WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 5, 2105-2124. Research Article ISSN 2277– 7105

AMELIORATIVE EFFECT OF *AFRAMOMUM MELEGUETA* (ALLIGATOR PEPPER) AGAINST PARAQUAT INDUCED TESTICULAR DAMAGE

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Article Received on
18 March 2020,

Revised on 08 April 2020,
Accepted on 28 April 2020,

DOI: 10.20959/wjpr20205-17442

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ABSTRACT

Aframomum Melegueta is essential in African communities and is well known for its culinary, medicinal and economic values. This study investigated the effect of co-administration of ethanolic extract of *Aframomum Melegueta* seeds (EEAMS) and paraquat on testicular parameters. Thirty male Wistar rats (125-200g) were divided into six groups (A-F) of five rats each. Group A(control) received feed and distilled water. Group B and C received 400mg/kg and 200mg/kg of EEAMS respectively. Group D received 20mg/kg of paraquat. Group E received a co-administration of 20mg/kg of paraquat and 400mg/kg of EEAMS. Group F received a co-administration of 20mg/kg of paraquat and 200mg/kg of EEAMS. Paraquat was administered trice a week while extract was administered once daily via oral route for 4 weeks. The animals were sacrificed 24hours after the last administration. Sera, testes and epididymis were collected. The testes were processed and semen analysis, SOD, MDA and sera testosterone tests carried out. Results showed that epididymal sperm count, motility, morphology and sera testosterone levels were reduced ($p<0.05$) in addition to higher levels of oxidative stress in rats that received paraquat alone. However, all parameters were improved in groups that received a co-administration of paraquat and EEAMS. Histopathological investigations of the testes

showed severe spermatogenic arrest and loss of interstitial and sertoli cells in rats that received paraquat alone. However co-treatment of animals with EEAMS showed ameliorative outcomes. Ethanolic seed extract of *Aframomum Melegueta* thus has a dose dependent ameliorative effects on testes following exposure to reactive oxygen species produced by paraquat.

KEYWORDS: *Aframomum Melegueta*, paraquat, testes, testosterone.

INTRODUCTION

Paraquat is an organic compound widely used as an herbicide. It acts on green plants by inhibiting photosynthesis while producing destructive reactive oxygen species at the same time. These actions are quick and non-selectively, effectively killing green plant tissue upon contact.[1] Paraquat exposure is not only toxic to plants but also adversely affects humans and farm animals by accumulating in the lungs, liver, kidneys and heart.[2] Animal exposure occurs by accidental or intentional swallowing, through contact with damaged skin or inhalation.[3] Studies on paraquat have shown that chronic exposure to humans can lead to lung and liver damage, kidney failure and parkinsonian lesions.[3] Paraquat toxicity occurs through excess production of free radicals which results in oxidative stress and tissue damage.[4] Reports have shown its toxic effects on both humans and experimental animals by its contribution to the development of neurological conditions,[5] as well as its effects on the reproductive system.[6,7] Testicular damage has also been reported to be associated with paraquat.[8] Anti-androgens like paraquat exert their anti-infertility effect by their action on the hypothalamus-pituitary-gonadal axis or direct hormonal effect on reproductive organs resulting in the inhibition of spermatogenesis.[9]

In recent years, the use of herbal products for therapeutic purposes have proved helpful in restoring normalcy to diseased tissues especially in countries with low income where access to costly drugs is limited,[10,11,12] although consumption of some in large quantities have been reported to be toxic.[13,14] *Aframomum melegueta* (Alligator pepper) is a tropical herbaceous perennial plant of the genus *Aframomum,* belonging to the family *Zingiberaceae* (ginger family).[15] It is widely grown across tropical Africa including Nigeria, Liberia, Sierra Leone, Ghana, Cameroon, Cote D`Ivoire and Togo.[15] It has long been used as a medicinal plant. Its seed is a rich source of essential oils such as gingerol (shagol and paradol) and it owes its pungency to these. It has equally been shown to contain alkaloids (piperine), and resins.[16] It is rich in fibers, the reason behind the fast regeneration of tissues and in wound healing.[17,18]

