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Allium Cepa (Onion) Extract Enhances and Protects Testicular Function and Architecture against Paraquat Induced Oxidative Damage.

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Abstract: *Allium cepa* (*A. cepa*) is consumed for its health benefits. This study investigated the therapeutic potentials of ethanolic extract of *A. cepa* bulbs against paraquat-induced testicular toxicity in animal model. Thirty Wistar rats were split into control and five test groups (n=5). Group A (Control) received feed and water; test groups (B-F) were treated orally as follows: group B (20 mg/kg b.w. of paraquat for 4weeks); group C (1000 mg/kg b.w. of *A. cepa* extract for 4weeks); group D (a co-administration of 20 mg/kg b.w. of paraquat and 100 mg/kg b.w. of *A. cepa* extract for 4weeks); group E (a co-administration of 20 mg/kg b.w. of paraquat and 1000 mg/kg b.w. of *A. cepa* extract for 4weeks); and group F (1000 mg/kg b.w. of *A. cepa* extract for 2weeks before co-administration with 20 mg/kg b.w. of paraquat for 2weeks). In the end, sperm count, morphology, motility, sera testosterone levels, malondialdehyde (MDA) and superoxide dismutase (SOD) levels as well as histology of the testes were assessed. Paraquat administration caused significantly ($P<0.05$) reduced sperm count, motility, alteration in sperm morphology and induced cell death. Oral gavage of paraquat also caused significant ($P<0.05$) decrease in serum testosterone and SOD levels with concomitantly elevated MDA levels. However, following the co-administration with ethanolic extract of *A. cepa* to experimental rats, there was an improvement in sperm parameters (count, motility and morphology) as well as in sera testosterone and SOD levels. It can be concluded that *A. cepa* exerts strong antioxidant effects in a dose-dependent manner in ameliorating testicular toxicity induced by paraquat in animal models.

Keywords: *A. cepa*, paraquat, testis, sperm, testosterone

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1. INTRODUCTION

Paraquat is an organic compound widely applied as a potent herbicide. As a herbicide, paraquat inhibits photosynthesis and defoliates green plants.[1,2] Paraquat is used in research to produce reactive oxygen species (ROS) as it undergoes a redox reaction in-vivo before it is oxidized to produce overwhelming amounts of reactive oxygen species (ROS).[3] Oral exposure of paraquat to animals is injurious and has been documented to cause acute respiratory distress syndrome (ARDS) and death.[4] The diluted form of paraquat used for spraying tends to be mild, which makes accidental exposure and contacts before dilution the primary source of human poisoning besides suicide/deliberate consumption. In laboratories, exposure of paraquat to experimental animals have shown that paraquat is highly toxic by the inhalation and oral routes.[5] It is also toxic to the skin, causing varying degrees of injuries.[5] Its contact with the eye results in visual problems, ocular irritations and injuries.[5] A gulp of few grams of paraquat even after inducing emesis immediately could lead to death due to the development of fibrous tissue in the lungs.[4] Exposure to large doses for several days leads to lung damage, kidney and heart failures as well as esophageal strictures[6], with accidental deaths and suicides from paraquat ingestion relatively common.[7] Testicular evaluations on experimental animals after paraquat exposure have shown significant alterations in not only the histoarchitecture but also in testicular parameters such as sperm count, sperm morphology, sperm motility and testosterone levels.[8,9,10] In female experimental animals, exposure to paraquat showed a significant alteration in ovarian weight and blood levels of luteinizing hormone and follicle-stimulating hormone and also caused a significant decrease in the number follicles, as well as alterations in the cells of corpus luteum and viability of the oocytes.[11] Decoctions of parts of grasses, herbs, shrubs, and trees have been tested on animals including man and have been found to have culinary and medicinal values.[12-14] While some have an abundance of antioxidants in their roots, stem, bark, leaves, fruits and seeds, some boast of large amounts of other substances which could provide protective and curative effects to humans ailments.[15-19] Some of these plants however, contain substances which when consumed at various doses have harmful effects on some systems of the body.[20,21] *A. cepa* (onion) is a widely used vegetable/spice deployed either alone or added to other plant extracts to arrest tissue injuries and dysfunctions in addition to its use as a medicinal and food source.[22] (*A. cepa* is commonly used in our daily diet and has been a source of high nutritional value due to its antithrombotic, hypolipidemic, hypotensive, diaphoretic, antibiotic, antidiabetic, antiatherogenic and anticancer properties).[23] These lifesaving properties of onion are ascribed to its organosulfur and phenolic constituents.[22] Previous research has shown that onion contains exogenous and endogenous antioxidants such as selenium, glutathione, vitamins A, B, and C, and flavonoids such as quercetin and isorhamnetin.[24] These antioxidants protect DNA and other important molecules from oxidation and damage, which would otherwise induce apoptosis. They thus could improve sperm health parameters, increasing the rate of fertility in men.[25] Researchers have documented the androgenic activity of *A. cepa* juice as well as its ability to recover spermatogenesis in *Toxoplasma gondii* infected rats.[22] Despite this listed therapeutic activities of onion, little is known about its ameliorative mechanism and therapeutic application against paraquat-induced testicular toxicities with limited literature on this available, thus warranting the need to examine the effects of such co-administration on the testes and testicular parameters in an animal model. This study is designed to investigate the therapeutic potentials of ethanol extract of *A. cepa* bulbs in ameliorating paraquat-induced testicular toxicity in animal models.

