



## Melatonin and *Allium Sativum* (Garlic) Protect Dibutyl Phthalate Influence on Spermogram of Rabbit Bucks

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### SUMMARY

This study was designed to evaluate the effects of melatonin and *Allium sativum* (garlic) on dibutyl phthalate (DBP) influence on spermogram of rabbit bucks. Forty two (42) rabbit bucks were used for this study, bucks were randomly divided into 7 groups of 6 bucks each. Group A was administered olive oil for 16 weeks, group B (olive oil + DBP for 16 weeks), group C (melatonin for 8 weeks, then olive oil + DBP for another 8 weeks), group D (garlic for 8 weeks, then olive oil + DBP for another 8 weeks), group E (olive oil + DBP for 8 weeks, then melatonin for another 8 week), group F (olive oil + DBP for 8 weeks, then garlic for another 8 weeks) and group G (olive oil + DBP for 8 weeks, then melatonin and garlic for another 8 weeks). The observation period lasted for 120 days, during which semen samples were collected weekly between the hours of 8.00 am to 10.00 am using artificial vagina (AV). There were significant differences ( $P < 0.05$ ) in mean reaction time, semen volume, sperm motility, sperm concentration, percentage live spermatozoa and percentage abnormal morphology between DBP exposed groups and treatment groups. Conclusion, DBP has adverse effects on spermogram but administration of melatonin and garlic has promising protective effects than therapeutic effect on rabbit bucks.

**Key words:** Melatonin; *Allium sativum*; Dibutyl phthalate; Spermogram; Rabbit Bucks

### INTRODUCTION

Several studies have linked declining reproduction especially male fertility to toxicants found in the environment, particularly endocrine-

disrupting chemicals (EDCs), such as phthalates (Wong and Cheng, 2011; Nordkap *et al.*, 2012). One of the phthalates, Dibutyl phthalate (DBP) has attracted special attention due to high

production volume in millions of tons annually (Swan and Elkin, 1999; Guerra *et al.*, 2010). As a result, human and animal exposure becomes inevitable with its attendant negative consequences on reproduction (Kolaric *et al.*, 2008; Guerra *et al.*, 2010; Zhou *et al.*, 2011; Wang *et al.*, 2012a, 2012b; Asghari *et al.*, 2015; Hamdy *et al.*, 2015; Rehani *et al.*, 2015). In addition, DBP was reported to increase generation of ROS within the testes, concomitantly decreasing antioxidant concentration, resulting to impaired spermatogenesis (Lee *et al.*, 2007; Zhou *et al.*, 2011).

Phthalates were used in the 1930s for the first time to replace unpleasant odour camphor, phthalates are used mainly for manufacturing of medical supplies such as blood storage bags and intravenous solution containers, food containers, food packaging materials, children toys, cars interiors, floor tiles, food wraps and plastic products. Approximately 3 million tons of phthalate are produced per annum around the globe. DBP is the most commonly used phthalates fulfill about 40% of total phthalate use. Blood storage bags usually have a high content of 20-40% DBP but primary source of exposure is through contaminated food (Shi *et al.*, 2012; Liaqat, 2018).

Phthalates are ubiquitous xenobiotics widely used in consumer products (Shea, 2003; Heudorf *et al.*, 2007) with epidemiological studies revealing its detection in urine, blood and breast milk of humans (Swan *et al.*, 2005, Main *et al.*, 2006; Fromme *et al.*, 2007).

There is a global paradigm shift in favour of organic livestock production through the use of botanicals to halt the deleterious effects of drug residues and hormones from edible animal tissues to humans. Botanicals like garlic have been reported to demonstrate a potent antioxidant action through improvement in semen concentration and serum antioxidant enzyme activities with no obvious pathology on the testes (Shinkut *et al.*, 2016a, 2016b). To achieve this

shift, it is important to compare the antioxidant potential of garlic due to its availability to a standard and a potent antioxidant, like melatonin in terms of protective and therapeutic effects on oxidative stress (OS)-induced infertility to establish if it could be a good substitute.

There is paucity of information on the use of antioxidants both as prophylactic and therapeutic measures in addressing OS-induced infertility in male animals. There is also dearth of information comparing the antioxidant potential of garlic with other standard antioxidants

The study aims to investigate the effects of melatonin and *Allium sativum* on dibutyl phthalate influence on spermogram of rabbit bucks.

