Title: THE AMIOLERATIVE ROLE OF ASCORBIC ACID ON POTASH-INDUCED PROSTATE INJURY USING SPRAGUE-DAWLEY RAT AS EXPERIMENTAL MODEL

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Abstract

The study is aimed at evaluating the effect of ascorbic acid on the prostate of Sprague-Dawley rats exposed to potash-induced prostate injury. This study was done with 40 male rats, divided into four (4; N=10) groups. Group A-D received 0, 3, 6 and 9 g/kg of Potash respectively orally. After 4 weeks of administering potash, five (5) rats from each group were randomly picked and euthanized. The prostates were harvested for histology, oxidative stress and hormonal milieu. Another four weeks were used to administer Ascorbic acid at 100 mg/kg orally to the remaining rats from each group and the prostates were harvested for analyses. Upon completion, the rats were euthanized and the above parameters were checked. Potash decreased rat prostate weight, caused a decrease in the testosterone level (TSH) and follicle-stimulating hormone (FSH), also induced oxidative stress which may lead to decline in reproductive function. Administering ascorbic acid was able to alleviate symptoms caused from the potash induction and support recovery. In conclusion, potash is a chemical substanceand studies have shown its negative effect on the reproductive activity in male, hence should bereduced or avoided for healthy lifestyle and amelioration was recorded upon administration of ascorbic acid.

Keyword: Potash, Ascorbic Acid, Prostate, Hormonal Milieu, Oxidative Stress.

INTRODUCTION

Potash refers to potassium compounds and potassium-bearing materials, most commonly potassium carbonate. The word "potash" originates from the Middle Dutch *potaschen*, denoting "pot ashes" in 1477. The old method of making potassium carbonate (K_2CO_3) was by collecting or producing wood ash (the occupation of ash burners), leaching the ashes, and then evaporating the resulting solution in large iron pots, which left a white residue denominated "pot ash" (Spears, 2017). Approximately 10% by weight of common wood ash can be recovered as potash (Jones, 2015). Later, "potash" became widely applied to naturally occurring minerals that contained potassium salts and the commercial product derived from them (Roy, 2020).Most of the world reserves of potassium (K) were deposited as sea water in ancient inland oceans. After the water evaporated, the potassium salts crystallized into beds of potash ore. These are the locations where potash is being mined today (Prugger, 2018). The deposits are a naturally occurring mixture of potassium chloride (KCl) and sodium chloride (NaCl), more commonly known as table salt (Smith, 2016). Over time, as the surface of the earth changed, these deposits were covered by thousands of feet of earth (Lin, 2019).Ascorbic acid has been proved to have great ameliorative properties on the toxicity in the testes in various studies conducted, shown in this report (Ododo, 2019).Ascorbic

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acid, usually referred to as vitamin C, is a powerful antioxidant (reducing) substance that works to combat pathogenic bacteria. It helps purify the responses and promotes collagen growth in the skin, capillaries, bones, and fibrous tissues (Li & Zhong, 2020). The majority of ascorbic acid is present in citrus fruits and vegetables like lemon, oranges, tomatoes, broccoli, etc. (Mahmoud, 2018).

However, humans are unable to make or store it. Therefore, it must be consumed through diet. Ascorbic acid occurs in three (3) states: Fully reduced as ascorbate (ASC), partially oxidized as semidehydroascorbate (SDA), and fully oxidized as dehydro-L-ascorbic acid (DHA) (Martin, 2003). Ascorbic acid (Vitamin C) is a powerful antioxidant that plays a protective role in the male reproductive system (Leite *et al.,* 2019).The aim of this study is to establish that Ascorbic acid plays an ameliorative role on the prostate of a Sprague-Dawley rat induced with potash.

MATERIALS AND METHODS

Drugs/ Substances

Researches were conducted on Animal Model Forty (40) male Sprague-Dawley rats, weighing between 160±40g were used. They were housed in a standard ventilated steel mesh plastic cages in the Animal House of the Anatomy Programme, College of Health Sciences, Bowen University, Iwo, Osun State, Nigeria. They were under standard room temperature and were left to acclimatize for a duration of two weeks before the commencement of the experiment. The method for administration of the substances (Potash and Ascorbic acid) was orally. All experimental procedures and techniques were approved by the Health Research Ethics Committee of the College of Health Sciences, Bowen University, Iwo, Osun State, Nigeria, coupled with strict compliances with the guiding principles for research involving animal models.