2. MATERIALS AND METHODS

2.1 Animal handling and Ethical Approval

This research was carried out between the 2nd of May and the 30th of August, 2019. Fifty-six (56) male Wistar rats (122-134g) were purchased from a local Animal House. The rats were housed in clean well-ventilated plastic rat cages, fed with standard rat chow and distilled water *ad libitum* and allowed to acclimatize for two weeks. Twenty-six (26) of these rats were used to determine the median lethal dose of paraquat and ethanol extract of *A. cepa*, while thirty (30) were used for the research proper. Ethical approval for this research was obtained from the Ethical Committee of the Faculty of Basic Medical Sciences.

2.2 Preparation of ethanol extract of A. cepa and its Acute Toxicity test

One kilogram of fresh *A. cepa* bulbs of the Bombay Red variety was purchased from a local Vegetable market. The bulbs were chopped into slices, oven-dried and subsequently ground into powder. This powdered form of *A. cepa* was used to produce ethanol extract of *A. cepa* using the method as described by Nagappan.[26] The median lethal dose (LD_{50}) of ethanol extract of *A. cepa* was determined using the method as described by Lorke[27] with a slightly modified protocol. LD_{50} of ethanol extract of *A. cepa* via oral route in adult male Wistar rats was found to be above 5000mg/kg.

2.3 Procurement and Acute Toxicity Study for Paraquat

Paraquat in the form of Paraquat Dichloride (276g/l) (Springfield Agro LTD, Apapa, Lagos) was used. The median lethal dose (LD_{50}) test for paraquat was carried out using the method as described by Lorke[27] with slight modification. In this study, two phases were deployed and 13 rats were used. These rats were carefully gavaged with graded doses of paraquat via the oral route. LD_{50} of paraquat via oral route was found to be approximately 50mg/kg.

2.4 Experimental Protocol

Thirty growing adult male Wistar rats (122-150g) from the acclimatized group were grouped into six groups (A-F) of five rats each. Group A (Control) received feed and water only. Group B received 20 mg/kg of paraquat for 4weeks. Group C received 1000 mg/kg of *A. cepa* extract for 4weeks. Group D received a co-administration of 20 mg/kg of paraquat and 100 mg/kg of *A. cepa* extract for 4weeks. Group E received a co-administration of 20mg/kg of paraquat and 1000 mg/kg of *A. cepa* extract for 4weeks, while Group F received 1000 mg/kg of *A. cepa* extract for 2weeks before co-administration with 20 mg/kg of paraquat for 2weeks. All administrations were through the oral route and once a day. During this experiment, the Guide for the Care and Use of Laboratory Animals was strictly followed.[28]

2.5 Termination of Experiment, Sacrificing of Animals and Sample Collection

This was a subacute test and lasted for 4 weeks. Twenty-four hours after the last administration of the extract and paraquat, rats were anesthetized using chloroform vapour and blood samples collected by the ocular puncture. Animal sacrifice and sample collection was carried out using the method as described by Ofoego *et al.*[10] The experimental animals were humanely sacrificed by cervical dislocation. The testes including their epididymis from each rat were quickly and carefully exposed, and retrieved following a midline abdominal incision. The testes together with their epididymis were weighed. The epididymis was then separated and the epididymal fluid was collected from the caudal part for semen analysis. The remains of the experimental animals were appropriately buried. The testes were fixed in freshly prepared Bouin's fluid.

2.6 Hormonal Assay

Sera samples were collected after centrifuging the blood samples at 2500 rpm for 10 minutes using Wisperfuge model 1384 centrifuge (Tamson, Holland), and were assayed for testosterone levels using a Microwell enzyme-linked immunoassay (ELISA) technique; using analytical grade agent (Syntron Bioresearch Inc., USA) as described by Ofoego *et al.*[10] Values obtained were documented and expressed in ng/ml.

