International Journal of Research and Reports in Gynaecology

4(3): 53-63, 2021; Article no.IJRRGY.75526

Consumption of Potash Adversely affects Sperm Quality and Sex Hormones of Male Wistar Rats

Augustine I. Airaodion[1*], Funmilola C. Oladele[2], Simeon O. Oluba[3], Ayodeji A. Adedeji[4], Aanu P. Agunbiade[1], Anthony U. Megwas[5], Emmanuel B. Ayita[6] and Ojo J. Osunmuyiwa[7]

[1]Department of Biochemistry, Federal University of Technology, Owerri, Imo State, Nigeria.
[2]Department of Medical Biochemistry, Ekiti State University, Ado-Ekiti, Nigeria.
[3]Department of Medical Laboratory Service, Clinical Biochemistry Unit, Inland Medical Centre, Ikare-Akoko, Ondo State, Nigeria.
[4]Department of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria.
[5]Department of Optometry, Federal University of Technology, Owerri, Imo State, Nigeria.
[6]Department of Biochemistry, Federal University, Oye-Ekiti, Ekiti State, Nigeria.
[7]Department of Biomedical Science and Public Health Technology, Margaret Mosunmola College of Health Science and Technology, Owo, Ondo State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Abdelmonem Awad M. Hegazy, Zagazig University, Egypt.
Reviewers:
(1) Dra. Patricia E. Marini, Universidad Nacional de Rosario, Argentina.
(2) Sava Micic, University Belgrade, Serbia.
Complete Peer review History: https://www.sdiarticle4.com/review-history/75526

Original Research Article

Received 10 August 2021
Accepted 20 October 2021
Published 12 November 2021

ABSTRACT

Background: Potash consumption is very common in Nigeria especially in the rural communities. It is often used as food additives in different doses without any regard for its toxicity.
Aim: This study sought to evaluate the effect of potash on the sperm quality and reproductive hormones in male Wistar rats.
Methodology: Thirty male Wistar rats were divided into five groups of six each. Animals in group A were administered distilled water while those in groups B, C, D and E were administered 250, 500, 750 and 1000 mg/kg body weight of potash for 28-days via oral route of administration. At the end

**Corresponding author: E-mail: augustineairaodion@yahoo.com;*

of the treatment period, blood samples were collected *via* cardiac puncture. Sperm quality and sex hormones were evaluated using standard methods.
Results: The results of this present study showed a significant ($p<0.05$) increase in the concentration of serum follicle stimulating hormone (FSH) at 500, 750 and 1000 mg/kg in a dose-dependent manner when compared with the control group. Administration of potash significantly reduced ($p<0.05$) the concentration of serum Luteinizing hormone (LH) in all the treatment groups when compared with the control group. There was a significant reduction ($p<0.05$) in the concentration of serum testosterone and seminal pH sequel to the administration of potash at 500, 750 and 1000 mg/kg body weight when compared with the control group. Administration of potash significantly reduced ($p<0.05$) the sperm count, motility and morphology of healthy male Wistar rats in all the treatment groups in a dose-dependent manner when compared with the control group.
Conclusion: The results of this study showed that potash has the propensity to compromise sperm quality and sex hormones in male Wistar rats. This does not automatically translate to such effect on humans. It is recommended to be studied in future studies before being investigated in clinical trials.

Keywords: Antifertility potential; potash; sex hormones; sperm quality.

1. INTRODUCTION

Potash is any of various mined and manufactured salts that contain potassium in water-soluble form, the name derived from pot ash, refers to plant ashes soaked in water in a pot, the primary means of manufacturing the product before the industrial era [1]. It is produced worldwide at amounts exceeding 30 million tonnes per year, mostly for use in fertilizers. Various types of fertilizer-potash constitute the single largest global industrial use of the element potassium. Potassium was first derived by electrolysis of caustic potash (aka potassium hydroxide), in 1807 [2]. The old method of making potassium carbonate (K_2CO_3) was by collecting or producing wood ash (an occupation carried out by ash burners), leaching the ashes and then evaporating the resulting solution in large iron pots, leaving a white residue called pot ash [3]. Approximately 10% by weight of common wood ash can be recovered as pot ash. Later, potash became the term widely applied to naturally occurring potassium salts and the commercial product derived from them [4]. Locally known as “kaun” or “Akanwu”, Potash is used commonly for culinary purposes. It is used for cooking pulses like beans, akidi (black Mexican beans), fiofio (cowpea beans etc. in order to tenderize the pulsesso easily [5]. “Kaun” is also added in ewedu and okro soup (a Nigerian delicacy) during preparations in order to increase the greenness and texture of the vegetables [6]. No data exist about the quantity or dosage of potash consumed in the average daily meal of Nigerians. Airaodion et al [7] observed that potash is hepatotoxic. Its nephrotoxicity has also been reported [8].

