male albino rats tested with neem (*Azadirachta indica*) seed extract. Also, Shaikh (2009) reported suppressive effect on the spermatogenesis, semen quality and seminiferous tubule diameter of male albino rat treated with low dose (0.6 mL/animal) of neem seed oil. In a fertility study using garlic (*Allium sativum*) bulb, Chakrabarti *et al.* (2003) reported that low doses of 0.25 g/mL and 0.5 g/mL induced instant immobilization of the epididymal sperm and human ejaculated sperm, respectively. In another study, methanol leaf extract of bush marigold (*Aspillia africana*) administered at 100, 200, 400 mg/kg b.wt./day for 52 days to rat induced antispermatogenic effect (Ruth, 2015). Aqueous extract of bark of *Carica papaya* has shown inhibition of sperm motility due to ultrastructural changes in epididymis (Manivannan *et al.,* 2004). Other plants reported to exhibit antispermatogenic properties include Mucuna urens ethanolic seed extract (Udoh & Ekpenyong, 2001; Etta et al., 2009); Piper nigrum fruit powder (Mishra & Singh, 2009); and Thevetia peruviana methanolic stem bark extract (Gupta *et al.,* 2011).

*Cymbopogon citratus*, commonly known as lemongrass, is a plant many people use as a spice. Common among users of this plant are Nigeria and Indonesia (Hasim *et al.,* 2015). *C. citratus,* among other spices, has been reported to contain phenolic compounds with strong antioxidant potential (Hasim *et al.,* 2015; Vazquez-Briones *et al.,* 2015). The plant is a perennial tropical grass capable of withstanding a wide range of temperatures and can thrive in warm, semi-warm, and temperate climates (Oviya *et al.*, 2016). *C. citratus* can grow up to 60 to 120 cm high with long green leaves and a very pleasant aroma and taste. The leaf essential oil is used in the food, perfumery, soap, cosmetic, pharmaceutical, and insecticide industries (Oviya *et al.,* 2016). The main constituents of the essential oil (citral, terpenes and geraniol-terpenic alcohol) have also been reported (Oviya *et al.,* 2016).

Phytochemical screening of *C. citratus* reveals the presence of carbohydrates, tannins, glycosides, alkaloids, flavonoids, phenols, steroids and phytosteroids (Umar *et al.*, 2016; Oviya *et al.,* 2016). The activities of these phytochemicals may induce adverse effects on the fertility of male albino rats, making the plant an efficient antifertility agent in the rat, and probably human. The plant *Mangifera indica*, commonly called mango in most countries of the world, is a tall plant with many leaves that originated from India and is now naturalised in West Africa (Okwu and Ezenagu, 2008). All the organs of the plant including stem, bark, roots and fruits are beneficial to man and are consumed mostly for medical or therapeutic reason (Abdelnaser & Shinkichi, 2010; Okwu & Ezenagu, 2008) but the fruit pulp is mostly consumed as dissert or processed into juice to be taken as fruit juice. Nunez-Selles (2005) reported that the extracts from leaves, seed kernel, fruit pulp, roots, bark and stem bark have been used extensively for medicinal purposes in many countries.

Evaluation of the phytochemical composition of mango stem bark and leaves reveals that the plant organs contain several bioactive compounds such as flavonoids, alkaloids, saponins, phenols and tannins as well as many water-soluble vitamins and mineral elements (Okwu & Ezenagu, 2008) making it effective in folklore medicine, especially for therapeutic purpose. *M. indica* has anti-oxidant, anti-inflammatory and immunomodulatory properties, making it efficient for use as a nutritional supplement (Nunez-Selles, 2005). Divyalashmi and Aruna (2017) reported antioxidant and antimicrobial properties of M. indica, along with numerous bioactive compounds, which inhibited bacteria across various zones and concentrations. Sekinat *et al*. (2007) investigated the antifertility effects of the methanolic leaf extract of *M. indica* using male Sprague Dawley rats and reported its effects on sperm motility, sperm count, morphology, live death ratio and epididymal volume of rats. While the reproductive effects of some medicinal plants have been reported, information on *Cymbopogon citratus* (DC.) Stapf and *Mangifera indica* (L.) is limited. Therefore, the aim of this study was to investigate the effects of varying doses of *Cymbopogon citratus* (DC.) Stapf and *Mangifera indica* (L.) ethanolic leaf extract as antifertility agent in male albino rat.