2.7 Semen Analysis

2.7.1. Sperm count

Investigation on sperm count was carried out using the method as described by the World Health Organization[29] with slight modifications. Briefly, a graduated cylinder was used to dilute the semen in sodium bicarbonate-formalin diluting fluid in the proportion of 1 in 20. A Pasteur pipette was then used to fill an Improved Neubauer ruled chamber with well-mixed diluted semen and allowed to stand for 3-5 minutes and allowed to settle. The 10X objective was used to view, with the condenser iris closed sufficiently to give good contrast, and the number of spermatozoa in 2 large squares (2 sq mm) counted. The number counted was multiplied by 10^6.

2.7.2. Sperm motility

Investigation on sperm motility was carried out using the method as described by World Health Organization [29] with slight modification. Briefly, a well-mixed drop of liquefied semen was placed on a slide, evenly distributed and covered with a cover slide. The 40X objective was used to observe and count. A total of 100 spermatozoa were counted out of which the number of motile ones was recorded in percentage.

2.7.3. Sperm Morphology

Investigation on sperm morphology was carried out using the method as described by World Health Organization [29] with slight modification. Briefly, a thin spread of liquefied well-mixed semen was made on a slide. While still wet, the spread was fixed with 95% v/v ethanol for 5-10 minutes and allowed to air-dry. The spread was subsequently washed with sodium bicarbonate-formalin solution to remove any mucus which may be present and rinsed with several changes of water. The spread was then covered with dilute (1 in 20) carbon fuchsin and allowed to stain for 3 minutes after which it was washed off with water. The spread was counterstained with dilute (1 in 20) Loeffler's methylene blue for 2 minutes and washed off with water, drained and allowed to air-dry. It was then examined for normal and abnormal spermatozoa using the 40X objective. The 100X objective was used to confirm abnormalities. One hundred sperm cells were identified and secluded, while the percentage of normal and abnormal morphology were evaluated and recorded. Sperms with altered morphology were apparent under the power x400. Morphologically normal sperm were distinguished with their characteristic typical sperm appearance. Any sperm that showed any form of defects such as short tail, no tail, abnormal head or middle piece, or any other form not in conformity with the normal appearance of a rat sperm was counted as an abnormal sperm.

2.8 Preparation of testicular homogenates and determination of Testicular malondialdehyde (MDA) concentration

We prepared the testes of the experimental animals for oxidative stress by applying the method as described by Balahoroğlu *et al.*[30] Briefly, the left testis of each experimental animal was homogenized in potassium phosphate buffer 10mM with a pH of 7.4 for estimation of MDA. Afterwards, we centrifuged the homogenates for 15 minutes using a cold centrifuge. The precipitate formed afterwards was used for the estimation of MDA. Spectrophotometric determination of testicular tissue MDA was carried out by measuring the thiobarbituric acid reactive substance (TBARS) spectrophotometrically using the method as described by Ohkawa *et al.*[31] We recorded the values obtained in nmol/g.

2.9 Evaluating SOD activities

We measured SOD activity in the testes of the experimental animals using the method as described by Sun *et al.*[32] Briefly, an assay reagent comprising of 0.3mM xanthine, 0.6mM Na_2EDTA, 0.15mM nitroblue tetrazolium (NBT), 0.4M Na_2CO_3, and 1 g/l bovine serum albumin was well mixed with 0.5 ml of crude testis homogenate. 50µl, 167 U/L of Xanthine oxidase was used to initiate the reaction. Nitroblue tetrazolium's (NBT) reduction by superoxide anion radicals was evaluated by quantifying the absorbance at 560nm. Results we obtained following evaluation of superoxide dismutase (SOD) activity in the homogenized testes were promptly recorded in U/ml.

2.10 Tissue processing/Histopathology

The right testes were processed by normal histological methods involved in hematoxylin and eosin staining methods of fixation, dehydration and clearing, impregnation, embedding, mounting and staining.[10] The micrographs of relevant stained sections were viewed with the aid of a light microscope (Olympus XS2-107BN, Japan) and subsequently taken with a photomicroscope (Olympus, Japan).

3. STATISTICAL ANALYSIS

The raw data results obtained from this study such as body weight, relative testicular weight and serum testosterone levels were analyzed using SPSS version 22 and values obtained were documented as mean ± standard deviation (SD) and mean ± standard error of mean (SEM) were applicable. Analysis of

variance (ANOVA) was further used followed by *Post hoc* Fisher LSD multiple to determine sources of variation. Data were considered significant at $P \leq 0.05$.

4. RESULT

4.1 Effect of Ethanol Extract of A. cepa (onion) and Paraquat on body weight of experimental animals

Table 1: Body Weight Analysis (g)

Group	Initial Body weight(g) Mean±SD	Final Body weight(g) Mean±SD	p-value	Weight difference(g) Mean±SD
A	130.00± 14.14	200.00± 28.28	0.090	70.00±14.14
B	150.00±14.14	190.00±14.14	0.295	40.00±28.28
C	146.67±11.55	193.33±11.55	0.073	46.67±23.09
D	140.00±20.00	163.33±15.28	0.020*	23.33±5.77
E	143.33±20.82	146.67±11.55	0.874	3.33±32.15
F	126.67±11.55	150.00±30.00	0.423	23.33±40.41

*Values are in Mean ± SD; (n = 5). * = p< 0.05 when initial weight was compared to final weight.*

Results we obtained as displayed in table 1 showed that there was an increase in the bodyweight of all experimental animals. The highest weight increase was seen in the control group (Group A) with the least observed in Group E.

