Available online at http://www.ajol.info/index.php/njbas/index
Nigerian Journal of Basic and Applied Science (June, 2019), 27(1): 67-75
DOI: http://dx.doi.org/10.4314/njbas.v27i1.9
ISSN 0794-5698

Phytomedical Potentials of *Chromolaena Odorata* Against Arsenic-Induced Testicular Toxicity In Wistar Rats

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ABSTRACT
The testicular, sperm and endocrine protective properties of *Chromolaena odorata* (CA) in arsenic treated rats were investigated using forty male wistar rats (190-200g) grouped into 4 (A to D) of ten rats each. Oral administrations for 2 weeks of 0.2ml corn oil (A), 2.5mg/kg of sodium arsenite (B), 200mg/kg ethanol leaf extract (ELE) of CA (C), 200mg/kg ELE of CA and 2.5mg/kg sodium arsenite given at 1 hour interval (D) were done. Twenty-four hours after final administrations, semen, blood biochemical and hormonal analyses were carried out after sacrifice of the rats. Results revealed that Group C's mean scrotal circumference, Left and Right testicular weights were highest across groups and significant compared to group B values ($p<0.05$). Group C had significantly ($P<0.05$) highest mean value of sperm motility across the groups just as group A was significantly ($p<0.05$) higher than those of groups B and D. Significantly low Testosterone and high luteinizing hormone concentrations were observed in group B. It was concluded in this study that ethanol leaf extract of *Chromolena odorata* had a profound scrotal, testicular, sperm and endocrine protective properties in arsenic-treated wistar strain albino rats.
Keywords: *Chromolaena odorata,* Reproductive toxicity, Arsenic, Testicular, Wistar rats

INTRODUCTION
Arsenic is among the most toxic heavy metals in the environment (ATSDR, 2005). Many systems within the body are affected by inorganic arsenic exposure. Some of these toxic effects range from skin lesions, cardiovascular, haematological, hepatic, reproductive and renal defects (Abernathy *et al*., 2003). Urothelial cytotoxicity, increased cell proliferation and ultimately tumour generation have been reported in rats that were exposed to arsenic. (Suzuki *et al*., 2008). Arsenic poisoning is one of the greatest risk factors contributing to reproductive failure in animals and humans (Jaishankar *et al*., 2014). Sources are largely from herbicides, insecticides and food preservatives (ATSDR, 2005). Arsenic in ground water is the major source of arsenic intoxication (Argos *et al*., 2012). The intoxicative effect could be through its thiol-reactive property in which arsenic compounds inhibit enzymes by altering proteins through its reaction with proteinaceous thiol groups (Sharma *et al*. 2014). Phytotherapeutic products from medicinal plants have become universally popular in the control of arsenic poisoning especially in developing countries (Leonardo *et al*., 2000). The plant, *Chromolaena odorata,* is known for its wide range therapeutic potentials. It is a perennial semi woody shrub, which belong to the family *Asteraceae*. The plant is native to central and South America and is now distributed through Africa and Tropical Asia (Burkill, 1997). In Nigeria, It is called *Akintola-ta-ku, Awo-lowo and Obiarakara in Yoruba, Igbo and Hausa Languages respectively.* It is a deep-rooted plant and can survive harsh climatic condition. Traditionally, the plant's leaf is used as a coagulant to stop bleeding (vasoconstriction) in fresh wounds and treatment of stomach ache (Sofowora, 1982). It has also been found to be an effective antidiarrhoea, astringent, antihypertensive, anti-inflammatory and diuretic (Iwu, 1993). The flowers of the plant contain flavanones (5, 7 – dihydroxy – 7 4 –dime-thoxy-flavanone, 4-tetramethoxy flavanone, and 4-hydroxy-5,6,7-trimethoxy flavanone), chalcones (2-hydroxy-4, 4,5,6,-tetramethoxylcalcone and 4, 2-dihydroxy-4,5,6-trimethoxychalcone) and

flavones (acacetin and luteolin) (Arene *et al.;*1985). Notable phenolic acids such as protocatechuic acid, p-hydroxy benzoic acid, P-Coumaric acid, ferulic acid and vanillic acids have been isolated from the leaves and identified (Phan *et al.,* 2001) together with essential oils (Bamba *et al.,* 1993; Chowdhury, 2002), Steroids (Ahmed *et al;* 1995) triterpenes and flavonoids (Arene *et al;* 1985). It has been generally accepted that plant's actions are traceable to the chemical components of the plant extracts (Phan *et al*., 2001). This plant, known for its vast therapeutic potentials has been used in humans and animals as treatment for various conditions, however, there is paucity of information on its sperm protective potential in rats exposed to sodium arsenite toxicity. This work sought to investigate the plant's sperm, testicular and endocrine protective properties in arsenic-exposed rats