## MATERIALS AND METHODS

### Experimental animals

Forty two (42) apparently healthy, New Zealand White rabbit bucks (*Oryctolagus cuniculus*), 10 - 12 month old with body weight of 1.80-2.00 kg were used for the study. The bucks were sourced from rabbit farms within Zaria and environs and treated with Ivermectin (Kepromec®) against endoparasites and helminthes infection. Also, penicillin-streptomycin (Penstrep) was used to treat against possible bacterial infection, before the commencement of the experiment. Water and feed were provided *ad libitum*, the bucks were housed in standard rabbit cages, one buck per cage.

### Chemical and *Allium sativum* Acquisition and Preparation

Di(*n-butyl*) phthalate DBP (CAS Number 84-74-2-technical grade-99% purity) was purchased from Sigma Aldrich USA. DBP was reconstituted in olive oil (Goya Extra Virgin Olive Oil, Sevilla, Spain) to form a solution of 50 % (w/v) as described by Oda and Waheeb. (2017) and administered to bucks at dosage of 750 mg/kg, with modification. Melatonin (MEL, 5 mg/Tablet, Nature made, USA) was dissolved in 10 ml of distilled water to make 0.5 mg/ml suspension daily before administration to the

animals (Umosen *et al.*, 2012). All preparations were administered orally to the animals using gastric tube. *Allium sativum* (garlic) bulb was sourced from Sabon Gari, Kaduna State, Nigeria.

### Experimental design

Forty two (42) rabbit bucks were randomly divided into seven (7) groups of six (6) bucks each, designated as groups A, B, C, D, E, F and G.

Group A: served as negative control, each buck received Olive oil only at 1.5 ml once a day, five consecutive days in a week for 16 weeks.

Group B: served as positive control, each buck received 1.5 ml Olive oil + DBP (750 mg/kg) once a day, five consecutive days in a week with no treatment for 16 weeks.

Group C: each buck received pretreatment with 0.5 mg/ml melatonin once a day, seven days a week for 8 weeks, followed by 1.5 ml Olive oil + DBP (750 mg/kg) once a day, five consecutive days in a week administered for another 8 weeks.

Group D: each buck received pretreatment with 5.0 % *A. sativum*, once a day, seven days a week for 8 weeks, followed by 1.5 ml Olive oil + DBP (750 mg/kg) once a day, five consecutive days a week administered for another 8 weeks.

Group E: each buck received 1.5 ml Olive oil + DBP 750 mg/kg once a day, five consecutive days a week for 8 weeks, followed by treatment with 0.5 mg/ml melatonin, once a day, seven days a week for another 8 weeks.

Group F: each buck received 1.5 ml Olive oil + DBP 750 mg/kg, five times a week for 8 weeks, followed by treatment with 5.0 % *A. sativum* seven days a week for another 8 weeks.

Group G: each buck received 1.5 ml Olive oil + DBP 750 mg/kg five consecutive days a week for 8 weeks, followed by treatment with 0.5 mg/ml melatonin + 5.0 % *A. sativum* seven days a week for another 8 weeks.

The rabbit bucks were acclimatized for 30 days before commencement of the study. All rabbits were fed diets corresponding to their groups as shown in TABLE 1, as described by Shinkut *et*

*al.* (2016a). The diets were of isonitrogenous and isocaloric values. Dried bulbs of *Allium sativum* were then weighed and added to the feed raw materials and ground together to form the experimental diets (5% or 5 kg of garlic was weighed and added to 95% or 95 kg of other feed ingredients to make up 100 kg of the experiment diet for garlic treatment groups). Approval for the study was sought and obtained from the Ahmadu Bello University Committee for Animal Use and Care with the approval number: ABUCAUC/2018/059. The study lasted for four months (120 days), during which semen samples were collected weekly, a total of three hundred and thirty six (336) semen samples were collected for laboratory analysis.

### Assembling the artificial vagina

The bucks were trained for semen collection during the acclimatisation period and semen collection was done using a specially designed artificial vagina for rabbits as described by Shinkut *et al.* (2016a). The artificial vagina (AV) was assembled as follows: a short plastic cylinder was obtained and a latex condom was used as a liner, whose end was cut off to allow both ends opened. A rubber band was used to fix the liner on the cylinder at one end, then glycerol was administered into the space between the cylinder and the rubber liner and the other end of the cylinder was fixed with another rubber band to assemble the AV. The assembled AV was placed in a beaker of warm water at 40°C, the warm water caused expansion of the glycerol within the liner and also provided the necessary pressure and temperature. Trace of water was wiped from the AV, a short test tube was attached to the end of the AV and the other end lubricated with non perfumed petroleum jelly for ease of penetration.

