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Oxidative Stress and Reproductive Damage Induced by Lead Acetate in Female Guinea Pig (*Cavia porcellus*): Curative Effects of Hydroethanolic Extract of *Spirulina platensis*

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Abstract: This study was aimed at evaluating the curative effects of Hydroethanolic Extract Of *Spirulina platensis* (HESP) on the reproductive function of female Guinea pig exposed to oxidative stress. Sixty females, 3-4 months old, weighing 300-400 g were divided into six groups (10 animals/group). The neutral control received distilled water, the negative control was treated with lead acetate at a dose 12 mg/kg.b.w while the positive control was given 12 mg of lead acetate/kg b.w and 100 mg of vitamin C. Groups 4, 5 and 6 were treated with lead acetate at a dose of 12 mg/kg b.w for the first 30 days and then received from the 31st day to the 90th day HESP at doses of 50,100 and 200 mg/kg.b.w respectively once daily. Results revealed that lead caused prominent toxic effects on fertility, deterioration of sex organs as well as a disruption of serum levels of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and progesterone. Lead acetate markedly increased tissues oxidative-stress marker (malondiadehyde), whereas it reduces the activities of antioxidant-enzymes, Superoxide Dismutase (SOD), total peroxidase and catalase. These changes were also accompanied by a significant ($p<0.05$) decrease of body weight gain. However treatment with HESP ameliorated lead acetate-induced anomalies by significantly ($p<0.05$) increasing the body weight gain, organ weights and feed intake. Also HESP let to a significant ($p<0.05$) improvement of fertility indices, a significant ($p<0.05$) increase in serum LH, FSH, progesterone hormones and an increase in antioxidant enzymes. Curative treatment brought about histological tissues protection and a significant ($p<0.05$) decrease of toxicity biomarkers (AST, ALT, creatinine and urea) and Malondiadehyde (MDA). In conclusion, lead acetate induced reproductive stress and administration of HESP can mitigate these adverse effects due to its antioxidant properties.

Keywords: Curative Effects, Lead Acetate, Oxidative Sress, Reproduction, *Spirulina platensis*

Introduction

Lead is one of the environmental pollutants that causes cancer (Flora *et al*., 2012; Patrick, 2006) imperiling human and animal health (Yildirim *et al*., 2011). It is used in different industrial processes which increase its presence in the environment (Flora *et al*., 2012). Also, lead exposure is associated with oxidative stress which leads to reproductive toxicity in both animal and human populations (Carocci *et al*., 2016; Dorea and Donangelo, 2006; Flora *et al*., 2012). Soheir (2015) mentioned that lead induced important damages on reproductive

functions. Additionally, Madiha *et al*. (2008) reported that lead induced biochemical and histological disruptions in many organs such as kidney, ovary, liver and endocrine organs due to the increased reactive oxygen species (ROS). Oxidative stress results from an imbalance in the body's oxidants and antioxidants in favor of the first and is known to induce cellular damage (Halliwell, 2007). Under normal circumstances, the body is endowed with effective antioxidant systems, maintaining the antioxidant/pro-oxidant balance. However, in extreme oxidative power, the body's antioxidant machineries are overwhelmed and necessitate exogenous antioxidants (Saxena and Garg, 2010).

In Africa, especially south of the Sahara, the use of plant extracts for the treatment and management of diseases has been in existence since ancient times. A larger number of these tropical plants and their extracts have shown beneficial therapeutic effects such as enhancing fertility and contraceptive compounds, antioxidant, anti-inflammatory, anti-cancer, anti-microbial and aphrodisiac (Raji *et al*., 2006).

Spirulina platensis is a blue green algae (Mycobacterium) belonging to the family Oscillatoriaceae (Cruchot, 2008). *Spirulina* is unique among blue-green algae because it has long history of safe use (Hayashi *et al*., 1996; Mazo *et al*., 2004). In central Africa, it is used as a primary food source and is currently grown at large scale in many countries for commercial purpose as a nutritional supplement for its high proteins, vitamins and mineral contents (Cruchot, 2008).

