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Androgenic And Antioxidant Activity Of Stelleria Media On Rat Following Sub-Chronic Exposure To Dichlorvos

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***Abstract**: This study aimed at investigating the possible use of Stelleria media as an antioxidant to ameliorate vital organ damage and spermatogenesis impairment induced by Dichlorvos following sub-chronic exposure of Sprague-Dawley rats at 25mg/kg/bw/day. The result revealed 10.73% loss in bodyweight, 20.16% loss in liver weight, 32.21% of testes weight, 29.91% epididymal weight indicative of systemic toxicity, damage to liver hepatocytes and reproductive organs respectively. Aspartate Transaminase and Alkaline Phosphatase were significantly ($p< 0.05$) elevated during the treatment period above control coupled with 14.53% Glucose reduction. Also elevated ($p<0.05$) were serum Urea, Creatinine, Total protein and Albumin. Histopathological liver micrographs of animals exposed to DDVP only revealed occurrence of binucleated hepatocytes, vacuolation, regeneration and proliferative activity associated with micronuclei. Testes of rats exposed to Dichorvos only showed inhibition of meiotic division, cellular degeneration, interstitial space devoid of Leydig cells, adluminal and luminal space showing loss of spermatogonia, sparsely populated with spermatocytes, maturing spermatozoa respectively and an empty lumen. It is concluded that Dichorvos is a systemic toxin, hepatotoxic, adversely affects the kidney and impairs spermatogenesis. With the widespread and unregulated use of this chemical in the developing countries caution in its use as an insecticide is hereby recommended.*
***Keywords:** androgenic, Dichlorovos, hepatocytes, Stellaria media, Spermatogenesis.*

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I. Introduction

Dichlorvos is an organophosphorus insecticide used worldwide for pest control in Agriculture, public, private, and animal health programmes. In animal studies cases of spermatozoa abnormalities in mice, birth defects in foetuses of rats injected with Dichlorvos have been reported[1, 2]. Increased levels of biomarker enzymes for hepatotoxicity and haematoxicity in mice exposed to Dichlorvos were also reported [3, 4]. Other toxicity studies of Dichlorvos on blood, liver and kidney have been reported in rats [5,6,7,8,9,10], while some reported using herbal extract of Zingiber officinale, Alstonia boonei, aqueous seed of Leea guineensis and vitamin C supplementation as antioxidant on Dichlorvos induced- toxicity in animal studies [1,6,11,12]. Moreso, [3] the reversal of all hepatic markers to near normal levels indicating the ameliorative effect have been reported for Lycopene against Dichlorovos treated rats. The antioxidant and therapeutic activity of Solanum lycopersicum on liver and reproductive functions of male rats exposed to Cypermethrin was also reported [13,14]. However, a lack of beneficial effect of Solanum lycopersicum on female rats and foetuses following exposure to Cypermethrin has been reported[15] . Stelleria media has been reported to contain high concentrations of Vitamin C, Genistein, Flavonoids, Tritepenoids, Saponins and Thiamin commonly used as postpartum depurative and circulatory tonic [16, 17]. The continued indiscriminate use of dichlorvos in developing countries requires the search for viable alternatives in mitigating the oxidative stress to the human vital organs. Based on the reports of widespread damage to many vital organs, its widespread use in homes, farms, offices and industries as a fumigant and the apparent paucity of literature on mitigating factors, this study was initiated to investigate the possible use of an indigenous herbal antioxidant to ameliorate vital organ damage and inhibition of spermatogenesis induced by dichlorvos using Sprague-Dawley rats as the model organism

II. Materials and Methods

2.1 Experimental Location

This study was carried out in the Research laboratory of Reproductive physiology and Genetics Unit of the Department of Animal and Environmental Biology. River State University, Nkpolu-Oroworukwo, Port Harcourt (Coordinates $4^{o}48^{'}14^{''}$ N $6^{o}59^{'}12^{''}$ E) and lasted for 35days.

2.2 Experimental Animals Management

Experimental animals consisted of twenty male Sprague- Dawley rats of mean weight 245.83±8.39g. The animals were purchased from the Department of Biochemistry, University of Port Harcourt, and allowed to acclimate for 14 days prior to the commencement of the experiment.

The chemical 2, 2- dichlorovinyl dimethyl phosphate (DDVP) was purchased from a reputable chemical store in Rivers State, Port Harcourt.

2.3 Experimental design and Procedure

The experimental animals were kept in a well-ventilated Animal House with 12 hour light: 12 hour dark condition and fed with standard rat chow and cool clean water ad libitum. Twenty adult male rats randomly selected into plastic cages and grouped as A to D, with five male rats per cage. Group A served as control. Group B were administered 25mg/kg/bw/day of DDVP and 5000mg/kg/bw/day of dried leaf ethanolic tincture of Stelleria media. Group C received 25mg/k/bw/day of DDVP and 5000mg/kg/bw/day of fresh leaf ethanolic tincture of Stelleria media. Group D received 25mg/kg/bw/day of DDVP only. The study was carried out according to the institutional animal care protocols at the River State University, Port Harcourt, Nigeria and followed approved guidelines for the ethical treatment of laboratory animals.