### Semen evaluation

Ejaculates obtained were subjected to routine evaluation as described by Zemjanis (1970). This includes: Reaction time, the visual or gross evaluation of the ejaculate soon after collection

**TABLE 1: Composition of Experimental Diets for the individual groups**

Composition (%)	Treatment groups						
	A	B	C	D	E	F	G
Maize	30.16	30.16	30.16	28.57	30.16	28.57	28.57
Groundnut cake	28.12	28.12	28.12	26.64	28.12	26.64	26.64
Rice offals	35.32	35.32	35.32	33.46	35.32	33.46	33.46
Crude <i>Allium sativum</i>	0.0	0.0	0.0	5.0/0	0.0	0/5.0	0/5.0
Vitamin premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Palm oil	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Bone meal	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Methionine	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100	100
Dry matter	89.50	89.50	89.50	87.89	89.50	87.89	87.89
Crude protein	16.81	16.81	16.81	18.75	16.81	18.75	18.75
Ether extract	1.27	1.27	1.27	1.10	1.27	1.10	1.10
Crude fibre	8.65	8.65	8.65	8.54	8.65	8.54	8.54
Nitrogen free extract	53.96	53.96	53.96	52.46	53.96	52.46	52.46
ash	7.20	7.20	7.20	8.65	7.20	8.65	8.65
ME(kcal/kg)	2,640.42	2,640.42	2,640.42	2,645.18	2,640.42	2,645.18	2,645.18

Metabolisable energy calculated according to formula of Ponzenga (1985):

$ME = 37 \times \%CP + 81 \times \%EE + 35.5 \times \%NFE$ . ME = Metabolizable energy; CP = Crude protein; EE = Ether extract; NFE = Nitrogen free extract; Crude *A. sativum* 5.0/0 = 5% *A. sativum* in diet for 8 weeks followed by 0% *A. sativum* in diet from 9-16 weeks. Crude *A. sativum* 0/5.0% = 0 % *A. sativum* in diet for 8 weeks followed by 5.0 % *A. sativum* in diet from 9-16 weeks

for volume and colour, as well as microscopic examination for motility, concentration, percentage live spermatozoa and morphological abnormalities.

**Reaction time (libido):** A matured doe (teaser) was introduced to the buck prior to semen collection and observed for sex drive. The time

in seconds it took the buck to sniff, groom and mount the female was recorded (Saleem, 2003).

**Volume:** volume of semen was measured directly from the calibrated tube used for the collection.

**Sperm progressive motility:** percentage of spermatozoa with forward progressive motility was estimated by diluting a drop of semen with 4

drops of normal saline on a prewarmed glass slide and cover with a clean cover slip. Microscopy observation was done under the  $\times 40$  objective lens.

*Spermatozoa concentration:* was determined using Neubauer haemocytometer, semen was aspirated into the red cell diluting pipette up to the 0.1 mark (25 $\mu$ l) and the volume made up to the 101 mark (5ml) with 10% normal saline which ensured thorough mixing by capillary action, the mixture was allowed to spread under the cover slip, placed tightly on the haemocytometer after few drops were discarded. The cells were allowed to settle before counting under  $\times 40$  objective lens, sperm cells were counted in 5 smaller squares of the improved Neubauer haemocytometer and the concentration determined using the following formula:

Number of sperm cells/ml = number of sperm cells counted in 5 smaller squares  $\times 5 \times 10^4 \times$  dilution factor(5000) (Bearden and Fuquay, 1992; Azawi and Ismaeel, 2012).

*Percentage livesperm cells:* A thin smear of semen was made on a clean grease free slide and stained with 2 drops Eosin-Nigrosin stain. This technique is based on the principle that Eosin-nigrosin penetrates and stains dead sperm cells, while live sperm cells repel the stain. Dead spermatozoa stained pinkish or reddish while live spermatozoa remained colourless. Two hundred (200) stained and unstained sperm cells were counted when the slide dried, using light microscopy at  $\times 40$  magnification and percentage of each estimated (Esteso *et al.*, 2006).

