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Original Research Article

Allium cepa ameliorates calcium carbide induced reproductive toxicity in male Wistar rats

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ABSTRACT

Calcium carbide, a popular substance used in commercial ripening of fruits has been shown to have deleterious effects on reproduction. The protective antioxidant and anti-inflammatory properties present in *Allium cepa* (*AC*) were harnessed in this study to counter the adverse effects of calcium carbide (CC) on the reproductive potentials of male albino wistar rats. Thirty sexually mature male albino rats were randomly divided into six groups (I, II, III, IV, V and VI) of five rats each. Group I (negative control) was administered distilled water. Group II received CC (0.25mg of calcium carbide per 20g of feed per rat). Group III received *AC* (100mg/kg) only, while the treatment Groups IV, V and VI received CC(0.25mg) + *AC* (100mg/kg), CC (0.25mg) + *AC* (200mg/kg) and CC (0.25mg) + *AC* (400mg/kg), respectively. Following administration, the animals were assessed for sexual behaviour (libido), antioxidant effects, gonadosomatic index, semen characteristics and testicular histomorphology. The result obtained revealed improved reproductive traits including libido, semen characteristics ($p<0.05$), remarkable anti-oxidative properties and gonadosomatic indices ($p<0.05$) in groups III, V and VI when compared to group II. The testicular histomorphology image showed ameliorative activities of *A. cepa* (V and VI) on the degenerative activities of calcium carbide observed in II. This study suggests, therefore, that *A.cepa* has an ameliorative effect on calcium carbide-induced reproductive toxicity in a dose dependent manner.

Keywords: *Allium cepa, calcium carbide, semen, gonadosomatic, libido*

INTRODUCTION

In recent years, the constant exposure of humans and animals to environmental contaminants has become a major public health concern. This has been attributed mainly to man's adaptation to certain 'trending' modern lifestyle and major global industrial revolution among other factors (Ng, 2014).These toxicants are not just harmful to the body in general but may offset the fine tuning of biochemical reactions that regulate the normal functioning of the reproductive system culminating in infertility (Skinner *et al.*, 2010).

Calcium carbide, an artificial fruit ripening agent which is gradually gaining wide acceptance by commercial fruit sellers here in Nigeria (Essien *et al.*,2018) and some other parts of the world (Siddqui & Dua,2010; Ur-Rahman *et al*.,2008), has been shown to have negative reproductive implications in both males (Ogbuagu and Oritsematosan, 2016) and females (Enitome *et al*., 2019).

Allium cepa, a common vegetable consumed daily in almost every household has great therapeutic effect (Benmalek *et al*., 2013) and has been confirmed to possess antioxidant properties (AshwiniSathishkumar,2014).This study therefore aims to investigate the effect of *A. cepa* on calcium carbide induced reproductive toxicity in male albino Wistar rats.

MATERIALS AND METHODS

STUDY LOCATION

This study was carried out at the Theriogenology and Veterinary Physiology laboratories of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

EXPERIMENTAL ANIMALS AND THEIR MANAGEMENT

Forty two males and six female Wistar rats aged eleven weeks and weighing between 104 and 120 g obtained from

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the Department of Physiology and Pharmacology, Michael Okpara University of Agriculture Umudike were used for the study. The animals were fed daily on poultry feed (finisher) containing 30% protein; water was given *ad libitum*. They were acclimatized for 2 weeks before the commencement of the study and were kept in well-ventilated aluminium cages at room temperature and under natural light/darkness cycles. They were maintained in accordance with the recommendation of the *Guide for the care and use of laboratory animals* (DHHS,1985)

CALCIUM CARBIDE (*CC*)

Calcium carbide was purchased from a welding shop at Ohiya Mechanic Village, Umuahia South Local Government, Abia State.