2.4 Data collection

Data was collected on body and organ weights, testicular histolopathology, hepatic toxicity biomarkers**.** The initial and final bodyweight of the animals were recorded in grams. The change in weight was determined by deducting the initial bodyweight from the final weight. By the end of the experimental duration (35 days) animals were euthanized by ethyl ether inhalation. Data was collected for the assessment of organosomatic and gonadosomatic indices.

2.4.1 Organosomatic index

Kidney, Liver, Spleen, and Heart were removed intact and freed from adhering tissues and weighed according to [18]. The weight recorded to the nearest 0.01gram

2.4.2 Gonadosomatic index

The reproductive organs including paired testes, paired epididymides, prostate and seminal vesicles were removed and weighed. Organosomatic and Gonadosomatic indices were calculated as reported by [19].

2.5 Biochemical Analysis of Liver Biomarkers

The samples were collected individually by cardiac puncture into sterile tubes and the serum separated at 2500 g for 10 min and stored for determination of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) [20] Alkaline Phosphatase (ALP) [21]. Total bilurubin (TB), Total protein (TP) and Creatinine were determined as reported in [22]. Albumin (ALB) concentration was assayed according to Sigma Diagnostics based on the procedure of [23].

2.6 Histopathological Evaluation of the Liver and Testis

Known weight of the liver and testis was fixed in Bouin's fixative and processed according to the protocol described by [24, 25] sectioned with a Digital Microtome Model A O Spencer No. 820 at 5um thick and stained with Hematoxylin and Eosin (H &E) Photomicrographs were generated with a digital Microscope Biosphere Miller B with an image processor DN2 – Microscopy Image processing Software [26] at X100 magnification.

2.7 Statistical Analysis

Data obtained from the study were subjected to one-way ANOVA and means and standard deviations calculated with SPSS version 20. Where there were significant differences, Pair-wise comparison was also conducted with Tukey test for mean separation.

III. Results

The phytochemical analysis of Stelleria media was carried out according to the method of [27]. The result are presented in Table 2 which shows a high content of Oxalate, Steroids, Flavonoids, Saponins, Phenols, Alkaloids Glycosides and Tannins

3.1 Phytochemical Analysis

Table 1: Phytochemical components of the leaf of Stelleria media

Stelleria media	Phytochemical Composition %
Oxalate	+++
Steroids	+++
Flavonoids	+++
Saponins	++
Phenols	++
Alkaloid	++
Tannin	++

Glycosides	+
Resins	-
Terpenoids	-
Triterpenoids	-
Phytate	+

3.2 The effect of Dichlorvos and Stelleria media on body weights

The effect of the administration of DDVP and Stelleria media on the mean body weights of Sprague-Dawley rats exposed for 35days is shown in fig 1a-d. Group A is the control with an initial body weight of 156.27±1.70g in week 1 to 250.91±5.57g in week 7 (fig 1a). There was also an increase in the body weight of animals in groups co-administered both dry and fresh Stelleria media tincture from 154.82±6.78g to 248.79±9.67g and 157.41±0.99g± to 240.15±1.36g. In group D administered DDVP alone, there was an initial increase in body weight from 151.89±4.12g in week 1 to 224.29±3.06g in week 3 which latter remained unchanged till the end of the experiment at week 7.