In addition to these, it has a high amount of tannin employed in the treatment of burns, wounds and pains in inflamed mucus.[19] Its medicinal uses also includes its use as an aphrodisiac, in measles and leprosy management, for regulating lactation and post-partum hemorrhage, as a purgative, and as an anthelminthic and hemostatic agent.[20] The seeds of *Aframomum melegueta* are also used for managing abdominal discomfort, as a carminative, for stomach ache[21] and as a stimulant, used in veterinary preparations.[22]

Cases of infertility in humans have been on the rise[23] and substantially contributes towards increased reports of family problems and marriage breakups. Male infertility in human's accounts for about 40-50% of infertility cases[24] and is commonly due to deficiencies in the semen.[25,26,27,28] Paraquat, an anti-androgen widely used by both subsistent and commercial farmers as a herbicide, has the ability to induce testicular damage.[9] This situation has shown a lot of difficulty to manage. The use of plant extracts rich in phytochemicals have however been used to provide a variety of soothing effects.[29] Limited literature exist on the effect of ethanolic extract of *Aframomum melegueta* seed on paraquat induced testicular damage hence the need to carry out this research aimed at investigating the effect of co-administration of ethanolic extract of *Aframomum melegueta* (Alligator pepper) seed and paraquat on the testes of adult male Wistar rats.

MATERIALS AND METHODS

Location and Duration of Research

This research was carried out in the Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University Nnewi, Anambra State. It lasted for 4 weeks (28 days).

Procurement, Housing and Management of Animals

Sixty growing male Wistar rats weighing between 125-200g were used as experimental animals in this study. They were procured from a private farm in Otolo Nnewi, Anambra State, Nigeria. These animals were kept in the research section of the Animal House of the Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus and allowed to acclimatize for a period of two weeks. They were housed in well ventilated stainless steel standard rat cages under room temperature (27-31^{o}C) throughout the course of the research. They were fed with standard rat feed (Top vital feed, Eastern Premier Feed Mills Ltd) and distilled water. The bedding of the cages was clean sawdust. The cages were cleaned every day to maintain a healthy environment.

Procurement of *Aframomum Melegueta* (Alligator pepper) fruits and Preparation of Ethanolic Extract of *Aframomum Melegueta* seed

Dry Alligator pepper fruits (pods) were procured from Nkwo Nnewi market in Nnewi, Anambra State, Nigeria and authenticated in the Department of Botany, Nnamdi Azikiwe University Awka, Anambra State. The seeds were removed and shed dried. The dried seeds were ground using a local grinder into a coarse form. 250g of the ground seeds was soaked in 1000mls of 98% ethanol (BDH England) for 48 hours after which it was sieved using porcelain cloth and further filtered using Whatman No. 1 filter paper into a clean glass beaker. The filtrate was concentrated using digital rotary evaporator (TT-S2 Techmel & Techmel USA) and was further dried using thermostat oven (DHG-9023A PEC medicals USA) into a semi-solid substance and was stored in a refrigerator (Nexus) at 4^{o}C.

Acute Toxicity Study for Ethanolic Extract of *Aframomum Melegueta* Seed

The median lethal dose (LD_{50}) test for ethanolic extract of *Aframomum Melegueta* seed was carried out in the Physiology laboratory of the Department of Human Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus. This was determined using the method as described by Lorke[30] with slight modification. In this study which was carried out in two phases, 13 rats were used. They received graded doses of the extract via oral route. LD_{50} of ethanolic extract of *Aframomum Melegueta* seed was found to be above 5000mg/kg.

Phytochemical analysis for ethanolic seed extract of *Aframomum Melegueta*

Phytochemical screening for ethanolic extract of *Aframomum Melegueta* seed was carried out using the method as described by Okwu.[31]

Procurement of Paraquat and Its Acute Toxicity Study

Paraquat in the form of Paraquat dichloride solution (128g/l) was purchased from the agro-allied section of New Market Owerri, Imo state, Nigeria. The median lethal dose (LD_{50}) test for paraquat was carried out in the Physiology laboratory of the Department of Human Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus. This was determined using the method as described by Lorke[30] with slight modification. In this study which was carried out in two phases, 13 rats were used. They received graded doses of paraquat via oral route. LD_{50} of paraquat via oral route was found to be 50mg/kg.

