

Original Research Article

Effects of Ethanol Extract of Tigernuts and Date Palm Fruits on Sperm Parameters Following Treatment with Doxorubicin

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Article History

Received: 12.06.2025

Accepted: 16.08.2025

Published: 15.11.2025

Journal homepage:

<https://www.easpublisher.com>

Quick Response Code



Abstract: Doxorubicin (DOX) is a potent chemotherapeutic agent widely used for treating various cancers, but is known for its detrimental side effects, including reproductive toxicity in males. The aim of this study to the effects of hydroethanol extracts of tiger nuts (*Cyperus esculentus*) and date palm (*Phoenix dactylifera*) against Doxorubicin (DOX) induced reproductive damage in male Wistar rats. A total of 35 rats were divided into seven groups with 5 rats per group and subjected to DOX-induced reproductive toxicity (15 mg/kg for three days), followed by administration of different doses of the plant extracts, for 27 days. On the 31st day animals were sacrificed and samples collected for the Biochemical analysis of reproductive hormone levels (FSH, LH, testosterone), sperm quality, and testicular histology. The DOX-only group showed significant reductions in sperm count, motility, hormone levels, and antioxidant status, with increased lipid peroxidation and abnormal testicular histology. Conversely, groups treated with tiger nuts and date palm extracts, particularly at high doses and in combination, demonstrated marked improvements in these parameters. Co-administration significantly restored hormone levels, enhanced antioxidant enzyme activity, improved sperm morphology and count, and reversed DOX-induced histopathological alterations. The findings confirm that tiger nuts and date palm possess strong antioxidant and fertility-enhancing properties capable of mitigating DOX-induced reproductive toxicity.

Keywords: Effects, Tiger Nuts, Date Palm, Sperm Parameters, Doxorubicin.

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INTRODUCTION

The inability to become pregnant after a significant duration of sexual activity without the use of contraception is known as infertility resulting from changes in reproductive factors (Santiago, *et al.*, 2000). According to the report by World Health Organization (WHO) (2003) the annual number of infertile couples globally was found to be 50-80 million. Infertility can have a feminine or masculine origin, with the male factor only present in one third of cases (Babakhanzadeh *et al.*, 2020). A multitude of causes and risk factors contribute to the increasing incidence of male infertility (Salonia *et al.*, 2021) which can be stratified as congenital, acquired, and idiopathic. Environmental or occupational exposure to toxic chemicals (Ma *et al.*, 2019) and various lifestyle factors eg, smoking (Taha *et al.*, 2012; Sharma *et al.*, 2016), alcohol consumption (Ricci *et al.*, 2017) recreational drug use (Gundersen *et al.*, 2015) obesity (Eisenberg *et al.*, 2013) and psychological stress (Nargund, 2015) are all potential risk factors for male infertility (Durairajanayagam, 2018). Doxorubicin

(DOX) is a widely used anthracycline antibiotic in chemotherapy due to its effectiveness against various cancers and its clinical application is often limited by its adverse effects, including reproductive toxicity. Studies have demonstrated that DOX induces oxidative stress, DNA damage, and apoptosis in testicular tissue, ultimately impairing spermatogenesis and hormonal balance in male reproductive systems (Gautam *et al.*, 2006; Zhang *et al.*, 2010). Doxorubicin impairs sperm motility, and strikingly impedes the process of spermatogenesis, leading to induction of testicular failure and eventually infertility (Hou, *et al.*, 2005; Mohan, *et al.*, 2021). Doxorubicin is an anthracycline chemotherapeutic drug that cures several types of cancers, including breast cancer. A young female who has breast cancer might often face a problem of infertility and premature oocyte after the treatment of chemotherapeutic drugs. In addition to this, they also induce female reproductive toxicity characterized as a premature ovarian failure (POF). Doxorubicin directly affects the follicles and oocytes. It causes some