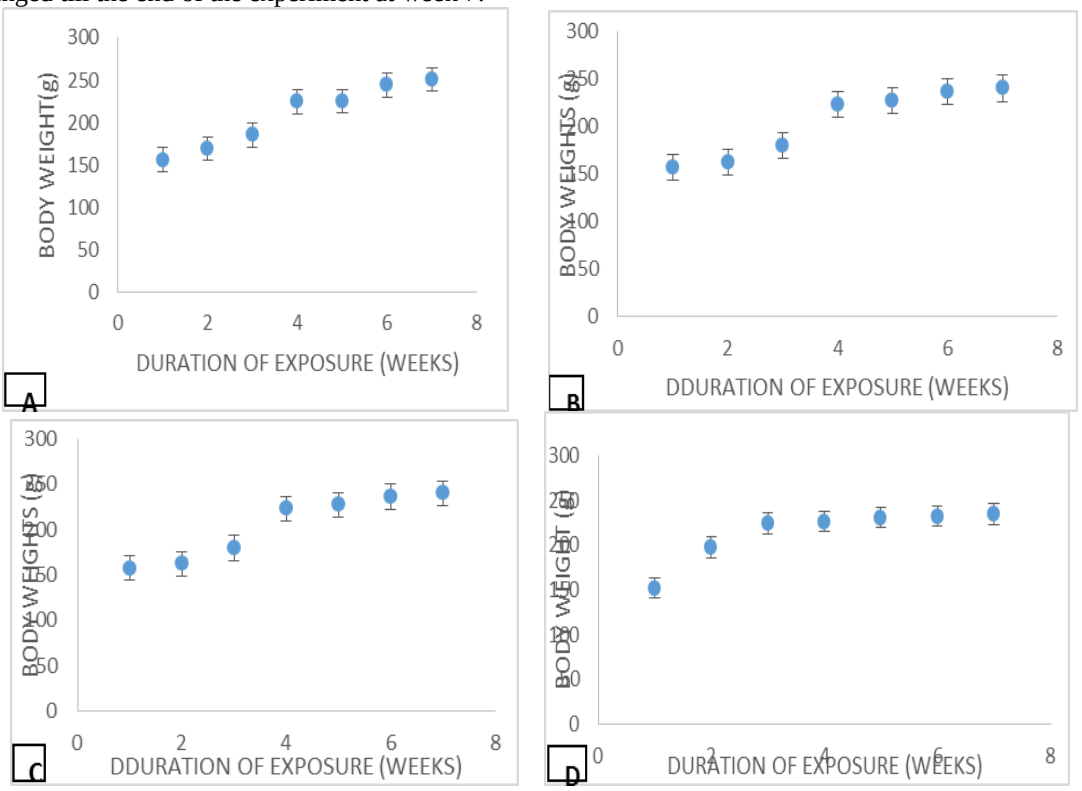

Fig.1 (a-d) Effect of co-administration of dry and fresh leaf tincture on the bodyweight of SD rat exposed (sub-chronic) to DDVP

3.3 Effect of Co-administration of DDVP and Stellaria media on organ weights

The effect of DDVP and Stelleria media tincture on Sprague-Dawley rat vital organs is shown in Fig.1(a-f) there was a 20.16% loss in liver weight 9.25% loss in heart weight in group D Similarly there was 32.21% reduction of testes weight and 29.91% epididymal weight loss in rats exposed to DDVP only. Negligible weight loss in testes (2.03%) of rats co-administered dried and fresh leaf of S.media ethanolic tincture was observed (Fig.2(a-h)

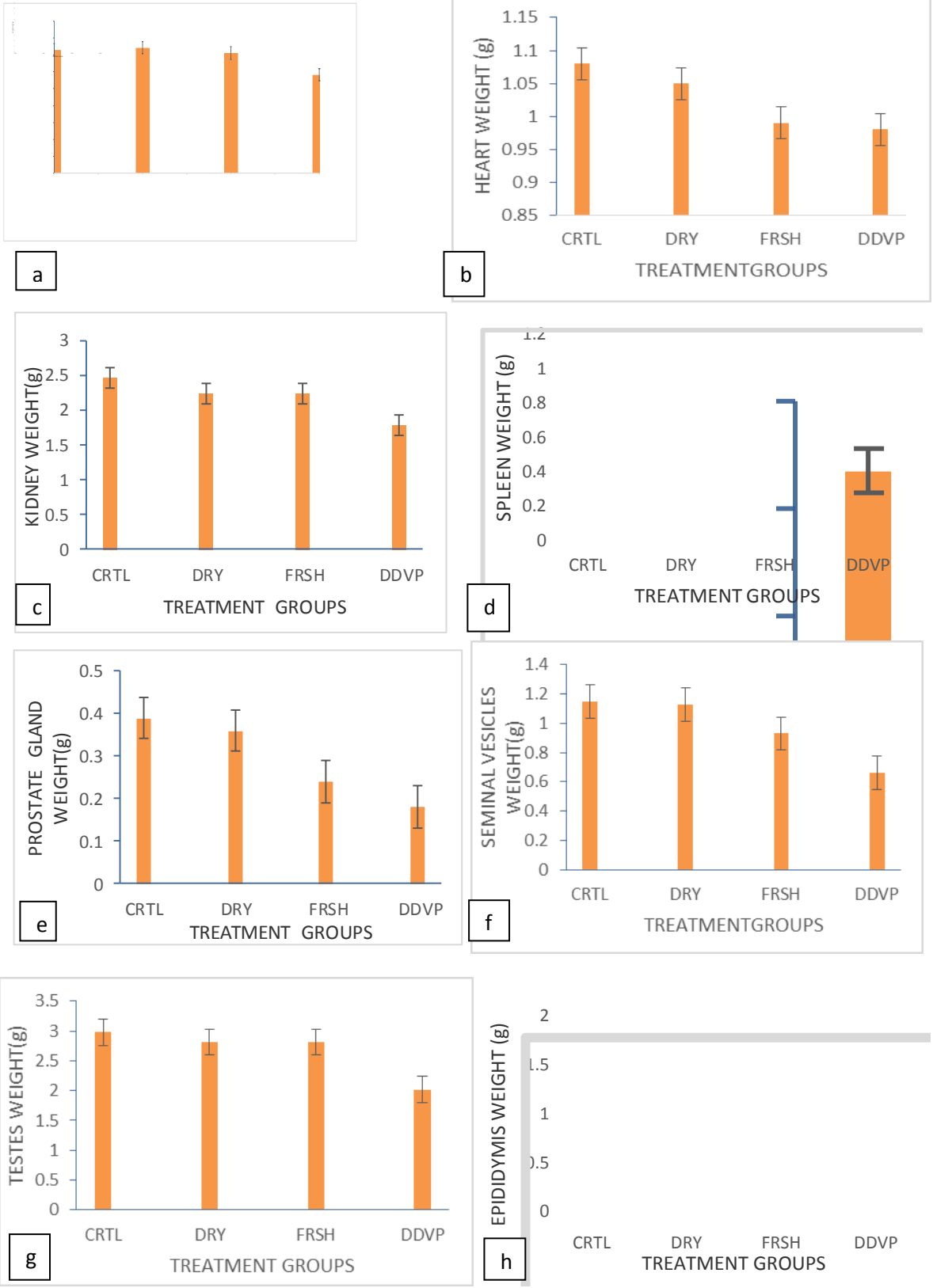

Fig.2 (a-h) Effect of DDVP on organ weights of rats co-administered Stelleria media tincture

Organosomatic indices genetically controlled and are species specific, there was no significant difference in the Cardiosomatic, Hepatosomatic, Renosomatic and Splenosomatic indices of control rats and those exposed to DDVP and co-administered S.media tincture. However, the bodyweight of the animals in groups D were significantly lower than those of groups A,B and C .