*Sperm abnormalities:* was determined by making a thin smear of the semen sample, on clean grease-free glass slide and stained with 2 drops of Eosin-Negrosin. Two hundred sperm cells were counted per slide using hand counter under light microscopy at  $\times 100$  magnification using oil immersion. All abnormal cell types were counted and recorded (Rekwot *et al.*, 1987).

#### **Data Analyses**

Data collected were expressed as mean  $\pm$  standard error of mean (SEM) and subjected to

repeated measures one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Graph Pad prism version 5.0 for windows 2003 from Graph pad prism software, San Diego, California ([www.graphpad.com](http://www.graphpad.com)) was used. Values of  $P \leq 0.05$ , was considered significant.

## **RESULTS**

### **Semen characteristics**

The mean values of reaction time, ejaculate volume, gross sperm motility, sperm concentration, percentage live sperm, and percentage abnormal sperm of rabbit bucks of treatment groups A, B, C, D, E, F and G are presented in Figures 1-6.

#### **Reaction time**

Reaction time for all the groups did not differ significantly up to the 6<sup>th</sup> week ( $P \leq 0.05$ ). However, reaction time showed significant differences from week 7 being higher for groups B, E, F, G than for groups A, C, D. The most significant difference occurred after week 8 when group B showed the highest reaction time up till the end of the study.

Note also, that group E, F and G had elevated reaction time which reduced significantly after week 8, as shown in Figure 1

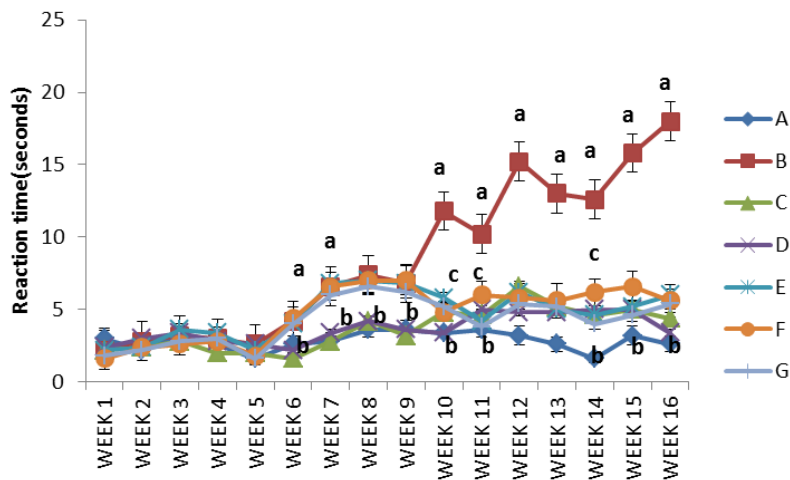
#### **Ejaculate volume**

Mean ejaculate volume (ml) for all the groups did not differ significantly until 6<sup>th</sup> week ( $P \leq 0.05$ ). However, ejaculate volume showed significant differences from week 9 being higher for groups A, C, D, E, F, G than for group B.

Note the ejaculate volume of group B remained significantly low from week 5 to the end of the study, as shown in Figure 2.

#### **Progressive motility**

Mean individual motility for all the groups did not differ significantly until 3<sup>rd</sup> week ( $P \leq 0.05$ ). However, individual motility showed significant differences from week 5 being higher for groups A, C, D, than for groups B, E, F, G. The most significant difference occurred after week 3 when



**FIGURE 1.** Mean Reaction time (seconds) of rabbit bucks administered {olive oil for 16 weeks (group A), olive oil + DBP for 16 weeks (group B), melatonin for 8 weeks, then olive oil + DBP for 8 weeks (group C), garlic for 8 weeks, then olive oil + DBP for 8 weeks (group D), olive oil + DBP for 8 weeks, then melatonin for 8 week (group E), olive oil + DBP for 8 weeks, then garlic for 8 weeks (group F) and olive oil + DBP for 8 weeks, then melatonin and garlic for 8 weeks (group G)} from week 1 to week 16 of the study. Groups with different letters show levels of significance. Number of animals per

(group E), olive oil + DBP for 8 weeks, then garlic for 8 weeks (group F) and olive oil + DBP for 8 weeks, then melatonin and garlic for 8 weeks (group G)} from week 1 to week 16 of the study. Groups with different letters show levels of significance. Number of animals per group = 6. group B showed significantly lower motility till the end of the study.