PURCHASE AND PREPARATION OF *ALLIUM CEPA* (*AC*) EXTRACT

Extraction of *A.cepa* was done following the procedures from previous studies. The extraction was also done in batches. Briefly, fresh *A.cepa* bulbs obtained from a local market were rinsed thoroughly in distilled water, air dried and the juice extracted using an electrically operated juice extractor. The juice was further sieved with a sieve cloth and the concentration of each batch of juice extracted was determined. Fresh *A. cepa* juice was prepared on weekly basis following the same procedure and kept at 4^{o}C to prevent it from losing its potency.

DETERMINATION OF THE CONCENTRATION OF *ALLIUM CEPA* (*AC*) EXTRACT

The concentration of each batch of onion juice extracted was determined by weighing 3 empty crucibles after which 5mL of the extract were measured into each crucible and then concentrated to dryness in a hot air oven. Once dried, the crucibles were reweighed and the later weight subtracted from the former weight and the mean was taken.

ACUTE TOXICITY TEST FOR CALCIUM CARBIDE

Six male albino rats were used. The rats were divided into two groups (A and B) of 3 rats each. The up and down method was used for this acute toxicity test. Group A rats received 2,500mg/kg calcium carbide while Group B rats received 1,000mg/kg calcium carbide. The substance was administered orally in both groups and the rats subsequently observed for 72 hours for behavioural changes, signs of toxicity and mortality.

ACUTE ORAL TOXICITY TEST FOR *ALLIUM CEPA*

Prior to acute oral toxicity test, the concentration of the *A cepa* (onion) juice was determined. Thereafter, six male albino rats were used for the test. The rats were divided into two groups (A and B) of three rats each. The up and down method was used for this acute toxicity test. Group A rats received 2000mg/kg of *A. cepa* while Group B rats received 5ml/kg of distilled water orally. The animals were observed for 72 hours for behavioural changes, signs of toxicity and mortality.

EXPERIMENTAL DESIGN

After the period of acclimatization, the animals were randomly selected and divided into six groups (I, II, III, IV, V and VI) with each group comprising five (5) Wistar rats. The carbide was mixed with feed while the onion juice was drenched orally.

Table 1: Experimental design

Group	Treatment	Dose
I	Distilled water (Control)	5ml/kg
II	Carbide only (***CC***)	0.25g in 100g of feed
III	*Allium cepa* only (***AC*$_1$**)	100mg/kg of *Allium cepa*(*AC)*
IV	Carbide + *Allium cepa* (***CC*+*AC*$_1$**)	0.25g of carbide + 100mg/kg of *AC*
V	Carbide + *Allium cepa* (***CC*+*AC*$_2$**)	0.25g of carbide + 200mg/kg of *AC*
VI	Carbide + *Allium cepa* (***CC*+*AC*$_3$**)	0.25g of carbide + 400mg/kg of *AC*

OBSERVATION OF LIBIDO

Libido in the rats was determined by physical observation. This was done by assessing each male's reaction and sexual behaviour or response when a female rat on oestrus was introduced. Based on the reactions, scores were allocated following the scoring pattern described by Chibundu (2005).

DETERMINATION OF ANTIOXIDANT LEVELS

Estimation of reduced glutathione (GSH) in Serum was as described by Ellman (1959), level of superoxide dismutases (SOD) activity in the serum was determined by the method of Sun *et al*. 1988 while the level of thiobarbituric acid reactive substance (TBARS) and malondialdehyde (MDA) production was measured in serum by the modified method as described by Draper et al., (1993). `

SEMEN COLLECTION AND ANALYSIS

At the end of the study the animals were humanely sacrificed by cervical dislocation and their testes carefully excised, trimmed of fat and weighed. The caudal epididymis was dissected and minced in pre-warmed normal saline (37°C). The epididymal washing was used to analyze the semen characteristics for each rat in each group. The macroscopic (colour, consistency, pH)and microscopic (sperm motility, live sperm proportion, sperm concentration and sperm morphology) of the collected semen samples were

evaluatedusing the methods described by Peter (2002) and Comaire *et al.*(1992).