## MATERIALS AND METHOD

### Collection and extraction of plant leaves

Leaves *C. citratus* and *M. indica* were harvested fresh from the University of Calabar Botanical Garden, transported in black nylon bags to the botanical herbarium of the Department of Botany, University of Calabar, for proper identification and authentication [Fayomi 164 (FHI) and A. A. 2171 (FHI) for *C. citratus* and *M. indica,* respectively]. Leaves were washed with clean, fresh water, air-dried, and further dried in the oven dryer at 42 °C for another 24 hours. The oven-dried leaf materials were crushed into powder separately using electric grinder and sieved into fine powder, stored in an air-tight bottle in the laboratory for future extraction. The sieved powder (50g) was subjected to extraction in a Soxhlet apparatus using ethanol as a solvent (Divyalashmi and Aruna, 2017).

**Experimental animal:** Twenty-five (25) albino male rats of 3 months old weighing 180-220 g were obtained from the Animal House of the Department of Zoology and Environmental Biology, University of Calabar. Rats were transported in plastic cages to the Department of Zoology and Environmental Biology laboratory for the experiment. Rats were kept under laboratory condition for 24 hours for acclimatization; they were then transferred into new cages and fed *ad libitum* with pelleted animal feeds for a period of 21 days.

The experimental rats were randomly assigned to three groups including the control. Each group contained 10 rats that were further subdivided into two containing 5 rats each. Group I (Control) were fed with distilled water.
Group II contained 10 rats, of two subgroups containing 5 rats each. One group was fed with 150 mg/kg of *C. citratus* extract, while the other group was fed 300 mg/kg of *C.*

*citratus* extract daily. Group III equally contained 10 rats, which were further subdivided into two subgroups, each containing 5 rats. One group was fed 150 mg/kg) of *M. indica* leaf extract, while the second subgroup was fed with 300 mg/kg of *M. indica* leaf extract daily.

**Semen collection and analysis:** After 21 days, the rats were starved for 24 hours and sacrificed under ketamine anaesthesia. Semen was collected from the caudal epididymis for analysis. The epididymis was sectioned into head and tail using a pair of fine scissors. The tail was collected in a small petri dish containing 1 ml of phosphate buffer saline maintained at 37 ˚C. The tail was pressed between forceps and punctured by means of needle. Sperms were allowed to be released into the phosphate buffer saline by gently shaking the petri dish occasionally for about 10-15 minutes. The spermatozoa thus formed were strained through ordinary plastic strainer to remove the tissue fragments and kept in an incubator maintained at 37˚C (Saba *et al.,* 2009; Oyeyemi and Ubiogoro, 2005).

**Determination of sperm count:** The prepared sperms suspension was thoroughly shaken to evenly disperse the sperms. A quantity of 0.05ml of the sample was diluted to 1 ml with formalin bicarbonate solution using white blood cell pipette. The formalin bicarbonate solution effectively immobilized the sperm cells to facilitate counting of the sperms. Counting was done on a haemocytometer using the usual RBS counting chamber (Ahmed *et al.,* 2013).

**Evaluation of sperm motility:** Sperm motility was evaluated according to (Ahmed *et al.,* 2013). A small drop of prepared spermatozoa suspension was placed on a microscope slide, pre-warmed for approximately 37˚ C then covered with a cover slip ringed with petrolatum. The microscope slide was observed under high power. Motility was evaluated by scanning 10 different fields containing at least 10 sperms. With respect to this, the sperm sample was rated as active motility, sluggish motility and no motility (dead sperms).

Sperm morphology was assessed by smears of the sperm suspension, prepared on clean slides in a manner identical to that for blood film. The smear was allowed to dry then stained with Leishman's stain. The morphology of stained sperm was observed under high magnification. Abnormalities were noted such that if 70 percent of the sperm had a head, tail and midpiece, it was considered that the animal had normal sperms (Ahmed *et al.,* 2013).

**Statistical analysis**

The data obtained was analysed using Predictive Analytical Software (PASw) version 27. Test for significance between the doses was performed using analysis of variance (ANOVA). A p-value < 0.05 was used as the criterion for statistical significance.