Note also, that group E, F and G had low motility which increased significantly after week 8, till the end of the study, as shown in Figure 3.

**Sperm concentration**

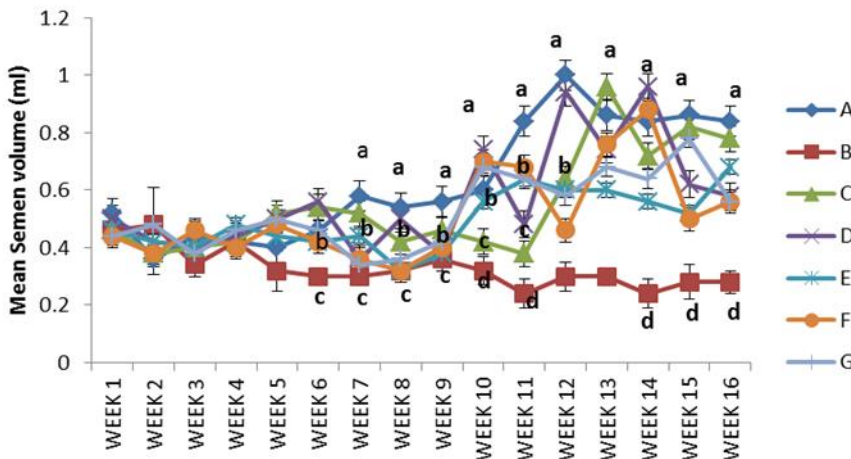
Mean sperm concentration ( $10^6/ml$ ) for all the groups did not differ significantly until 6<sup>th</sup> week ( $P \leq 0.05$ ). Sperm concentration was significantly high from week 6, being higher for groups A, C, D, than for groups B, E,

F, G. The most significant difference started at week 6 when group B showed significantly lower concentration till the end of the study.

Note also, that group E, F and G had sperm concentration which increased significantly after week 9, till the end of the study, as shown in Figure 4.

**Percentage live spermatozoa**

Percentage live spermatozoa for all the groups did not differ significantly until 6<sup>th</sup> week ( $P \leq 0.05$ ). Percentage live spermatozoa was significantly high at week 8, being higher for groups A, C, D, than for groups B, E, F, G. The most significant difference started at week 6 when group B showed significantly lower percentage live spermatozoa till the end of the study. Note also, that group E, F and G had sperm concentration which increased



**FIGURE II.** Mean semen volume (ml) of rabbit bucks administered {olive oil for 16 weeks (group A), olive oil + DBP for 16 weeks (group B), melatonin for 8 weeks, then olive oil + DBP for 8 weeks (group C), garlic for 8 weeks, then olive oil + DBP for 8 weeks (group D), olive oil + DBP for 8 weeks, then melatonin for 8 week (group E), olive oil + DBP for 8 weeks, then garlic for 8 weeks (group F) and olive oil + DBP for 8 weeks, then melatonin and garlic for 8 weeks (group G)} from week 1 to week 16 of the study. Groups with different letters show levels of significance. Number of animals per group = 6.

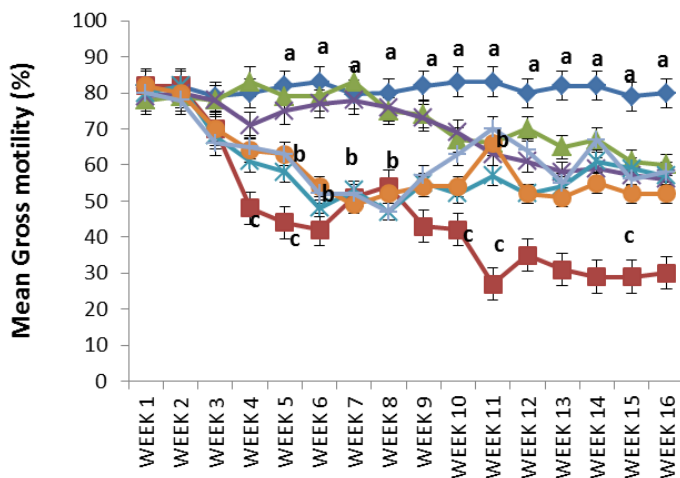
significantly after week 9, till the end of the study, as shown in Figure 5.