DETERMINATION OF GONADOSOMATIC INDEX (GSI)

The individual live weight of the rats in each group was taken prior to sacrifice and their testicular weights measured. The data obtained was used in calculating the gonadosomatic index for each rat as described by Parmeswaran *et al.,* (1974)

$$GSI = \frac{\text{Paired testicular weight}}{\text{Live weight of the rat}} \times 100$$

TESTICULAR HISTOMORPHOLOGICAL STUDIES.

This was carried out as described by John and Alan, (1977). Testicular tissues of three randomly selected rats from each of the groups were fixed in Bouin's fluid for 6 h and transferred into 10% formalin. They were dehydratedwith varying percentage of ethanol. Sections were cleared in xylene and embedded in molten wax. Thin sectionswere cut (5 μm), stained with hematoxylin and eosin, and microscopically analyzed.

DATA ANALYSIS

Data obtained from the experiment were expressed as Mean ± SEM (standard error of mean). Data obtained were analysed using the Statistical Package for Social Sciences (SPSS) version 20 by one-way Analysis of variance (ANOVA). Multiply comparisons were made with least square difference (LSD) as post hoc test. Values at $P<0.05$ were considered statistically significant.

RESULTS

ACUTE TOXICITY TEST FOR CALCIUM CARBIDE

After 72 hours no mortality was recorded in any of the groups. However, animals in group B (1.0g/kg) were alert, active and and apperantly normal some animals in group A (2.5g/kg CC) appeared dull,were off feed and exhibited nervous signs.

ACUTE TOXICITY TEST FOR *ALLIUM CEPA*

The animals were observed for 72 hours and there was no observable sign of toxicity in the two groups post administration of extracts in both groups. This informed the dosage for the study.

***ALLIUM CEPA* CONCENTRATION**

The concentration of the *A.cepa* determined for each batch prior to administration gave a range of 86 – 100mg/mL

EFFECT OF CALCIUM CARBIDE AND *ALLIUM CEPA* ON LIBIDO

The group administered with *A. cepa* only recorded the highest libido score followed by the calcium carbide inducted groups treated with 200, and 400 mg/kg of the *A. cepa* juice compared with the normal control group, whereas the untreated carbide group recorded the least libido score.

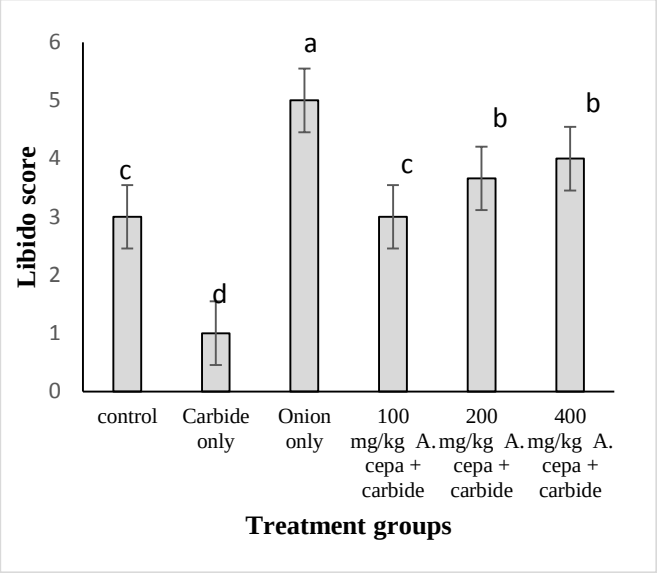

Figure 1: Sexual behaviour (libido) in *Allium cepa (AC)* and Calcium Carbide *(CC)* Treated Male Wistar Rats

EFFECT OF CALCUIM CARBIDE AND *ALLIUM CEPA* ON SEMEN CHARACTERISTICS

The result of the study above is represented in Table 3. The semen parameters such as colour, consistency, progressive motility, spermatozoa live proportion, spermatozoa concentration, were significantly ($p<0.05$) improved in the *A. cepa*-treated groups in a dose-dependent manner compared with the normal control group, whereas, these parameters were significantly ($p<0.05$) reduced in the calcium carbide untreated group. Also the calcium carbide treated experimental rats produced watery semen with significantly increased pH and reduced progressive motility. The treatment also significantly reduced the spermatozoa concentration, with significant increase in number of morphologically abnormal spermatozoa when compared with the control group.