Experimental Design and Protocol

Thirty male albino Wistar rats from the acclimatized group were shared into six (A-F) groups of five animals each. Group A served as the control. They were fed with normal rat feed and distilled water *ad libitum* throughout the experiment. Group B received oral administration of 400mg/kg of ethanolic extract of *Aframomum Melegueta* seed for the entire duration. Group C received oral administration of 200mg/kg of ethanolic extract of *Aframomum Melegueta* seed for the entire duration. Group D received oral administration of paraquat twice a week (Mondays and Fridays) for 4 weeks. Group E received a co-administration of 20mg/kg of paraquat and 400mg/kg of ethanolic extract of *Aframomum Melegueta* seed for 4 weeks, while Group F received a co-administration of 20mg/kg of paraquat and 200mg/kg of ethanolic extract of *Aframomum Melegueta* seed for 4 weeks. All extracts were administered once a day throughout the duration of the experiment while all paraquat administrations were twice a week (Mondays and Fridays) for 4 weeks. The dosage of paraquat used in this study was established following comparative study of previous literature from scholarly works.[8,32,33] The animals were weighed once every week using weighing balance (Camry Model: J1103759). This experiment was carried out with strict compliance to the guide for the care and use of laboratory animals.[34] This experiment was a sub-acute test and lasted for 4 weeks.

Termination of Experiment and Sample Collection

Twenty-four hours after the last administration, the animals were anesthetized using chloroform vapour and blood samples collected through ocular puncture and stored in plain specimen bottles. Sera was collected after centrifuging these blood samples and used for testosterone hormone assay.

After collection of blood samples, the animals were further euthanized with chloroform vapour and sacrificed via cervical dislocation. A midline abdominal incision was immediately made and their testes together with the epididymis were quickly excised, weighed and fixed in freshly prepared Bouin's fluid.

Estimation of Oxidative Stress Level

The preparation for estimating oxidative stress in the testes was carried out as described by Balahoroğlu *et al*.[35] Malondialdehyde (MDA) was evaluated using the method as described by Wasowich *et al*,[36] while superoxide dismutase (SOD) tests were carried out using the method as described by Sun *et al*.[37]

Spermatological Studies

Semen Collection

The epididymis was separated after retrieval of testis from the scrotum. The epididymal fluid (semen) was collected from the caudal part for assessment of sperm count, motility and morphology.

Sperm Count Assessment

Investigations on sperm count was carried out using the method as described by World Health Organization[38] with modifications. 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 transfer the well mixed diluted semen into an improved Neubauer ruled chamber and allowed to stand for 3-5 minutes. The 10x objective of a light microscope (Olympus XS2-107BN, Japan) was used to view and the number of spermatozoa in 2 large squares (2 sq. mm) of the Neubauer ruled chamber counted. The number counted was multiplied by 10^6.

Assessment of Sperm Motility

Investigation on sperm motility was carried out using the method as described by World Health Organization[38] with modification. A well-mixed drop of liquefied sperm was placed on a slide evenly distributed and covered with a cover slide. The 40x objective of a light microscope (Olympus XS2-107BN, Japan) was used to observe and count. A total of 100 spermatozoa were counted out of which the number of motile ones were recorded in percentage.

Assessment of Sperm Morphology

Investigation on sperm morphology was carried out using the method as described by World Health Organization[38] with modification. A thin smear of liquefied well-mixed semen was made on a slide. While still wet, the smear was fixed in 10% diluted neutral buffered formalin with the ratio 1:20 and with the aid of light microscope (Olympus XS2-107BN, Japan) at x400 magnification. The smear was counterstained with dilute (1 in 20) Loeffle's methylene blue for 2 minutes and washed off with water. The smear was drained and allowed to air-dry. The smear was examined for normal spermatozoa using 40x objective. A count of 100 spermatozoa was made and the percentage showing normal morphology was deduced. Morphological changes were clear under the power of x400.

Histopathological Evaluation

The fixed testes were processed at the Histology section of the Department of Anatomy, Faculty of Basic Medical Science, Nnamdi Azikiwe University, Nnewi Campus. This histological method of processing tissue involved various stages of preparing, cutting, staining and examination of slides for histological report. Slides were photographed using a photomicroscope (Olympus, Japan).