Spirulina platensis is a rich source of provitamin A or beta carotene and Superoxide Dismutase (SOD) enzyme. These antioxidants are very effective for prevention of various harmful effects of heavy metals and chemicals (Qureshi *et al*., 1996). *Spirulina* is considered as a valuable additional food source of macro and micro nutrients including amino acids, chlorophyll, gamma-linoleic acid, carotenoids, Vitamins A, B1, B2, C, E and trace elements such as iron, iodine, selenium and zinc (Mazo *et al*., 2004). *Spirulina platensis* possess potent antiviral (Hayashi *et al*., 1996), antioxidant (Dartsch, 2008; Ramadan *et al*., 2008), anticancer (Qureshi *et al*., 1996) antihyperlipidemic, probiotic, antidiabetic, antiobesity effect (Iwata and Inayama, 1987; Cruchot, 2008) and strengthens immune system (Liu *et al*., 1991; Estrada *et al*., 2001). These properties were largely related to *Spirulina`s* phycobili protein (Dartsch, 2008) and phycocyanin (Estrada *et al*., 2001; Wu *et al*., 2005). *Spirulina* is gaining more attention from medical scientists as a nutraceutical and pharmaceutical substances (Khan *et al*., 2005). Thus, *spirulina* can be used both in nutritional as well as therapeutic strategies (Qureshi *et al*., 1996) and it may inhibit lipid peroxidation as it is a cocktail of antioxidants (Ramadan *et al*., 2008).

Therefore, the present study was undertaken to investigate the reproductive toxicity of lead acetate and to evaluate the curative potential of *Spirulina platensis* against lead acetate induced oxidative reproductive stress.

Materials and Methods

Animals

Sixty (60) female cavies (*Cavia porcellus*) (4_ months old, with a mean body weight of 350±5.3g) were obtained from the Teaching and Research Farm of the University of Dschang. Males were used only as sires and were not treated. Throughout the trial (90 days), males and females were fed with experimental ration.

Housing, Feeding and Prophylactic Measures

These animals were identified using numbered ring attached on the ear and housed in clean room previously disinfected with CRESYL®. The animals were managed intensively, housed in identical cages measuring 100 cm×80cm×60cm (length, width and height) and maintained on the normal diets as shown in Table 1. The cages had mesh openings to provide ventilation.

Preparation of Plant Extract

Spirulina platensis was collected from Lake Chad in June 2017. The plant material was shade-dried, ground to obtain fine powder which was macerated in ethanol (70°) for 72 h. After filtration, the filtrate was concentrated under vacuum to remove ethanol and further dried using a freeze dryer to obtain fine powder.

Table 1: Composition and chemical characteristics of the feed

Ingredients	Quantities (%)
Maize	26.50
Wheat bran	3.00
Kernel cake	12.00
Soy beans cake	5.00
Cotton cake	6.00
Premix 5% *	5.00
Fish meal	2.00
Palm oil	2.00
Sea shells	2.00
Salt	0.50
Rice bran	15.00
Total (kg)	100.00
Chemical characteristics (calculated)	
Metabolisable energy (kcal/kg)	2600.00
Crude proteins (% DM)	19.00
Crude cellulose (% DM)	14.18
Calcium (% DM)	1.26
Phosphorus (% DM)	0.55
Sodium (% DM)	0.27
Lysine (% DM)	1.01
Methionine (% DM)	0.40

*Premix: Mineral Nitrogen Mineral Complex: DM: dried matter

Chemicals

Lead acetate was obtained from commercial sources (Trust Chemical Laboratories, United Kingdom; P.NO AIPL/20140112UN/2915.2990). Vitamin C was also obtained from commercial sources; (Shalina, Nariman point, Mumbai, India. A/Em/At: Plot No. E-2, M.I.D.C. Jejuri; Tal: Purandar. Dist : Pune, Maharashtra, India).