EFFECT OF CALCIUM CARBIDE AND *ALLIUM CEPA* ON SPERM MORPHOLOGY

Calcium carbide administration resulted in a significant ($p<0.05$) increase in sperm morphological abnormalities which were in turn significantly reduced in a dose-dependent manner following the administration of *A. cepa* juice extract. The least significant (1.37±0.35%) reduction in total abnormal spermatozoa obtained was at the dose of 400 mg/kg body weight (Table 3).

Table II: Semen characteristics of *Allium cepa (AC)* and Calcium Carbide *(CC)* treated Male Wistar Rats

TREATMENT GROUP	Control	Carbide only	*A. cepa* only	100 mg/kg *A. cepa*+ Carbide	200 mg/kg *A. cepa*+ Carbide	400 mg/kg *A. cepa*+ carbide
Semen colour (1–2)	1.00±0.00^{b}	1.00±0.00^{b}	1.00±0.00^{b}	1.00±0.00^{b}	1.33±0.33^{b}	2.00±0.00^{a}
Semen consistency (1-4)	3.00±0.00^{b}	1.00±0.00^{d}	4.00±0.00^{a}	2.00±0.00^{c}	2.33±0.33^{c}	4.00±0.00^{a}
Semen pH	6.71±0.02^{c}	7.38±0.03^{a}	6.83±0.05^{b}	6.63±0.00^{c}	6.72±0.05^{c}	6.68±0.00^{c}
Individual sperm cell motility (1-4)	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
Spermatozoa progressive motility (%)	70.30±0.43^{bc}	57.66±1.88^{d}	72.45±2.85^{bc}	67.00±0.62^{c}	76.67±4.97^{ab}	82.94±0.77^{a}
Spermatozoa live proportion (%)	84.70±1.98^{a}	70.13±1.15^{c}	76.70±3.24^{b}	74.95±0.05^{bc}	85.13±2.24^{a}	89.36±0.38^{a}
Spermatozoa concentration ($\times10^{6}$/C.Ep)	119.87±2.85^{a}	81.78±15.35^{b}	129.15±2.30^{a}	109.14±1.77^{a}	119.04±0.50^{a}	121.77±0.59^{a}
Normal spermatozoa proportion (%)	98.03±0.38^{ab}	87.44±0.52^{d}	98.93±0.45^{a}	95.35±0.01^{b}	97.32±0.49^{b}	98.27±0.35^{ab}

Note: Values are presented as mean± S.E. where means on the same row with different superscripts a-e are significantly (P<0.05) different.

Table III: Spermatozoa morphology of *Allium cepa (AC)* and Calcium Carbide *(CC*) Treated Male Wistar Rats

TREATMENT GROUP	Control	Carbide only	*A. Cepa*only	100 mg/kg *A. cepa* + carbide	200 mg/kg *A. cepa* + carbide	400 mg/kg *A. cepa* + carbide
Detached head (%)	0.13±0.06^{d}	1.20±0.01^{a}	0.00±0.00^{d}	0.95±0.08^{b}	0.98±0.00^{b}	0.68±0.13^{c}
Twisted tail (%)	0.10±0.10^{c}	1.01±0.02^{a}	0.00±0.00^{c}	0.38±0.07^{b}	0.10±0.03^{c}	0.09±0.01^{c}
Curled sperm cell (%)	0.05±0.05^{de}	0.98±0.06^{a}	0.01±0.00^{e}	0.48±0.12^{b}	0.34±0.06^{bc}	0.25±0.05^{cd}
Small head proportion to body size (%)	0.09±0.04^{c}	3.04±0.09^{a}	0.00±0.00^{c}	0.60±0.02^{b}	0.52±0.20^{b}	0.30±0.17^{bc}
Bent neck (%)	0.04±0.02^{c}	0.90±0.11^{a}	0.05±0.01^{c}	0.59±0.21^{b}	0.10±0.04^{c}	0.00±0.00^{c}
Proximal cytoplasmic droplets (%)	1.06±0.16^{bc}	3.42±0.55^{a}	0.33±0.10^{cd}	1.25±0.19^{b}	0.73±0.19^{bcd}	0.02±0.01^{d}
Distal droplets (%)	0.48±0.24^{bc}	1.99±0.22^{a}	0.66±0.33^{bc}	0.72±0.10^{b}	0.37±0.11^{bc}	0.01±0.01^{c}
Total abnormal sperm cell (%)	1.97±0.38^{cd}	12.55±0.52^{a}	1.06±0.45^{d}	4.62±0.01^{b}	3.17±0.49^{c}	1.37±0.35^{d}