MATERIALS AND METHODS

Preparation of Sodium arsenite

Sodium arsenite (Ioba, Chemie Co. India) solution was prepared and a dose of 2.5mg/kg body weight was administered according to the guidelines for *in vivo* assays in rats (Preston *et al*., 1987). Freshly prepared stock solution was used for the experiment.

Plant Collection and extraction

The leaves of *Chromolaena odorata* were collected from the Postgraduate College Environs, University of Ibadan and authenticated at the Department of Botany, Faculty of Science, University of Ibadan, Ibadan, Nigeria with voucher number UIH-22425. Freshly obtained *Chromolaena odorata* leaves were dried at room temperature after which the dried leaves were ground into fine powder for cold extraction process. This was carried out by soaking the ground leaves in n-hexane for 48 h to de-fat the leaves. The ground materials were then sieved out, air-dried and then soaked in 96% ethanol for 72 h. The soaked particles were removed and the supernatant was collected. Both the n-hexane and ethanol fractions of the extract were concentrated using rotary evaporator at 40^0C. The ethanol fraction was then used to dose the animals using anti-oxidant free corn oil as the vehicle.

Phytochemical screening

This was carried out using the methods previously described by Trease and Evans (1983) and Harbourne (1983). The metabolites tested for were alkaloids, anthraquinones, cardiac glycosides, tannins, flavonoids, saponins, phenols and steroids.

Experimental animals

Forty male Albino rats weighing between 190-200g were used. They were obtained from the experimental animal house of the Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. All the animals received humane care according to the criteria outlined in the Public Health Service Policy on Humane Care and the Use of Laboratory Animals US Department of Health and Humane Services.

Experimental Protocol

The animals were grouped into 4 (A to D) of ten rats each. All animals were kept in plastic laboratory cages under controlled conditions of temperature (25 ± 2°C), relative humidity (50 ± 15%) and normal photoperiod (12 h light and 12 h dark). The rats were allowed to acclimatize for a period of two weeks before the commencement of the experiment. They were fed on a standard diet (commercial pelletized diet from Grand Cereals, Jos, Plateau State, Nigeria) and provided water *ad libitum*. All animals were weighed weekly from 8^{th} week to 12^{th} week.

Preparation of plant fraction

Ethanol extract suspensions were freshly dissolved in antioxidant-free corn oil, which served as a vehicle. Suspensions were administered orally (per os) at a dose of 200mg/kg body weight of the ethanol leaf extract. Prepared suspensions were kept at room temperature.

Experimental protocol
Group A animals were administered 0.2ml of corn oil, animals in group B were given 2mg per kg body weight of sodium arsenite while group C received 200mg/kg of the ethanol leaf extract only and Group D were served 200mg/kg ethanol leaf extract and sodium arsenite at 1 h interval. All administration was carried out orally for 8 weeks and animals were sacrificed 24 h after the final administration.

Blood Collection and Biochemical assay
The rats were anaesthetized using diethylether in a desiccator and blood samples were collected via the retro-orbital venous plexus into sterile sample tubes without anticoagulant. It was allowed to settle, and separated by centrifuging at 500 rpm for 10 minutes. Supernatant serum was collected and analyzed using Randox specific kits for levels of the creatine kinase (CK), acid phosphatase, lactate dehydrogenase (LDH), using an automated analyzer, ATAC 8000 (Elan diagnostic, CA USA). Serum testosterone and luteinizing hormone concentrations were also determined using automated analyzer

Semen collection and analysis
After blood collection, a mid caudoventral abdominal incision was made on the rats with sterilized scissors, permitting instant access to the testis once pushed upward from the scrotum. The testes were then separated from the epididymides. The right and left epididymides were trimmed off the body of the testes and semen sample was collected from the tail of the epididymis through a stab incision made with a scalpel blade (Ola-Davies and Ajani 2016 a) from where semen was milked out onto a warm glass slide for evaluations.