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genotoxic effects (Mohan, *et al.*, 2021). Previous study revealed that the volume of sperm reduces significantly in the group administered with 400mg of monosodium glutamate. This reduction in the volume of sperm could be due to substance found in MSG (Gbaranor, *et al.*, 2024). Erection is aid by nitric oxide (NO) and its binds with the heme iron guanylate cyclase and activates the enzyme, guanylate cyclase. Guanylate cyclase converts GTP into cGMP which closes C2⁺ channels (Gbaranor, *et al.*, 2024a). Also, Gbaranor, *et al.*, (2024) revealed that smoothies increase the level of nitric oxide significantly. Previous study by Gbaranor, *et al.*, (2021) revealed that, most people in the rural areas and some in the urban areas depends on herbal medicine each time they have medical issues. WHO (2007), whose report, revealed that 60% of the world's populations depend on herbal and traditional medicine, and 85% of the world's developing countries use traditional medicine in caring for diseases (Shuaib, M. *et al.*, 2023). African culture cherished traditional medicine and is an important component of African Traditional Religion (ATR) (Gbaranor, *et al.*, 2024b). Traditional medicine has been used across the globe and it has been seen to be cheap, effective, available and affordable in our society. Tradition medicine has become major source of income to the traditionalists due to the fact that majority of the populace now depends on it for treatment of various illness (Gbaranor, *et al.*, 2024b). Date palm and Tiger nuts may improves sperm count or increases sperm concentration, sperm motility, sperm morphology and other reproductive parameters due to the phytochemical contents. There are reports suggesting the ameliorating effect of various plant extracts and other bioactive compounds on the testicular toxicity induced by Adriamycin (doxorubicin). In view of the aforementioned, this study set out to evaluate the effect of ethanol Extract of Tigernuts and Date palm on sperm parameters in Wistar rats as animal model.

MATERIALS AND METHODS

Collection, Identification and Authentication

Fresh fruits of tiger nuts and date palm was obtained from mile one market, Port Harcourt in August, 2024. The plant was identified and authenticated at Plant Science and Bio-technology department Rivers State University by a person, with Herbarium Number: **RSUPbH01**

Plant Extraction

The extraction was done using the maceration method. Tiger nut fruits and Date palm fruit were purchased and was air dried, it was then coarsely grinded and weighed in to a maceration jar and was soaked with Ethanol. The soaked plant fruit were allowed to stand for three days (72 hrs) with continuous stirring, every morning using a stirring rod. Thereafter, the macerated fruits were filtered to remove the shaft and re filtered for three times using a cellulose paper (whatman No 1) filter paper. To ensure a clear menstruum. The menstruum was mounted on the water bath at a temperature of 50 degree

Celsius in other not to denature the extract, the extract yield was then stored for the experimental purpose.

Acute Toxicity Test (LD₅₀ Determination)

LD₅₀ of the extract were determined using the method described by Lorke (1983). LD₅₀ was determined to be 5000 mg/kg/bwt. The extract's various dosages were then calculated, using 10% for low and 40% for high, respectively. As a result, the dosage was established, with 500mg/kg/bwt being the low dose and 2000 mg/kg/bwt being the high dose. This was done separately for tiger nuts and date palm respectively.

Experimental Animal

A total of 35 males Wistar rats weighing 120-140g were used for the study. Male rats were sourced from animal house, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria. The animals were kept in cages and maintained at their natural condition. The animals were kept clean in a disinfected cage with saw dust as their beddings in animal house and were allowed to acclimatized for two weeks in their new environment, with free access to food and water.

Experimental Design

Thirty-five (35) male rats were used for this study. They were randomly selected and group into 7 groups with 5 rats per group. The animals were weighed before commencement of administration and after administration.