EFFECT OF CALCIUM CARBIDE AND *A.CEPA* ON SERUM ANTIOXIDANT LEVELS

The result of the antioxidant activity of *A. cepa* as presented in Table 4 showed significant (p<0.05) differences between the serum level of Superoxide Dismutase (SOD), Malondialdhyde (MDA), and glutathione (GSH) between the treated and the untreated groups. All the carbide inducted groups ii, iv, v and vi recorded significant reduction in the SOD and GSH mean values with the least significant (p<0.05) reduction in the untreated carbide group, whereas the mean values of serum SOD and MDA in group iii were comparable with the normal control group. Similarly,

Table IV. Antioxidant Activities in *Allium cepa (AC)* and Calcium Carbide *(CC)* Treated Male Wistar Rats

	SOD	MDA	GSH
Control	1.63±0.01[a]	16.16±0.06[d]	4.98±0.03[b]
Carbide only	0.67±0.05[d]	27.16±0.96[a]	4.08±0.04[e]
***A. cepa* only**	1.71±0.01[a]	16.72±0.24[cd]	5.40±0.12[a]
100 mg/kg *A.cepa* + carbide	0.86±0.03[c]	19.90±0.80[b]	4.60±0.01[d]
200 mg/kg *A.cepa* + carbide	0.97±0.02[c]	19.08±0.24[b]	4.78±0.03[cd]
400 mg/kg *A.cepa* + carbide	1.16±0.09[b]	18.37±0.04[bc]	4.95±0.02[bc]

Note: Values are presented as mean± S.E. where means on the same column with different superscripts a-e are significantly (P<0.05) different

EFFECT OF CALCIUM CARBIDE AND *ALLIUM CEPA* ON GONADOSOMATIC INDEX (GSI)

The highest significant increase in the size of the testes relative to the body weight was obtained in the group administered with 400 mg/kg of the *A. cepa* juice extract compared to the other control groups (I-III). On the other hand, the treatment groups, negative control treated with distilled water and onion only, values were significantly (P <0.05) higher than positive control treated with Carbide only.

EFFECT OF DIFFERENT TREATMENTS ON TESTICULAR HISTOMORPHOLOGY

Photomicrograph of testis of experimental rats in group I (distilled water; Fi3A) showed normal differentiation and good structural organization of spermatogenic cells (arrows) while that of group ii (calcium carbide alone; (Fig 3B) showed multifocal depletion of spermatocytes (arrows) and multifocal degeneration of spermatids (arrow heads), Conversely testis of group III (*A. cepa* only; Fig 3C) showed marked proliferation and differentiation as well as good structural organization of spermatogenic cells (arrows). Concurrent administration of calcium carbide and graded doses (100, 200 & 400mg of *A. cepa* Fi3 D-F) showed a dose dependant regeneration of spermatogenic cells when compared to the normal control (Fig 5A).