## RESULTS

**Sperm count:** Figure 1 shows the results of mean values of sperm count of rats fed *C. citratus* at the low dose (272.00±7.47x$10^6$/ml) and high dose (297.00±3.62x$10^6$/ml) groups, which were significantly higher ($p<0.05$) than that of *M. indica* (113.20±12.51x$10^6$/ml and 27.60±3.67x$10^6$/ml for low and high dose groups, respectively). There was a significant difference ($p<0.05$) between the low dose and high dose of both plants.

### Sperm motility and sperm morphology

**Total sperm motility (%):** Total sperm motility observed in the high dose and low dose groups of *C. citratus* were 96.80±0.80% and 96.00±0.00%, respectively, significantly higher ($p<0.05$) than those of rats fed *M. indica* leaf extract (24.80±16.08% and 37.80±2.56% for high and low dose groups, respectively). Total sperm motility was not significantly different ($p>0.05$) between the high dose and low dose groups of *C. citratus-fed rats, but the high dose of M. indica-fed* rats was significantly higher ($p<0.05$) than the low dose group.

The mean percentage values of active sperm motility of rats fed *C. citrus extract at the high dose and low dose were significantly higher ($p<0.05$) than those of the M. indica* group (Figure 2). The values of active sperm motility observed in the high dose and low dose groups of *C. citratus* were 93.80±1.07% and 90.80±0.37%, respectively, significantly higher ($p<0.05$) than those of rats fed *M. indica* leaf extract (14.60±14.10% and 18.20±1.93% for high and low dose groups, respectively). Active sperm motility was not significantly different ($p>0.05$) between the high dose and low dose groups of *C. citratus* fed rats but the high dose of *M. indica* fed rats was significantly higher ($p<0.05$) than the low dose group.

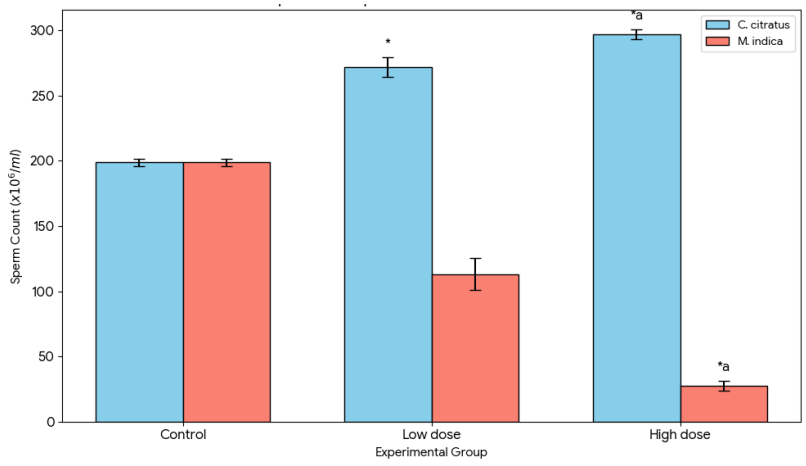


**Figure 1:**
Comparison of sperm count between *C. citratus* and *M. indica* leaf extracts
*Values are expressed as mean ±SEM, n = 5;*
** = significantly different from value in the next column at $p<0.05$*
*a = significantly different from Low dose within the same group at $p<0.05$*