Experimental Designs

Forty (40) male Sprague-Dawley rats were used and divided into four (4) groups of ten rats pergroup (n=10) as follows: Group A, served as the healthy control ~~and received 0g/kg of Potash~~. Groups B-D were administered ~~, served as low, medium and high doses received~~ 3, 6 and 9g/kg of Potash respectively for a period of 4 weeks. ~~The animals were randomly selected without bias into the respective groups.~~ The doses chosen for this study were similar from a previous study. ~~Each animal has its own canula and the respective syringe. Also, error due to parallax was avoided.~~ Upon completion of each treatment routine, five (5) rats from each group were randomly selected and euthanized using 1 ml of Ketamine as anesthetic. The remaining 5 rats in each group were given 100 mg/kg of Ascorbic acid for another period of 4 weeks. Upon completion, the animals were sacrificed and their prostate was obtained for histological findings and oxidative stress markers, blood was taken for hormonal parameters. The prostates were excised and hormonal milieu were analyzed. The Prostates were harvested were fixed in 10% Formal saline fluid for histological purpose.

Histological Procedures

For the histological analysis, Hand E was used as stains. The sections were made at 5microns before photomicrography at X 400 magnification. The harvested Prostates were kept in a universal bottle containing Bouin's fluid. Tissues were processed for microscopic examination making use of a standard protocol and 5µm thick paraffin section were made.

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Commented [A5]: First list the materials used: chemicals/Reagents, equipment/instrument all with their suppliers and model numbers where applicable. State where the potash and Vit. C were obtained from and how the various doses were prepared and stored.

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Prostate Weight

The prostates were weighed using a sensitive weighing scale balance and recorded, before fixing in 10% Formal Saline solution.

Blood Collection

About 2mls of blood were collected through cardiac puncture into heparinized bottles. This were immediately put into the ice filled cooler, then into the refrigerator for preservation. The blood was later taken and centrifuged for about 20 min. After been separated from the red cells, it was stored in bottles and sealed before being put in the refrigerator.

Statistics

The data acquired from all groups were compiled and statistically analyzed using One Way of variance (ANOVA) and Student t-test method on SPSS software version 22. LSD multiple test range was used to compare means obtained after each treatment with control measurements. The result of the data was expressed as Mean ±SEM (standard error of mean) were $p<0.05$ was taken as significant.

RESULTS

Effect of Potash and Ascorbic Acid on Oxidative Stress Markers of Adult Male Sprague-Dawley Rats