Infertility is defined as the inability to achieve pregnancy after 12 months of unprotected intercourse [9]. Male infertility is found in 50% of infertile couples [10]. According to Speroff and Fritz [11], 55% of the reasons for infertility are found to be male-related and 35% to be female-related, while 10% constitutes infertility of unknown origin [11]. Some of the etiologies of decreasing male fertility can be related to declining androgen levels, reduced sexual activity, perturbations in sperm quality, especially, motility, morphology, and DNA integrity [12]. Gonadotropin releasing hormone (GnRH) secreted by the hypothalamus enhances the release of gonadotropins i.e. follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland [13]. LH is a glycoprotein that regulates testosterone synthesis by the extratubular Leydig cells. The other gonadotropic hormone, FSH controls spermiocytogenesis and spermiogenesis by regulating both the germinal epithelium and Sertoli cells [14]. The concentrations of these hormones are under negative feedback regulation by the gonads [15]. Testosterone is responsible for normal growth, development of male sex organs, and maintenance of secondary sex characteristics. An elevated intratesticular concentration of Testosterone is an indication of sperm production, and function. Testosterone boosts motility of sperm and epididymis function [16]. Inability of the pituitary gland to secrete FSH and LH will result in disruption of testicular function leading to infertility [17].

Semen is an organic fluid that contains spermatozoa. It is secreted by the gonads and other accessory sex organs of male, and can

fertilize female ova. In humans, semen is composed of several components besides spermatozoa: proteolytic and other enzymes as well as fructose which is the major energy source of spermatozoa, and provide a medium through which they can move or "swim" [18]. Male infertility can be evaluated by the analysis of semen and hormonal profile [19]. This study sought to evaluate the effect of potash on the sperm quality and reproductive hormones in Wistar rats.

2. MATERIALS AND METHODS

2.1 Experimental Design

Potash was locally sourced in a market in Owerri, Imo State, Nigeria and was carefully preserved to avoid contamination. Thirty (30) healthy male Wistar rats *(Rattus norvegicus)* weighing between 145 and 160 g were used for the experiment. They were acclimatized for seven (7) days during which they were fed *ad libitum* with standard feed and drinking water and were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health [20]. They were randomly divided into five (5) groups of six (6) rats each. Animals in group A were administered distilled water while those in groups B, C, D and E were administered 250, 500, 750 and 1000 mg/kg body weight of potash for twenty-eight (28) days via oral route of administration. At the end of 28 days of treatment, animals were anaesthetized using diethyl ether and were sacrificed and blood samples were collected *via* cardiac puncture.

2.2 Determination of Sperm Quality

The cauda epididymis were separated from both testes and tinged with 2 mL of normal saline then teased the cauda epididymis of each rat. The suspension was mixed through a metallic net to avoid any other tissue contamination. Sperms counts were done with the aid of hemocytometer according to the method of Eliasson [21]. Motility of spermatozoa was determined according to the methods of Tijee and Oentoeng [22]. Sperm abnormality and seminal pH were determined according to the method of Airaodion et al. [23]

2.3 Determination of Male Reproductive Hormones

The serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were assayed using Enzyme-Linked Immunosorbent Assay (ELISA) according to the methods described in Manafa et al. [24].

2.4 Statistical Analysis

The data collected were analyzed with one way analysis of variance (ANOVA) using SPSS software version 25 and were expressed as Mean±SEM. Duncan's multiple ranges was used to separate the means. Differences were considered to be statistically significant when ($p<0.05$). Graph pad prism software version 8.0.2 was used to plot the graph.

3. RESULTS

The results of this present study were presented in mean ±SEM with n = 6. This showed that administration of potash to 28healthy male Wistar Rats significantly increased ($p<0.05$) the concentration of serum follicle stimulating hormone (FSH) (Fig. 1) at 500, 750 and 1000 mg/kg body weight in a dose-dependent manner when compared with the control group. The increase was more significant in 1000 mg/kg body weight, with no significant different at 250 mg/kg body weight and the control group. Administration of potash significantly reduced ($p<0.05$) the concentration of serum Luteinizing hormone (LH) (Fig. 2) in all the treatment groups (250, 500, 750 and 1000 mg/kg body weight) when compared with the control group. The reduction was more significant at both 750 and 1000 mg/kg body weight which are statistically similar to each other. There was significant reduction ($p<0.05$) in the concentration of serum testosterone (Fig. 3) and seminal pH (Fig. 8) after administration of potash at 500, 750 and 1000 mg/kg body weight when compared with the control group. The reduction was more significant at 1000 mg/kg body weight, with no significant different at 250 mg/kg body weight. Administration of potash significantly reduced ($p<0.05$) the sperm count (Fig. 4), sperm motility (Fig. 5) and sperm morphology (Fig. 7) of healthy male Wistar rat in all the treatment groups (250, 500, 750 and 1000 mg/kg body weight) in a dose-dependent manner when compared with the control group. The reduction was more significant at 1000 mg/kg body weight.