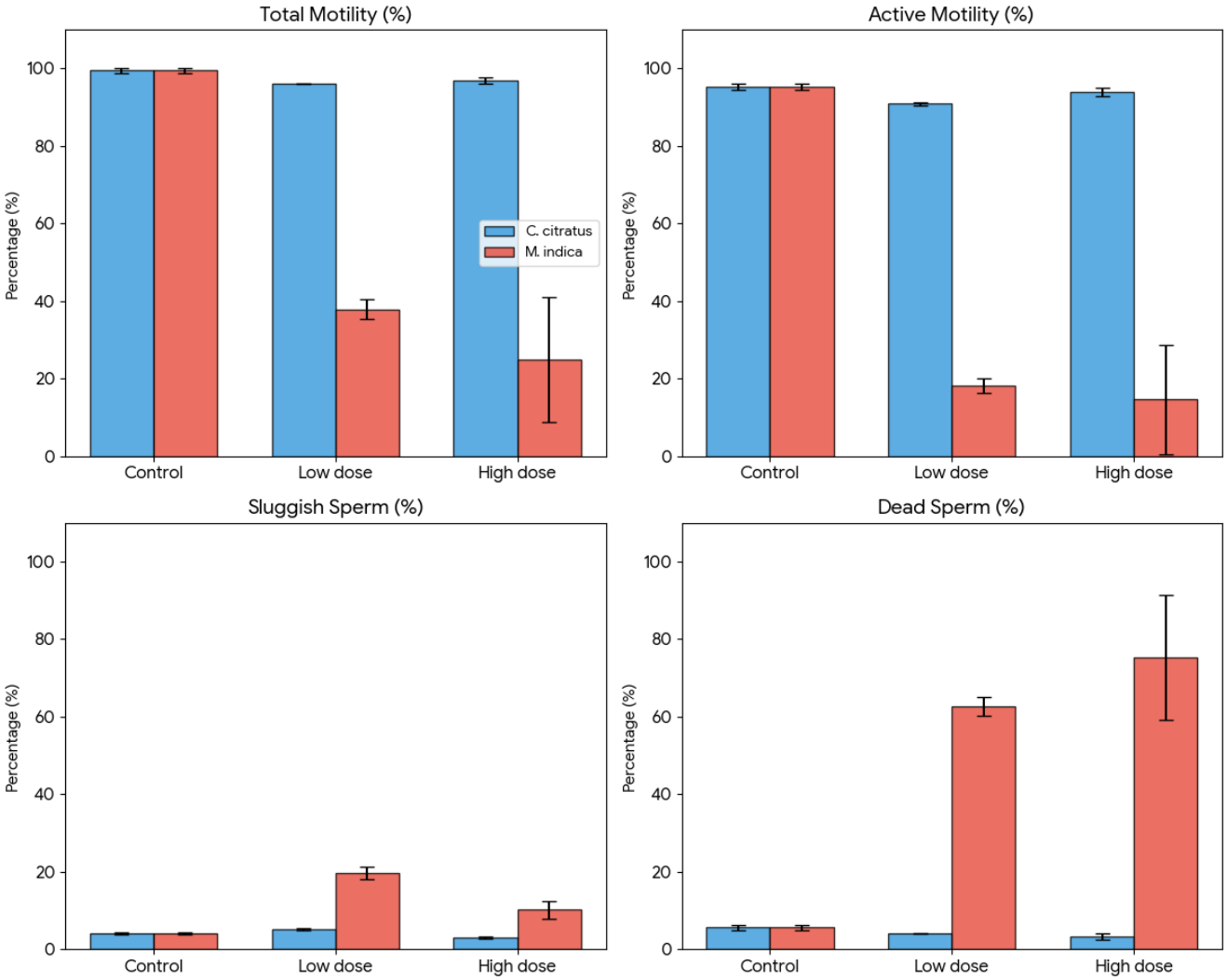


**Figure 2:**
Comparison of sperm motility indices between *C. citratus* and *M. indica* leaf extracts
*Values are expressed as mean ±SEM, n = 5*
** = significantly different from value in the next column at p<0.05*
*a = significantly different from Low Dose within the same group at p < 0.05*