4.2 Effect of Co-administration of ethanol extract of A. cepa and paraquat on the relative testicular weight on Adult male Wistar rat.

Table 2: Effect of co-administration of ethanol extract of onion and paraquat on the relative testicular weight of adult male Wistar rat.

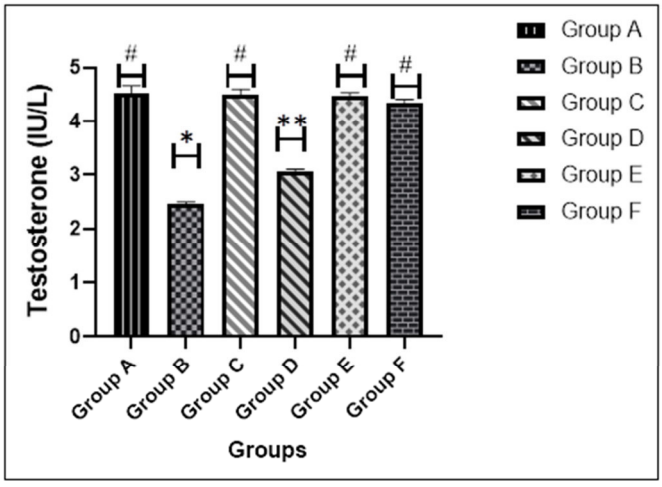
Groups	Relative Testicular weight (g) Mean±SEM	p-value	f-value
A	3.07±0.51		2.310
B	2.97±006	1.000	
C	3.11±0.23	1.000	
D	2.46±0.49	1.000	
E	2.10±0.67	0.607	
F	2.96±0.38	1.000	

Values are in Mean ± SEM; (n = 5).

Result obtained from this study and presented in table 2 shows that but paraquat and allium cepa extract administration did not cause any significant difference in the relative testicular weight in the test groups when compared with the relative testicular weight of group A (control).

4.2 Effect of Co-Administration of Ethanol extract of onion and paraquat on the serum testosterone level on Adult male Wistar Rat.

Results obtained following evaluations on sera testosterone levels showed that paraquat administration altered testosterone levels. Testosterone levels were statistically lower ($p< 0.05$) in Groups B and D when compared to Control (Group A) with the lowest levels of testosterone observed in Group B (that received paraquat alone). We observed that other test groups had significantly higher levels of testosterone when compared to Group B, as shown in Fig1.

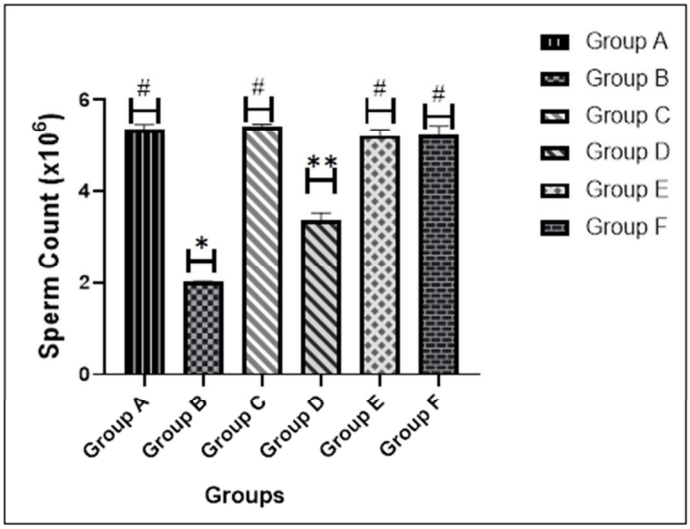


(and ** = p< 0.05 when compared to control; # = p< 0.05 when compared to group B that took 20mg/kg of paraquat for 4weeks).*

Fig 1: Effect of Co-Administration of ethanol extract of onion and paraquat on the relative testosterone level.

4.3 Effect of Co-Administration of Ethanolic extract of onion and paraquat on the percentage sperm count in Adult male Wistar Rat.

Microscopic investigations we carried out showed that sperm count was affected following administration of paraquat. Significantly lower ($p< 0.05$) sperm counts were observed in Groups B and D, as seen in Fig. 2. The lowest sperm count was observed in Group B (that took 20mg/kg of paraquat for 4weeks), with all other test groups showing significantly higher sperm counts when compared to Group B.

