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Effect of methanolic extract of *Gmelina arborea* fruit on semen traits, testicular morphometry and histopathology in Cadmium exposed rabbit bucks

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INTRODUCTION

Heavy metals were since classified among major causes (Adedokun et al., 1989; Madrid et al., 2002) of environmental contamination threatening animal health and limiting productivity. Heavy metals occur as natural constituents of the earth crust, and are per-

Among heavy metals, Cadmium (Cd) is one of the common pollutants with deleterious reproductive and physiological impact in the biological system. This study was designed to evaluate the detrimental effects of Cd on the seminal and histopathological parameters of the testes; and to explore the therapeutic potentials of the methanolic extract of Gmelina arborea (MEGA) fruit in averting such reproductive damages. To achieve this, a total of 45 rabbit bucks aged between 24-28 weeks and weighing between 1.41-1.43 kg were used. The rabbit bucks were assigned to 5 treatments groups (control, Cd-only, Cd + 300 mg MEGA, Cd + 600 mg MEGA and Cd + 900 mg MEGA) in a completely randomized design. The rabbits were dosed with 3 mg CdCl2 /kg feed for 7 days followed by MEGA for 56 days after every 72 hours before the commencement of semen collection. The result of semen evaluation indicates that semen volume, semen motility, semen concentration, total ejaculate and viability were significantly (P < 0.05) reduced by Cd compared to the control group. Libido, body weight and testis density of the Cd-only rabbit group were also significantly (P < 0.05) reduced. Histopathological examination also revealed severe testicular damage due to Cd. However, treatment with MEGA significantly (P < 0.05) reversed the deleterious reproductive effects caused by Cd. In conclusion, Cd drastically affected the testis of rabbit bucks and treatment with MEGA alleviated these deleterious effects.

Effet de l'extrait méthanolique du fruit de *Gmelina arborea* sur les caractères du sperme, la morphométrie testiculaire et l'histopathologie chez le lapin exposé au cadmium

RÉSUMÉ

SUMMARY

Parmi les métaux lourds, le cadmium (Cd) est l'un des polluants les plus communs avec un impact néfaste sur la reproduction et la physiologie dans le système biologique. Cette étude a été conçue pour évaluer les effets néfastes du Cd sur les paramètres séminal et histopathologique des testicules; et d'explorer les potentiels thérapeutiques de l'extrait méthanolique de Gmelina arborea (MEGA) en évitant de tels dommages reproductifs. Pour ce faire, un total de 45 lapins âgés de 24 à 28 semaines et pesant entre 1,41 et 1,43 kg ont été utilisés. Les mâles de lapin ont été assignés à 5 groupes de traitement (contrôle, Cd-seul, Cd + 300 mg MEGA, Cd + 600 mg MEGA et Cd + 900 mg MEGA) dans un design complètement randomisé. Les lapins ont reçu 3 mg de CdCl2 / kg de nourriture pendant7 jours, suivis de MEGA pendant 56 jours après toutes les 72 heures avant le début du prélèvement de sperme. Le résultat de l'évaluation du sperme indique que le volume de sperme, la motilité du sperme, la concentration du sperme, l'éjaculation totale et la viabilité étaient significativement (P <0,05) réduits par le Cd par rapport au groupe témoin. La libido, le poids corporel et la densité des testicules du groupe de lapins uniquement au Cd étaient également significativement réduits (P <0,05). L'examen histopathologique a également révélé des lésions testiculaires graves dues au Cd. Cependant, le traitement avec MEGA de manière significative (P <0,05) a inversé les effets nocifs sur la reproduction causés par le cadmium. En conclusion, Cd a considérablement affecté les testicules des lapins et le traitement par MEGA a atténué ces effets délétères.

> sistent environmental contaminants since they cannot be degraded or destroyed. Although these elements are lacking in abundance they are not lacking in significance. The impact of toxic metals contamination on animals result in serious economic losses, thus, there is an increasing concern about environmental pollutants emanating into the livestock production systems.

Cadmium (Cd) is one of these known heavy metals that is most significant in public health (Orisakwe, 2014), probably because of its high toxicity even in relatively low concentration (Wierzbicka et al., 2007), wide range of organ toxicity and long elimination half-life (Patrick, 2003). Cd has high rates of soil to plant transference compared with other non-essential elements, and plants accumulate large amounts of Cd from low Cd content soils more avidly than they do other heavy metals such as lead and mercury (Satarag et al., 2003). Through plant uptake of Cd, this toxic metal of increasing environmental concern enters the food chain of livestock in significant amounts. Farm animals such as rabbits, goats, sheep and cattle feed on these plants and plants products which have absorbed and accumulated these toxic elements from the soil over time. Cd affects the central and peripheral nervous systems, haemopoietic system, cardiovascular system, kidneys, liver, and reproductive systems (Patra et al., 2011). Given the known adverse effects of Cd which emanates into the food chain of our animals, there is need for therapeutic intervention.