Table 2: Effect of DDVP on Organosomatic indices of Sprague-Dawley Rats

ORGANOSOMATIC INDICES (%)					
	Body Weight(g)	CARDIOSOMATIC	HEPATOSOMATIC	RENOSOMATIC	SPLENOSOMATIC
A	250.91±5.57 [ab]	0.51±0.64	3.69±0.41	1.16±0.15	0.51±0.12
B	248.79±9.676 [b]	0.49±0.15	3.45±0.34	1.04±0.27	0.48±016
C	240.15±6.11 [a]	0.49±0.09	3.30±0.61	1.04±0.09	0.47±0.05
D	224.29±3.06 [ab]	0.46±0.55	3.23±0.24	1.00±0.16	0.45±0.64
Anova F-value	4.1	0.16	1.31	0.62	0.16
SIG	0.03	0.92	0.31	0.61	0.92

*Values are in Mean ±SD

There was no significant difference in the Gonadosomatic indices of animals exposed to DDVP and co-administered S. media tincture when compared to the control and group D administered Dichlorvos only.

Table 3: Effect of DDVP on Gonadosomatic Indices of Sprague-Dawley Rats

GROUPS	BW	PROST	SEM. VES	LTW	RTW	PTW	LEP	REP	PEW
A	250.91±5.57 [ab]	0.18±0.08	0.54±0.19	0.72±0.10	0.69±0.08	1.42±0.14	0.38±0.11	0.39±0.11	0.76±0.22
B	248.79±9.676 [b]	0.17±0.09	0.54±0.17	0.71±0.04	0.68±0.06	1.39±0.08	0.37±0.09	0.37±0.64	0.74±0.15
C	240.15±6.11 [a]	0.11±0.02	0.31±0.12	0.68±0.21	0.59±0.16	1.27±0.36	0.29±0.04	0.28±0.05	0.58±0.09
D	224.29±3.06 [ab]	0.09±0.02	0.28±0.12	0.66±O.O6	0.64±0.79	1.31±1.30	0.28±0.08	0.30±0.09	0.58±0.17
ANVA-F	4.11	1.80	3.74	0.19	1.00	0.51	1.43	1.69	1.58
SIG	0.03	0.19	0.04	0.90	0.42	0.68	0.28	0.22	0.24

*Value are Mean ±SD. * PROST(prostate), SEM.VES(seminal vesicle), LTW(left testicular weight), RTW(right testicular weight), PTW(paired epididymal weight), LEP(left epididymal weight), REP(right epididymal weight), PEW(paired epididymal weight).

The effect on kidney biomarkers of sub-chronic exposure of SD rat to DDVP and co-administration of S. media ethanolic tincture is shown on Table 4.Urea was significantly ($p<0.05$) in group C while DDVP only (Group D) was significantly ($p<0.01$) elevated when compared to control, Creatinine, Total Protein were equally elevated in group D while Serum Albumin was significantly ($p<0.05$) increase in group D administered DDVP only.

Table 4: Effect of Co-administration of Dichlorvos and Stelleria Media ethanolic tincture on Kidney Biomarkers in SD rats

PARAMETERS	UR(mg/dL)	CR(mg/dL)	T P(g/dL)	T.BIL(µmol/L)	ALB(g/dL)
CRTL	5.67±0.41[c]	138.40±14.03[ab]	88.40±2.51[b]	13.32±3.04[a]	38.33±5.03[a]
DRY	5.64±1.52[c]	133.67±8.08[ab]	83.60±2.97[a]	10.60±1.51[a]	39.00±1.87[a]
FRSH	6.52±1.07[b]	144.20±41.00[b]	82.00±6.93[b]	14.18±4.63[a]	39.80±1.93[a]
DDVP	8.54±1.28[a]	154.40±18.18[a]	79.33±3.61[a]	15.00±6.25[a]	34.60±3.85[a]
ANVA-F	5.86	0.53	1.36	1.07	2.66
SIG	0.01	0.03	0.02	0.39	0.08

*Values are Mean±SD. Values with same superscript letters are not significantly different, whereas those with different superscript letters are significantly different ($P < 0.05$).