**Percentage sperm abnormalities**

Percentage sperm abnormalities for all the groups did not differ significantly until the 3<sup>rd</sup> week ( $P \leq 0.05$ ). However, percentage abnormal sperm showed significant differences from week 8, being higher for groups B, E, F, G than for

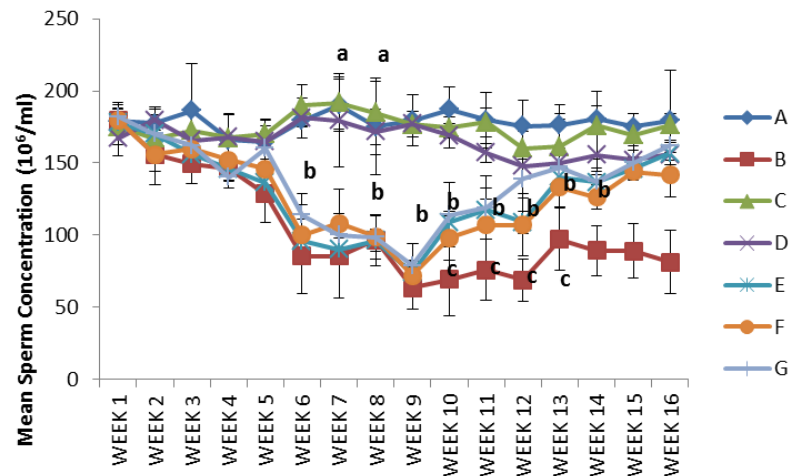
groups A, C, D. The most significant difference occurred after week 9, when group B showed the highest percentage sperm abnormalities up till the end of the study.

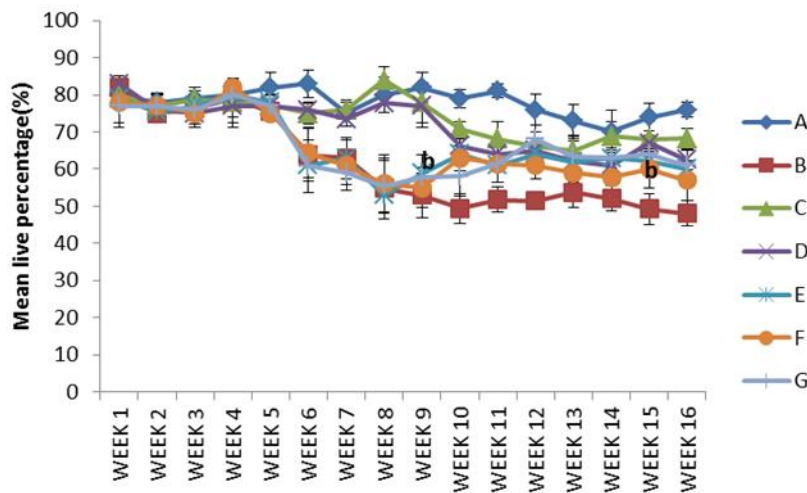
Note also, that group E, F and G had elevated percentage sperm abnormalities which reduced significantly after week 9, till the end of the study, as shown in Figure 6.



**FIGURE III.** Mean progressive motility (%) of rabbit bucks administered {olive oil for 16 weeks (group A), olive oil + DBP for 16 weeks (group B), melatonin for 8 weeks, then olive oil + DBP for 8 weeks (group C), garlic for 8 weeks, then olive oil + DBP for 8 weeks (group D), olive oil + DBP for 8 weeks, then melatonin for 8 week (group E), olive oil + DBP for 8 weeks, then garlic for 8 weeks (group F) and olive oil + DBP for 8 weeks, then melatonin and garlic for 8 weeks (group G)} from week 1 to week 16 of the study. Groups with different letters show levels of significance. Number of animals per group = 6.