Statistical Analysis

Raw data obtained such as body weights, relative organ weights and testicular parameters were analyzed using the SPSS (version 21) software package. All the results obtained were expressed as Mean value ± SEM in each group. All the tested parameters were subjected to statistical analysis using Paired T-test and the one-way analysis of Variance (ANOVA). Differences between means were regarded significant at $P<0.05$.

RESULT

Phytochemical Findings

The phytochemical analysis of ethanolic extract of *Aframomum Melegueta* seed revealed the presence of tannin, saponin, flavonoid, steroid, terpenoids, cardiac glycoside, alkaloid and phenol as shown in table 1.1.

Table 1.1: Phytochemical present in Ethanolic extract of *Aframomum Melegueta* seed.

Constituents	Results
Tannin	+
Saponin	+
Flavonoid	+
Steroid	+
Terpernoids	+
Cardiac glycoside	+
Alkaloid	+
Phlobatannis	-

+ = present

- = not present

Physical Observations of Experimental Animals

Experimental animals were apparently normal within the two weeks of acclimatization. However, during the experiment proper, difficulty in breathing and loss of appetite was evident in the group that received paraquat only. The groups receiving alligator pepper alone or in combination with paraquat were apparently normal and fed well.

Effect of Co-Administration of Ethanolic Extract of *Aframomum Melegueta* seed and Paraquat on Body Weight of Adult Male Albino Rats

During the course of this experiment, the weight of the experimental animals were taken on a weekly basis. The values obtained showed that there was significant difference in the body weights of the experimental animals when comparing the initial mean body weights to their mean final body weights in all the groups as shown in table 1.2.

Results also showed that there was a significant decrease in weight differences in group D (that received paraquat alone) when compared with group A (control) as shown in table 1.3.

Table 1.2: Body Weight Analysis (g).

Groups	Initial Body Weight (g) Mean ± SEM	Final Body Weight (g) Mean ± SEM	p-value	T-test
A	162.50 ± 17.68	217.50 ± 10.61	0.058	-11.00
B	167.67 ± 2.89	216.67 ± 14.43	0.057	-4.000
C	168.33 ± 7.64	208.33 ± 14.43	0.044*	-4.619
D	208.33 ± 14.43	186.67 ± 11.65	0.128	1.664
E	185.00 ± 13.23	225.00 ± 25.00	0.057	-4.000
F	167.67 ± 2.89	208.33 ± 14.43	0.076	-3.413

Table 1.3: Weight difference Analysis (g).

Groups	Weight difference (g) Mean ± SEM	p-value	F-value
A	55.00 ± 7.07		6.914
B	40.00 ± 17.32	1.000	
C	40.00 ± 15.00	1.000	
D	-21.67 ± 22.55	0.007*	
E	40.00 ± 17.32	1.000	
F	31.67 ± 16.07	1.000	

Effect of Co-Administration of Ethanolic Extract of *Aframomum Melegueta* seed and Paraquat on Relative Testicular Weight of Adult Male Albino Wistar Rats

Results obtained from this study showed that there was no significant difference in relative testicular weights in the test groups when compared with group A (control) as shown in table 1.4.

Table 1.4: Effect of co-administration of Ethanolic extract of *Aframomum Melegueta* (Alligator Pepper) seed and paraquat on the relative testicular weight of adult male albino wistar rats.

Groups	Relative testicular weight (g) Mean ± SEM	p-value	f-value
A	1.69 ± 0.31		1.947
B	1.50 ± 0.07	1.000	
C	1.41 ± 0.07	1.000	
D	1.60 ± 0.12	1.000	
E	1.80 ± 0.30	1.000	
F	1.46 ± 0.15	1.000	

Effect of Co-Administration of Ethanolic Extract of *Aframomum Melegueta* (Alligator Pepper) seed and Paraquat on Serum Testosterone Level of Adult Male Albino Wistar Rats

Result obtained from this study and presented in table 1.5 showed that there was significantly lower sera testosterone levels in group D when compared to the Group A (control).

Table 1.5: Effect of co-administration of Ethanolic extract of *Aframomum Melegueta* (Alligator Pepper) seed and paraquat on serum testosterone levels of adult male albino wistar rats.