Doxorubicin 15mg/kg/bw was administered into all the groups except control for 3days to induced reproductive toxicity. Thereafter, on the 4th day, hydro-ethanol extracts of both tiger nuts and date palm were administered to evaluate the ameliorative effects of the fruit's extracts on the doxorubicin induced reproductive toxicity. Induction of reproductive toxicity through doxorubicin was done by an established method by Asiwe *et al.*, (2024) and Jaiyeoba-Ojigho, *et al.*, (2025) respectively.

Group 1 received 1ml of distilled water plus feeds 30 days

Group 2 received 15mg/kg/bw doxorubicin plus feeds for 3days

Group 3 received 15mg/kg/bw doxorubicin for 3 days and low dose (500mg/kg/bw) of tiger nuts for 27 days plus feeds

Group 4 received 15mg/kg/bw doxorubicin for 3 days and low dose (500mg/kg/bw) of date palm for 27 days plus feeds

Group 5 received 15mg/kg/bw doxorubicin for 3 days and high dose (2000mg/kg/bw) of tiger nuts for 27 days plus feeds

Group 6 received 15mg/kg/bw doxorubicin for 3 days and high dose (2000mg/kg/bw) of date palm for 27 days plus feeds

Group 7 received 15mg/kg/bw doxorubicin for 3 days + high dose (20000mg/kg/bw) of tiger nuts + high dose (20000mg/kg/bw) of date palm for 27 days plus feeds. The study lasted for 30 days and on the 31st day, the animals were sacrificed after being anaesthetized and samples tissues and blood were collected for biochemical analysis.

15 mg/kg of doxorubicin, was used as documented by Jaiyeoba-Ojigbo, *et al.*, (2025)

Samples Collection

Treatment of animals was done for 30 days. On the 31st day, animals were sacrificed. Each animal was anaesthetized in desiccator with cotton wool soaked in about 10ml chloroform and the needed tissues and organs were removed: Blood, and left testes, were collected and weighed immediately with sensitive weighing balance. After weighing, testes, was stored in formalin for Histology examination.

Sperm Analysis: Sperm analysis was done by Chehrei, *et al.*, 2017 method.

Ethical Considerations

Ethical Approval was gotten from the Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University Research Ethics Committee.

Statistical Analysis

Statistical analysis was performed on data sourced from this experiment using mean $\pm$ SEM and statistical analysis were done using ANOVA. The multiple comparison test was equally done when obtained disparity were significant statistically using p less than 0.05. SPSS version 23 was used.

RESULTS

The study showed the effects of tiger nut and date palm on the progressive semen motility percentage. The outcome of our finding showed that the doxorubicin, low dose of tiger nut, date palm and high dose of date palm group significantly $p < 0.05$ decreased when compared to the control group, While the high dose of tiger nut and the co administered group of tiger nut and date palm showed no significant difference. Also

there was a significant $p < 0.05$ increase on the low and high dose of tiger nut, high dose of date palm (Fig. 1). Revealed the effects of tiger nut and date palm on the non progressive semen motility percentage. The result revealed that the doxorubicin, low dose of date palm, high dose of tiger nut and date palm significantly $p < 0.05$ increased when compared to the control group, While the low dose tigernut, high dose of tiger nut, date palm and co-administered group significantly decreased significantly $p < 0.05$ decreased when compared to the doxo rubicin group (Figure 2). The sperm count as seen in figure 3 above, showed a significant $p < 0.05$ decrease in the doxorubicine group, low dose of date palm and tigernut, and high dose of date palm when compared to the control group, Whereas the high dose of tiger nut, and co-administered group of tiger nut and date palm significantly increased significantly $p < 0.05$ decreased when compared to the doxo rubicin group. Results showed the result for the normal morphology of the semen following induction of infertility and treatment with varying dose of tigernut and date palm the result showed a significant $p < 0.05$ decrease in the doxorubicine group, low dose of tiger nut, and date palm when compared to the control group, However the low dose of tiger nut, high dose of date palm, tiger nut and co-administered group of tiger nut and date palm significantly increased significantly $p < 0.05$ decreased when compared to the doxo rubicin group (Figure 4). Results showed the result for the abnormal morphology of the semen following induction of infertility and treatment with varying dose of tigernut and date palm the result showed a significant $p < 0.05$ increase in all the treated groups except the co-administered group when compared to the control group, However the low dose of tiger nut, high dose of, tiger nut and co-administered group of tiger nut and date palm significantly increased significantly $p < 0.05$ decreased when compared to the doxorubicin group (Figure 5). Also, the results showed the result for sperm volume of the semen following induction of infertility and treatment with varying dose of tigernut and date palm the result showed a significant $p < 0.05$ decrease in the group treated s except the co-administered group doxorubicin when compared to the control group, However the other treated groups significantly increased $p < 0.05$ when doxorubicin group.