The result of the evaluation of the effect of DDVP and co-administration of ethanolic extract of S.media tincture on Liver biomarkers is presented in Table 4. The serum concentration of Aspartate Transaminase was significantly ($p<0.05$) elevated in group B and C administered dry and fresh extracts respectively, while the increase in the concentration in group D was significant at $p<0.01$. There was no difference in the total cholesterol, the level glucose dropped from 3.58±0.33 to 3.06±0.93 while Alanine aminotransferase increased significantly ($p<0.05$) from 45.80±13.63 in the control to group C co-administered fresh S.media tincture and significantly higher 66.00±13.34 in group D administered Dichlorvos only ($p< 0.01$)

Table 5: Effect of Co-administration of Dichlorvos and ethanolic extract of Stelleria Media on Liver Biomarkers in Male SD rats

PARAMETERS	GLU(mmol/L)	T.CHO(mmol/L)	AST(U/L)	ALT(U/L)	ALP (U/L)	LDH (U/L)
CRTL	3.58±0.33	3.22±0.68	71.80±20.57 [c]	12.40±5.55	45.80±13.63[c]	77.2±22.62 [a]
DRY	3.57±0.42	2.93±0.61	93.80±26.81 [b]	13.00±2.55	46.67±5.86[c]	75.8±20.46 [a]
FRSH	3.96±1.17	2.92±0.82	95.20±24.52 [b]	13.67±3.06	57.80±13.41[b]	42.66±21.13 [b]
DDVP	3.06±0.93	2.52±0.23	101.67±27.09 [a]	13.80±5.26	66.00±13.34[a]	40.60±14.70 [b]
ANVA-F	0.98	1.06	1.25	0.98	2.36	1.008
SIG	0.43	0.40	0.03	0.96	0.04	0.02

*Values are Mean±SD.Values with same superscript letters are not significantly different, whereas those with different superscript letters are significantly different ($P < 0.05$).

The effect of sub-chronic exposure of male SD rats on the Liver and the functions is shown on Plate 1 (A-D). The normal liver polygonal cell architecture of hepatocytes, sinosoids, Kupffer cells and the associated hepatic portal vein are present in Plate 1A which served as the control. Plate 1 B shows some evidence of injury indicated by the presence of micronuclei .The injured hepatocytes appeared to have been regenerated only very few sinosoids are free of infiltration. Plate 1C shows an epithelium with a number of binucleated cells and dividing cells at various stages of mitotic division. Plate 1D shows epithelium with a large number of micronuclei, picnotic hepatocytes, very few dividing cells, infiltration of the sinosoids and the presence of Kupffer cells. Vacuolated, binucleated hepatocytes, regenerative cells, dysplastic, proliferative activities associated with micronuclei.

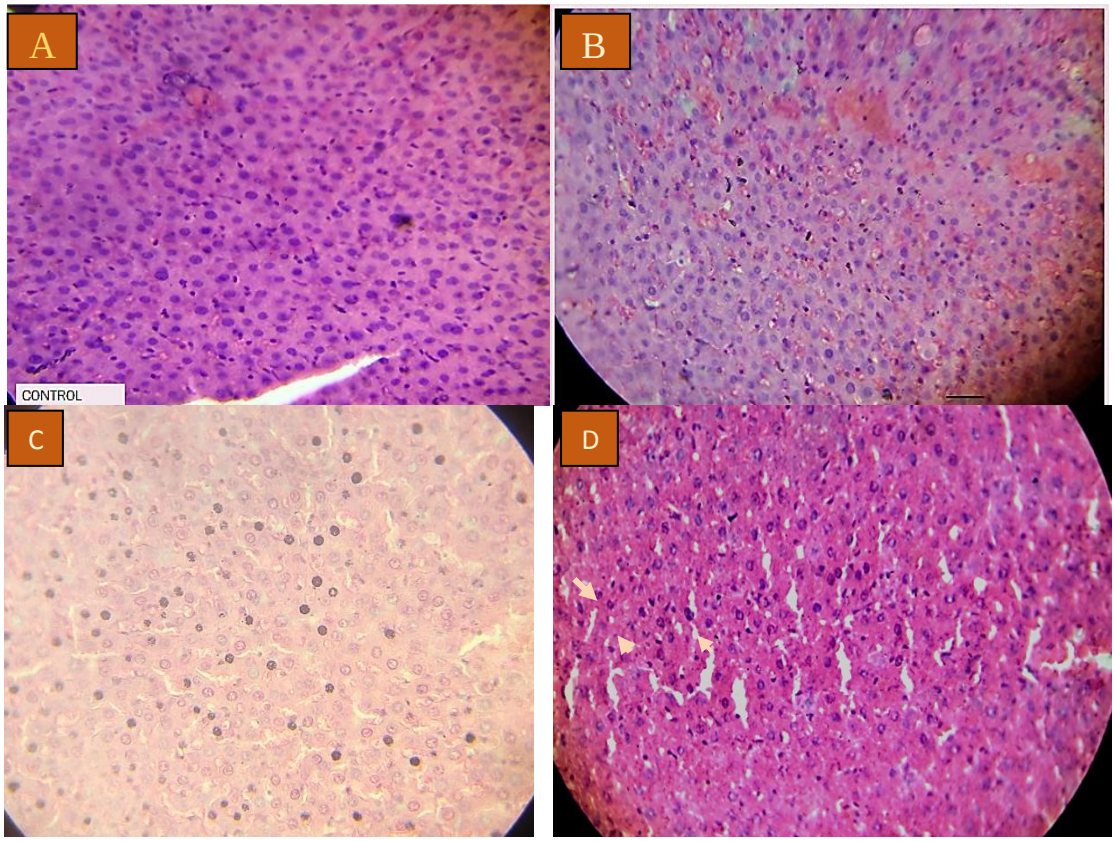

Plate 1 (A-D): Liver Function of Rats Co-administered Dichlorvos and Stelleria Media Tincture