Percentage motility
This was evaluated using a drop of semen with drop of 2.9% buffered sodium citrate on a warm glass slide covered with a glass slip and viewed at a magnification of ×40. Only sperm cells moving in unidirectional motion were included in the motility rating, while sperm cells moving in circles, in backward direction or pendulating movement were excluded (Zemjanis, 1977).

Morphological abnormalities
Semen dropped on warm glass slide was mixed with a drop of warm Wells and Awa stains. A thin smear was then made of the mixture for morphological studies (Zemjanis, 1977).

Percentage liveability
Mixture of a drop of semen and one drop of warm Eosin-Nigrosin stain on a warm slide was used to make a thin smear. The smear was air dried and observed under the microscope. The ratio of the *in vitro* dead sperm cells was observed and it is based upon the principle of Eosin penetrating and staining the dead autolysing sperm cells whereas viable sperm cells repel the stain.

Data analysis
Data was presented as the mean ± standard error of the mean (SEM). Statistical analysis was by analysis of variance (ANOVA) using least significant difference model Statistical package used was Graphpad prism (version 6.0). $P<0.05$ was considered significant

RESULTS
Phytochemical screening of ethanol leaf extract of *Chromolaena odorata* revealed the presence of alkaloids, anthraquinones, cardiac glycosides, tannins, flavonoids, saponins, phenols and steroids (Table 1).

Scrotal and Testicular Biometry
The mean scrotal circumference, left testicular weight (LTW) and right testicular weight (RTW) in group B (arsenite-exposed) were lower than groups A (Control) treated Corn-oil, C (treated 200mg/kg extract) and D (treated 200mg +

Sodium arsenite (SA) but were only significant when compared with group A ($p<0.05$). There were consistent higher mean values of other scrotal and testicular parameters obtained in group C (treated 200mg/kg extract) compared to groups A (Control), B (Arsenic group) and D (treated 200mg + SA) although the changes were not significant ($P>0.05$) (Table II)

The Arsenic group B showed consistent decreased mean values in almost all the parameters measured when compared across the groups.

There were observable slight increases in the mean values of all the parameters (LTL being significant at $p<0.05$) in Group D (treated 200mg + SA) when compared with group B (treated arsenite only) (Table 2).

Percentage sperm motility and Livability

The mean percentage sperm motility in Group A (treated with corn-oil) was significantly ($p<0.05$) higher than those in groups B (Arsenic group), and D (treated with 200mg + SA). However group C (treated with 200mg/kg extract) had the highest mean value when compared across the groups. The Animals exposed to sodium arsenite (group B) had a significantly decreased sperm percentage motility compared to groups A and C; whereas, there was a compensatory and significant increase ($p<0.05$) in sperm percentage motility of group D rats (treated 200mg + SA) when compared to group B (Arsenic exposed). The sperm percentage livability of group B rats (Arsenic group) was the lowest although there were no significant changes ($p>0.05$) in the mean values obtained across the groups (Figure 1).

Table 1: Phytochemical screening of Ethanol leaf extract of *Chromolaena odorata*

Constituents	Qualitative test	Observation
Alkaloids	Dragendoff's	+ve
Anthraquinones	Borntragers	-ve
Cardiac glycosides	Killer-killanins	+ve
Tannins	Ferric chloride	+ve
Flavonoids	Ferric chloride	+ve
Saponins	Frothing	+ve
Phenols	Sodium hydroxide	+ve
Steroids	Salkoski's	+ve

KEY: +ve = detected; - ve= not detected

Table 2: Scrotal and Testicular Biometry of wistar strain Albino rats in different treatment groups

Parameters	Corn oil Group (A)	Sodium Arsenite Group (B)	200mg/kg leaf extract Group (C)	200mg/kg leaf extract + Sodium Arsenite Group (D)
Scrotal circumference (cm)	6.67±0.38	5.03±0.39*	6.92±0.70*	6.78±0.21
Scrotal length (cm)	3.57±0.18	3.28±1.16	4.08±0.45	3.28±0.31
Left testis weight (g)	1.07±0.04	0.76±0.04*	1.14±0.04*	0.98±0.10
Left testis diameter (cm)	3.30±0.06	3.13±0.09	3.35±0.08	3.20±0.17
Left testis length (cm)	2.27±0.12	2.30±0.06	2.43±0.15	2.48±0.95
Right testis weight (g)	1.06±0.06	0.76±0.05*	1.18±0.05*	0.98±0.11
Right testis diameter (cm)	3.20±0.11	3.00±0.09	3.37±0.11	3.00±0.82
Right testis length (cm)	2.27±0.12	2.30±0.07	2.40±0.14	2.50±0.07