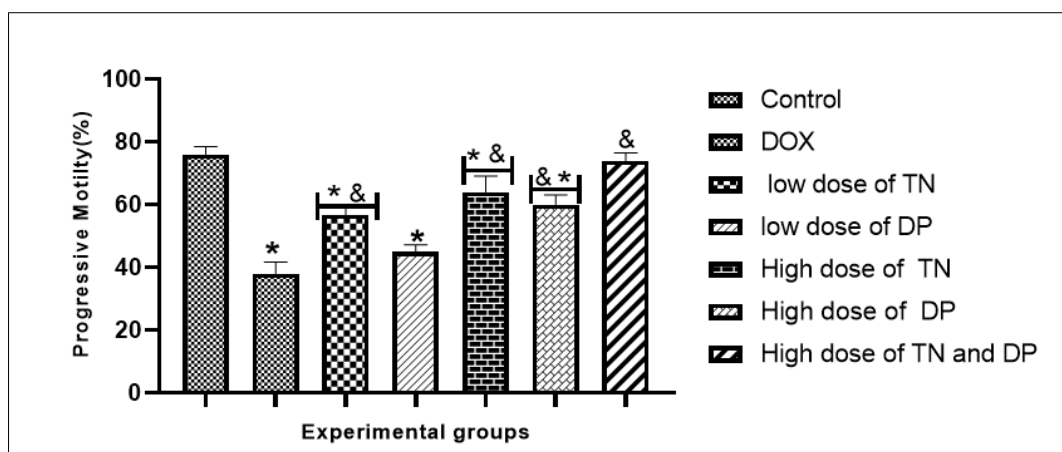


Figure 1: Shows the result of one month administration of Tiger nut and Date palm on progressive sperm motility in male wistar rat following doxorubicin induced fertility toxicity and treatment with high and low doses of tiger nut date palm. Data are expressed as Mean $\pm$ SEM, $p < 0.05$: *significant compared to control and &when compared to doxorubicin group