Indeed, researchers have evaluated varieties of botanically derived compounds to elucidate the scientific basis for their claimed potential effects. Gmelina arborea (White teak) is one of such plants and belongs to the Verbinaceae family. It has been screened and reported to possess derived compounds that have beneficial effect on immunity, fertility, antioxidant defence, increased metabolic activity, detoxifying and cleansing properties (Wansi et al., 2012). Therefore, investigations into the pharmacological validation of this less explored plant will go a long way in handling physiological and reproductive defects in animals arising from Cd toxicity through ingestion or inhalation. Hence, this study was designed to evaluate the spermatogenic, morphometric and histopathological effect of the fruit of Gmelina arborea on the testis of Cd induced toxicity in rabbit bucks.

MATERIALS AND METHODS

LOCATION OF STUDY

The experiment was carried out at the Rabbitary Unit of the Teaching and Research Farm of the University of Benin, Benin City. The University of Benin is in Ugbowo and Ugbowo is situated in Ovia-North, Edo State, Nigeria.

PROCUREMENT OF GMELINA ARBOREA FRUITS

The fruits of *Gmelina arborea* were acquired at the Forestry Department of the defunct Nigerian Newsprint Manufacturing Company (NNMC), Oku-Iboku in Akwa Ibom State. The acquired fruits were authenticated by a botanist, split, air-dried, finely ground and stored in an air tight container.

PREPARATION OF FRUIT EXTRACTS

The ground *Gmelina arborea* fruits was weighed and extraction was carried out with 99 % methanol in a soxhlet apparatus. The extract obtained was concentrated by recovery of methanol. The solvent was recovered using rotary vacuum evaporator and the concentrated extract was preserved in an airtight bottle.

EXPERIMENTAL MATERIALS AND MANAGEMENT

A total of forty-five (45) Composite rabbit bucks aged 24 - 28 weeks and weighing between 1.41-1.43 kg were used for this study. The rabbits were managed intensively in a hutch. They were quarantined for 2 weeks during which they were treated with Ivomec[®] injection for the control of haemoparasite, internal and external parasites. The rabbits were allowed *ad libitum* access to water and feed (commercial growers' diet).

EXPERIMENTAL DESIGN

The treatment protocols consisted of 5 groups: group 1 (control), group 2 (3 mg of $CdCl_2/kg$ feed/ day for 7 days), group 3 (3 mg of $CdCl_2/kg$ feed/ day for 7 days + 300 mg/kg body weight of MEGA fruits for 56 days at 72 hours interval), group 4 (3 mg of $CdCl_2/kg$ feed/day for 7 days + 600 mg/kg body weight of MEGA fruits extract for 56 days at 72 hours interval) and group 5 (3 mg of $CdCl_2/kg$ feed/day for 7 days + 900 mg/kg body weight of MEGA fruits extract for 56 days at 72 hours interval). Each treatment group consisted of 9 rabbits per group in a completely randomized design.

DATA COLLECTION AND EVALUATION

SEMEN COLLECTION

Two weeks prior to semen collection, the rabbit bucks were trained to serve an Artificial vagina (AV) using a teaser rabbit doe. On the 57th day following the administration of MEGA, the bucks under study were placed on a semen collection schedule of twice per week. One ejaculate was collected from each rabbit buck once between 08:00 to 13:00 h (local time) on Mondays and Thursdays for 3 consecutive weeks. Prior to semen examination, the collected semen samples were placed in a water bath at 37 °C.

Estimation of semen traits and libido

Semen evaluation involved the estimation of both microscopic and macroscopic indices. Ejaculate volume was read-off directly in millilitres from a calibrated glass collection tube attached to the AV. Sperm motility percentage score was subjectively assessed in a drop of fresh semen on a warm glass slide covered with a warm cover slip and examined using a Light microscope at x40 magnification. Sperm cell concentration $(\times 10^6/\text{mm}^3)$ was determined using the improved Neubauer haemocytometer at a dilution of 1 in 100 in a solution of 45 mL normal saline and 5 mL formalin. Total sperm (×10⁶/mm³ per ejaculate) was determined by multiplying the semen ejaculate volume by the sperm cell concentration. Morphological examination of the semen was done by performing different counts of the morphologically normal and abnormal sperm cell types on eosin/nigrosin stained preparations. Examples of morphological abnormalities observed were: double-head, elongated head, pyriform head, bent head, bent tail, bent mid-piece, coiled tail, double tail, headless and tailless spermatozoa. All those with normal morphology were recorded as N while the total number of the counted spermatozoa were recorded

as T. The percentage normal sperm morphology was calculated as (N/T 100). The rate of live sperm count (viability) was assessed in the same smears used for sperm morphological examination, where the eosin stains dead sperm pink and nigrosin allows live (colourless or transparent) to be displayed.