DISCUSSION

The testis is the major male reproductive organ, and is responsible for spermatogenesis and hormone (testosterone) production. Libido or sexual behaviour of male animals has a direct relationship with the amount of testosterone and semen quality. In this study, highest and least libido scores were observed in the groups administered with *A. cepa* only and carbide only, respectively while there was a dose-dependent increase of the libido scores within the treated groups. The observed effect may be attributed to testosterone potentiating effect of onion. A previous report (Ahkigbe & Igeh, 2012) that *A. Cepa* has aphrodisiac activity is in agreement with this present study.

In addition, administration of *A. cepa* increased serum antioxidants (superoxide dismutase, glutathione reductase) activities and significantly reduce the level of malondialdehyde in serum of treated rats. Oxidative stress has been implicated in many pathologic conditions and the reproductive organs like testes are not an exception (Sharma *et al.*, 2018). Extent of oxidative damage is ascertained by measuring malondialdehyde (MDA) levels, reactive oxygen species (ROS) generation, alterations in antioxidant defences, and the extent of protein oxidation. *Allium cepa* contains quite an array of phytoconstituents including quercetins anthocyanin and vitamins which help in free radical scavenging and inhibition of oxidases such as

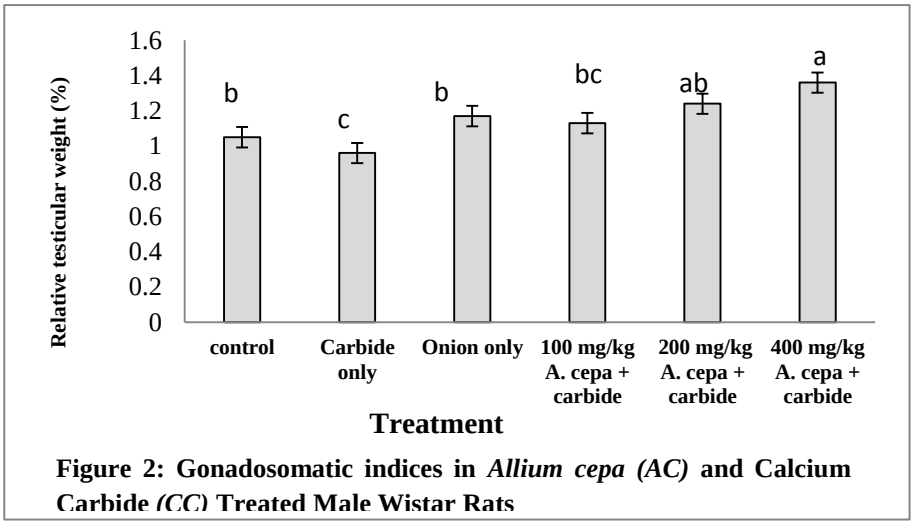

Figure 2: Gonadosomatic indices in *Allium cepa (AC)* and Calcium Carbide *(CC)* Treated Male Wistar Rats

lipoxygenase (Ahmed *et al*., 2017). Quercetin, an important flavonoid, has a beneficial effect on health due to its antioxidant activities as it is involved in scavenging free radicals such as superoxide radicals generated by xanthine/xanthine oxidase. Studies by Mi *et al*. (2007) on the effect of quercetin on oxidative damage in cultured chicken spermatogonial cells showed quercetin to have no deleterious effect on spermatogonial cells at doses of 1 mg/mL and 10 mg/mL.This finding is in agreement with the results of this present study.