*Refers to values along the row with significant difference ($P<0.05$)

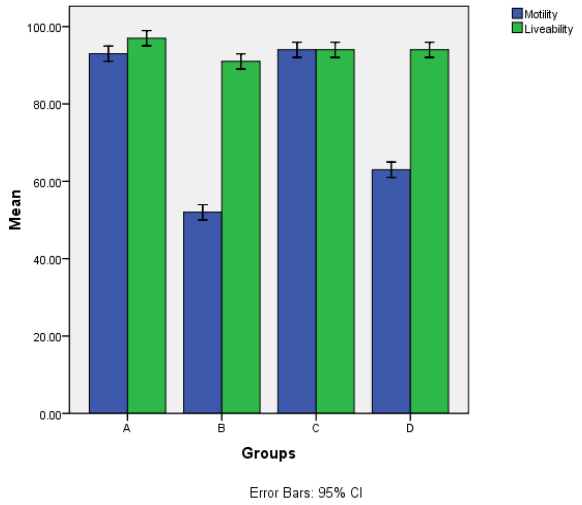

Figure 1: Percentage sperm motility and Liveability of wistar strain Albino rats in different treatment groups

Biochemical assays

The mean LH concentration of group B rats was the highest and was significant compared with those of groups A, C and D just as that of group D was higher compared to that of group A. The converse was observed with mean testosterone concentration. Group B rats had significantly lowest testosterone compared with groups A, C and D. The acid phosphatase enzyme level was significantly higher in group D compared with groups A,B and C while Lactate dehydrogenase(LDH) concentration of group A was higher significantly compared to groups B, C and D. The serum Creatine kinase (CK) level of group B was higher significantly when compared with group C. (Table IV).

DISCUSSION

The mean scrotal circumference of arsenic-exposed animals (group B), was significantly ($p<0.05$) lower than other treatment groups. This has a negative implication on scrotal health with scrotal circumference being a significant correlate of fertility in animals (Bezerra *et al*., 2009), it implies that arsenite is capable of inducing scrotal pathology and hence precipitates infertility in animals (Ola-Davies *et al*., 2014). It is known that scrotal circumference is highly related to semen quality and reproductive soundness (Bongso *et al*., 1982). It is strongly correlated to body weight (Bezerra *et al*., 2009), testicular weight, whole epididymal sperm reserves (Ugwu, 2009). Therefore, the results obtained in the current study in which the arsenic-treated group (B) showed a consistent decreased mean values in scrotal circumference corroborate earlier report (Fouad *et al.,* 2014) showing that arsenite could induce male reproductive toxicity, reduction of testicular weight and scrotal circumference in experimental animals.

Table 3: Hormones and Enzymes profile of wistar strain Albino rats in different treatment groups

PARAMETERS	**Corn oil Group (A)**	**Sodium Arsenite Group (B)**	**200mg/kg leaf extract Group (C)**	**200mg/kg leaf extract + Sodium Arsenite Group (D)**
LH (ng/ml)	11.67 ± 0.88^{ab}	17.25 ± 0.48^{a}	13.75 ± 0.76^{a}	15.50 ± 0.87^{ab}
TESTO (ng/ml)	1.50 ± 0.06^{abc}	1.03 ± 0.03^{a}	1.28 ± 0.09^{ab}	1.23 ± 0.10^{ac}
ACID PHOS (U/l)	1.57 ± 0.12^{a}	1.45 ± 0.13^{b}	1.55 ± 0.19^{c}	1.87 ± 0.05^{abc}
ACID MNOS(U/l)	0.60±0.06	0.78±0.11	0.65±0.06	0.65±0.06
LDH(U/l)	17.67 ± 1.86^{abc}	25.50 ± 1.85^{a}	23.50 ± 1.04^{b}	25.50 ± 1.32^{c}
CK(U/l)	15.33±0.67	18.75 ± 1.75^{b}	15.00 ± 0.71^{b}	17.00±1.73

Key: TESTO = Testosterone; LH = Luteinising Hormone; ACID PHOS = Acid Phosphatase; LDH = Lactate Dehydrogenase; CK = Creatine Kinase

Mean values with same superscripts along the rows are significantly different ($p<0.05$

The significant increase observed in the mean scrotal circumference, left testis weight (LTW) and right testis weight (RTW) of group C (treated with 200mg/kg extract) compared to groups A (Control), B (Arsenic group) and D (treated with 200mg + SA) indicates that the extract of *Chromoleana odorata* at 200mg/kg body weight possesses a pro-fertility property.