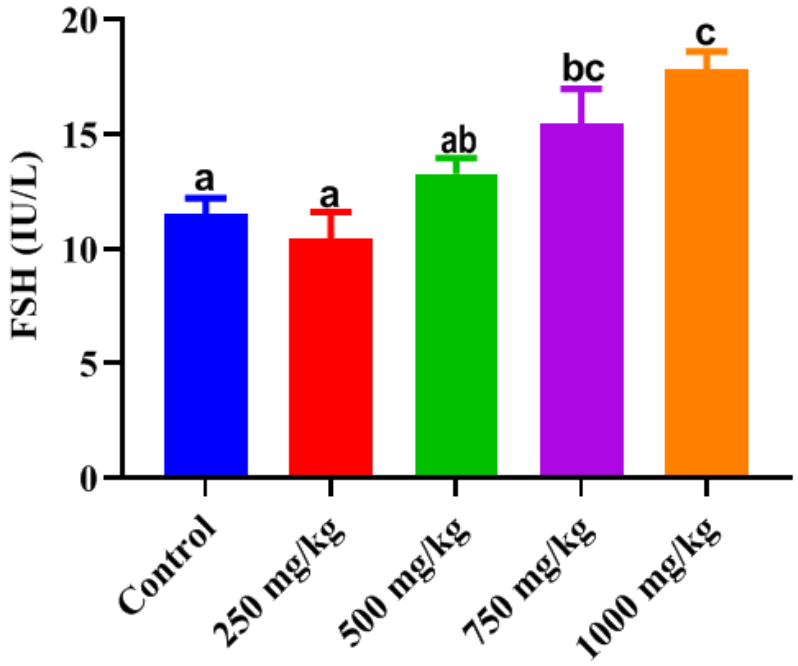

Fig. 1. The effect of potash on the Concentration of Follicle stimulating hormone (FSH) in healthy male Wistar rat. Values with the same superscripts do not differ significantly from each other whereas values carrying different superscripts are significantly different from each other ($p<0.05$)

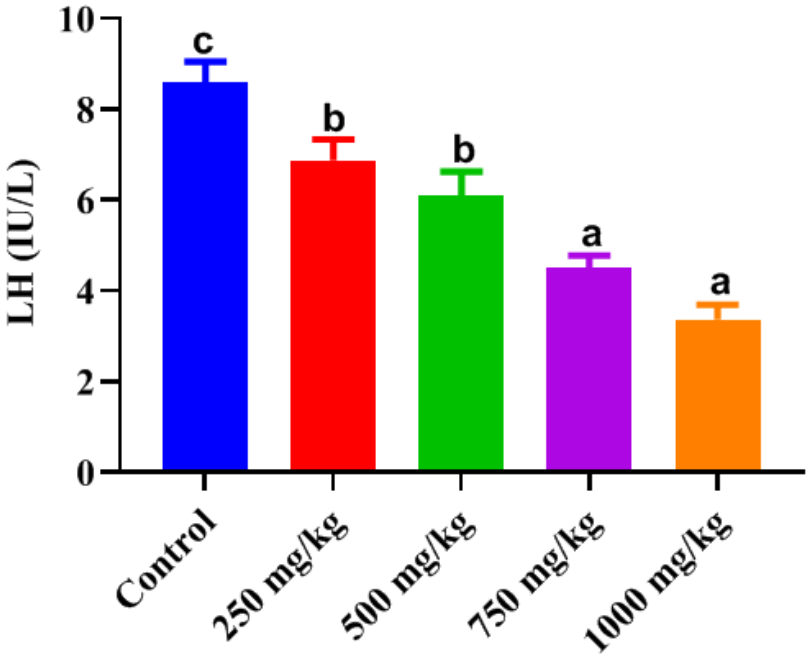

Fig. 2. The effect of potash on the Concentration of Luteinizing hormone (LH) in healthy male Wistar rat. Values with the same superscripts do not differ significantly from each other whereas values carrying different superscripts are significantly different from each other ($p<0.05$)