Experimental Design

Female Guinea pigs were divided into six groups of 10 animals each as follow:

- **Group I:** neutral control received distilled water for 90 days.
- **Groups II**: negative control received 12mg/kg/b.w of lead acetate diluted in distilled water for 90 days.
- **Group III:** positive control treated for the first 30 days with lead acetate (12 mg/kg b.w.) and then received 100 mg /kg/bw of vitamin C from the 31st day to the 90th day.
- **Group IV:** females treated for the first 30 days with lead acetate (12 mg/kg.b.w) and then treated with 50 mg /kg/bw of HESP from the 31st day to the 90th day.
- **Group V:** females treated for the first 30 days with lead acetate (12 mg/kg.b.w) and then received 100 mg/kg/b.w of HESP from the 31st day to the 90th day.
- **Group VI:** females administered with lead acetate for the first 30 days (12 mg/kg.b.w) and then received 200 mg /kg/bw of HESP from the 31st day to the 90th day.

All the females were treated for 90 days including 60 days of pregnancy. After 30 days, mating was done by placing 2 non-treated males into cages containing five treated females. At the end of the trial, female animals were sacrificed under ether anesthesia for blood collection. The blood was collected from the ventral aorta and stored at room temperature. Serum was collected 12 hours later for the estimation of reproductive hormones levels, serum hepatic enzymes (ALT, AST), oxidative stress and renal markers (creatinine and urea). Fetuses and organs were also collected and weighed. After the sacrifice, the remains of animals were carbonized into sanitary pits

Evaluation of Fertility of Treated Guinea Pigs

After sacrifice, the following measurements were registered: number of pregnant cavies, number and weight of fetuses. These characteristics were used to evaluate the rates of fertility, viability and mortality indices.

Biochemical Analysis

- Hormonal assay

  Serum content in reproductive hormones (FSH, LH and progesterone) were determined using appropriate commercial kits (ELISA AccuDiagTM, Diagnostic Automation Inc).
- Toxicity markers

  The levels of total proteins, creatinine, urea, total cholesterol, AST and ALT in the serum were determined using CHRONOLAB kit following the manufacturer's protocol.
- Antioxidant markers

  Superoxide dismutase (SOD), total peroxidase, Catalase activity and Malondialdehyde (MDA) were measured using the spectrophotometer (GENESYS 20.0) and according to the methods described respectively by (Nilsson *et al*., 1989; Misra and Fridovich, 1972; Sinha, 1972; Habbu *et al*., 2008).

Evaluation of Reproductive Organs Weight

After dissection, the female's gravid uterus, empty uterus and ovaries were collected and washed with normal saline to separate the surrounding fat and connective tissues. After drying, the weight was recorded with the aid of an electronic scale of 160 g capacity and 10^{-3}g precision.

Histology

The ovaries of each female were fixed in Bouin's solution for one week, embedded in paraffin, cut at 5 µm and stained with Harris haematoxylin and eosin. The tissue sections were observed under a light microscope (Leica DM 750, X10 and X 40) for morphology harmony and cellular integrity.

Statistical Analysis

Values were presented as Mean±SEM. ANOVA was performed, for comparison with post-hoc Duncan test to compare the level of significance between the controls and experimental groups. Probability values less than 0.05 ($p \leq 0.05$) was considered statistically significant. Statistical analyses were performed with SPSS for Windows software program 20.0

Results

Feed Intake, Body Weight and Relative Weight of Reproductive Organs

The final body weight, body weight gain and feed intake highly decreased in the negative control (T0-) group as compared to the neutral control (T0). However, the values of these characteristics increased in vitamin C and *Spirulina platensis*-treated group (Table 2).

Fertility, Sex Organ Weight and Embryo Characteristics

The fertility rate, viability rate, ovary weight, the full and empty uterus weight, litter size and weight decreased significantly ($p<0.05$) in lead-treated females. However, the co-treatment of lead and HESP increased these parameters compared to the positive control (T+) animals (Table 3).

Reproductive Serum Hormones

Lead acetate-treated group (T-) showed a non-significant ($p>0.05$) decrease in serum level of LH and progesterone compared to the control (T0). HESP group showed a significant ($p<0.05$) increase in progesterone and a significant decrease in FSH and LH compared to T0 but similar to those of the positive control (T+) group (Table 4).