Although there were no observable significant ($p>0.05$) changes in all the testicular indices measured across the groups except for LTW (left testis length) and RTW (right testis length), there were consistent higher mean values obtained in group C (treated 200mg/kg extract only) than groups A (Control), B (Arsenic group) and D (treated with 200mg +SA). Also, group D showed consistent higher mean values compared to arsenic treated group B. This also suggests that extract of *Chromoleana odorata* at 200mg/kg body weight may offer some ameliorative effects against arsenic toxicity in rats. This is similar to a report by Ola-Davies and Ajani (2016a) in which ethanol leaf extract of *Ageratum conyzoides* protected spermatozoa against arsenic reproductive toxicity in wistar strain albino rats.

The pro-fertility potential of *Chromoleana odorata* extract also reflected in the mean percentage motility of group C (treated 200mg/kg extract) being the highest mean value when compared across the groups. This agrees with the findings of Oyeyemi and Ajani, (2014) in which extract of *Mormodica charantia* enhanced sperm motility in wistar strain albino rats. The Arsenic treated group B had a significantly lowest sperm percentage motility across the groups; whereas, there was always a significantly increase ($p<0.05$) in group D rats (treated 200mg + SA). This increase might be attributed to the inclusion of 200mg/kg leaf extract of *Chromolaena odorata* having a sort of ameliorative effect against arsenic toxicity. This is similar to a report by Ola-Davies and Ajani (2016b) in which ethanol leaf extract of *Pistia stratiotes improved sperm motility and* protected spermatozoa against arsenic reproductive toxicity in wistar strain albino rats.

The phytochemical analysis (Table 1) revealed the presence of tannins in *Chromolaena odorata* and this could have interfered with the absorption of arsenic thereby minimizing the effect of arsenate toxicosis. Also, flavonoids contained in the plant provided antioxidant effects against the toxicosis (Bhattacharya, 2017). In addition, the flavonoids might have inhibited actions of pro-inflammatory endogenously-produced substances thereby decreasing the adverse inflammatory degenerative effect of toxicosis induced by arsenite (Roy *et al,* 2014).

The arsenic-exposed group B had the lowest percentage mean value of live spermatozoa although this was not adversely affected by the treatment across the groups. This finding is similar to a report by Ola-Davies *et al*. (2014) in which male wistar strain albino rats were exposed to arsenite and fractions of *Spondia mombin.*

The significantly lowest testosterone concentration of arsenic-treated group could be linked to the reduced scrotal circumference which is indicative of reduction in testicular mass hence reduced capacity of the testis to perform its exocrine (spermatogenesis) and endocrine (steroidogenesis) function. Reduction in testosterone removed negatively feedback to LH production hence, compensatorily, luteinizing hormone (LH) production increased in the group to stimulate the Leydig cells to produce more testosterone. This probably explains the significantly high LH concentration in the arsenic group compared to all other groups. The increased testosterone concentration of group D compared with group B indicate ameliorative property of the extract against arsenic reproductive toxicity (Oloye *et al.*, 2017). The testosterone levels in all the groups fell within the reported range of 2-48 nmol/l expected for the wistar rat within experimental laboratory (Heywood, 1980). However, a prolonged exposure to arsenic could further damage the testis and eventually lead to azoospermia.

The higher concentrations of creatine kinase in group B compared to other groups may be the reaction of the rat to toxicity of arsenite as observed by Ola-Davies *et al.* (2017). This might be made possible due to myolysis of the cardiac muscle and other skeletal muscles induced by toxicosis caused by arsenite in the exposed, untreated group (B). Also, in groups B and D (both exposed to arsenic), LDH levels were higher significantly when both were compared with the control. This might be due to the fact that hepatotoxicity, myolysis and genotoxicity triggered production and release of LDH (Garg, 2008; AlForkan *et al.*, 2016).