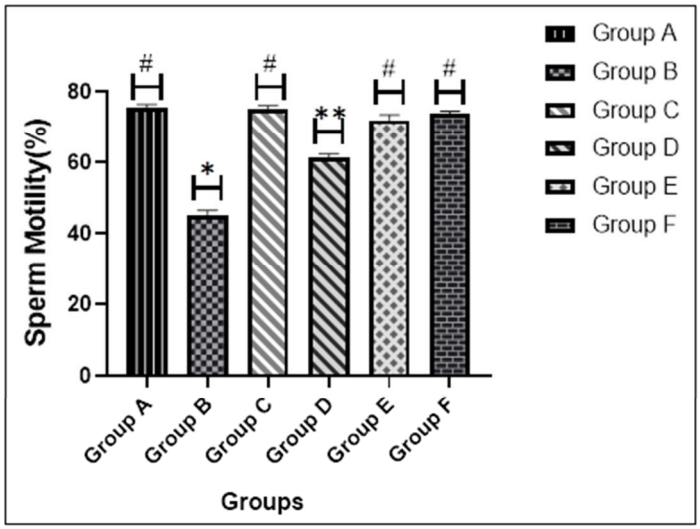


(and ** = p< 0.05 when compared to control; # = p< 0.05 when compared to group B that took 20mg/kg of paraquat for 4weeks)*

Fig 2: Effect of Co-Administration of ethanol extract of onion and paraquat on the Sperm Count

4.4 Effect of Co-Administration of Ethanol extract of onion and paraquat on the percentage of sperm motility in Adult male Wistar Rat.

We observed that paraquat administration caused significantly lower percentages in sperm motility in Groups B and D ($p< 0.05$) when compared to Control (Group A). However, when we compared all other test groups with Group B, we observed significantly higher percentages in active sperm motility in these test groups as presented in Figure 3.

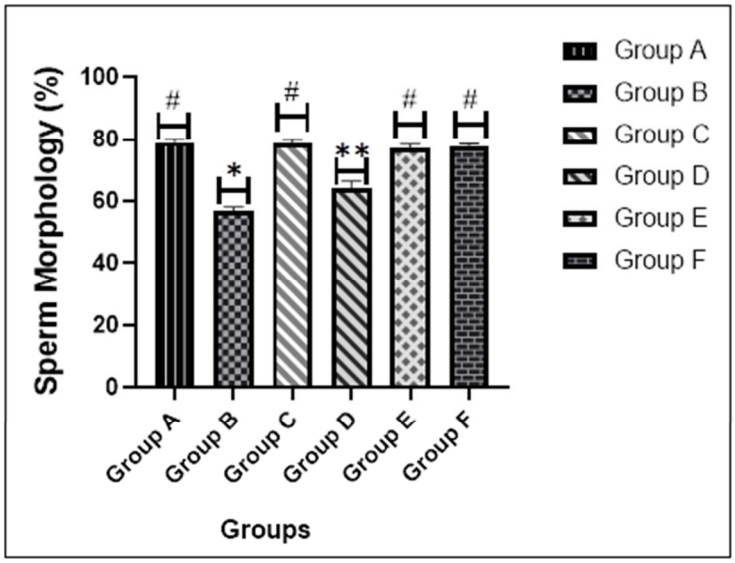


(and ** = p< 0.05 when compared to control; # = p< 0.05 when compared to group B that took 20mg/kg of paraquat for 4weeks).*

Fig 3: Effect of Co-Administration of Ethanol extract of onion and paraquat on the percentage sperm motility

4.5 Effect of co-administration of ethanol extract of onion and paraquat on the percentage of sperm morphology in adult male Wistar rats.

Observations on sperm morphology showed altered sperm morphology in some test groups following oral paraquat administration. There were significantly lower percentages in normal sperm morphology in Groups B and D ($p< 0.05$) when compared to the Control group (Group A). Values obtained in other test groups showed significantly higher percentages in normal sperm morphology when compared with Group B, as shown in Fig 4.