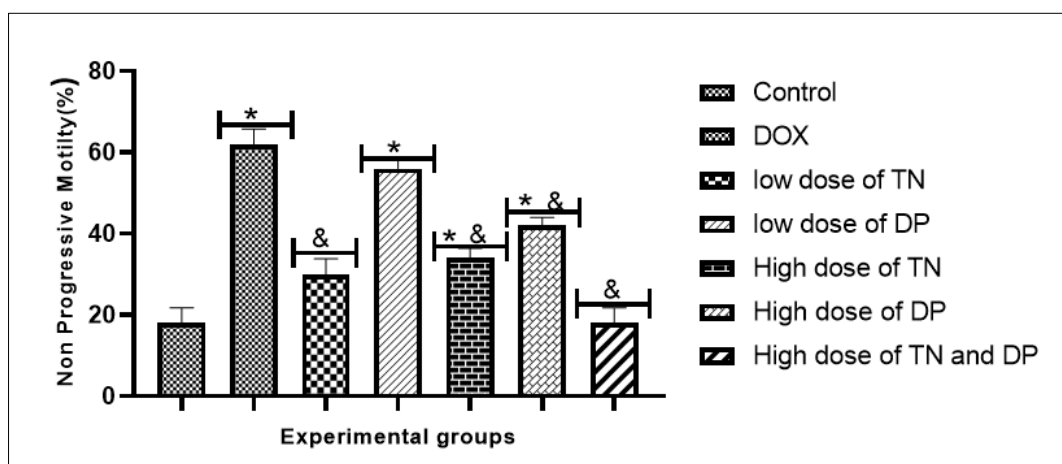


Figure 2: Shows the result of one month administration of Tiger nut and Date palm on non progressive sperm motility in male wistar rat following doxorubicin induced fertility toxicity and treatment with high and low doses of tiger nut date palm. Data are expressed as Mean $\pm$ SEM, $p < 0.05$: *significant compared to control and &when compared to doxorubicin group

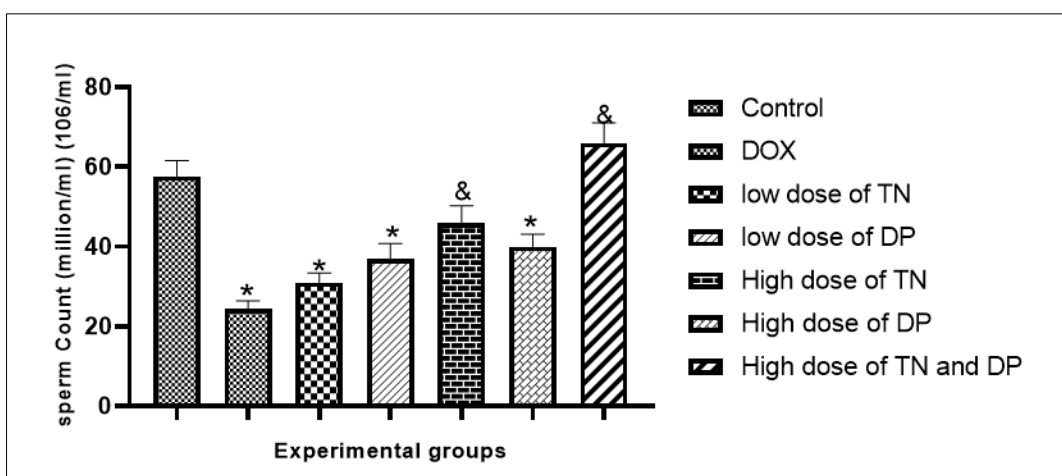


Figure 3: Shows the result of one month administration of Tiger nut and Date palm on sperm count in male wistar rat following doxorubicin induced fertility toxicity and treatment with high and low doses of tiger nut date palm. Data are expressed as Mean $\pm$ SEM, $p < 0.05$: *significant compared to control and &when compared to doxorubicin group