Groups	Serum testosterone (ng/ml) Mean ± SEM	p-value	f-value
A	5.76 ± 1.20		4.695
B	3.81 ± 1.53	1.000	
C	4.28 ± 1.71	1.000	
D	1.69 ± 0.60	0.030*	
E	3.43 ± 0.29	0.617	
F	2.00 ± 0.54	0.050	

Effect of Co-Administration of Ethanolic Extract of *Aframomum Melegueta* (Alligator Pepper) and Paraquat on Oxidative Stress Level (evaluated by the use of MDA marker) of Adult Male Albino Wistar Rats

Result obtained from this study and presented in table 1.6 showed insignificant higher oxidative stress levels in all test groups when compared with group A. Group D which received 20mg/kg of paraquat had the highest oxidative stress level when compared with group A.

Table 1.6: Effect of co-administration of Ethanolic extract of *Aframomum Melegueta* (Alligator Pepper) seed and paraquat on oxidative stress level of adult male albino wistar rats.

Groups	MDA (nmol/g wet tissue) Mean ± SEM	p-value	f-value
A	1.07 ± 0.03		3.697
B	1.07 ± 0.15	1.000	
C	1.84 ± 0.69	0.972	
D	2.31 ± 0.36	0.110	
E	1.73 ± 0.54	1.000	
F	1.76 ± 0.14	1.000	

Effect of Co-Administration of Ethanolic Extract of *Aframomum Melegueta* (Alligator Pepper) seed and Paraquat on Anti-Oxidative Stress Level (evaluated by the use of SOD marker) of Adult Male Albino Wistar Rats

Result obtained from this study and presented in table 1.7 showed that there was significant lower anti-oxidative stress level in group D when compared to group A. Group D which received 20mg/kg of paraquat had the lowest anti-oxidative stress level.

Table 1.7: Effect co-administration of Ethanolic extract of *Aframomum Melegueta* (Alligator Pepper) seed and paraquat on anti-oxidative stress level of adult male albino wistar rats.

Group	SOD (u/ml) Mean ± SEM	p-value	f-value
Group A	17.71 ± 1.61		4.697
Group B	17.75 ± 1.44	1.000	
Group C	17.90 ± 3.36	1.000	
Group D	5.78 ± 0.77	0.000*	
Group E	12.92 ± 2.12	0.105	
Group F	11.92 ± 2.12	0.099	

Effect of Co-Administration of Ethanolic Extract of *Aframomum Melegueta* (Alligator Pepper) seed and Paraquat on Sperm Count of Adult Male Albino Wistar Rats

Result obtained from this study and presented in table 1.8 showed that there was a significantly lower sperm count value in group D and group F when compared with group A (control group).

Table 1.8: Effect co-administration of Ethanolic extract of *Aframomum Melegueta* (Alligator Pepper) seed and paraquat on sperm count of adult male albino wistar rats.

Groups	Sperm count (10^6) Mean ± SEM	p-value	f-value
A	51.00 ± 1.53		11.583
B	42.00 ± 2.31	0.144	
C	49.00 ± 3.79	0.733	
D	14.33 ± 2.33	0.000*	
E	41.67 ± 5.84	0.131	
F	29.67 ± 6.06	0.000*	

Effect of Co-Administration of Ethanolic Extract of *Aframomum Melegueta* (Alligator Pepper) seed and Paraquat on Sperm Motility of Adult Male Albino Wistar Rats

Result obtained from this study and presented in table 1.9 showed that there was a significantly lower percentage levels of sperm motility in groups D, E and F when compared with the group A (control).

Table 1.9: Effect co-administration of Ethanolic extract of *Aframomum Melegueta* (Alligator Pepper) seed and paraquat on sperm motility of adult male albino wistar rats.

Groups	Sperm motility (%) Mean ± SEM	p-value	f-value
A	78.67 ± 1.76		20.622
B	71.67 ± 4.41	0.070	
C	73.00 ± 2.08	0.142	
D	51.00 ± 0.58	0.000*	
E	64.33 ± 2.85	0.000*	
F	52.67 ± 1.45	0.000*	

Effect of Co-Administration of Ethanolic Extract of *Aframomum Melegueta* (Alligator Pepper) seed and Paraquat on Sperm Morphology of Adult Male Albino Wistar Rats

Result obtained from this study and presented in table 1.10 showed that there was significantly lower percentages of normal sperm in groups D and F when compared with the control group (group A).