Libido was estimated by observing the reaction time (seconds) which elapsed between exposure of a buck to a doe and the first copulation (serving the AV).

Evaluation of testicular morphometry and body weight

At the end of experiment, the experimental rabbits were sacrificed using *captive bolt* followed by immediate exsanguinations. Thereafter, the testis, liver and kidney were harvested and measured. Testis, epididymis and vas deferens were carefully separated and freed of tunica albuginea and all adhering connective tissues. The length of each testis was measured using Vernier caliper. Testis circumference, length of epididymis and vas deferens were determined with a measuring tape in centimetres. The testes and epididymis weights were measured on an electronic scale and expressed in grammes. Testis volume (g/cm³) was determined volumetrically using the Archimedes principle of water displacement in a measuring cylinder. The testes density was derived thus:

Testes density=
$$\frac{\text{Testes weight (g)}}{\text{Testes volume (cm3)}}$$

The initial and final body weights of rabbits were taken at the start and end of the study respectively using Camry table scale.

HISTOPATHOLOGICAL STUDIES

Histopathological examination was carried out at the College of Veterinary Medicine Laboratory, Michael Okpara University of Agriculture, Umudike, Nigeria. The testes recovered from testicular morphometry were transversely cut and fixed in Bouin's fluid for 24 hours. The tissues were washed in ascending grades of ethanol (50 %, 75 % and 100 %) and cleared with xylene. They were embedded in paraffin wax with a Shandon Duplex automatic tissue processor and then sectioned using microtome at 4-5 µ thickness. Dewaxed sections were stained with Haemotoxylin and Eosin (H&E). The slides were covered with DPX (Distyrene, Plasticizer, and Xylene) mountant to increase refractive index of the stained preparation and then covered with slides to prevent scratches. All sections were examined under light microscope using × 400 magnification. Photomicrographs of the testicular tissues were taken with Olympus photomicroscope for observation and documentation of histopathology.

STATISTICAL ANALYSIS

The data generated was subjected to statistical analysis of variance (ANOVA) procedure of GenStat 12th edition at 5 % probability level. Occurrence of significant means was separated using Duncan Multiple Range Test (DMRT) of the same statistical software.

RESULTS AND DISCUSSION

The results of semen evaluation and testicular morphometric parameter of Cd exposed bucks treated with different levels of MEGA fruit are presented in **Table I** and **II** respectively.