Table 2: Effects of HESP on body weight, body weight gain and feed intake in females Guinea pig treated with lead acetate

	Controls			Doses of Spirulina(mg/kg.bw)			
Parameters	0(T0) (n=6)	Lead (T-) (n=6)	Vit C(T+) (n=6)	50(T1) (n = 6)	100(T2) (n = 6)	200(3) (n = 6)	p
Initial body	333.71 ±30.64	330,21 ±72.71	331.87 ±53.99	353.28 ±59.35	325.12±68.7	338,12±68.88	0.30
Final body	524,00 ±79.64	476.33 ±125.20	489.66 ±68.6	487.80 ±103.71	516.11 ±121.17	479.83 ±45.22	0.81
Body gain	191.11 ±23.67	146,00 ±61.02	158.79 ±89.63	134.52 ±51.70	191.12 ±68.06	141.33 ±52.12	0.07
Feed intake	18294.45 ±70.20	18187.83 ±13.09	17264.67 ±13.15	17303.85 ±11.65	17778.77 ±13.21	18357.98 ±20.32	0.17

n: number of animals, T0: neutral control; T-: negative control 12 mg lead acetate/kg bw; T+: positive control lead acetate 12 mg /kg bw and 100 mg vitamine C; T1: lead+HESP 50 mg /kg bw; T2: lead+HESP 100 mg /kg b.w. T3: lead+HESP 200 mg/kg bw; HESP: hydroethanolic extract of *Spirulina platensis*; P = probability value

Table 3: Effect of HESP on fertility, sex organ weight and fetus indices in female Guinea pig treated with lead acetate

	Controls			Doses of spirulina (mg/kg.bw)			
Characteristics of reproduction	0 (T0) (n = 6)	Pb (T-) (n = 6)	VitC (T+) (n = 6)	50(T1) (n = 6)	100(T2) (n = 6)	200(T3) (n = 6)	p
Fertility index (%)	75.00±46.29^{a}	42.85±53.40^{b}	57.14±22.8^{ab}	71.42±48.79^{a}	62.5±51.7^{ab}	37.5±51.75^{b}	0.02
No. of fetuses/dam	1.66±0.57	1.33±0.57	1.50±0.54	1.40±0.54	1.8±0.83	1.33±0.57	0.37
Viability index (%)	100.00±0,00	80.12±57.04	100.00±0,00	100.00±0,00	88.88±49.8	100.00±0,00	0.61
Gravid uterine weight (g)	30.39±29^{ab}	33.66±42^{ab}	29.94±37.29^{b}	56.56±65.11^{a}	66.8±67.16 ^{a}	29.71±3.20^{b}	0.03
Empty uterine weight (g)	3.62±3.43	3.14±1.92	2.50±2.60	4.50±3.93	6.19±3.77	4.59±1.86	0.16
Ovaries weight	0.066±0.02	0.046±0.00	0.07±0.01	0.06±0.01	0.06±0.01	0.053±0.02	0.16
Fetal weight (g)	24.82±15.70^{b}	23.66±21.90^{b}	33.33±11.67^{b}	51±45.21 ^{ab}	66.4±44.86^{a}	22.33±9.0à^{b}	0.01
mortality index (%)	0.00±0,00	19.87±3.66	0.00±0,00	0.00±0,00	11.11±4.09	0.00±0,00	0.15

number of animal,. T0 : control; T- :negative control 12 mg lead acetate/kg b.w; T0+: positive control 12 mg lead acetate/kg b.w with 100mg of vitamine C ; T1: lead+50 mgHESP/kg b.w; T2: lead+100 mg HESP/kg b.w. T3: lead+200 mg HESP/kg b.w. HESP: hydroethanolic l extract of *Spirulina platensis*; P=probability value

Table 4: Effects of HESP extract on serum level of progesterone, luteinizing hormone and follicle stimulating hormone in female Guinea pig treated with lead acetate