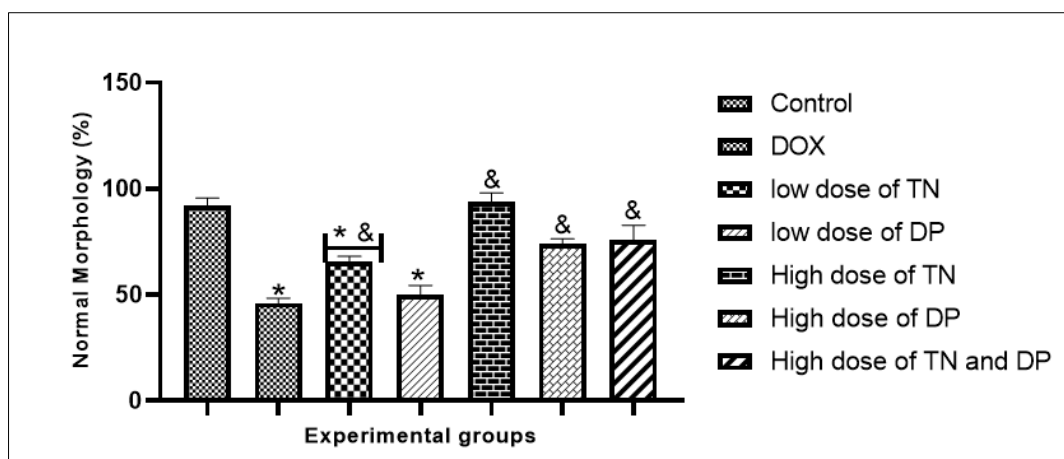


Figure 4: Shows the result of one month administration of Tiger nut and Date palm on normal morphology in male wistar rat following doxorubicin induced fertility toxicity and treatment with high and low doses of tiger nut date palm. Data are expressed as Mean $\pm$ SEM, $p < 0.05$ *: significant compared to control and &when compared to doxorubicin group

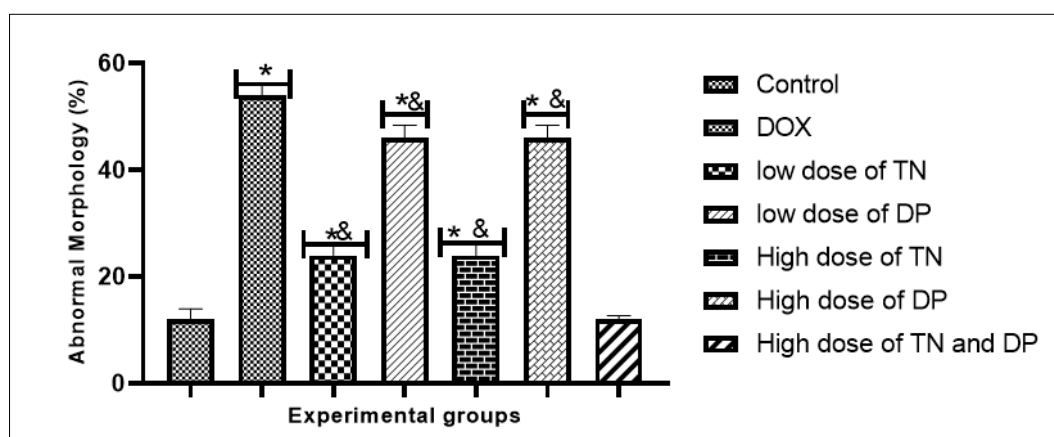


Figure 5: Shows the result of one month administration of Tiger nut and Date palm on normal morphology in male wistar rat following doxorubicin induced fertility toxicity and treatment with high and low doses of tiger nut date palm. Data are expressed as Mean $\pm$ SEM, $p < 0.05$ *: significant compared to control and &when compared to doxorubicin group