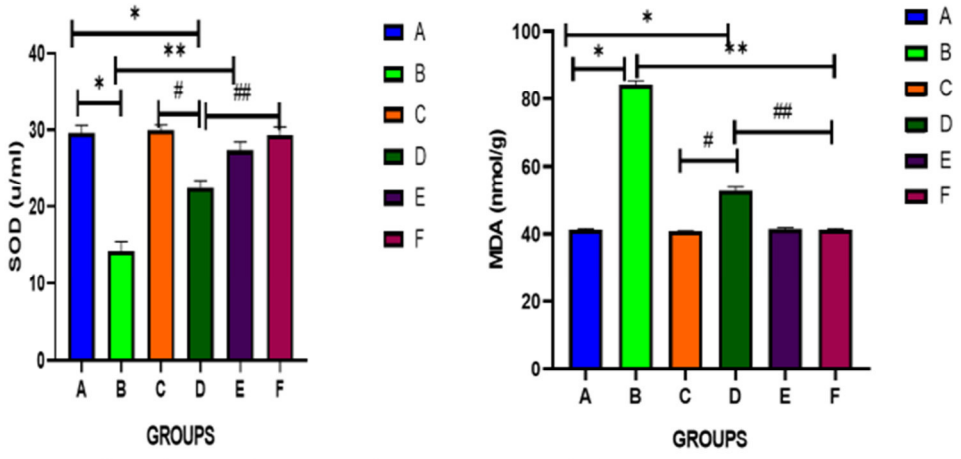


(and ** = p< 0.05 when compared to control; # = p< 0.05 when compared to group B that took 20mg/kg of paraquat for 4weeks)*

Fig 4: Effect of co-administration of ethanol extract of onion and paraquat on the percentage sperm morphology of adult male Wistar rats

4.6 Effect of Co-administration of ethanol extract of *A. cepa* and paraquat on the Testicular Oxidative stress levels (malondialdehyde and superoxide dismutase) in Adult male Wistar rat.

Analysis of malondialdehyde (MDA) and superoxide dismutase (SOD) showed that MDA was statistically higher and SOD significantly lower in the group that received 20mg/kg of paraquat alone when compared with Control group. However, administration of *A. cepa* extract negated the effect of the paraquat. There was a statistically significant difference between the control group and groups B and D. While Group B showed a statistically significant difference from groups C, D, E and F. Statistically significant differences were also observed between Group C and D as well as between Group D, E and F as shown in Fig. 5. On the other hand, administration of *A. cepa* to the experimental animals in groups C, D, E and F irrespective of the paraquat administered, caused an improvement in the antioxidant marker SOD in the testes of the experimental rats. SOD showed a statistically significant difference between the control group A and B as well as with the group D. Among the test groups, statistically significant difference existed between group B and C group D, Group E and Group F. Group C was also statistically different from group D. Group D also varied significantly from group F as shown in Fig. 5.

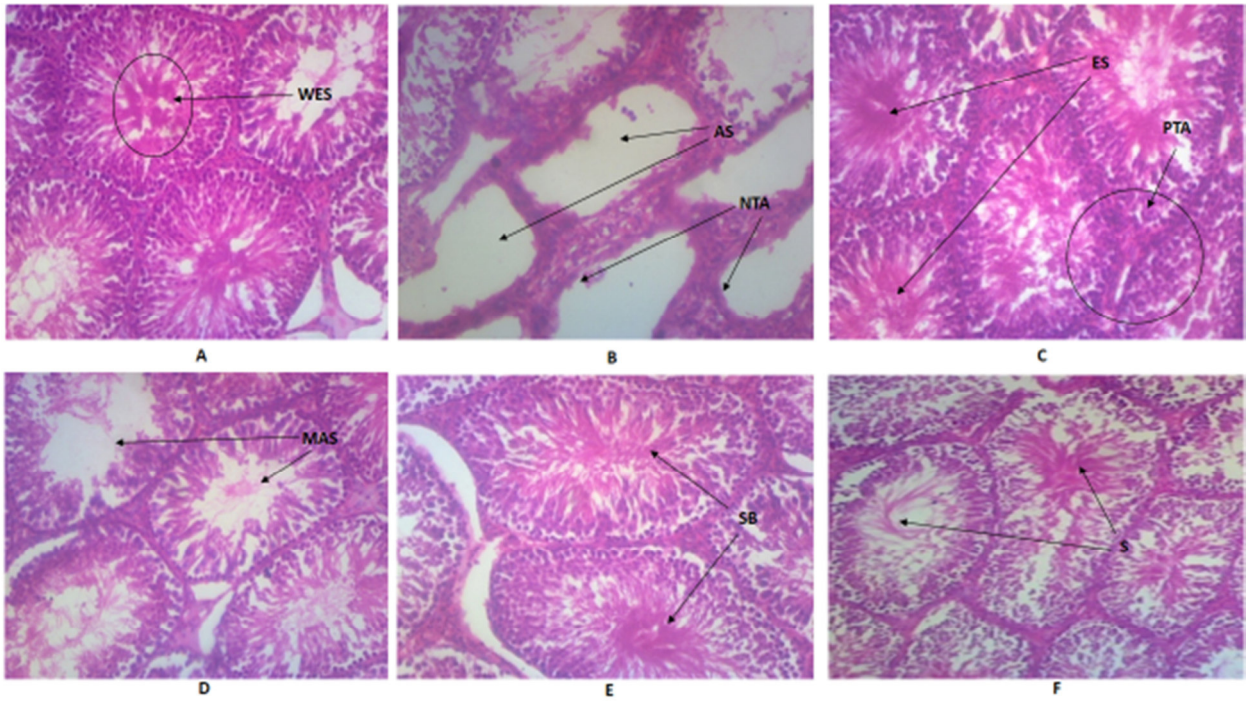


(= p< 0.05 when compared to control; ** = p< 0.05 when compared to group B that took 20mg/kg of paraquat for 4weeks; # = p< 0.05 when compared with group C; ## = p< 0.05 when compared with group D).*