	Controls			Doses of *Spirulina platensis* (mg/kg.bw)			
Serum hormones	0(T0) (n = 6)	Lead(T-) (n = 6)	Vit C(T+) (n = 6)	50(T1) (n = 6)	100(T2) (n = 6)	200(T3) (n = 6)	p
FSH (µM/min/g)	34.06±7.80^{b}	35.70±7.19^{a}	35.18±4.18^{a}	34.21±6.47^{b}	29.47±3.97^{b}	35.17±4.95^{a}	0.07
LH (µM/min/g)	81.50±17.31^{a}	75.00±18.10^{a}	30.00±8.86^{b}	63.41±12.91^{ab}	30.50±9.38^{a}	74.25±6.74^{b}	0.02
Progesteron (µM)	28.15±8.61^{a}	15.56±8.38^{b}	17.86±9.69^{ab}	14.15±2.90^{b}	22.03±4.40^{ab}	16.77±10.69^{b}	0.03

n:number of animals, T0: neutral control; T-: negative control 12 mg lead acetate/kg bw; T+: positive control lead acetate 12 mg /kg bw and 100 mg vitamine C; T1: lead+HESP 50 mg /kg bw; T2: lead+HESP 100 mg /kg b.w. T3: lead+HESP 200 mg/kg bw; HESP: hydroethanolic extract of *Spirulina platensis*; FSH: folliculo-stimulating hormone, LH: luteinizing hormone

Table 5: Effects of different levels of HESP on oxidative stress biomarkers in female Guinea pig treated with lead acetate

	Controls			Doses of spirulina (mg/kg.bw)			
Parameters	0(T0) (n = 6)	Lead(T-) (n = 6)	Vit C(T+) (n = 6)	50(T1) (n = 6)	100(T2) (n = 6)	200(T3) (n = 6)	p
SOD (µM/min/g)	5.49±1.46^{a}	3.71±1.62^{b}	6.87±1.9^{ab}	4.87±2.12^{b}	5.10±2.26^{ab}	6.68±0.91^{ab}	0.02
CAT (µM/min/g)	16.51±3.20^{a}	12.77±2.89^{b}	14.37±2.39^{ab}	17.73±1.59^{a}	16.58±5.75^{a}	15.39±5.47^{ab}	0.05
MDA (µM)	0.36±0.00^{b}	0.58±0.18^{a}	0.44±0.16^{ab}	0.37±0.03^{b}	0.45±0.15^{ab}	0.28±0.03^{b}	0.00
POD (µM/min/g)	36.84±19.54^{b}	21.15±11.26^{c}	49.21±21^{ab}	61.74±12.62^{a}	63.42±29.36^{a}	43.48±11.95^{ab}	0.00

n:number of animals,. Means values for each parameter in the same row, with different superscripts (a,b) differ significantly ($p \leq 0.05$). T0: neutral control; T-: negative control 12 mg lead acetate/kg bw; T+: positive control lead acetate 12 mg /kg bw and 100 mg vitamin C; T1: lead+HESP 50 mg /kg bw; T2: lead+ HESP 100 mg /kg b.w. T3: lead+HESP 200 mg/kg bw; HESP: hydroethanolic extract of *Spirulina platensis*, SOD: superoxyde dismutase; CAT: catalase; MDA: Malondialdehyde; POD: peroxidase; P=probability value

Table 6: Effects of different levels of HESP on toxicity biomarkers in female Guinea pig treated with lead acetate

	Controls			Doses of spirulina (mg/kg.pc)			
Parameters	0(T0) (n = 6)	Lead(T-) (n = 6)	VitC(T+) (n = 6)	50(T1) (n = 6)	100(T2) (n = 6)	200(T3) (n = 6)	p
AST (UI/L)	63.54±6.33^{b}	159.54±28.9^{a}	121.25±9.20^{ab}	122.90±10.3^{ab}	116.95±9.90^{ab}	114.2±10.7^{ab}	0.02
ALT (UI/L)	68.68±4.71^{b}	119.93±7.23^{a}	80.25±5.74^{b}	86.78±3.70^{b}	100.50±5.10^{ab}	90.12±8.02^{ab}	0.17
Creatinine (mg/dl)	0.44±0.15^{b}	1.20±0.36^{a}	0.77±0.38^{ab}	1.31±0.63^{a}	0.80±0.22^{ab}	0.70±0.60^{ab}	0.00
Urea (mg/dl)	35.11±1.10^{b}	51.74±8.85^{a}	46.20±8.50^{ab}	46.19±7.98^{ab}	44.60±8.76^{ab}	45.69±7.33^{ab}	0.03
Total proteins (mg/dl)	3.42±0.73^{cd}	3.25±1.00^{d}	5.25±1.02^{ab}	4.01±0.78^{bcd}	4.83±0.79^{abc}	6.06±0.93^{a}	0.01
Total cholesterol (mg/dl)	29.90±6.87^{a}	6.09±1.49^{b}	24.16±6.90^{a}	31.85±4.95^{a}	19.34±5.42^{ab}	30.70±9.26^{a}	0.03