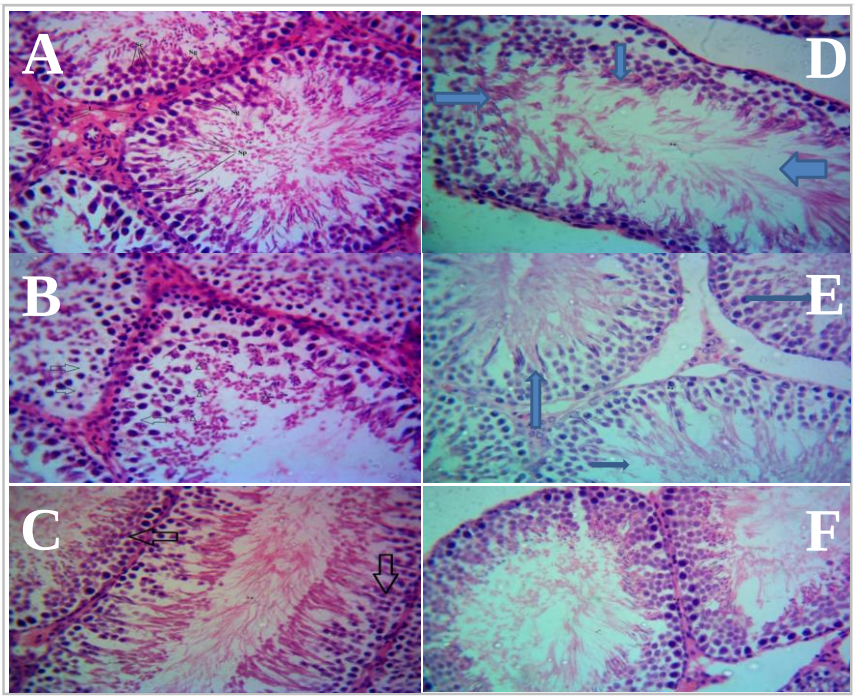

Figure IV. Histomorphology of experimental rats. Fig 3A control distilled water), positive controls Figs 3B and 3C treated with carbide only and *A. cepa* only respectively and Figs3 D-F treated with a combination of calcium carbide & graded doses(100, 200 & 400) of *A. cepa* respectively.

Results of gonadosomatic index (GSI) suggests that carbide has a deleterious effect on the testes as the GSI of the group treated with carbide alone were significantly lower than both the control and the *allium cepa (AC*) treated groups. Interestingly, there was dose dependant mitigation of this deleterious effect such that highest value of GSI was obtained from the group administered with 400 mg/kg of *AC* extract. This is consistent with previous studies (Yousef *et al*., 2005; Khattab *et al.,* 2010) that reported reduced body weight gain and testes weight in rats following exposure to aluminium. Arsenics and other heavy metals which are often impurities in low grade carbide could be responsible for these effects as testicular degeneration and depletion of germ cell layers have been documented as toxic effect of arsenics. Carbide treatment led to reduced semen quality as seen in massive reduction of sperm count and increased sperm abnormalities. This might be as a result of damage to the germinal epithelium of the seminiferous tubules by carbide. There could also have been degeneration of already existing sperm cells leading to reduced cell population. Administration of *A. cepa* juice reduced these adverse effects in a dose-dependent manner. This is in agreement with a similar study by (Ogbuagu & Oritsematosan, 2016) who reported a dose-dependent negative reproductive outcome including increased level of spermatozoa abnormality following consumption of CaC_2 in feeds.

The result of the histomorphological studies revealed that carbide administration resulted in a depletion of spermatocytes and multifocal degeneration of spermatids. *Allium cepa* administration was however able to ameliorate this problem as observed in the *A.cepa* treated groups. This may be mainly due to the antioxidant activity of *A. cepa* juice extract which may have prevented oxidative stress damage to the sperm cells as well as to the testicular tissues. This agrees with the finding of Ige *et al*., (2012) that *A. cepa* juice prevented oxidative stress induced by chronic lead acetate exposure. Similarly, in another study, administration of *A. cepa* was found to increase the activity of testicular antioxidants (superoxide dismutase, glutathione reductase, and catalase) and significantly reduce the level of malondialdehyde in testicular tissue of rats (Sharma, *et al.*, 2018) thereby protecting the testes from effect of toxicants

CONCLUSION

In conclusion, from this study, it can be deduced that carbide has deleterious effects on the reproductive parameters of animals which affects their reproductive performance and consumption of fruits ripened with carbide could pose a potential danger to reproductive performance in males. More so, regular consumption of onion or incorporating into animal feed can ameliorate this effect.

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