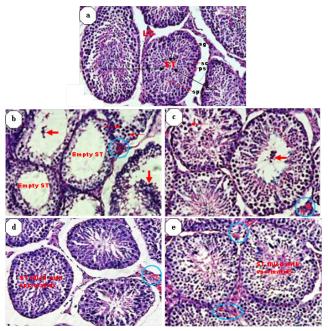


Figure 1. A photomicrograph of the testis of Cd exposed rabbits administered MEGA fruit stained with H&E, \times 100. (a) A control rabbit showing normal appearance of spermatogenic cells; spermatogonia (sg primary spermatocyte (ps), spermatid (sp), and Sertoli cells (sc). Leydig cells in interstitial tissue are noticed (LC). (b) Cross section of Cd-only treated rabbit showing: degeneration of spermatogénic cells, aggregation of necrotic spermatozoa at the seminiferous tubules or necroiic spermatozoa at the seminiferous tubules (STs) lumen (thick arrow), interstitial haemorrhage (encircled), empty STs (c) Section of rabbit treated with Cd+300 mg/kg MEGA showing; aggregation of few necrotic spermatozoa at the ST, mild intersti-tial haemorrhage (encircled), STs partially filled with spermatozoa, pyknotic nuclei (thin arrow). (d – e) sections of rabbits treated with Cd+600 and Cd+900 mg (kg MECA showing; mild vacualation interstitial mg/kg MEGA showing; mild vacuolation, interstitial haemorrhage (encircled), STs containing sperms and Sertoli cells are capped by tufts of late spermatids attached by their heads in the lumen (Microphotographie du testicule de lapins exposés au Cd auxquels on a administré un fruit MEGA coloré au H & E, x 100. (a) Un lapin témoin présentant un aspect normal de cellules spermatogènes; les spermatogonies (sg), les spermatocytes primaires (ps), les spermatides (sp) et les cellules de Sertoli (sc). Les cellules de Leydig dans le tissu interstitiel sont remarquées (LC). (b) Coupe transversale de lapin traité uniquement au Cd: dégénérescence des cellules spermatogènes, agrégation des spermatozoïdes nécrotiques au niveau des tubes séminifères (flèche épaisse), hémorragie interstitielle (encerclée), ST vides (c) Coupe de lapin traité avec Cd + 300 mg / kg MEGA montrant; agrégation de quelques spermatozoïdes nécrotiques à la ST, hémorragie interstitielle légère (encerclée), ST partiellement remplis de spermatozoïdes, noyaux pycnotiques (flèche fine). (d - e) des coupes de lapins traitées avec des indices MEGA de Cd + 600 et de Cd + 900 mg / kg; vacuolisation légère, hémorragie interstitielle (encerclée), ST contenant des spermatozoïdes et des cellules de Sertoli sont coiffées par des touffes de spermatides tardives attachées par leur tête dans la lumière).

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Parameters	Control	Cd	Cd+300 MEGA	Cd+600MEGA	Cd+900 MEGA	SEM	
Volume (ml)	1.17ª	0.40 ^b	0.51 ^b	0.43 ^b	0.66 ^b	0.16	
Motility (%)	74.48ª	20.25 [⊳]	80.62ª	88.54ª	74.15ª	8.22	
Concentration (x10 ⁶ /mm ³)	251.70ª	22.50°	119.20 ^b	254.20ª	97.50 ^b	30.00	
Total ejaculate (x10 ⁶ /mm ³)	294.40ª	9.50°	54.90 ^b	102.70 ^b	66.40 ^b	38.30	
Viability (%)	72.00ª	55.00 ^b	74.67ª	80.33ª	73.00ª	3.87	
Normal morphology (%)	70.33 ^{ab}	64.67 ^b	69.67 ^{ab}	73.00ª	74.33ª	2.30	
Reaction time (sec)	13.84 ^b	23.19ª	13.66 ^b	10.65 [⊾]	13.33 ^₅	2.07	

Table I. Semen characteristics and reaction time of cadmium exposed rabbit bucks administered MEGA							
fruit (Caractéristiques du sperme et temps de réaction des lapins de lapin exposés au cadmium administrés au MEGA).							

In the present study, the volume of the ejaculate significantly (P<0.05) decreased in all Cd treatments in comparison with the control group. This decrease in semen volume could probably be the result of the damage caused to the accessory glands which are responsible for the production of seminal plasma. Such presumed damage by Cd to the accessory glands has previously been described in rabbits, rats and mice by Phelps and Laskey (1989), Rehm et al. (2008) and Zakaria and Al-Busadah (2015). Furthermore, sperm viability, motility and concentration of Cd-only group significantly (P<0.05) decreased when compared with other treatment groups in this study. In contrast to the result of this study, Doyle et al. (1974) found that when CdCl₂ was administered to 4-month-old ram-lambs at a dose of 60 mg/kg feed for 191 days, sperm concentration was not affected. Also, Lymberopoulos et al. (2000) and Doyle et al. (1974) reported that sperm viability, grade motility, percentage live/dead ratio and the number of morphologically abnormal spermatozoa were not affected by Cd treatment.

Interestingly, the therapeutic intervention in Cd intoxicated rabbit bucks with MEGA fruits effectively attenuated the impaired reproductive effects of Cd by improving the sperm concentration, motility, viability, morphology as well as libido. Ripened fruit of Gmelina arborea have been traditionally reported to be effective as an aphrodisiac, treating sexual debility in male and habitual abortion in female (Rohit et al., 2012). Ahemen et al. (2016a) evaluated the sperm production rate, gonadal and extragonadal sperm reserves of rabbits fed varying levels of *Gmelina arborea* leaf meal (GALM). From the result obtained, they concluded that GALM up to 15 % inclusion in diet might generally support reproduction in rabbit bucks. In a somewhat similar finding, Ahemen et al. (2016b) also investigated the effect of aqueous extract of Gmelina arborea (AEGM) leaves on sperm production in rabbits. They documented that up to 400 ml/L of the extract significantly improved semen characteristics. The testes are endocrine glands hence damage to the tissue will result in abnormal endocrine responses. Indeed, the MEGA

Table II. Body weight and testicular morphometry of cadmium exposed rabbit bucks administered MEGA fruit (Poids corporel et morphométrie testiculaire des lapins de lapin exposés au cadmium administrés par MEGA).