Fig 5: Effect of co-administration of ethanol extract of onion and paraquat on the SOD and MDA levels in adult male Wistar rats

4.7 *Histological Findings*

Our observations on the histological slides of the testis we processed using a light microscope (Olympus XS2-107BN) showed reduced density of sperm bundles and general thinning of the walls of the seminiferous tubules, a sign of reduced spermatogenesis and loss of Sertoli cells and interstitial cells of Leydig (cells saddled with the responsibility of nurturing sperm cells and production of testosterone). Treatment with graded doses of ethanol extract of *A. cepa*, however ameliorated these conditions with improved spermatogenesis and increase in the density of sperm bundles as presented in Figure 6.



(Group A: Control; Group B: received 20mg/kg of paraquat for 4weeks; Group C: received 1000mg/kg of A. cepa extract for 4weeks; Group D: received a co-administration of 20mg/kg of paraquat and 100mg/kg of A. cepa extract for 4weeks; Group E: received a co-administration of 20mg/kg of paraquat and 1000mg/kg of A. cepa extract for 4weeks; Group F: received 1000mg/kg of A. cepa extract for 2weeks before co-administration with 20mg/kg of paraquat for 2weeks).

Fig 6: Photomicrographs of the histology of rat testes showing the effect of co-administration of ethanol extracts of *A. cepa* and Paraquat.

In figure 6, group A slides showed normal testicular architecture and well enhanced spermatogenesis (WES). Slides of group B had evidence of severe damage of testicular tissue with the arrest of spermatogenesis (AS) and necrosed testicular histology (NTA). Testicular slides of group C showed enhanced spermatogenesis (ES) and well preserved testicular architecture (PTA), while photomicrograph of group D testes showed mild arrest of spermatogenesis (MAS). However, photomicrograph of group E testes showed sperm bundles (SB) and enhanced spermatogenesis. Photomicrograph of group F testes showed seminiferous tubules with improved spermatogenesis (S).

5. DISCUSSION

This research evaluated the effect of oral co-administration of paraquat and ethanolic extract of *A. cepa* on the testes and testicular parameters in male Wistar rat model. Results we obtained showed that co-administration of paraquat and *A. cepa* had no significant effects on the relative testicular weights of the experimental animals. However, oral administration of paraquat caused significant reductions in sperm parameters (count, motility and morphology), sera testosterone levels, as well as marked alterations in the testicular histoarchitecture. Paraquat also caused a significant increase in MDA levels and significant reduction in superoxide dismutase (SOD) levels. Interestingly, co-administration with *A. cepa* extract negated the effect of the paraquat in a dose-dependent manner, bringing all parameters investigated to control or near control levels following co-administration with 1000mg/kg of ethanolic extract of *A. cepa*. Both farmers and laborers use paraquat as a multi-purpose contact herbicide. However, it is also really harmful to animals and human beings upon contact, causing varying degrees of injuries. It has been documented to affect the reproductive tract in both males and females, causing deleterious effects that range from reduced production of sex hormones to loss of germ cells, thus causing infertility.[33,9,11] This deleterious effects of paraquat is detailed via the creation of volumes of reactive oxygen species (ROS) that readily overwhelms the defense systems of the experimental animals and produces oxidative stress.[3,11] One of the fallouts of oxidative stress is tissue injury. We observed oxidative stress in the testes of experimental animals used in this research because MDA levels were higher ($p< 0.05$), with the activity of superoxide dismutase significantly decreased in homogenates of the testes of experimental animals that received paraquat alone. MDA is an oxidative stress maker formed as a secondary product during lipid peroxidation.[34] Lipid peroxidation is a biochemical process where oxidants (free radicals) attack and breakdown lipids resulting in the formation of lipid peroxyl radicals and hydroperoxides.[35] Oxidative stress causes alterations and damages to proteins, lipids, lipoproteins and DNA in tissues[34] which we believe is behind the loss of specific functions of the cellular linings of the seminiferous tubules, the loss of the supporting cell of the seminiferous tubules (Sertoli cells and Leydig cells) and the resultant malfunctioned spermatogenesis and lowered testosterone production observed in this research. Loss of Leydig cells leads to reduced sera levels of testosterone while the loss of Sertoli cells (which usually nourishes and facilitates spermatogenesis) causes a reduction in the number of sperm cells. Paraquat has also been documented to cause cellular apoptosis[36-38] via alterations in the expression of the genes of the Bcl-2 family (Bcl-2, Bcl-X_L, Bax, and Bad) that regulates apoptosis. It does this by promoting the inhibition Bcl-2 and Bcl-X_L while simultaneously increasing the expression of Bax