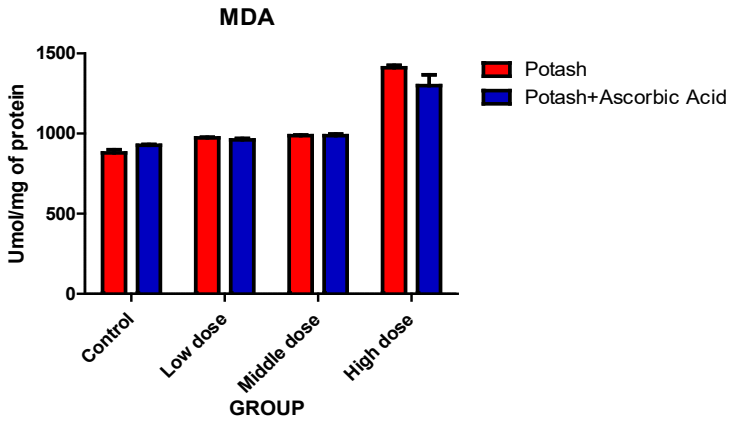

Figure 1

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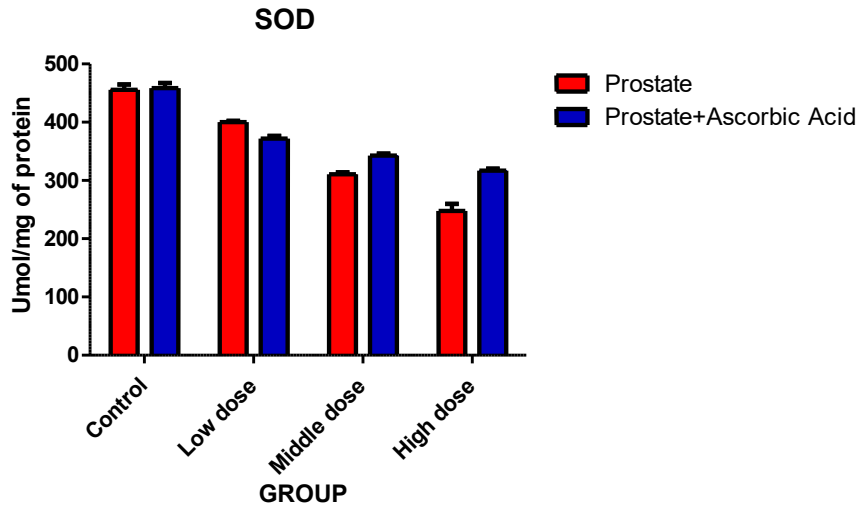


Figure 2

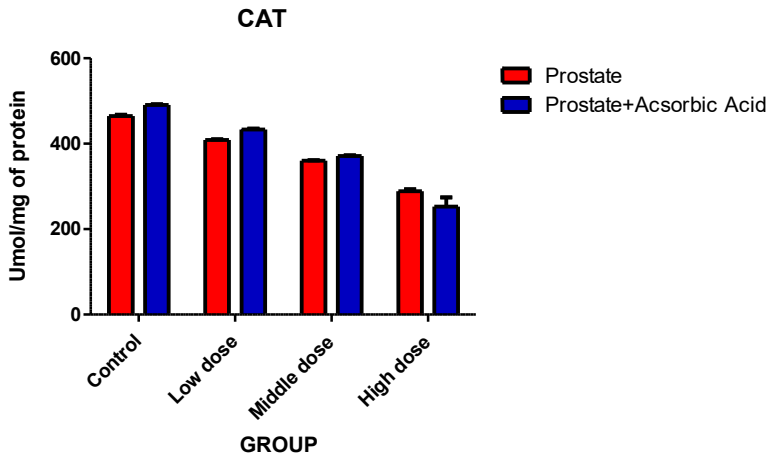
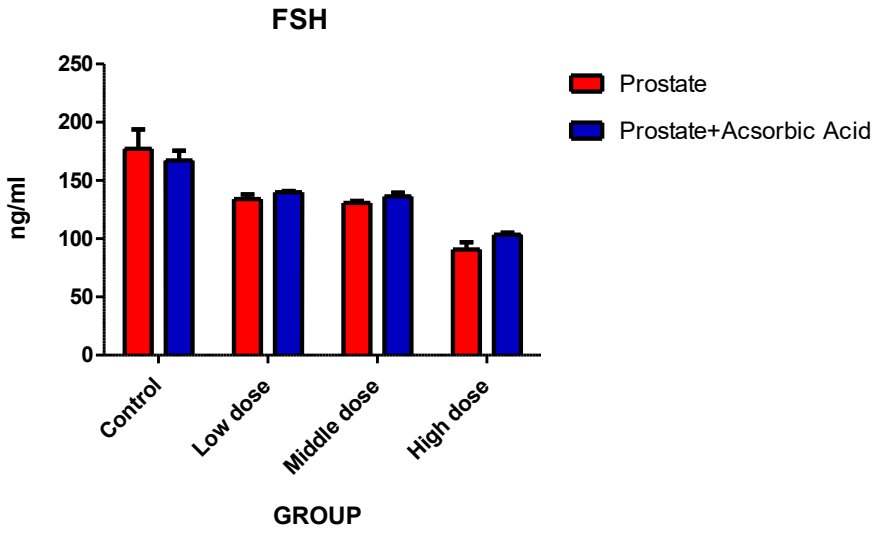