The effect of DDVP and co-administration of Stelleria media dry and fresh leaf tincture on spermatogenesis in SD rat is presented in Plate 2(A-D).The control group A shows a normal seminiferous epithelium with full complement of spermatogenic elements.The mitotic spermatogonia are seen lining the basement membrane,closely followed by the primary spermatocytes at different stages of division.The interstitial cells of Leydig are intact and so are the Setoli cell. The lumen is filled with matured spermatozoa following spermiogenesis. Plate 2 B also shows an epithelium undergoing normal spermatogenesis.The spermatogonia appear to be actively dividing as no cellular degeneration is observed. Primary spermatocytes are seen to have divided into secondary spermatocytes and to produce fully formed spermatozoa following elongation and differentiation.The lumen is filled with mature cells and the interstitial cells of Leydig are intact.Plate 2C shows inhibition of meiotic division and cellular degeneration.Plate 2D shows interstial space devoid of Leydig cells(arrow) the seminiferous epithelium with adluminal space showing loss of spermatogonia(arrow head),luminal space sparcely populated with maturing spermatozoa(+) and an empty lumen.

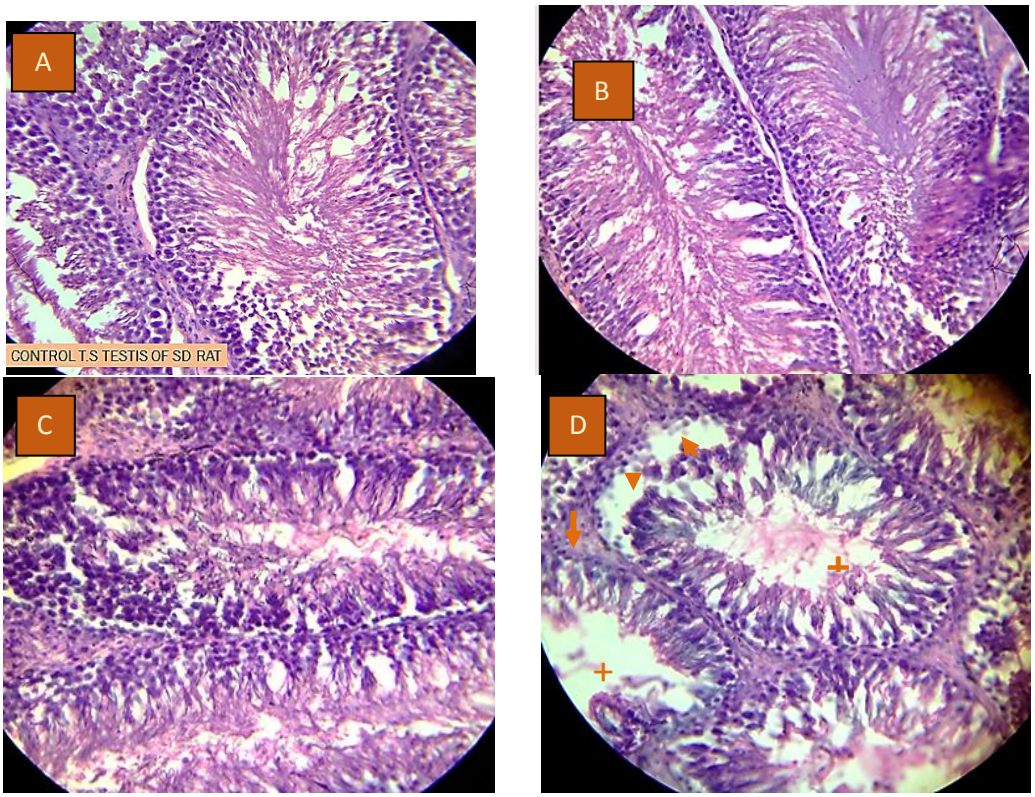

Plate 2 (A-D): The Effect of Co-administration of Stelleria Media Tincture on Spermatogenesis of Sprague-Dawley Rat exposed to Dichlorvos Plate 2A is control showing normal seminiferous epithelium,2B is epithelium from group co-administered dried leaf ethanolic tincture of Stelleria media 2C shows seminiferous epithelium from group co-administered fresh leaf ethanolic tincture of S.media ,2D is seminiferous epithelium of the group administered DDVP only.

IV. Discussion

The result of this study revealed that DDVP induced over 10.73% loss in bodyweight in the group administered DDVP only, 4.3% in group C co-administered fresh leaf ethanolic tincture of 5000mg/kg/bw/day of S.media along with 25mg/kg/bw/day, while there was no change in the bodyweight of group B administered dry leaf tincture of S. media. The loss in weight is indicative of systemic toxicity. There was no significant change in the weights of vital organs Liver, Kidney and Heart and Spleen. However, there was a 20.16% loss in liver weight in group D indicating loss or damage of the liver hepatocytes. There was no change in liver weight in groups B and C co-administered S.media with DDVP. This shows that DDVP is a systemic as well as hepatotoxic toxin and the lack of change in weight in groups B and C gives a signal that at 5000mg/kg/bw/day S.media is hepatoprotective. This is in line with its phytochemical components including flavonoids, Saponins, tannins which act as anti-oxidants against DDVP-induced oxidative stress. Similarly percentage weight loss in heart (9.25%) was recorded in group D indicating possible loss of the mitochondrial cells. The loss in weight of both the liver and the heart coupled with the 14.53% reduction Glucose might be a proof that this insecticide causes damage to the mitochondria thus inhibiting Gluconeogenesis.