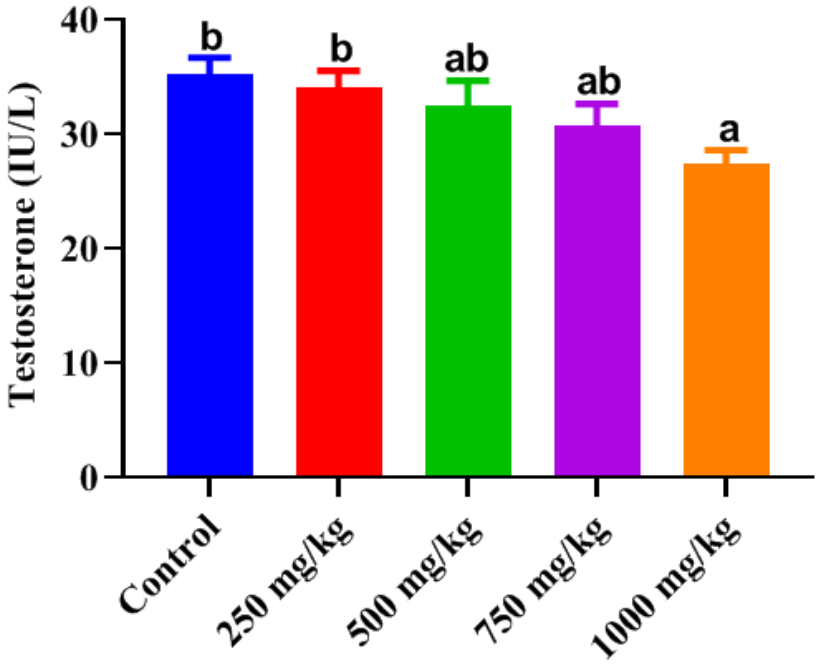

Fig. 3. The effect of potash on the Concentration of Testosterone in healthy male Wistar rat. Values with the same superscripts do not differ significantly from each other whereas values carrying different superscripts are significantly different from each other (p<0.05)

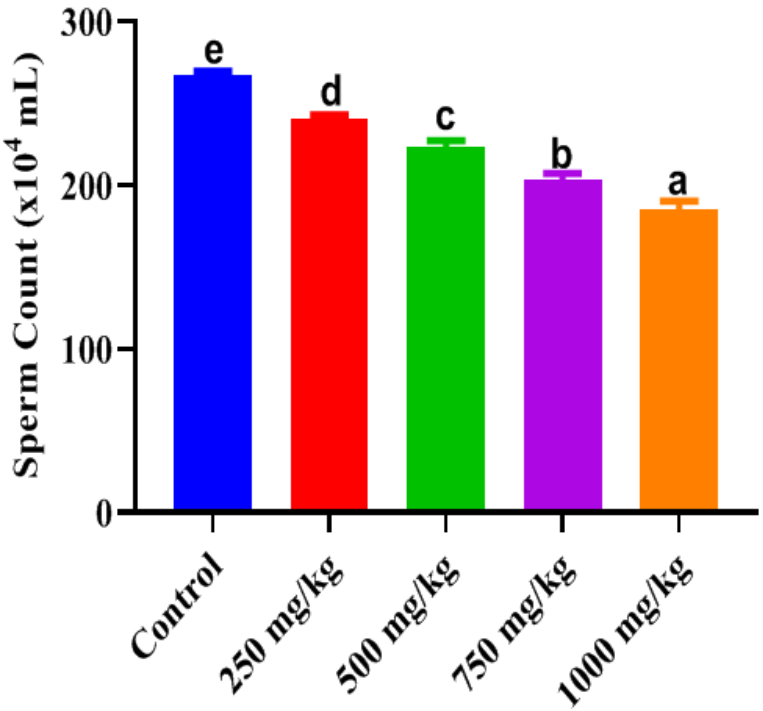

Fig. 4. The effect of potash on sperm counts in healthy male Wistar rat. Values with the same superscripts do not differ significantly from each other whereas values carrying different superscripts are significantly different from each other (p<0.05)

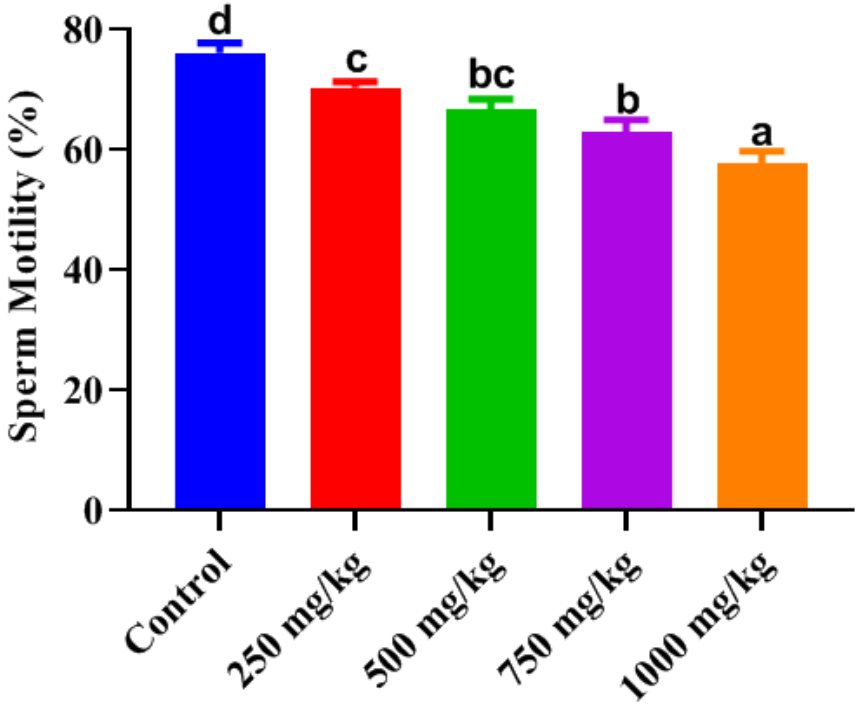

Fig. 5. The effect of potash on sperm motility in healthy male Wistar rat. Values with the same superscripts do not differ significantly from each other whereas values carrying different superscripts are significantly different from each other ($p<0.05$)