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Parameters	Control	Cd	Cd+300 MEGA	Cd+600 MEGA	Cd+900 MEGA	SEM	
Initial body weight (kg)	1.43	1.42	1.43	1.40	1.42	0.08	
Final body weight (kg)	1.67	1.53	1.65	1.65	1.60	0.06	
Body weight gain (kg)	0.23ª	0.12 ^b	0.21ª	0.25ª	0.18ª	0.05	
Paired testis length (cm)	5.53ªb	5.00 ^b	5.57 ^{ab}	6.00ª	5.60 ^{ab}	0.27	
Paired testis circumference (cm)	6.03	5.23	6.00	6.10	5.97	0.40	
Paired testis weight (g)	2.57	2.10	2.53	3.00	2.57	0.43	
Paired testis volume (cm ³)	2.63	1.57	2.60	3.07	2.53	0.50	
Paired testis density (g/cm ³)	0.99 ^b	1.35ª	0.98 ^b	1.00 ^b	1.03 ^b	0.06	
Paired epididymis length (cm)	16.73ªb	14.17 ^b	16.33ªb	19.50ª	17.87ª	1.00	
Paired epididymis weight (g)	1.10 ^b	0.87 ^b	0.90 ^b	1.57ª	1.00 ^b	0.08	
Paired vas deferens length (cm)	17.07	16.03	18.77	16.87	18.63	1.72	
^{a,b} Moons booring different letters of	superscript with	in the same re		(P < 0.05)			

^{a,b}Means bearing different letters of superscript within the same row differ significantly (P < 0.05).

in this study could have restored the integrity of the Cd-compromised testis by exerting stimulatory effect on the testicular organ and hypothalamus-pituitary gonadal axis with consequent improvement of semen characteristics. However, from the result of this study, levels exceeding 600 mg/kg of MEGA may become counterproductive by possibly producing a suppressive effect on the hypothalamus or exerting a direct toxic effect on the testis. Thus, a negative feedback action could have been established at a higher dose of MEGA which may have led to the observed reduction in semen traits beyond 600 mg/kg.

While semen analysis appears to be the cornerstone in the evaluation of testicular function, testicular morphometry has also been associated with testicular function (Tijani et al., 2014). Measurement of testicular size is critical in the evaluation of reproductive capacity of the male, since seminiferous tubules (the spermatogenic region of the testis) occupy approximately 80 % of the testicular volume (Jones et al., 2007). França and Russell (1998) reported a sharp decline in testicular morphometric parameters due to massive cellular loss from seminiferous epithelium. Daily sperm output (DSO) is highly correlated with testis weight in rabbits (Amann, 1970) and high correlations have been reported between testis size and Sertoli cell numbers (Brackett, 2004). Thus, animals with superior testicular morphometric indices would be expected to have higher DSO, Sertoli cell numbers and daily sperm production (DSP). Studies have also indicated that the longer the segment of epididymis present, the greater the likelihood of pregnancy with sperm obtained from the male specie (Jones et al., 2007). So, an estimate of spermatogenic cell capacity can also be provided by the assessment of testicular morphometry. The data for testicular morphometry here in, further shows the severity of Cd to the testicular tissues which led to significant (P > 0.05) decrease in testis density. Results of previous researchers noted weight reductions in testes, epididymis and accessory sex glands (El-Neweshy et al., 2013; Saeed, 2013; Zakaria & Al-Busadah, 2015) after Cd exposure. In the present finding, *Gmelina arborea* fruit extract has been shown to significantly (P<0.05) enhance testis density index. It is also indicated that rabbits that received 600 mg/kg body weight of MEGA exhibited superior significant improvement in epididymis weight compared to other treatment groups. Furthermore, in comparison with Cd-only treated rabbit group, the epididymis length of the rabbits in Cd + 600 and Cd + 900 groups showed higher significant improvement but not significantly (P<0.05) different from the Control and Cd + 300 treatment groups. Ahemen et al. (2016a) documented significantly (P<0.05) increased morphometric parameters for rabbits receiving 15 % Gmelina arborea leaf meal in their diets. They also concluded that Gmelina arborea leaf meal up to 15 % in diet did not adversely affect sperm production and sperm reserves in rabbit bucks.