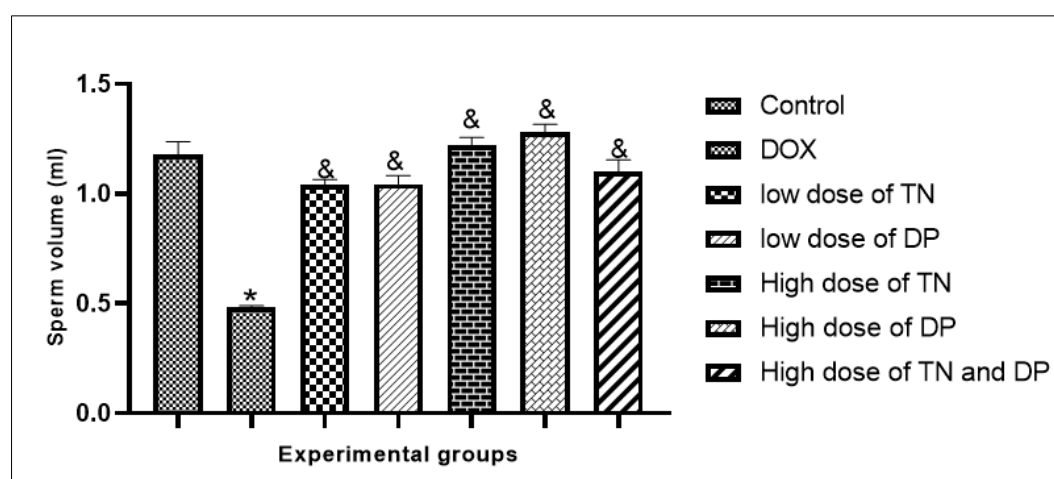


Figure 6: Shows the result of one month administration of Tiger nut and Date palm on sperm parameters in male wistar rat following doxorubicin induced fertility toxicity and treatment with high and low doses of tiger nut date palm. Data are expressed as Mean $\pm$ SEM, $p < 0.05$ *: significant compared to control and &when compared to doxorubicin group

DISCUSSION

The study revealed significant decreased in the progressive motility of sperm in the groups treated with doxorubicin, low dose of tiger nuts, low dose date palm, high dose of tiger nuts and high dose of date palm when compared with the control group. This decrease could be due to the bioactive substance that are found in the fruit extracts of tiger nuts and date palm. The decrease in the levels of sperm progressive motility in the doxorubicin treated group is higher than other fruits extract treated groups and this could be due to the toxic nature of doxorubicin to sperm parameters and thus may affect fertility in male. Again, this decreased in the levels of sperm progressive motility is in consonant with previous studies that doxorubicin lowers sperm motility, count, viability, and functional maturity while also decreasing semen volume (Ijaz, *et al*, 2024; Babalola, *et al.*, 2023). However, the levels of progressive motility of sperm in the low dose of tiger nuts, high dose of tiger nuts, high dose of date palm and in the co-administered high dose of tiger nuts and date palm treated groups is significantly increased when compared with the doxorubicin trated group. This increase in the progressive motility of sperm shows that both tiger nuts and date palm contain phytochemicals that could improve fertility by enhancing the progressive motility of sperm. Again, in the co-administered high dose of tiger nuts and date palm treated groups, the levels of sperm progressive motility is neither increase nor decrease significantly when compared with the control and this may be due to time or dose dependent.

In the groups treated with doxorubicin, low dose of date palm, high dose of tiger nuts, high dose of tiger nuts and high dose of date palm have significantly increase in the levels of the number of non-progressive motility of sperm when compared with control. However, the levels of increase in the non-progressive motility of sperm in the group treated with doxorubicin is higher than other groups treated with tiger nuts and date palm respectively. This may be due to mitochondrial damage and oxidative stress thus, impairing the energy production required for sperm movement. In the groups treated with low dose of tiger nuts, high dose of tiger nuts, high dose of date palm and the co-administered high dose of tiger nuts and date palm groups have significantly decrease in the levels of non-progressive motility of sperm when compared with the group treated with doxorubicin and this decrease in non-progressive sperm motility could be due to bioactive substance found in both date palm and tiger nuts that may cause the reduction in the non-progressive motility of sperm. However, the levels of non-progressive motility of sperm in the treated groups: low dose of tiger nuts, high dose of tiger nuts, high dose of date palm and the co-administered high dose of tiger nuts and high dose of date palm is significantly lower when compared with the group treated with doxorubicin and this may suggests that the fruits tiger nuts and date palm contain natural bioactive substance that may decrease the non-

progressive motility of sperm and thus enhance fertility in male.