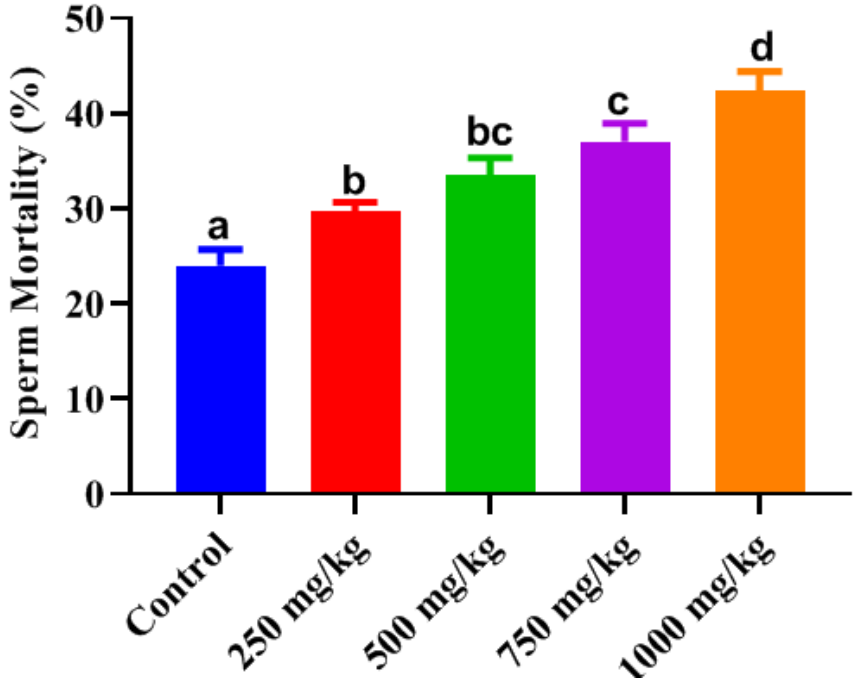

Fig. 6. The effect of potash on sperm vitality in healthy male Wistar rat. Values with the same superscripts do not differ significantly from each other whereas values carrying different superscripts are significantly different from each other ($p<0.05$)

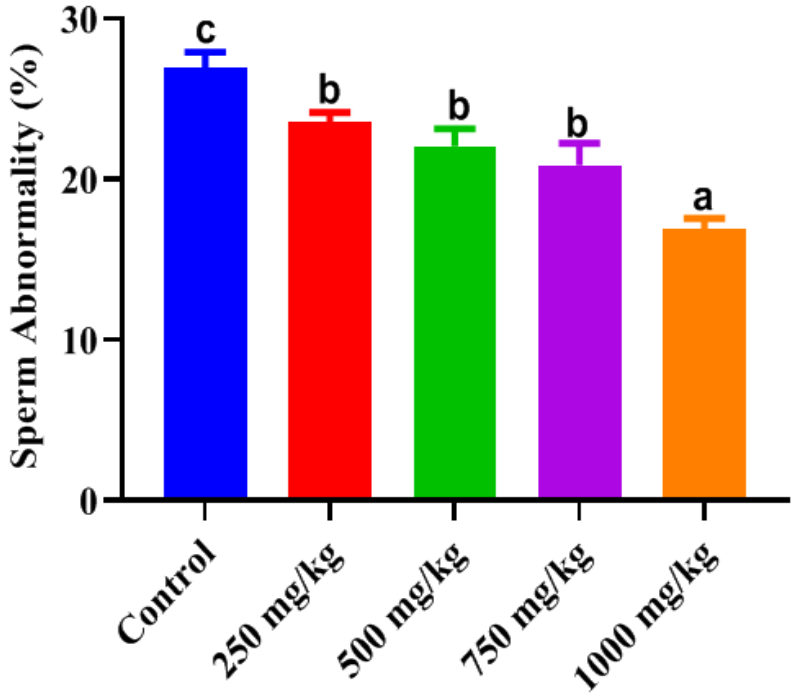

Fig. 7. The effect of potash on sperm morphology in healthy male Wistar rat. Values with the same superscripts do not differ significantly from each other whereas values carrying different superscripts are significantly different from each other ($p<0.05$)