The result of this study showed that $CdCl_2$ administration significantly decreased the body weight of rabbit bucks. Past reviews (Zeng et al., 2003; Amara et al., 2008) have likewise demonstrated a decrease in body weight after a loss in appetite; a primary mani-

festation of CdCl₂ administration. Sajjad et al. (2014) opined that decrease in body weight in post pubertal Cd treated male animals may be cognate with the greater accumulation of Cd in the hypothalamus of these animals. Therefore, in this study, it could be presumed that the interference of Cd with the function of the hypothalamus may have impeded the synthesis of androgens which plays a role in anabolism. Nevertheless, the administration of MEGA to Cd treated rabbits significantly (P<0.05) increased the body weight gain of rabbit bucks. The body weight gain peaked at the 600 mg/kg MEGA treated group and declined afterwards to 0.18 kg in the 900 mg/kg MEGA treated group. The result on body weight gain followed a somewhat similar trend with the result of semen concentration, motility, viability, libido and epididymis weight observed in this study. This is in line with the report of Bezerra et al. (2009) and Ansa and Imasuen (2015) who observed a positive correlation between body weight and reproductive indices.

The histopathological examination of the testicular tissue revealed the true position of the reproductive potentials of Cd exposed rabbits. In agreement with the results of Lymberopoulos et al. (2000), EL-Shahat et al. (2009) and El-Refaiy and Eissa (2013), the current study showed marked generalized disorganisation of the architectural structure of the seminiferous tubules (STs), a decrease in thickness of germ cell layer, widening of the central STs lumen, prominent germ cell population necrosis and leakage of blood into the interstitial spaces causing haemorrhage and oedema. Sertoli cells were abnormal in number and shape when compared to the control group. In the lumen of the STs, some tubules contained a large number of intraluminal collection of degenerated, necrotic, desquamated spematogenic cells and a very small number of spermatozoa were observed. Other tubules showed complete absence of sperm in the lumen, multiple vacuoles and sloughing of all layers of STs. The appearance of the testis resemble that of the immature testis and there were hallmark of degeneration and could indicate an end of reproduction.

Histopathologically, the microscopic observations of the testis in the control group showed normal appearance of STs, spermatogenic cells and interstitial cells (Figure 1a). The rabbits treated with Cd (Figure 1b) showed many histopathological changes. All the rabbits in this group developed severe testicular lesions, haemorrhage, widening of the central tubules lumen and germ cell necrosis. Multiple vacuoles were seen within the tubules. Furthermore, Cd inhibited spermatogenesis which was visible in the absence of the advance stages of sperm production. Indeed, the results of these histopathological changes are harmonious with the decreased semen parameters recorded in this study. Thus it appears that Cd damaged the STs, probably by causing a cessation of spermatogenesis; interfered with the testicular vasculature and blood flow; and probably gave rise to an increase in the absorption of dead spermatozoa by the epididymis, resulting in their disappearance from the reproductive tract. Blanco et al. (2007) reported that even low doses of $CdCl_2$ (1) mg/kg for one month) induced lack of spermatogenesis and severe necrosis of the testes of rats. Moreover, Santos et al. (2004) reported that endothelial damage of the small blood vessels, oedema and haemorrhage of the rat testes can be demonstrated by using just a single parenteral dose of Cd chloride at 2–4 mg/kg. Several studies focusing on Cd-related changes in testicular histopathology have implicated testicular blood vessel damage, followed by the degeneration of spermatopoietic epithelial, as the main cause of Cd toxicity (Thompson & Bannigan, 2008; Messaoudi et al., 2010).

Treatment with Gmelina arborea fruit extract for 56 days following Cd exposure attenuated the deleterious reproductive effects inflicted by Cd, restoring the spermatogonial and Sertoli cells with concomitant improvements in sperm count, motility and abnormality rates. The shrunken ST nearly retained their normal architecture and contain sperm and Sertoli cells which are capped by tufts of late spermatids attached by their heads in the lumen. The normal spermatogenic columns are almost recognizable. Though, the interstitial spaces are widen and the spernatogonia resting on a thin basement membrane. Although the exact mechanism by which MEGA exerts its therapeutic potency is not understood, it is presumed to apparently be via hormone mediated effects elicited through its phytochemical components that act as gonad-stimulating compounds, thus, maintaining normal hormonal levels and consequently restoring fertility in the Cd exposed rabbit bucks.

CONCLUSION

From the results of this finding, it has been observed that cadmium is toxic to the testis, thus, decreases seminal characteristics. However, the methanolic extract of *G. arborea* fruit remediated the toxic effect of cadmium to the testis by restoring normal spermatogenesis.