Figure 3

Effect of Potash and Ascorbic Acid on Hormonal Milieu of Adult Male Sprague-Dawley Rats



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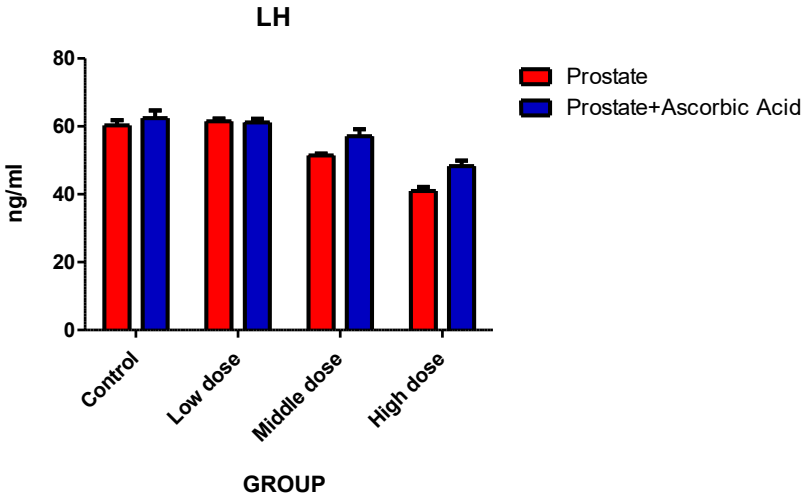

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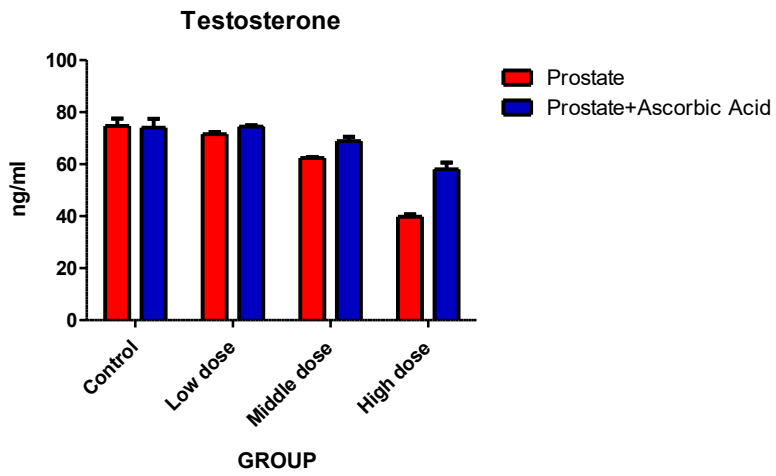

Figure 6

EFFECT OF POTASH AND ASCORBIC ACID ON THE PROSTATE OF A MALE SPRAGUE-DAWLEY

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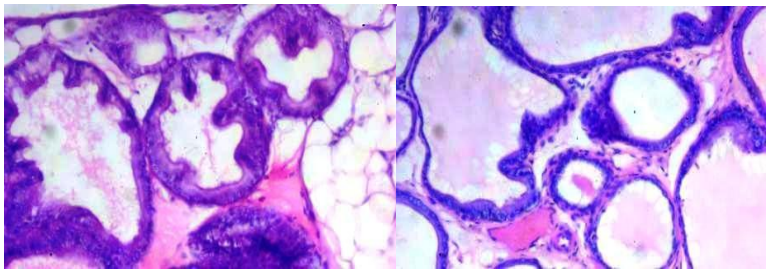

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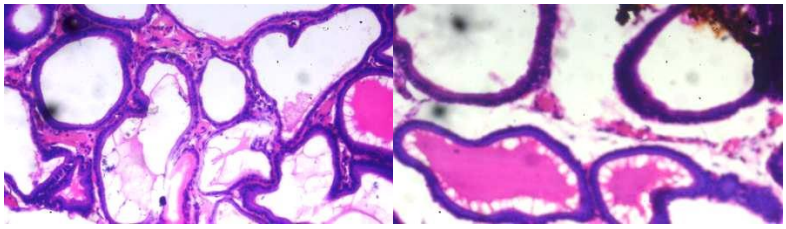