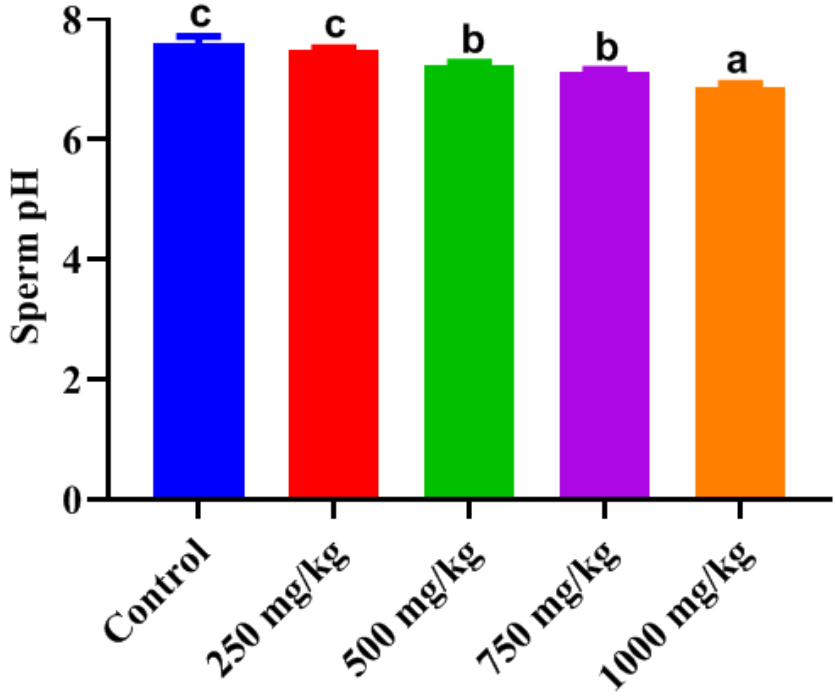

Fig. 8. The Effect of Potash on Seminal pH in healthy male Wistar rat. Values with the same superscripts do not differ significantly from each other whereas values carrying different superscripts are significantly different from each other ($p<0.05$)

4. DISCUSSION

Potash has been used from time immemorial as an agent for fertility control and suppression of sexual desire because it has been reported to cause the destruction of sperm cells accumulated in the male gonad [25]. It has been revealed that endocrine factors from brain highly regulate testicular functions, and hypothalamus-pituitary- testis circuit is core unit for maintaining endocrine balance and fertility, of which hypothalamus secrets Gonadotropin Releasing Hormone that stimulates pituitary gland to produce gonadotropic hormones i.e., luteinizing and follicle stimulating hormone [26,27].This present study was designed to ascertain the effect of potash on the male fertility of 28 healthy male Wistar rats. Potash at higher dosages (500, 750 and 1000 mg/kg body weight) was observed to increase the stimulation of follicle stimulating hormone in the serum of healthy male Wistar rats. This increase might have been possible due to the suppressing effect on feedback inhibition of anterior pituitary reported by Fattahi et al. [28], suggesting that potash may have direct effect on the anterior pituitary, which invariably led to increase stimulation of FSH in the serum. And likewise, some of the hormones released by Sertoli cells might have influenced the increase in the secretion of FSH, because FSH plays a significant role on Sertoli cells as a result of the feedback on the pituitary gland [29]. This study is in accordance to the findings of Mann and Lutwak-Mann [30] who found that tubular damage is always accompanied with an increase in serum FSH. Therefore, increase in the serum FSH level observed in this study suggested that administration of potash at higher dosages could have caused tubular damage and the testicles of the animals to have insufficient capacity for normal spermatogenesis.

Decrease in the level of luteinizing hormone in the serum after administration of potash in all treated groups might be as result of its inhibitory effect on sex hormones secretion. Studies conducted by researchers revealed that some substances with estrogenic activities have an inhibitory property on sex hormone secretion [29,31-33].They exert a direct inhibitory action on the testis, pituitary gland, causing changes in gonadotrophins concentrations and thus subsequent spermatogenic impairment, and changes in the concentration of neurotransmitter [33]. This study corresponds with the research conducted by Airaodion and coworker where administration of ethanolic extract of *J. curcas* leaves significantly reduced serum LH level in healthy male Wistar rat in a concentration dose dependent manner [29]. Reduction in the level of serum testosterone in this study at higher dosages (500, 750 and 1000 mg/kg body weight as a result of decrease in serum level of testosterone from the testicles and hormonal imbalance which will eventually affect spermatogenesis and fertility in experimental animals. Likewise, decreased levels of luteinizing hormone recorded in this study and the damage of Leydig cells might account for reduced testosterone production. Bo et al. [34] reported that decreased levels of serum testosterone can stimulate the release of gonadotropin-releasing hormone (GnRH) through a negative feedback mechanism and reduced seminal fluids secretion from seminal vesicles [34].This is similar with the previous findings of Airaodion and colleagues were *Carica papaya* leaves at higher dosages reduced serum level of testosterone [35].