BIBLIOGRAPHY

- Adedokun, JA, Emofureta, WO&Adedoji, OA 1989, 'Physical, mineralogical and chemical properties of harmattan dust at Ile-Ife, Nigeria', Applied Climatology, vol. 40, pp.161-169.
- Ahemen, T, Abu, AH & Akuba, JO 2016a, 'Effect of Gmelina arborea leaf meal on sperm production and sperm reserves in rabbit bucks', International Journal of Livestock Research, vol. 6, no. 4, pp. 98-104.
- Ahemen, T, Abu AH & Haaga, E 2016b, 'Testicular morphometry, sperm characteristics and viscera organ weights of rabbits following the administration of aqueous extract of *Gmelina arborea* leaves', *International Journal of Livestock Research*, vol. 6, no. 6, pp. 60-68.
- Amann, RP 1970, Sperm production rates In: Johnson, AD, Gomes, WR & VanDermark, NL. (eds), The testis, vol. 1. New York. Academic Press, p. 433-482.
- Amara, S, Abdelmelek, H, Garrel, C, Guiraud, P, Douki, T, Ravanat, J, Favier, A, Sakly, M, & Ben Rhouma, K 2008, 'Preventive effect of zinc against cadmium-induced oxidative stress in rat testis', *Journal* of Reproductive Development., vol. 54, pp. 129-134.
- Ansa, AA & Imasuen, JA 2015, 'Effect of human menopausal gonadotrophin on testicular morphometry, gonadal and extragonadal sperm reserves of rabbit bucks', World Rabbit Science., vol. 23, pp. 121-127.
- Bezerra, FQ, Aguiar Filho, LM, Freitas Neto, LM, Santos Junior, ER, Chaves, RM, Azevedo, EM, Santos, MH, Lima, PF & Oliveira, MA 2009, 'Body weight, scrotal circumference and testosterone concentration in young

Boer goat mates born during the dry or rainy seasons', *South African Journal of Animal Science*, vol. 39, no. 4, pp. 301-306.