n:number of animals, each value represents mean ± standard error mean. Means values for each parameter in the same row, with different superscripts (a,b) differ significantly ($p \leq 0.05$). T0: neutral control; T-: negative control 12 mg lead acetate/kg bw; T+: positive control lead acetate 12 mg /kg bw and 100 mg vitamine C; T1: lead+HESP 50 mg/kg bw; T2: lead+HESP 100 mg/kg b.w. T3: lead+HESP 200 mg/kg bw; HESP: hydroethanolic extract of *Spirulina platensis*, AST: aspartate aminotransferase ALT: alanine aminotransferase; P=probability value

Oxidative Stress Biomarkers

The serum concentration of MDA significantly ($p<0.05$) increased, whereas SOD, CAT and total peroxidases activities reduced significantly ($p<0.05$) in the negative control compared to T0 group. The reverse trend was observed in HESP-treated groups (Table 5). Yet, the mean values of oxidative stress indicators in the HESP-treated groups were not significantly ($p>0.05$) different from positive control (T0+).

Toxicity Biomarkers

The serum levels of ALT, AST, creatinine and urea significantly ($p<0.05$) increased in stressed (T-) cavies as compare to the control (T0). The contrary was recorded with serum total protein and cholesterol (Table 6). Administration of HESP significantly ($p<0.05$) decreased the serum levels of ALT AST, creatinine, urea and increased total proteins and cholesterol. The results recorded in vitamin C treated animals were similar.

Histological Finding in the Ovaries

The histopathological examination of ovaries of T0, showed a normal tissue and ovocyts, ovaries structure of stressed non-treated Guinea pigs exhibited evident signs of tissular injury such as atrophy and degeneration of the follicular cell and loss of vacuolars in the stroma with reduced presence of oocytes. HESP-treated animals (Fig. 2) showed improvement in ovary tissue structure and increased follicular cells compared to untreated animals and similar to vitamin C group.

Discussion

Exposure of animals to heavy metals is detrimental to their reproductive potential (Carocci *et al*., 2016; Dorea and Donangelo, 2006). Results of this study showed that there was a decrease in feed consumption, body weight and body weight gain in females Guinea pigs treated only with lead acetate compared to the control group which received distilled water and those co-exposed to lead acetate and hydroethanolic extract of *Spirulina platensis*. The reduction due to lead acetate can be explained by the fact that lead acetate could have had an inhibitory effect on the central nervous system which control feed intake (Madiha *et al*., 2008). The increase in these parameters with the administration of *Spirulina platensis* can be explained by the stimulation of the hypothalamus which might have provoked an increase in appetite and therefore in feed intake and body weight (Fox, 1999). Also, lead might have affected numerous other physiological functions including the female reproductive function (Flora *et al*., 2012).