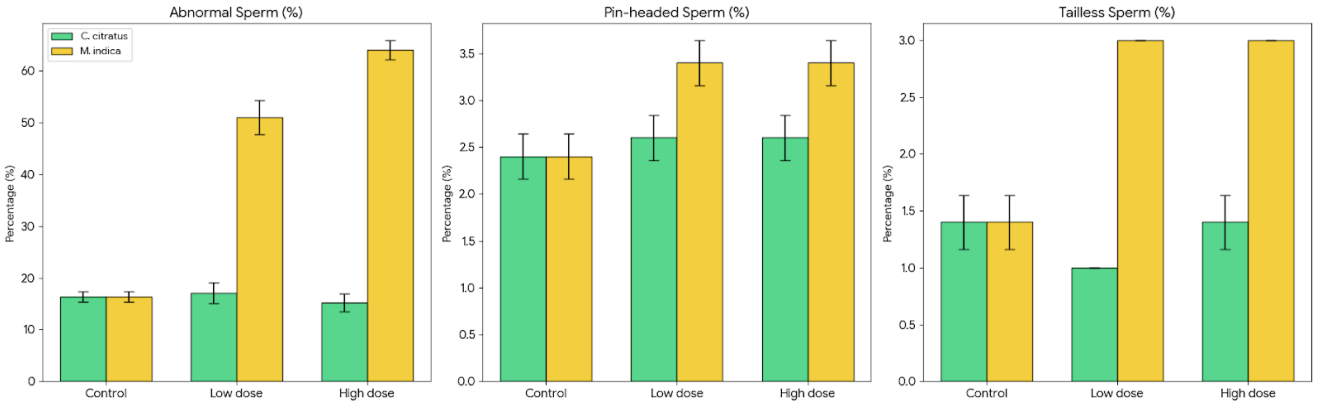


**Figure 3:**
Comparison of percentage sperm morphology indices between *C. citratus* and *M. indica* leaf extracts
*Values are expressed as mean ±SEM, n = 5*
** = significantly different from value in the next column at p<0.05*
*a = significantly different from Low Dose within the same group at p<0.05*

The mean percentage values of sluggish sperm motility of rats fed *C. citrtus* extract at the high dose and low dose were significantly lower (p<0.05) than those of *M. indica* group (Figure 2). The values of sluggish sperm motility observed in the high dose and low dose groups of *C. citratus* were 3.00±0.32% and 5.20±0.37%, respectively, significantly (p<0.05) lower than those of rats fed *M. indica* leaf extract (10.20±2.25% and 19.60±1.63% for high and low dose groups, respectively). Sluggish sperm motility was not significantly different (p>0.05) between the high dose

and low dose groups of *C. citratus-fed rats, but the high dose of M. indica-fed* rats was significantly lower ($p<0.05$) than the low dose group.

The mean percentage values of dead sperm cells of rats fed *C. citrtus* extract at the high dose and low dose were significantly lower ($p<0.05$) than those of *M. indica* group (Figure 2). Dead sperm cells observed in the high dose and low dose groups of *C. citratus-fed* rats were 3.20±0.80% and 4.00±0.00%, respectively, significantly lower ($p<0.05$) than those of rats fed *M. indica* leaf extract (75.20±16.08% and 62.60±2.50% for high and low dose groups, respectively). Values of dead sperm cells were not significantly different ($p>0.05$) between the high dose and low dose groups of *C. citratus-fed* rats but the high dose of *M. indica-fed* rats was significantly higher ($p<0.05$) than the low dose group

The mean percentage values of abnormal sperm of rats fed *C. citrtus* extract at the high dose and low dose, as presented in Figure 3, were significantly lower ($p<0.05$) than those of the *M. indica* group. Abnormal sperm values observed in the high dose and low dose groups of *C. citratus-fed* rats were 15.20±1.77% and 17.00±2.00%, respectively, significantly lower ($p<0.05$) than those of rats fed *M. indica* leaf extract (64.00±1.87% and 51.00±3.32% for high and low dose groups, respectively). Values of abnormal sperm cells were not significantly different ($p>0.05$) between the high dose and low dose groups of *C. citratus-fed rats, but the high dose of M. indica-fed* rats was significantly higher ($p<0.05$) than the low dose group. Values for pin-headed and tailless sperm cells were not significantly different ($p>0.05$) between the two plants (C. citratus and M. indica) and between the treatment groups (Figure 3).

## DISCUSSION

Daily administration of 150 and 300 mg/kg body weight of *C. citratus* and *M. indica* leaf extracts increased or reduced sperm count of male albino rats. As *C. citratus* extract significantly ($p<0.05$) boosted sperm count, *M. indica* extract significantly reduced ($p<0.05$) the sperm count with the lowest percentage recorded in the high dose group compared to the low dose. Potential changes in rat spermatogenesis and sperm parameters, resulting to significant decrease in sperm count, have been observed in rats treated with *Boswellia papyrifera* and *Boswellia carterii* incense (Ahmed *et al.,* 2013). Also, ethanolic extract of *Lagenaria breviflora* Roberts (the name of the botanist who first described the plant) has demonstrated a significant reductive effect on sperm count, indicating that the fruit extract can reduce or inhibit spermatogenesis (Saba *et al.,* 2008). The process of sperm formation and maturation has been affected in various ways by several herbal plants. This could cause delay or total disruption of reproduction (Ahmed *et al.,* 2013; Saba *et al.,* 2008). The alkaloid extract of Carica papaya seeds affects reproductive functions in male Wistar rats, leading to reduced sperm cell counts, sperm cell degeneration, and testicular cell lesions (Udoh *et al*., 2005). The higher alkaloid activity in M. indica extract could be responsible for the reduced sperm counts at low and high doses compared with those in *C. citratus* recorded in this study. Sperm count is an important index that accounts for a male's ability or inability to impregnate the female partner. Low sperm count is hindrance to conception, and herbal plants possess the ability to promote this condition.