It was revealed in this study that administration of potash has negative effect on male fertility by reducing sperm pH at 500, 750 and 1000 mg/kg body weight, sperm count, sperm motility, and significantly increased sperm vitality in all treatment groups. This might due to ability of potash to inhibit steroid hormone biosynthesis as well as impairment of spermatogenesis and toxic to the sperm [36]. The reduction in sperm count also indicated that potash possesses the propensity to inhibit the activity of ATPase in all tissue of the animals [37], which eventually led to the suppression of energy metabolism. And If ATPase activity is inhibited, it could suppress the motility rate of sperm, because ATP is the main energy source of sperm and this could directly have negative impact on sperm motility. A decrease in pH makes the medium of seminal plasma to become acidic which in turn makes sperms highly fragile, thus leading to higher rate of mortality. The result of this study is similar to our previous finding where administration of ethanolic extract of *J. curcas* leaves significantly reduced sperm pH, sperm count, sperm motility and significantly increased sperm mortality [29]. Reduction in sperm abnormality in this study showed that administration of potash did not generally affect sperm fertility. This is in accordance with the findings of Rabiu and Abubakar [25] that consumption of potash has a little effect on sexual desire among experimental animals. The study has shown that active sperm cells, number of viable cells, total sperm count and normal sperm morphology are generally

affected by consumption of the potash extract.

5. CONCLUSION

Potash consumption is very common in Nigeria especially in the rural communities. It is often used as food additives in different doses. The results of this study showed that potash has the propensity to compromise sperm quality and sex hormones in male Wistar rats. This does not automatically translate to such effect on humans. However, its regular consumption at high doses should be discouraged. And further study using human model is recommended.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Davy H. On some new phenomena of chemical changes produced by electricity in particular the decomposition of the fixed alkalies, and the exhibition of the new substances that constitute their bases; and on the general nature of alkaline bodies". Philosophical Transactions of the Royal Society of London. 2008;98:32.
2. Knight D. Humphry Davy; Science and Power. Oxford: Blackwell. 1992;66.
3. Dennis K. "Potash". 2005 Minerals Handbook. United States Geological Survey. 2006;58.1.
4. The World Potash Industry. Past, Present and Future. New Orleans, LA: 50th Anniversary Meeting; the Fertilizer Industry Round Table; 2000.
5. Iweka FK, Dic-Ijiewere OE, Oaikhena F, Bankole JK, Festus OO, Dada FL. The Effect of Potash On Liver Function Of Wister Rats. International Journal of Herbs and Pharmacological Research. 20165(1):13–20.
6. Okpala B. Benefits of Kaun Potash (Akanwu). Blog by Blessing Okpala. Global Food book Recipes for life; 2015. Availble:https://globalfoodbook.com/benefits-of-kaun-potash-akanwu/
7. Airaodion AI, Emaleku SA, Osunmuyiwa OJ, Megwas AU, Ayita EB, Simeon O. Oluba SO. Hepatotoxicity of Potash (Kaun) in Wistar Rats. International Journal of Health, Safety and Environment. 2021;05(02):68-73.
8. Airaodion AI, Emaleku SA, Osunmuyiwa OJ, Megwas AU, Ayita EB, Simeon O. Oluba SO, Adedeji AA. Nephrotoxic Nature of Potash (Kaun) in Wistar Rats. International Journal of Health, Safety and Environment. 2021;7(04):830-837.
9. Agarwal A, Majzoub A, Esteves SC. Infertility, recurrent pregnancy loss and sperm DNA fragmentation, have we found the missing link. Avi Harlev. 2016;5:935-941.
10. Cooper TG, Noonan E, von-Eckardstein S, Auger J, Baker HWG, Behre HM. World Health Organization reference values for human semen characteristics. Human Reproduction. 2010;16:231–245.
11. Speroff L, Fritz MA. Female infertility, Chapter 27, Clinical Gynecologic Endocrinology and Infertility, Eighth Edition. Lippincott Williams & Wilkins, syf. 2010;11:57.
12. Sartorius G, Nieschlag E. Paternal age and reproduction. Hum. Reprod. Update. 2010;16:65-79.
13. De Krester DM. Endocrinology of Male Infertility. Brit Med Bullet. 1979;35:187-192.
14. Amelar RD. Infertility in man. F. A Davis Company, Philadelphia, U.S.A. 1966;30-53.