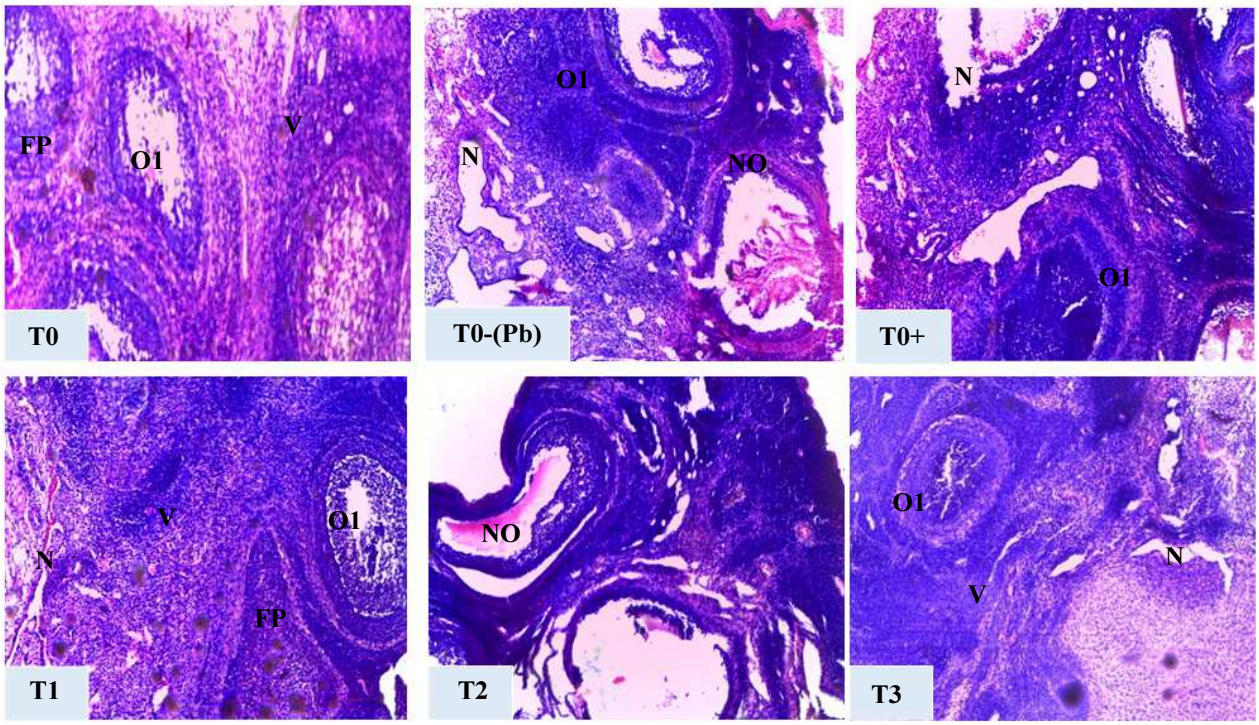

Fig. 1: Histology of ovary: T0: control; T-: Negative control: 12 mg lead acetate/kg b.w; T+: poitive control 12 mg lead acetate/kg b.w with 100mg of vitamin C; T1: Lead+50 mg HESP/kg b.w; T2:* lead+100 mg HESP/kg b.w. T3: Lead+200 mg HESP/kg b.w. HESP: Ethanolic extract of *Spirulina platensis* O1: Primary ovocyte; FP: Primairy follicule; NO: Necrosis ovocyte; V: Vascularisation; N: Nécrosis

The current study also revealed that lead acetate caused a decrease of fertility rate, fetal weight and viability, ovaries and uterus weight in lead exposed female cavies. Administration of HESP led to higher fertility indices, weight of foetuses and consequently increase in uterus weight. This increase can be due to the presence of antioxidants in *Spirulina* (Khan *et al*., 2005). Antioxidants are known to have the capacity of stabilizing or deactivating free radicals before they attack cells and are also absolutely able of maintain normal cellular conditions and well-being (Gertrude, 2008). Lead acetate also induced female reproductive toxicity by disrupting endocrine and many other biochemical mechanisms (Abdou and Newairy, 2006). This study indicated that the serum concentrations of LH and progesterone decreased in lead acetate treated female cavies as compared to control. These results agree with those of Dearth *et al*. (2002) who reported that prepubertal females exposed maternally to low levels of lead exhibited suppressed circulating levels of estradiol. In addition, Franks and Laughlin (1989) and Foster *et al*. (1996) reported that exposure to lead decreases the plasma progesterone concentration. Many epidemiological studies also found that reproductive impairments may develop in females even with low-to-moderate blood lead level, including intrauterine growth restriction, preterm delivery (Srivastava *et al*., 2001) and spontaneous abortion. (Tang *et al*., 2003). The present results support the hypothesis that the action of lead on female's fertility may be due in part to the down-regulation of progesterone, LH and estradiol (the main female sex hormones) by activating their metabolizing enzymes. Therapy treatment of lead-stressed female Guinea pigs with *Spirulina platensis* attenuated the hormones disruption by increasing their levels. The correction of hormones damage in female Guinea pigs treated with HESP can be due to the phytosterols, saponins, polyphenols and flavonoids present in the extract. Many studies have shown that these compounds increase the level of reproductive hormones (Estrada *et al*., 2001; Khan *et al*., 2005).