CONCLUSION

The study showed that 200mg/kg extract of *Chromoleana odorata* had a profound scrotal, testicular and sperm protective properties in arsenic-treated wistar strain albino rats and therefore could be incorporated into animal feeds especially in the chronically exposed population.

REFERENCES

Abernathy, C.O., Thomas, D. J., Calderon, R. L. (2003). Health effects and risk assessment of arsenic. *Journal of Nutrition*, **133**(suppl1):1536S–1538S

Ahmed, M. I. ; Rekhate, D. H. ; Dhore, R. N. ; Honmode, J. ; Sarde, P. P., (1995). Nutritive value of water hyacinth [*Eichhornia crassipes*] hay in sheep. *Indian Journal of Animal Nutrition*, **12**(3): 187-188.

Al-Forkan, M., Islam S., Akter, R., Shameen, A.S., Khaleda, L., Rahman, Z. and Salma, C.D.U. (2016). A sub-chronic exposure study of arsenic on haematological parameters, liver enzyme activities, histological studies and accumulation pattern of arsenic in organs of Wistar albino rats, *Journal of Cytolology Histolology* (pages 1-7) Available from: S5:1 http://dx.doi.org/10.4172/2157-7099.S5-006 accessed 15th August, 2016.

Arene, O.B., Nwankiti, A.O. and Okagor, N.(1985). The chemical basis of the pathology of the yam tuber. In: Osuji, G. (Ed). Advances in Yam Research. The Biochemistry and Technology of yam tuber. Biochemical Society of Nigeria. Anambra State University of Technology Nigeria. Pp 251-258

Argos, M., Ahsan, H., Graziano, J.H. (2012). Arsenic and human health: epidemiologic progress and public health implications. *Reviews on Environmental Health*, **27**(4):191–195.

ATSDR, (2005). Agency for Toxic Substances and Disease Registry. Toxicological Profile for Arsenic (update). Atlanta, Georgia, pp. 1-357.

Bamba, D, Bessiere, J.M, Marion, L, Pelissier, Y and Fouraste, I. (1993). Essential oil of *Eupatorium odoratum*. *Plant Medica*, **59**: 184-185.

Bezerra, F.Q.G., Aguiar Filho, C.R., Freitas Neto, L.M., Santos Junior, E.R., Chaves, R.M., Azevedo, E.M.P., Santos, M.H.B., Lima, P.F. and Oliveira, M.A.L., (2009). Body weight, scrotal circumference and testosterone concentration in young Boer goat males born during the dry or rainy seasons. *South African Journal of Animal Science*, **39** (4):301- 306.

Bhattacharya, S. (2017). Medicinal plants and natural products in amelioration of arsenic toxicity: a short review. *Pharmaceutical Biology*. 55(1); 349-354.

Bongso T.A., Jainudeen M.R., Zahrah A.S., (1982). Relationship of scrotal circumference to age, body weight and onset of spermatogenesis in goats. *Theriogenology*, **18** (5): 513 – 524.

Burkill, H.M. (1997) The useful plants of west tropical Africa. Families M-R. Royal Botanic Gardens, Kew.

Chowdhury, S. R. , Chowdhury, S. D. and Smith, T. K.. (2002). Effects of dietary garlic on cholesterol metabolism in laying hens. *Poultry Science*. **81**:1856–1862.

Fouad, H.K., Chiman, H.S. & Saleem, S.Q. (2014), Magnetic Field Effect on Growth and Antibiotic Susceptibility of *Staphylococcus aureus*. *Journal of Al-Nahrain University*, **17**, 138-143.

Garg, S.K. (2008) Veterinary Toxicology. CBS publishers and Distributors New Delhi-110002.

Harborne, J. B. (1989). General Procedures and Measurement of Total Phenolics. *Plant Phenolics*, 1–28.

Heywood, L.H. (1980). Testosterone levels in the male laboratory rat: variation under experimental conditions. *International Journal of Andrology,***3**(5): 519-529

Iwu, M.M., (1993). Modalities of Drug Administration: Hand Book of African Medicinal Plants. CRC Press Inc., Florida, pp: 309-330.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. I*nterdisciplinary Toxicology*, **7**(2), 60–72.