and Bad genes.[38] The genes Bcl-2 and Bcl-X_L block programmed cell death, while Bax and Bad genes aid progression towards apoptosis.[39] This probably complemented the lipid peroxidation we observed (via higher MDA levels observed) thereby causing cells lining the seminiferous tubules to condense, and fragment as seen in the histology slides of experimental animals that received paraquat alone. *A. cepa* is a vegetable used all over the world to prepare assorted meals and in various herbal remedies. Studies on *A. cepa* (Onion) have reported its safe, effective, and inexpensive use as remedies for numerous health conditions due to its anticarcinogenic properties, antiplatelet activity, antithrombotic activity, antiasthmatic and antibiotic effects.[40,41,42,43] Onions are rich in two chemical groups documented to be beneficial to humans by acting as antioxidants. These are the flavonoid group and the alk(en)yl cysteine sulphoxides (ACSOs) group.[44] Types of flavonoids found in *A. cepa* are the anthocyanins and quercetin that gives varieties of onion their colour. The flavor part of *A. cepa* is produced by the ACSOs. Other phytochemicals present in onion are the thiosulphonates, and the sulphides.[44] The quercetin and anthocyanins (flavonoids) components of onion has been documented to have a powerful radical scavenging activity (RAS), chain-breaking activity, H_2O_2-scavenging and reducing capacity.[44,45] Essential oil of onion (sulfur-containing compounds – allyl propyl disulfide) has also been documented to reduce serum levels of thiobarbituric acid reactive substances (TBARS). TBRARS are formed when MDA (an end product of lipid peroxidation) reacts with thiobarbituric acid (TBA).[46] TBA is an organic compound used as a reagent in assaying malondialdehyde (MDA). It is competently documented that antioxidant components of *A. cepa* do increase glutathione peroxidase activities.[47] This increase in the synthesis of glutathione (GSH), an endogenous tripeptide thiol we believe directly protected the testicular tissues from the deleterious effects of free radicals. The antioxidant property of *A. cepa* was observed in this research as homogenates prepared from the testes of the experimental group treated with *A. cepa* extract expressed an increase in the superoxide dismutase (SOD) levels. SOD is a potent detoxification enzyme that serves as the first line of defense (antioxidant) against reactive oxygen species (ROS).[48] This enzyme catalyses' the detoxification of superoxide radicals and breaks down hydrogen peroxides into harmless molecules[48], consequently rendering the harmful superoxide anion harmless. Insufficiencies in SOD have been fingered in many health conditions that affect the immune system, blood vessels, nervous system and accelerate ageing. [48,49,50] The dose-dependent ability of *A. cepa* to increase the SOD levels in the testis as we observed could be the reason why the functionality of the testes was preserved, via improved testicular architecture, sperm parameters (sperm count, motility and morphology) and sera testosterone levels in all groups co-administered with paraquat and ethanolic extract of *A. cepa*. These findings agree with the works of Helen *et al* [47], El-Soud and Khali[46] and Benkeblia[45] that documented improvements in various tissues following administration of *A. cepa* extracts.

6. CONCLUSION

This present study shows that ethanolic extract of *A. cepa* caused a dose-dependent ameliorative effect on testicular tissues exposed to the deleterious effect of paraquat. While paraquat increased the tissue levels of MDA and induced oxidative stress which led to reductions in the volume of sperm bundles in the seminiferous tubules and sera levels of testosterone as well as increased the percentages of deformed sperm, administration of *A. cepa* produced a dose-dependent protective and curative effect, restoring these parameters to levels closer to normal values. Use of *A. cepa* in our meals is thus highly recommended.

7. AUTHORS CONTRIBUTION STATEMENT

Experimental Design was done by Ofoego Uzozie Chikere, Eze Ejike Daniel, Nweke Elizabeth Obioma, Rabiu Mohammed Karimah and Obiesie Ifechukwu Justicia; Experiments was carried out by Ofoego Uzozie Chikere, Obiesie Ifechukwu Justicia, Mbagwu Ikechukwu Smart and Okafor Emeka Christian. Data analysis was carried out by Mbagwu Ikechukwu Smart, Eze Ejike Daniel and Rabiu Mohammed Karimah. Manuscript preparation and Proof reading was done by all Authors. All authors read and approved the final manuscript as this manuscript represents our honest work.

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9. CONFLICT OF INTEREST

Conflict of interest declared none.

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