The sperm count from this study, showed a significant $p < 0.05$ decrease in the doxorubicin group, low dose of date palm and tigernut, and high dose of date palm when compared with the control group. The decreased in sperm count levels in the doxorubicin treated gruoup is higher than other treated groups with tiger nuts and date palm and this could be due to the toxic nature of the anti-cancer drug on sperm cells and could lead to infertility. Again, it could be that doxorubicin caused damage to spermatogenic cells (the stem cells responsible for sperm production). This agreed with previous study that revealed that, the organs known to be vulnerable to doxorubicin toxicity is male germ cells (Mohan, *et al.*, 2021; Zi, *et al.*, 2021). In the group treated with high dose of tiger nuts and in the group co-administered with high dose of tiger nuts and high dose of date palm shows significant increase in the levels of sperm count when compared with the group treated with doxorubicin. This shows that administration of tiger nuts and date palm may preserve testicular tissues against doxorubicin toxicity. Again, tiger nuts is rich in arginine, which improves blood flow and supports testicular function and date palm increases sperm count due to flavonoids and phenolic compounds that protect against testicular damage and boost spermatogenesis.

The study revealed significant decrease in the normal morphology of sperm in the doxorubicin treated group and in the groups treated with low dose of tiger nuts and low dose of date palm when compared with the control group. Again, there is greater reduction in number of sperm with normal morphology in the doxorubicin treated group than when treated with tiger nuts and date palm. However, there is significant increase in the normal morphology of sperm in the low dose of tiger nuts, high dose of tiger nuts, high dose of date palm and in the co-administration of high dose of tiger nuts and high dose of date palm when compared with the doxorubicin treated group and this decrease in the normal morphology of sperm could be due to exposure to doxorubicin agreed with previous study by Mustafa *et al.*, (2022).

There was high number of abnormal morphology of sperm in the doxorubicin treated group when compared with the control group and other treated groups with tiger nuts and date palm. This abnormal morphology could be abnormalities in the sperm head, midpiece and tail malformation and this could arise from the toxic nature of the drug doxorubicin that caused damage to sperm DNA. Also, the number of abnormal morphology of sperm significantly increased in the groups treated with low dose of tiger nuts, low dose of date palm, high dose of tiger nuts and high dose of date palm when compared with control group and significantly decrease when compared with the doxorubicin treated group. The study also revealed that

there is reduction in the number of sperm cells with abnormal morphology in the groups treated with low dose of tiger nuts, low dose of date palm, high dose of tiger nuts and high dose of date palm when compared with doxorubicin treated group. This shows that doxorubicin induces reproductive toxicity in the exposed rats and the toxicity was ameliorated with treatment with both tiger nuts and date palm fruits extract. This ameliorative effect offered by tiger nuts and date palm may be due to the bioactive substance found in the fruits. There was neither increase nor decrease in the group treated with high dose of both tiger nuts and date palm when compared with the doxorubicin treated group.

Following induction of infertility with doxorubicin and treatment with varying dose of tigernuts and date palm the result showed a significant decrease in sperm volume in the doxorubicin treated group when compared with the control group. The study also revealed significantly increase in sperm volume in the groups treated with low dose of tiger nuts and date palm, high dose of tiger nuts and date palm and the co-administered group of tiger and date palm. This increase in sperm volume when treated with tiger nuts and date palm is due to their antioxidant, hormonal and nutritional properties of tiger nuts and date palm. Tiger nuts increase seminal plasma through enhanced function of accessory sex glands and date palm stimulates androgenic activity, leading to better glandular secretions and increased semen volume.

CONCLUSION

This study revealed that doxorubicin an anti-cancer drug contain agents that are inimical to the reproductive parameters thus may affect reproductive parameters. This study also, revealed the ameliorative effects of both tiger nuts and date palm fruits extract on doxorubicin induced reproductive toxicity in males Wistar rats. This fruits extracts contained bioactive substance that may function to improve sperm parameters and thus, may enhance fertility. Date palm and Tiger nuts may improves sperm count or increases sperm concentration, sperm motility, sperm morphology and other reproductive parameters due to dose dependents and also due to the phytochemical contents of both fruits extract.

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Cite This Article: Gbaranor K. B & Mgbe M. E (2025). Effects of Ethanol Extract of Tigernuts and Date Palm Fruits on Sperm Parameters Following Treatment with Doxorubicin. *EAS J Pharm Pharmacol*, 7(6), 101-108.