15. Jarow JP. Endocrine causes of male infertility. Urol Clin North Am. 2003;30:83-90.
16. Gray EN. Testosterone, Sexual Function, and Cognition. J. Clin Endocrinol Metab. 2005;90(7):3838 –3846.
17. Weinbauer GF, Nieschlag E. Gonadotropin control of testicular germ cell development. Adv Exp Med Biol. 1995;317:55-65.
18. Ali M, Al-Dosary UH, Ahmed MR, Saranya R, Babu SR, Choudhry AR. Y-STR Profiling of Semen Stain Evidences of Azoospermic Individuals. International Journal of Forensic Science and Pathology. 2015;3(11):210-214.
19. Jungwirth A, Diemer T, Dohle GR, Giwercman A, Kopa Z, Tournaye H, Krausz C. Guidelines for the investigation and treatment of male infertility. Eur Urol. 2012;61(1):159-163.
20. NAS. National Academy of Science Guide for the Care and Use of Laboratory Animals. Eighth Edition; 2011.
21. Eliasson R. Analysis of Semen. In progress in infertility. Eds. S.J. Behrman and R.W. Kistner, Little Brown and company Boston. 1975; II(33):693.
22. Tijee DY, Ontoeng S. The viscosity of Human Semen and the percentage of motile spermatozoa. Fertile Steril. 1968;19:562-565.
23. Airaodion AI, Ogbuagu EO, Ekenjoku JA, Okoroukwu VN, Ogbuagu U, Airaodion EO. Antifertility effect of ethanolic leaf extract of *Carica papaya* in male Wistar rats. Merit Research Journal of Medicine and Medical Science. 2019;7(10):374-381.
24. Manafa PO, Mouneke IG, Ekuma-Okereke O, Ebugosi RS, Chukwuma GO, Ibe NC, Chukwuanukwu RC, Ogbuowelu OS, Okocha EC, Nwene KE, Manafa VI, Manafa CC. Levels of testosterone, progesterone and follicle stimulating hormone in male sickle cell subjects in Nnamdi Azikiwe University Teaching Hospital, Nnewi. Acta Scientific Medical Sciences. 2019;3(11):11-20.
25. Rabiu A, Abubakar IS. Role of potash extract (Jar Kanwa) in suppression of male sexual desire: A study among Wister rats. Ann. Afr. Med. Res. 2020;2:81.
26. Foresta C, Selice R, Ferlin A, Arslan P, Garolla A. Hormonal treatment of male infertility: FSH. *Reprod. Biomed*., Online [Internet]. 2007;15(6):666-672.
27. Tendayi HM, Ndayisenga J, Nshutiyimana JC, Nyiramahirwe S, Mukanshuti J, Karenzi V, Rutayisire R. Relationship Between Sperm Quality and Male Reproductive Hormones Among Male Partners with Fertility Complications: Attending CHUB. Rwanda J. Med. Health Sci. 2020;3:3.
28. Fattahi E, Parivar K, Jorsaraei SGA, Moghadamnia AA. The effects of diazinon on testosterone, FSH and LH levels and testicular tissue in mice. Iran. Reprod. Med. 2009;7(2):59-64.
29. Airaodion AI, Ayanleke IA, Agunbiade AP, Ogbuagu EO, Airaodion EO, Ogbuagu U. Antifertility Propensity of *Jatropha curcas* Linn. Leaves on Male Wistar Rats. Int. J. Res. Rep. Gynaecol. 2020;3(2):21-29.
30. Mann T, Lutwak-Mann C. Male reproductive function and composition of semen: General considerations. Springer Verlag, Berlin, Germany. 1981;ll.
31. Heneweer M, Houtman R, Poortman J, Groot M, Maliepaard C, Peijnenburg A. Estrogenic effects in the immature rat uterus after dietary exposure to ethinylestradiol and zearalenone using systems biology approach. Toxicol. Sci. 2007;99:303-314.
32. Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, Vandenbergh JG, Walser-Kuntz DR, VomSaal FS. *In vivo* effects of bisphenol A in laboratory rodent studies. Reprod. Toxicol. 2007;24:199-224.
33. Airaodion AI, Ngwogu AC, Megwas AU, Ekenjoku JA, Ngwogu KO. Effect of common household insecticides used in Nigeria on rat male reproductive hormones. Int. J. Res. Rep. Gynaecol. 2019;2(1):1-8.
34. Bo C, Zhao W, Jia Q, Yang Z, Sai L, Zhang F, Du Z, Yu G, Xie L, Zhang Z. Effects of α-zearalanol on spermatogenesis and sex hormone levels of male mice. Int. J. Clin. Exp. Med. 2015;8(11):20002-20013.
35. Airaodion AI, Ngwogu AC, Megwas AU, Ekenjoku JA, Ngwogu KO. Effect of common household insecticides used in nigeria on rat male reproductive hormones. International Journal of Research and Reports in Gynaecology. 2019;2(1): 1-8.
36. Ekenjoku JA, Airaodion AI, Okoroukwu VN, Ogbuagu EO, Ogbuagu U. Oral

administration of ethanolic extract of *Vernonia amygdalina* leaves might impact negatively on fertility in male Wistar rats. Asian Journal of Medical Principles and Clinical Practice. 2019;2(3):1-8.

37. Lakhsman J, Changamma C. Antispermatogenic effect of *Vernonia amygdalina* seed extract on steroidogenesis in albino rats. Int. J. Pharm. Pharm. Sci. 2013;5(1):213-225.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/75526