Table 1.10: Effect co-administration of Ethanolic extract of *Aframomum Melegueta* (Alligator Pepper) seed and paraquat on sperm morphology of adult male albino wistar rats. (Normal sperm).

Groups	Sperm morphology (%) Mean ± SEM	p-value	f-value
A	78.67 ± 2.03		10.632
B	74.33 ± 3.84	0.201	
C	79.33 ± 2.33	0.842	
D	61.00 ± 0.58	0.000*	
E	72.33 ± 1.45	0.074	
F	65.67 ± 1.76	0.000*	

Histopathological Findings

Plate 1: Testis of group A (control) H&E (x400) shows photomicrograph of group 1 (control) with normal testicular architecture and seminiferous tubules lined with Sertoli cell (SC) and well enhanced spermatogenesis (WES).

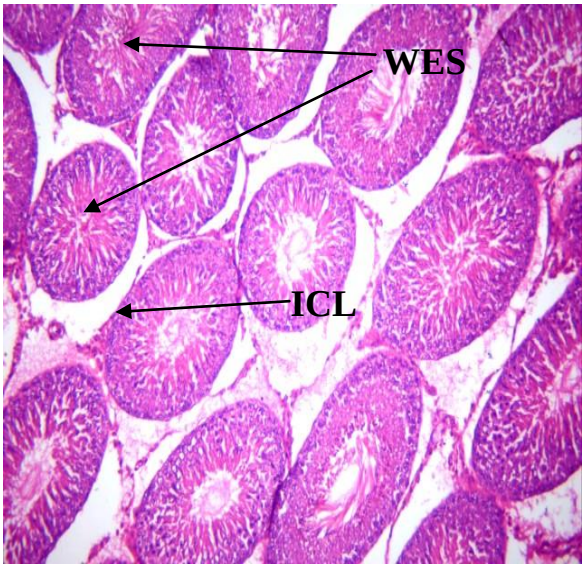

Plate 2: Testes of Group B H&E (x400) shows photomicrograph of group B showing testicular tissue with seminiferous tubules lined with interstitial cells of the Leydig (ICL) well enhanced spermatogenesis (WES).

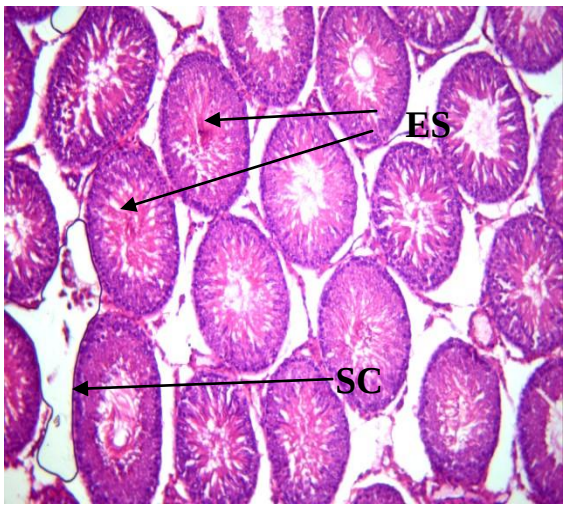

Plate 3: Testes of Group C (H&E) (x400) shows photomicrograph with normal testicular tissue, Sertoli cells (SC) and enhanced spermatogenesis (ES).

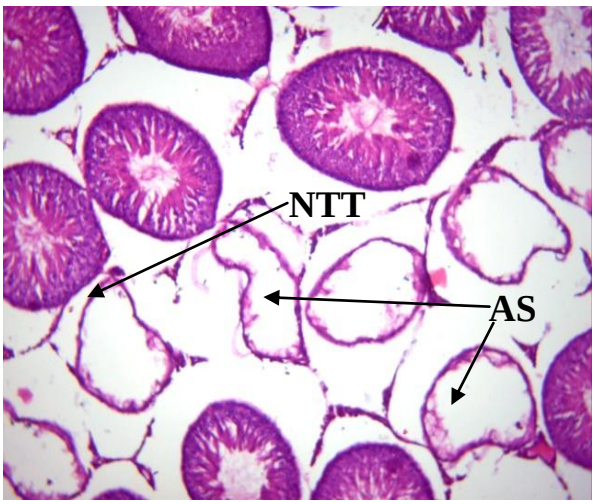

Plate 4: Testes of Group D (H&E) (x400) shows photomicrograph severe effect on the testicular tissue with arrest of spermatogenesis (AS), necrosis of testicular tissue (NTT).