The hepatocellular enzymes (AST, ALT) and the levels of creatinine, urea, total proteins and cholesterol are used to evaluate the function of the liver and kidney. The decrease in their activities in the liver and the kidney could be expected to occur associating with the pathology involving necrosis of theses organs. Rahman *et al*. (2001) suggested that the decrease in these parameters might show the stressed conditions of the treated animals. The present study indicated a decrease in the total proteins and cholesterol which could be attributed in part to the damaging effect of lead acetate on liver and kidney cells as confirmed by the increase in the activities of serum AST, ALT, creatinine and urea. However, administration of HESP

restored theses parameters towards normal which may be due to its anti-oxidative and hepato-protective activities that scavenged the reactive oxygen species due its content. Spirulina is an important herbal medicine that has antioxidant properties and scavengers of oxidative stress (Mazo *et al*., 2004; Iyyapu *et al*., 2006). A significant increase in Malondiadehyde level was observed in female cavies exposed to lead acetate in the present study. These results are in line with the observations of Adibmoradi *et al*. (2015) after lead acetate administration. The significant increase in MDA level suggests that oxidative stress is a major mechanism of the effect of lead in organism. However, MDA level decreased significantly in a dose dependent manner in HESP-treated. Therefore, it can be concluded that HESP plays a protective role through its antioxidant activity. The decrease in MDA concentration might be linked to the increase of antioxidants production in the HESP-treated groups. The activities of SOD, CAT and POD in the tissue decreased in females exposed to lead acetate treatment cavies, nevertheless, restoration in spirulina-treated females suggested that spirulina has protective effects against oxidative stress induced by lead acetate (Khan *et al*., 2005). Therefore, it can be concluded that HESP plays a curative role through its antioxidant activity.

Histological analysis can also be used to examine the morphological changes in ovary to reflect possible effect of lead on the ovaries cells and reproductive hormones. Analysis of the micrographs revealed gross structural disintegration in the cortex when compared with the control. This disintegration is evidenced by the presence of large opened spaces. Loekle *et al*. (1983) described these open spaces as areas of tissues disintegration. The micrographs of HESP cavies showed minimal alterations with reduced fat deposition as compared to the lead acetate-treated cavies. These findings confirmed the curative effect of *Spirulina platensis* against the histological changes in lead acetate and its ability to improve the functional efficiency of the uterus and ovary.

Conclusion

The hydroethanolic extract of *Spirulina platensis* minimizes the adverse effects of lead-induced reproductive stress in adult female's Guinea pigs due to its antioxidant properties. Thus, the hydroethanolic extract of *Spirulina platensis* as well as vitamin C could be used as curative alternative to alleviate the effects of reproductive stress induced by lead acetate in female reproductive system.

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Author's Contributions

Ngoula Ferdinand and Tchoumboue: Supervised and designed the project, cross checked the draft of the manuscript and finally approved for submission.

Deutcheu Nienga Sorelle and Ngoula Ferdinand: Designed the project, conducted the experiment, collected, analyzed data and wrote the first draft of the manuscript.

Mabou Nguemo Jasmine Laura, Manfo Tsague F. Pascal: Assisted in the conduction of the experiment, collected data.

Vemo Bertin Narcisse and Ngoumtsop Victor Herman: Conducted laboratory analysis of experiment.

Ngouateu Kenfack Omer Bébé and Manfo Pascal: Rechecked the draft of the manuscript.

Conflict of Interests

The authors declare that they have no financial or personal conflict which may have inappropriately influenced them in writing this article.

Ethical Consideration

Experimental protocols used in this study were approved by the Ethical Committee of the Department of Animal Science of the University of Dschang-Cameroon (ECDAS-UDs 26/07/2017/UDs/FASA/DSAES) and was in conformity with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986.

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