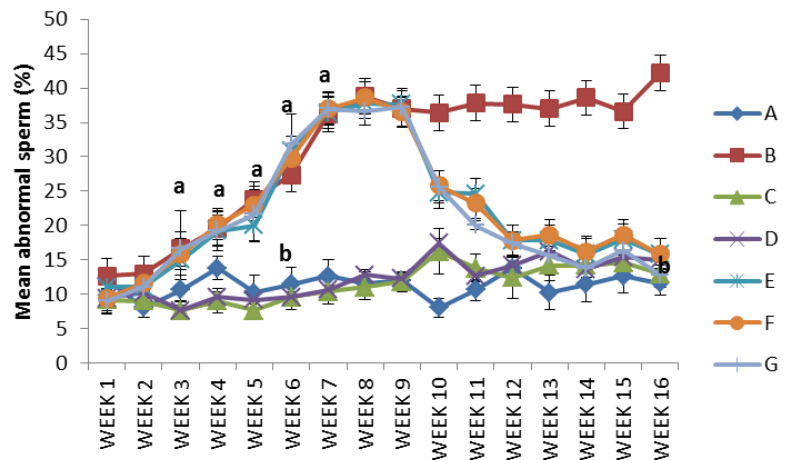
**Figure IV.** Mean sperm concentration ( $10^6/ml$ ) of rabbit bucks administered {olive oil for 16 weeks (group A), olive oil + DBP for 16 weeks (group B), melatonin for 8 weeks, then olive oil + DBP for 8 weeks (group C), garlic for 8 weeks, then olive oil + DBP for 8 weeks (group D), olive oil + DBP for 8 weeks, then melatonin for 8 week (group E), olive oil + DBP for 8 weeks, then garlic for 8 weeks (group F) and olive oil + DBP for 8 weeks, then melatonin and garlic for 8 weeks (group G)} from week 1 to week 16 of the study. Groups with different letters show levels of significance. Number of animals per group = 6





**FIGURE V.** Mean live percentage (%) of rabbit bucks administered {olive oil for 16 weeks (group A), olive oil + DBP for 16 weeks (group B), melatonin for 8 weeks, then olive oil + DBP for 8 weeks (group C), garlic for 8 weeks, then olive oil + DBP for 8 weeks (group D), olive oil + DBP for 8 weeks, then melatonin for 8 week (group E), olive oil + DBP for 8 weeks, then garlic for 8 weeks (group F) and olive oil + DBP for 8 weeks, then melatonin and garlic for 8 weeks (group G)} from week 1 to week 16 of the study. Groups with different letters show levels of significance. Number of animals per group = 6

**Figure VI.** Mean abnormal sperm (%) of rabbit bucks administered {olive oil for 16 weeks (group A), olive oil + DBP for 16 weeks (group B), melatonin for 8 weeks, then olive oil + DBP for 8 weeks (group C), garlic for 8 weeks, then olive oil + DBP for 8 weeks (group D), olive oil + DBP for 8 weeks, then melatonin for 8 week (group E), olive oil + DBP for 8 weeks, then garlic for 8 weeks (group F) and olive oil + DBP for 8 weeks, then melatonin and garlic for 8 weeks (group G)} from week 1 to week 16 of the study. Groups with different letters show levels of significance. Number of animals per group = 6.



**DISCUSSION**

Good sex drive (libido) of rabbit bucks and high quality semen are required to achieve maximum productivity either through artificial insemination (Rodriguez- De Lara *et al.*, 2008) or natural mating (Saleh *et al.*, 2010). In this study group B, bucks presented a lower sexual drive (increased reaction time) compared to the treatment groups and negative control (Figure 1). This possibly corresponds to a decrease in testosterone (T)

concentration in the same group B in similar fashion with other groups. This corroborates the role of T in enhancing male sexual character (ie libido) which is reflected by the reaction time measured.

The observed decreased in semen volume in group B from week 6 compared to other treatment groups may have been influenced by the corresponding decrease in concentration of testosterone (T) and follicle stimulating hormone (FSH) in the study (Figure 2). Even though other groups values falls within the normal range of 0.3-0.8ml as reported by Compos *et al.* (2014).



Sperm progressive motility gives the most significant information about the quality of semen. This is because when the semen is deposited in the female tract during mating or AI, motility becomes indispensable for the sperm cells to undergo the needed transformation (capacitation) before reaching the site of fertilization. The significant decrease in sperm progressive motility of DBP treated groups observed in this study (Figure 3), may be due to rapid loss of intracellular adenosine triphosphate (ATP) and damage of the sperm membrane caused by the effect of DBP (De Lamirande and Gagnon, 1992; Dokmeci, 2005). DBP induced free radicals (FR), caused mitochondrial damage this may have reduced the energy available in the cell and thus impede the movement of sperm (De Lamirande and Gagnon, 1992; De Lamirande *et al.*, 1997; 1998), impaired motility causes a smaller number of sperm reaching the oocyte which in turn greatly reduces the likelihood of fertilization. (Whittington *et al.*, 1999; Kao *et al.*, 2007). However, for the groups that were later treated after exposure and those pretreated before exposure to DBP, they exhibited better sperm motility though not as the negative control (group A) but far better than the positive control (group B). This by implication shows that administration of melatonin and garlic had protective and therapeutic effects on sperm motility.