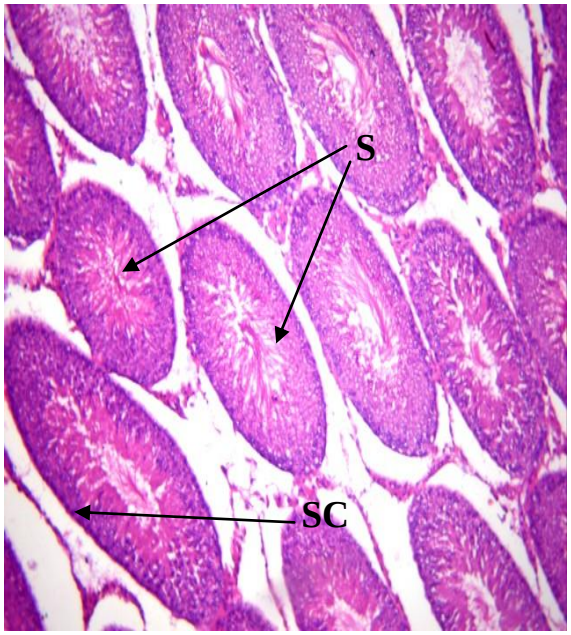

Plate 5: Testis of group E (H&E) (x400) shows photomicrograph apparently normal testicular tissue with Sertoli cells (SC) and spermatogenesis (S). The overall feature are consistence with normal testicular tissue.

Plate 6: Testis of Group F (H&E) (x400) shows photomicrograph with moderate spermatogenesis (MS) and testicular tissue lined with interstitial cells of Leydig (ICL).

DISCUSSION

Herbicides such as paraquat affects the male reproductive system at sites such as testes, accessory sex glands and to a large extent, the central nervous system including the neuroendocrine system.[39,8] Studies on paraquat have shown its deleterious effects on the reproductive system of males.[6,7,8]

The use of the *Aframomum Melegueta* (Alligator pepper) seed in medicinal concoctions has been in use especially in rural areas where there is limited access to affordable modern medicine. Medicinal applications of *Aframomum Melegueta* seed abound and have been reported.[20,21,22]

Result from this study on body weight revealed that all experimental groups had increases in body weight with the exception of the group that received 20mg/kg of paraquat alone that showed a significant loss of weight when compared with Control. The reason for the increase in the Control group may be physiological due to the high fiber and carbohydrate content in the rat feed. The reason for the increase in Groups B, C, E and F may also be physiological due to the high fiber and carbohydrate content in the rat feed in addition to the alligator pepper extract which is also rich in fiber. This is in agreement with the findings of Obike *et al*.[40] which reported body weight gain in experimental animals that received *Aframomum Melegueta* extracts. However, weight loss in group D that received 20mg/kg of paraquat alone might be due to the effect of paraquat being able to cause amongst other things loss of appetite and cell death by free radicals induced by Nicotinamide Adenine Dinucleotide Phosphate (NADPH) depletion.[1]

Findings from this study showed that there was no significant difference in relative testicular weight in all the test groups when compared to the control group.

Investigations on serum testosterone levels in the current study showed that serum testosterone level was significantly lower in group D which received 20mg/kg of paraquat. There were however higher levels of serum testosterone in all the other groups when compared to Group D. The lower testosterone levels observed in group D might be as a result of the deleterious effect of paraquat which produces volumes of free radicals that reacts with macromolecules to produce reactive oxygen species (ROS) that cause damage to various organs including the testis and also inhibits biochemical activities such as enzyme and hormone synthesis. This is in agreement with the finding of Ofoego *et al*.[8] In their study,

paraquat decreased serum testosterone levels significantly. The higher testosterone levels observed in groups that received a co-administration of paraquat and graded doses of *Aframomum Melegueta* seed extract might be as a result of the phytochemicals present in *Aframomum Melegueta* seed extract especially niacin which has been documented to improve testicular parameters and increase serum testosterone levels.[41] This is achieved by possibly inhibiting the oxidative effect of paraquat, terminating the chain reaction which could lead to the production of free radicals.[42] This too is in agreement with the works of Mbongue *et al.*[41] and Akpanabiatu *et al.*[43] In their respective works, *Aframomum Melegueta* extracts caused a significant increase in serum testosterone levels of experimental animals.