Leonardo, D.C.L., Franco, A., Gustavo, A.T.L., Luciano, M.A., Lius, F.M.E.S., Gabriele, P.D.S., Isabela, D.M.A., José, F.N.N., Israel, F., Karla, K., (2000). Toxicological evaluation by *in vitro* and *in vivo* assays of an aqueous extract prepared from *Echinodorus macrophyllus* leaves. *Toxicology Letters* **116**, 189-198.

Ola-Davies, O. E and Ajani, O. S (2016 a). Effects of Ageratum Conyzoides on Semen Characteristics and Sperm Morphology in Rats Exposed to Sodium Arsenite *African Journal of Biomedical Research,* **19***:* 55-60.

Ola-Davies, O. E and Ajani, O. S (2016b). Semen characteristics and sperm morphology of Pistia stratiotes Linn. (Araceae) protected male albino rats (Wistar strain) exposedto sodium arsenite. *Journal of Complementary and Integrative Medicine*. DOI 10.1515/jcim-2015-0033

Ola-Davies, O. E, Ajani. O S and Oyeyemi, M.O., (2014): Spermatozoa morphology and characteristics of *Spondias mombin.* L. (*anacardiaceae*) protected male wistar rats exposed to sodium arsenite. *Journal of Veterinary Medicine and Animal Health,* **6** (2): 63-66

Ola-Davies, O.E., Biobaku, K.T. Okediran, B.S. and Adah, A.S.(2017). Evaluation of the "Antidotal" Potential of Mangifera indica L. leaves extract on sodium arsenate exposed male Wistar rats using some biochemical markers. *Ceylon Journal of Science,* **46**(1): 97-102

Oloye, A. A., Ola-Davies, O. E, Oyeyemi, M. O (2017) Haemogram and Hormonal profile of WAD buck treated with leaf ethanol extract of Spondias mombin. *Sokoto Journal of Veterinary Sciences,* **15** (3). 85-90

Oyeyemi, M. O and Ajani, O. S. (2014). Spermatozoa characteristics, serum biochemistry and hematological profile of male albino rats (wistar strain) treated with *Mormodica charantia. International Journal of Applied Agricultural Research,* **9**(1): 41-52.

Phan, T.T., L. Wang, P. See, R.J. Grayer, S.Y. Chan and S.T. Lee, (2001). Phenolic compounds of chromolaena odorata protect cultured skin cells from oxidative damage: Implication for cutaneous wound healing. *Biological and Pharmaceutical Bulletin,* **24**: 1373-1379.

Preston, R.J., Dean, B.J., Galloway, S., Holden, H, McFee, A.F., Shelby, M., (1987): Mammalian *in vivo* cytogenetic assays: analysis of chromosome aberrations in bone marrow cells. *Mutation Research*, 157-165.

Roy, A,, Das, A,, Das, R[,], Haldar S[,] , Bhattacharya S[,] , Haldar, P. K. (2014). Naringenin, a citrus flavonoid, ameliorates arsenic-induced toxicity in Swiss albino mice. *Journal of Environmental Pathology, Toxicology and Oncology*, **33**(3):195-204.

Sharma, B, Singh, S., Siddiqi, N. (2014), Biomedical implications of Heavy metals

induced imbalances in Redox system, *Biomed research international.* https://www.ncbi.nlm.nih.gov/pmc/articles/PMC 4145541 (Accessed on May 28, 2018).

Suzuki S., Arnold, L., Ohinishi, T., Cohen, S.(2008). Effects of inorganic arsenic on the rat and mouse urinary bladder. *Toxicological Sciences,* **106(**2), 350-363

Sofowora A: (1982) Medicinal plants and traditional medicines in Africa . John Wiley and Sons Ltd, Nigeria ,pp 64-79.

Trease, E.G. and Evans, W.C.. (1983). Textbook of pharmacognosy, 14th Ed. W.B. Saunders Company Ltd. 24 – 28 Oval Road London NWI TDX, UK. Pp, 13 – 511.

Ugwu S.O.C., (2009). Relationship between scrotal circumference, in-situ testicular measurements and sperm reserves in the West African Dwarf bucks. *African Journal of Biotechnology*, **8**(7): 1354 – 1357.

Zemjanis R. (1977). Collection and evaluation of semen. In: Diagnostic and therapeutic technique in animal production. 3rd edition. The Williams and Wilkins company, Baltimore 139-180.