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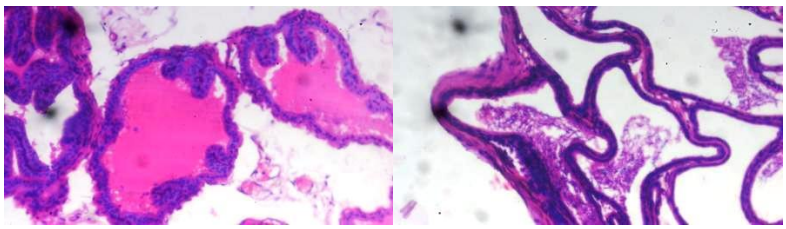

D1 PRO **D12 PRO**

Photomicrograph of a prostatic section stained by Hematoxylin and Eosin showing prostate glands lined by inner cuboidal epithelium (black arrow) and outer basal epithelial cells. Most of the glands contain eosinophilic secretions and corporal amylacea while other glands are thrown into papillary infoldings (white arrow), normal stroma and smooth muscles fibers are seen (slender arrow). No infiltration of inflammatory cells, no atypia or malignancy is seen seen.

~~RESUTS AND~~ DISCUSSION

In figure 1, The control group has relatively moderate MDA levels. As the dose increases, the MDA levels fluctuate, with the highest being in the medium and high-dose groups. The reduction in MDA levels with ascorbic acid treatment is more noticeable in the higher dose groups. MDA is a marker of oxidative stress. Higher MDA levels suggest increased lipid peroxidation. The reduction in MDA with ascorbic acid suggests that it has an antioxidant effect, reducing oxidative stress in these groups. The variation in MDA levels among doses indicates a dose-dependent response to the treatment.[11] In figure 2, The control group showed moderate superoxide dismutase (SOD) levels. With increasing doses, SOD levels declined, suggesting oxidative stress. However, ascorbic acid supplementation enhanced SOD activity, particularly in low and medium doses, indicating its antioxidative role in protecting cells from oxidative damage.[12] In figure 3 Catalase (CAT) levels decrease with increasing dose, indicating oxidative stress.[13] Ascorbic acid supplementation elevates CAT activity, particularly in low and medium doses, suggesting its antioxidative role in mitigating oxidative damage.[14] In figure 4, Follicle-stimulating hormone (FSH) levels decline with increasing doses, suggesting impaired reproductive function.[19]However, ascorbic acid mitigates this reduction, indicating its protective role.[20] Figure 5, Luteinizing hormone (LH) levels decline with increasing doses, suggesting dose-dependent reproductive impairment.[21]However, ascorbic acid administration partially restores LH levels, indicating its protective role in hormonal balance.[22] Figure 6, Testosterone levels decline with increasing doses, indicating a negative impact on endocrine function.[23] Ascorbic acid supplementation partially restores testosterone levels, suggesting its protective role against oxidative stress-induced hormonal disruption.[24]

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This discussion is grossly deficient and does not qualify to be regarded as based on sound scientific evidence

CONCLUSION

In the Potash group, a dose-dependent significant increase was observed when treatment groups were compared to control. Similar significant increase was recorded in when medium and high doses were compared to low dose and when high dose was compared to medium dose. However, the reverse was recorded in the Intervention group, as significant decrease was recorded when treatment groups were compared to control in a dose-dependent manner. Also, significant decrease was recorded when medium and high doses were compared to low dose and when high dose was compared to medium dose.

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General comments

There has been an attempt to present an experiment as a scientific writing. However, there are serious gaps and deficiencies in the reporting style that must be addressed. Some of the issues have been raised in the in-text comments.

The concept is commendable but huge revision is required to bring the write-up toacceptable minimum standards for publication as a research paper.

The introduction and experimental design (materials and methods section) needs to be re-visited and results presented must reflect the design of the study

The discussion must address all results presented with evidences from literature to support or otherwise, the opinions/interpretations of the results by the authors.

The manuscript currently has no conclusion. See in-text comments.

CONFLICT OF INTEREST

Authors declared no conflict of interest.

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