The result of this study indicates that both extracts of *C. citratus* and *M. indica* significantly ($p<0.05$) affected the sperm motility of male albino rats. However, prominent effect was recorded in the rats group fed *M. indica* revealing that the plant is more effective in hindering sperm motility parameters. Moreover, the high dose group (300 mg/kg) gave the lowest percentage motility with the corresponding highest percentage of dead sperm cells indicating that the extract induced adverse effect on sperm motility of tested rats. On the other hand, *C. citratus* extract boosted sperm motility and reduced percentage of dead sperm cells indicating that the extract has a positive effect on sperm motility. Also, *M. indica* at the dose of 300mg/kg b. wt caused significant decrease ($p<0.05$) in sperm motility compared to the low dose (150mg/kg b.wt) and the *C. citratus* groups. On the other hand, the result revealed that *C. citratus* extract at a high dose (300 mg/kg body weight) increased total sperm motility and percentage sperm activity with a corresponding reduction in percentage of dead sperm cells revealing its efficacy in enhancing spermatogenesis compared to low dose and the *M. indica* groups. The presence of calcium could have promoted this sperm activity (motility) (Luconi *et al.,* 2006).

Sperms with abnormal morphology are shown to be non-motile or less motile; the abnormalities which according to Thomas and Thomas (2001) arise as a result of a fundamental case with the process of sperm maturation where abnormal sperm cells are matured from damaged seminiferous tubules. Nirmal *et al.* (2017) and Hasim *et al.* (2015) reported that *C. citratus* extract and *M. indica* extract, respectively, have an antioxidant effect, and testicular membranes are rich in lipid (fatty acids), which are prone to oxidative injury. Similarly, it has been considered that damage to the cell membrane (lipid peroxidation) may contribute to gonadal dysfunction (Emanuele & Emanuele, 2001). The testicular cell membrane is stabilised by vitamin A, which is responsible for reducing lipid. It could therefore be reasonable to accept that abnormal sperm cells were increased due to this effect in the high dose group, as the antioxidant effect of both plant extracts (C. citratus and M. indica) may be similar to that of vitamin A present (Cheel *et al.*, 2005; Nakamura *et al.*, 2003). According to Martinez *et al.* (2000), the extract of M. indica L. (Vimang) has shown effective scavenging activity against hydroxyl radicals and hypochlorous acid, acting as an iron chelator. According to Martinez et al. (2000), the extract of M. indica showed a significant inhibitory effect on the peroxidation of rat-brain phospholipids and on DNA damage induced by bleomycin or copper-phenanthroline systems. The ethanol extract of *C. citratus* leaves is potentiated as an antioxidant because it is inhibitory against free radical DPPH (2,2-diphenyl-1-picrylhydrazyl), and this activity is significant in spermatogenesis and sperm activity (Emanuele & Emanuele, 2001). This may be true of the chemical content of *C. citratus* extract (5.157 to 6.012 mg/kg) (WHO, 2004) and *M. indica* leaf extract (3.82 mg/100g) (Okwu & Ezenagu, 2008) that yielded high sperm motility. The results of this study are consistent with Ogedengbe *et al.* (2016). In the case of *C. citratus* extract, the increase in total and active sperm count with a

corresponding reduction in dead sperm may be attributed to the ability of *C. citratus* extract to mitigate testicular injury thereby re-vitalizing the cells while the reverse is the case of *M. indica* leaf extract. Khillare and Shrivastav (2003) reported a linear decrease in percentage motility, becoming zero at a 3-mg dose within 20 seconds following increase in concentration of the leaf extract. There is a clear indication therefore, that many African herbal plants are capable of causing fertility effect in animals.

In conclusion, both *C. citratus* and *M. indica* leaf extracts serve as empirical evidence to support the claims of their ability to influence fertility. However, *C. citratus* can boost sperm count while *M. indica* decreases sperm count. *C. citratus* enhances spermatogenesis while *M. indica* hinders spermatogenesis. These effects were dose-dependent.

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