Aspartate Transaminase (AST) and Alkaline Phosphatase (ALP) were significantly ($p< 0.05$) elevated during the treatment period above control levels and those of the groups co-administered dry and fresh leaf ethanolic tincture of S.media.The same trend was observed in serum total protein and creatinine ($p<0.05$) respectively and urea ($p<0.01$). Foreign compounds are predominantly bio-transformed in the liver by the action of metabolizing enzymes including microsomal enzymes, aminotransferases and oxygenases. Earlier investigation reveals that pesticides generated free radicals which have been implicated in the induction of liver lesions [13,14] in rats exposed to Deltamethrin [18] as well as induction of metabolite-related disorders. The elevated levels of ALT, AST, ALP, urea, albumin and total bilurubin as well as the reduction in total protein level in this investigation is characteristic of impaired liver function reflected as an initial elevation of the biomarker enzyme levels. Similar observations were made by other researchers [4,5,8] in rodents. A number of lesions were observed in the Histopathological micrographs of the experimental animals exposed to DDVP only (Plate 1d), occurrence of binucleated cells, vacuolation, micronuclei, regeneration and proliferative associated with micronuclei is an indication of pathological conditions as well as genotoxic damage involving chromosomal instability. High frequency of micronuclei is a biomarker of liver cirrhosis Micronuclei are chromosomes fragments left out during nuclear division. Chromosome breakage and the subsequent instability is suspected to be induced mostly by clastogenic chemicals and toxicants. Plate 1a and b show normal hepatocyte architecture due to the anti-oxidant property of the dried leaf ethanolic tincture of S.media while C shows liver hepatocytes undergoing restoration.

The reduction in the weight of testes (32.21%) and epididymides (29.91%) of rats exposed to DDVP only is a strong indicative of its reproductive toxicity. Negligible weight loss in testes (2.03%) of rats co-administered dried and fresh leaf of S.media ethanolic tincture shows that this herb possesses potent anti-oxidant activity thereby amerliorating the toxic effect of Dichlorvos. Similar protective effects have been reported in Solanum lycopersicum following chronic exposure of SD rat to Cypermethrin [13, 14].Histopathological evaluation of the testes of Plate 2B and C show seminiferous epithelium that can hardly be differentiated from the control (Plate2A).This is a strong indication that Stelleria media is androgenic based on the phytochemical analysis in Table 1 and its restorative effect on spermatogenesis in animals in groups B and C co-administered dried and fresh leaf tincture respectively. This property is novel having not been described before in literature.

Changes in the levels of plasma LDH_4 have been utilized diagnostically for detection of testicular damage has been investigated as a marker for post-meiotic germ cell activity [14].In this investigation 48.45% decrease in the serum concentration of Lactic acid dehydrogenase concentration was observed and Histopathological assessment of Plate 2D shows inhibitory effect of DDVP on meiotic cell division, differentiation and maturation processes. In view of these observations, it is concluded that Dichorvos is a systemic toxin, hepatotoxic, adversely affects the kidney and impairs spermatogenesis. With the widespread and unregulated use of this chemical in the developing countries caution in its use as an insecticide is hereby recommended

References

[1]. Bhawana, S., Priyanka S., Joshi, S.C. (2017). Ameliorating effects of Aqueous Extract of Zingiber officinale Against Dichlorvos Induced Liver Toxicity in Male Albino Rats. International Journal of Pharmaceutical Sciences Review and Research; 45(2): 173-181.

[2]. Ojo, O.A., Oyinloye, B.E., Ajiboye, B.O., Ojo, A.B., Akintayo, C.O., Okezie, B. (2014) Dichlorvos Induced Nephrotoxicity in Rat Kidney: Protective Effects of Alstonia Boonei Stem Bark Extract. International Journal of Pharmacognosy; 1(7): 429-437.

[3]. Abu El-Saad, A.M., Ibrahim, M.M., Hazani, A.A., El-Gaaly G.A. (2016).Lycopene attenuates dichlorvos induced oxidative damage and hepatotoxicity in rats. Human and Experimental Toxicology; 35(6): 654-665.

[4]. Zeycan, Y., Ismail C., Vedat, T. (2009). Hematotoxic and hepatotoxic effects of dichlorvos at sublethal dosages in rats. Environmental Toxicology; 24(2): 128-132.

[5]. Edem, V.F., Owoeye O., Akinyoola B.S., Rahaman S., Akang E.E., Arinola, G.O. (2012). Histological changes in liver and lungs of rats exposed to dichlorvos before and after vitamin supplementation. European Journal of Anatomy; 16(3):190-198.

[6]. Holy, B., Kenanagha B., Onwuli D.O. (2015) Haemato-pathological effects of dichlorovos on blood puncture and liver cells of albino rats. Journal of Toxicology and Environmental Health Sciences; 7(2): 18–23.