Sperm concentration is an important indicator of spermatogenesis (Wang *et al.*, 2004) the observed reduction in sperm concentration in DBP exposed groups from week 6 of the study (Figure 4), may have resulted from the direct effect of DBP on testicular Leydig's and Sertoli's cells, causing a decrease in T production (Al-Thani *et al.*, 2003), which is a prime regulator for sperm production (Steinberger, 1975). Phthalate are reported to disrupt spermatogenesis and induce mitochondria dysfunction in gonocytes (Suna *et al.*, 2007) and also decrease T concentration (Park *et al.*, 2002). This may have led to the decreased level of serum FSH in the

DBP treated group, a hormone involved directly in maintaining spermatogenesis in conjunction with testosterone (Plant and Marshall, 2011). When sperm concentration and motility decreases, it may imply a decrease in fertility (Narayana *et al.*, 2002). This finding corroborates similar studies by Hamdy *et al.* (2015), Oda and Waheeb. (2017). Groups treated with melatonin and garlic, after exposure to DBP shown improvement in sperm concentration, this may be attributed to the fact that melatonin and garlic exerted a reversal effect on DBP effect on sperm concentration. Furthermore, the observed no significance decrease in sperm concentration of groups pretreated with melatonin and garlic before exposure to DBP could be an indication of their protective effect against DBP exposure. The concentration of melatonin in the testes is similar to that of other tissues in the body (Malm *et al.*, 2017), studies have shown that melatonin has high antioxidant properties and the potential to trap free radicals (Malm *et al.*, 2017). Hence the high sperm concentration observed in group C despite exposure to DBP, may be due to anti-apoptotic and antioxidant effect of melatonin (Armagan *et al.*, 2006; Saadat *et al.*, 2014; Mohammadghasemi and Jahromi, 2018). Saponin present in garlic is reported to positively influence libido and spermatogenesis (Francis *et al.*, 2002; Shinkut, 2015). Like-wise, flavonoid is reported to have positive influence on antioxidant concentration (Shinkut, 2015), that may have been responsible for the high sperm concentration in group D despite exposure to DBP.

The observed improvement in percentage live spermatozoa in groups exposed to DBP (E, F and G) before treatment with melatonin and garlic could be that they exerted some levels of therapeutic effects against DBP. More importantly, groups pretreated before exposure to DBP (C and D) showed that DBP did not significantly influence its outcome, we therefore deduce that they exerted protective effects better

than therapeutic effect on percentage like spermatozoa.

The normal morphology of sperm cell of head, midpiece and tail is very strategic for the spermatozoa to accomplish the task of fertilization in the female genital tract. Whatever affects the structural integrity of sperm cell will definitely compromise its motility within the female genitals, invariably affects its normal function of fertilization thereby impacting negatively on the fertility of the animal. Dobrzynska *et al.* (2009; 2012), reported that administration of phthalates causes degeneration of spermatogonia and spermatocytes, this may have been responsible for the observed increased in percentage abnormal spermatozoa in the DBP exposed groups (B, E, F and G) above normal (Figure 5). Kuzminsky *et al.* (1996) reported the average abnormalities of rabbit semen to be 18.2%. Our finding is in parallel with other studies (Wang *et al.*, 2015; Dobrzynska, 2016). There was a drastic reduction in percentage abnormal spermatozoa in groups exposed to DBP before treated with garlic and melatonin (E, F, and G), from week 10 of the study, we may attribute this to the effect of the treatment and for group pretreated before exposure (C and D), they maintained a steady low % of abnormal spermatozoa, this could be that melatonin and garlic had a protective effect on the testes preserving it from the menace of DBP on the testicular tissue.

Conclusion: DBP has negative effects on spermogram of rabbit bucks, but administration of melatonin and garlic has promising protective and ameliorative effects.

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