This current study also revealed that there was higher levels of oxidative stress as evaluated by the use of MDA marker in group D that received 20mg/kg of paraquat when compared with the group A (control). Group B that received 400mg/kg of the extract alone had an oxidative stress level almost similar to the control group. Comparing the groups that received doses of both paraquat and Alligator pepper extract with the control group, the oxidative stress level was higher although not as high as the Group D that received paraquat alone. This high level of oxidative stress in Group D could be due to the fact that paraquat has the ability to produce destructive reactive oxygen species.[1]

For the anti-oxidative stress levels as evaluated by the use of SOD marker in this current research, there was a significant lower level in Group D that received 20mg/kg of paraquat when compared to the group A (control). There was however insignificant differences in levels of SOD in other experimental groups when compared to Control. This shows that the Alligator pepper seed extract had a cushioning effect on the stress levels which was induced by paraquat in Groups E and F, due to the presence of phytochemicals abundant in the extract particularly flavonoids and phenols which are antioxidants. This result is also in agreement with that of Oyinloye *et al.*[44] who documented that treatment of experimental animals with *Aframomum Melegueta* showed a significant reversal effect on their oxidative stress levels induced by cadmium administration.

Findings from this research also showed that administration of paraquat alone caused a significant reduction in sperm parameters (count, motility and morphology) when compared with that of control group. Paraquat produces destructive and harmful radicals that have been documented to cause deleterious effects on reproductive parameters.[9,8] This is in agreement with work done by D'Souza[6] and Ofoego *et al.*[8] who reported reduction in sperm

parameters (count, motility and morphology) of experimental animals after exposure to paraquat. The cause for the reduced sperm parameters in group F might be due to the overwhelming deleterious effect of paraquat despite the administration of 200mg/kg of the seed extract of *Aframomum Melegueta* which seemed to have a less potent cushioning effect. The dose dependent potency of *Aframomum Melegueta* seed extract was evidenced in the group that received a co-administration of paraquat and 400mg/kg of the seed extract of *Aframomum Melegueta* where higher values in testicular parameters similar to the Control group was documented.

Histopathological results obtained from this study revealed varying degrees of testicular histo-architectural alterations such as arrest of spermatogenesis, focal loss of both Interstitial cells of Leydig and sertoli cells in group D that received 20mg/kg of paraquat. This result agrees with that of Ofoego *et al*.[8] and D'Souza *et al*,[6] where administration of paraquat caused necrotic testicular tissues with testicular atrophy. The groups treated with only ethanolic extract of *Aframomum Melegueta* seed showed testicular tissue with seminiferous tubules lined with interstitial cell of Leydig, sertoli cells and enhanced spermatogenesis. This shows that the extract has the ability of enhancing spermatogenesis. Findings from group 5 that was received a co-administration of 20mg/kg of paraquat and 400mg/kg of the extract and group 6 that received a co-administration of 20mg/kg of paraquat and 200mg/kg of the extract showed testicular tissue with apparently normal testicular architecture and enhanced spermatogenesis. This shows that *Aframomum Melegueta* seed extract has to a great extent a dose dependent ameliorative effect on paraquat induced testicular damage. This may be attributed to the antioxidant activity of the constituents which could have conferred an ameliorating effect on the testes, cancelling out the effects deleterious effects of paraquat.

CONCLUSION

Results from this study shows that ethanolic extract of *Aframomum Melegueta* (Alligator pepper) seed has dose dependent ameliorative effect on the testes following testicular exposure to reactive oxygen species produced by oral ingestion of paraquat.

ACKNOWLEDGEMENTS

We wish to express our appreciation to Mr Ezekafor E. of the Department of Human Physhiology, and Mr Ubammadu CE of the Department of Medicine both of Nnamdi Azikiwe University, Nnewi Campus for their help in animal handling and statistical analysis respectively.

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