[7]. Ajiboso, S.O.O., Gbate, M., Ajari, O.I., Adeyemo, S.O. (2012). Sub Chronic Inhalation Toxicity Studies of 2,2-Dichlorovinyl Dimethyl Phosphate (DDVP) in Albino Rats. Advances in Biological Research; 6(4): 133-140

[8]. Ezeji, E.U., Dominica, O., Angela, C., Udebuani, J. N., Okereke, K. and Obasi, O. (2015). Effect of Dichlorvos on the fertility of adult male albino rats. Nature and Science; 13(12): 1-5.

[9]. Kingsley, C.K., Solomon, N.I., Odudu, A. (2016). Haematological, Biochemical and Antioxidant Changes in Wistar Rats Exposed to Dichlorvos Based Insecticide Formulation Used in Southeast Nigeria. Toxics 4(4): 28

[10]. Agarwal, S., Mittal, S., Bajaj, H., Chauhan, B., Kumar, R. (2016). Pyrexia: Pathophysiology and use of herbal drugs. International Journal of Pharmaceutical Sciences Review and Research; 7(4): 1-8.

[11]. Oyebanji B. O., Saba A. B., Oridupa O.A. (2012). Anti-inflammatory and analgesic effects of methanol extract of Stellaria media (L.) Vill. leaf. African Journal of Biomedical Research; 15: 29 – 34

[12]. Ajiboye, B.O., Salawu, S.O., Okezie, B., Oyinloye, B.E., Ojo, A.O., Onikanni, S.A, Oso, A.O., Asoso, O.S. and Obafemi T.O. (2014). Mitigating potential and antioxidant properties of aqueous seed extract of Leea guineensis against dichlorovos-induced toxicity in Wistar rats. Journal of Toxicology and Environmental Health Sciences; 6(7): 132-146.

[13]. Obulor, A.O. & Orlu, E.E. (2018a). Inhibitory Activity of Lycopene on Cypermethrin-induced hepatotoxicity and Liver injury in male Sprague-Dawley rats. Journal of Pharmacy and Biological Sciences, 13(1), 63-73.

[14]. Obulor, A.O. & Orlu, E.E. (2018b). Evaluation of the therapeutic effect of Lycopene on Cypermethrin-induced reproductive toxicity in rats. Journal of Environmental Science, Toxicology and Food Technology, 12(3), 10-16.
[15]. Obulor, A.O.,Orlu, E.E., Daka, E.R., Oruambo, I.F., Ebere, Nwabueze. (2018). Cypermethrin-Induced Anti-Fertility and the ameliorating effect of Lycopene Supplementation in Female rats. International Journal of Agriculture and Veterinary Science, 11(3), 57-63
[16]. Dong, Q., Huang, Y., Qiao, S.Y. (2007). Studies on chemical constituents of Stelleria media: China Journal of Chinese Material, 32(11), 1048-1051.
[17]. Hu, Y.M., Wang, H., Ye, W.C., Qian, L. (2009). New triterpenoid from Stelleria media. Natural Product Research, 24(14), 1278-1278.
[18]. Orlu, E.E. (2014). Deltamethrin-induced Alterations in Sperm Morphology and Spermatogenesis Impairment in Adult Sprague-Dawley Rats. Research Journal of Applied Sciences, Engineering and Technology, 7, (11), 2324-2331.
[19]. Orlu, E.E. & Egbunike, G.N. (2009). Daily sperm production of the domestic fowl (Gallus domesticus) as determined by quantitative testicular histology and homogenate methods. Pakistan Journal of Biological Sciences, 12, 1359-1364.
[20]. Bergmeyer, H.U., Herder, M. & Rej, R. (1986a). International Federation of Clinical Chemistry (IFCC) Scientific Committee, Analytical Section: Approved recommendation (1985) on IFCC methods for the measurement of catalytical concentration of enzymes, Part 2. IFCC method for aspartate aminotransferase. Journal Clinical Chemistry and Clinical Biochemistry, 24, 497-510.
[21]. McComb, R.B. & Bowers, G.N. (1972). Study of optimum buffer conditions for measuring alkaline phosphatase activity in human serum. Clinical Chemistry, 18, 97-104.
[22]. Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R. J. (1951). Total protein estimation by Lowry's method. Journal of Biology and Chemistry, 193, 265
[23]. Doumas, B.T., Watson, W.A. & Biggs, H.G. (1971). Albumin standards and measurement of serum-albumin with bromocresol green. Clinical Chimica Acta Journal, 31, 87-92.
[24]. Orlu, E.E. & Egbunike, G.N. (2009). Daily sperm production of the domestic fowl (Gallus domesticus) as determined by quantitative testicular histology and homogenate methods. Pakistan Journal of Biological Sciences, 12, 1359-1364.
[25]. Orlu, E.E. & Obulor, A.O. (2014). Investigation of the effect of aqueous leaf extract of Acanthus montanus on spermatogenesis in Swiss mice. Journal of Pharmacy and Biological Sciences, 9(3), 44-49.
[26]. Orlu, E.E. & Ogbalu, O.K. (2012). Partial Inhibitory Effect of Ethanol Extract of Lepidagathis alopecuroides (Vahl) on Spermatogenesis in Sprague-Dawley Rats. International Journal of Animal and Veterinary Advances, 4(3), 214-220.

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