- Blanco, A, Moyano, R, Vivo, J, Flores-Acuna, R, Molina, A, Blanco, C, Aguera, E & Monterde, JG 2007, 'Quantitative changes in the testicular structure in mice exposed to low doses of cadmium', *Environmental Toxicology and Pharmacology*, vol. 23, pp. 96–101.
- Doyle, JJ, Pfander, WH, Grebing, SE & Pierce, JO 1974, 'Effect of dietary cadmium on growth, cadmium absorption and cadmium tissue levels in growing lambs', *The Journal of Nutrition*, vol. 104, pp. 160–166.
- El-Neweshy, MS, El-Maddawy, ZK & El-Sayed, YS 2013, 'Therapeutic effects of date palm (*Phoenix dactylifera* L.) pollen extract on cadmiuminduced testicular toxicity', *Andrologia*, vol. 45, no. 6, pp. 369-378.
- El-Refaiy, AI & Eissa, FI 2013, 'Histopathology and cytotoxicity as biomarkers in treated rats with cadmium and some therapeutic agents', *Saudi Journal of Biological Sciences*, vol. 20, no. 3, pp. 265–280.
- El-Shahat, AE, Gabr, A, Meki, AR & Mehana, ES 2009, 'Altered testicular morphology and oxidative stress induced by cadmium in experimental rats and protective effect of simultaneous green tea extract', *International Journal of Morphology*, vol. 27, no. 3, pp. 75 7-764.
- França, LR & Russell, LD 1998, The testis of domestic animals In: Regadera, J & Martinez-Garcia, F. (Eds.). Male reproduction. A multidisciplinary overview. Madrid: Churchill Livingstone. pp. 197-219.
- Jones, RC, Dacheux, JL, Nixon, B & Ecroyd, HW 2007, 'The role of the epididymis in sperm competition', Asian Journal of Andrology., vol. 9, pp. 493–499.
- Lymberopoulos, AG, Kotsaki-Kovatsi, VP, Taylor, A, Papaioannou, N & Brikas, P 2000, 'Effects of cadmium chloride administration on the macroscopic and microscopic characteristics of ejaculates from Chios ram-lambs', *Theriogenology*, vol. 54, pp. 1145-1157.
- Madrid, L, Diaz-Barrientos, E & Madrid, F 2002, 'Distribution ofheavy metal contents of urban soils in parks of Seville', *Chemo-sphere*, vol. 49, pp. 1301–1308.
- Messaoudi, I, Banni, M, Said, L, Said, K & Kerkeni, A 2010, 'Evaluation of involvement of testicular metallothionein gene expression in the protective effect of zinc against cadmium-induced testicular pathophysiology in rat', *Reproductive Toxicology (Elmsford, NY).*, vol. 29, no. 3, pp. 339–345.
- Orisakwe, OE 2014, 'Lead and Cadmium in public health in Nigeria: Physicians Neglect and Pitfall in Patient Management', North American Journal of Medical Sciences., vol. 6, no. 2, pp. 61–70.
- Patra, RC, Rautray AK & Swarup, D 2011, 'Oxidative stress in lead and cadmium toxicity and its amelioration', Veterinary Medicine International, vol. 1, pp. 9-15.
- Patrick, L 2003, 'Toxic metals and antioxidants: part II, the role of antioxidants in arsenic and cadmium toxicity', Alternative Medicine Review, vol. 8, no. 2, pp. 106-128.
- Phelps, PV & Laskey, JW 1989, 'Comparison of age related changes in vivo and in vitro measures of testicular steroidogenesis after acute cadmium exposure in the Sprague Dawley rat', J. Toxicol. Environm. Health., vol. 27, pp. 95-105.
- Rehm, S, White, TE, Zahalka, EA, Stanislaus, DJ, Boyce, RJ & Wier, PJ 2008, 'Effects of food restriction on testis and accessory sex glands in maturing rats', *Toxicol Pathol.*, vol. 36, pp. 687–94.
- Rohit, K, Vaibhav, P, Manodeep, C & Jagadish, K 2012, 'Phytochemical and pharmacological profile of *Gmelina arborea*: An overview', International Research Journal of Pharmacy, vol. 3, no. 2, pp. 61-64.
- Saeed, BT 2013, 'Effects of cadmium on sperm parameters, histological and hormonal changes in testes of mature rats', *Iraqi J. Embryos and Infertility Researches*, vol. 3, no. 6, pp. 45-51.
- Sajjad, S, Malik, H, Farooq, U, Rashid, F, Nasim, H, Tariq, S, & Rehman, S 2014, 'Cadmium chloride toxicity revisited: effect on certain andrological, endocrinological and biochemical parameters of adult male rabbits', *Physiol Res.* vol. 63, no. 4, pp .505-512.
- Santos, FW, Oro, T, Zeni, G, Rocha, JB, doNascimento, PC & Nogueira, CW 2004, 'Cadmium induced testicular damage and its response to administration of succimer and diphenyl diselenide in mice', *Toxicol*ogy Letters., vol. 152, pp. 255-63.

- Satarag, S, Baker, JR, Urbenjapol, S, Haswell-Elkins, M, Reilly, PE, Williams, DJ & Moore, MR 2003, 'A global perspective on cadmium pollution and toxicity in non-occupationally exposed population', *Toxicology Letters*, vol. 137, no. 1–2, pp. 65–83.
- Thompson, J & Bannigan, J 2008, 'Cadmium: toxic effects on the reproductive system and the embryo', *Reproductive Toxicology*, vol. 25, no. 3, pp. 304–315.
- Tijani, KH, Oyende, BO, Awosanya, GO, Ojewola, RW, Lawal, AO & Yusuf, AO 2014, 'Scrotal abnormalities and infertility in West African men: A comparison of fertile and sub-fertile men using scrotal ultrasonography', African Journal of Urology, vol. 20, no. 4, pp. 180–183.
- Wansi, SL, Nyadjeu, P, Nguelefack, TB, Fodouop, SFK, Donatien, AA & Kamanyi, A 2012, 'In vivo antioxidant and vasodilating activities of

Gmelina arborea (Verberaceae) leaves hexane extract', Journal of complementary and integrative medicine, vol. 9, no. 1, pp. 42-50.

- Wierzbicka, MH, Przedpełska, E, Ruzik, R, Ouerdane, L, Połeć-Pawlak, K, Jarosz, M, Szpunar, J & Szakiel, A 2007, 'Comparison of the toxicity and distribution of cadmium and lead in plant cells,' *Protoplasma*, vol. 231, no. 1-2, pp. 99-111.
- Zakaria, A & Al-Busadah, KA 2015, 'Pentoxifylline efficiency in protecting testes against cadmium toxicity', *Journal of Animal and Veterinary Advances*, vol. 14, pp. 18-29.
- Zeng, X, Jin, T, Zhou, Y & Nordberg, GF 2003, 'Changes of serum sex hormone levels and MT mRNA expression in rats orally exposed to cadmium', *Toxicology*, vol. 186, pp. 109-118.