integrity Research Journals

Journal of Animal Science and Veterinary Medicine
Volume 4(4), pages 145-150, August 2019
Article Number: 00EB05B26
ISSN: 2536-7099
https://doi.org/10.31248/JASVM2019.155
https://integrityresjournals.org/journal/JASVM

Full Length Research

Evaluation of antidotal potentials of hydroethanolic extract of *Spirulina platensis* on teratogenicity of *Cavia porcellus* induced by exposure to lead acetate

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Received 27th July, 2019; Accepted 22nd August, 2019

ABSTRACT: Lead acetate is a compound that is teratogenic and could cause hazards in pregnant humans and economic loses in livestock. This study aims to evaluate the protective and curative effects of hydroethanolic extract of *Spirulina platensis* (HESP) on embryo-toxicity in guinea pig exposed to lead acetate. Eighteen females, aged 4 months old and weighing 350±5.3 g were divided into 8 groups of 10 guinea pigs each. The negative control (group 1) received by gavage distilled water and positive control (group 2) was exposed to lead acetate at a dose of 12 mg/kg.bw alone. While Groups 3, 4 and 5 co-exposed for a period of 90 days to 12 mg/kg bw of lead acetate and 50, 100 and 200 mg/kg.bw of hydroethanolic extract of *Spirulina platensis* respectively. The animals of groups 6, 7 and 8 were also exposed for the first 30 days to lead acetate, then 50, 100 and 200 mg /kg.bw of HESP from the 31st to the 90th day. At the end of the trial, female animals were sacrificed for determine the teratogenicity. Lead acetate caused a significant decrease of placental and fetal weight, number of implantation sites and gravid uterine weights. In the other hand, it increased the incidence of fetal anomalies when compared to the negative control. HESP significantly reduced ($p< 0.05$) the lead acetate-induced abnormities by increasing fetal number, weight, viability and reduced skeletal anomalies. Treatment of female guinea pigs with hydroethanolic extract of *Spirulina platensis* whether for protective or curative at dose of 100 mg/kg.bw, attenuated lead induced reproductive disrupted, fetal malformation and fetal mortality. The Hydroethanolic extract of *Spirulina platensis* due to some phytochemical principles rich in antioxidant properties, could possibly mitigate the adverse effects of lead acetate.

Keywords: Guinea pig, lead acetate, *Spirulina platensis*, teratogenicity.

INTRODUCTION

In the last decades, the increase in population has led to the compelling need to increase food production. The development of high-yielding crop varieties and the formulation of more potent pesticides to aid in the elimination of pests that destroy crops are therefore imperative (Hayes and Laws, 1998). These pesticides contain heavy metals which affect animals or humans either directly by exposure or indirectly in contaminated food and water consumption (ATSDR, 2007).

Many heavy metals are genitotoxic, teratogenic and embryo toxicity. Heavy metal exposure pollutants during the time of periconceptional period, conception, implantation, placentation and organogenesis at pregnancy were reported to be adversely (Quansah and Jaakkola, 2009). Embryo toxicity and teratogenicity are side effects of irrational therapy with various drugs during pregnancy (Franciszek, 2000). These side effects can occur in high doses close to the toxic level for the dam or the pregnant

animal. Lead is a toxic metal in humans and animals (Siddiqui et al., 2002; Ahamed et al., 2008; Ahamed et al., 2011). This is considered as one of the most hazardious environmental pollutants that could affect the homeostasis of embryo through exposure of the mother (Clarkson et al., 1985; Ahamed et al., 2008). Evidence of the harmful effects of lead on the organism in general and reproduction in particular have been widely studied (Clarkson et al., 1985; Tas et al., 1996). The exposure of rodents to lead has increased the tendency to fetal death, growth retardation, resorption and congenital malformations (Gale et al., 1980). Induction of oxidative stress is one of the mechanisms implicated in lead induced fetal-toxicity (Siddiqui et al., 2002; Ahamed et al., 2008). Medicinal plants such as *Spirulina platensis* due to its antioxidants activity could include protection to animals exposed to heavy metals from oxidative stress.

Spirulina platensis is a rich source of provitamin A or beta carotene and superoxide dismutase (SOD) enzyme. These antioxidants are very effective for the prevention and reversal of various toxic effects of heavy metals and xenobiotics (Asadpour et al., 2013). Spirulina is also 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 (Abdel-Daim et al., 2013). Spirulina is gaining more attention from medical scientists as a neutriceutical and pharmaceutical agent (Asadpour et al., 2013). Thus, Spirulina might offer both nutritional as well as therapeutic uses. It may inhibit lipid peroxidation of fetal tissues as it constitutes a cock tail of antioxidants. The present study evaluates the antidotal potentials of hydroethanolic extract of *Spirulina platensis* (HESP) against lead induced fetal development alteration in guinea pigs.

MATERIALS AND METHODS

Animals

Eighty (80) female guinea pigs (*Cavia porcellus*) aged 4 months old, with a mean body weight of 350±5.3 g were obtained from the Teaching and Research Farm of the University of Dschang. These animals were maintained on the normal diets under ambient temperature, 12/12hours light/dark cycle, in well ventilated, and proper hygienic conditions. Males were used only as sires and were not treated. Throughout the trial (90 days), male and female cavies were maintained according to the National Research Council Institute of the Laboratory of Animal resources.

Preparation of plant extract

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

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, Inde. A/Em/At: Plot No. E-2, M.I.D.C. Jejuri; Tal: Purandar. Dist: Pune, Maharashtra, Indi).

Experimental design

Female guinea pigs were divided into eight groups of 10 animals in each comparable in terms of weight. Groups were constituted as shown in Table 1. All the females were treated for 90 days by oral administration (gavage). After 30 days, in both preventive and curative treatments timed 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. The numbers of implantation sites and viable fetuses examined under 400x magnification and resorptions were calculated. All viable fetuses were weighed, measured and preserved in 95% ethanol during 3 days then potassium hydroxide was introduced for a period of 5 days. To identify skeletal anomalies, the fetuses were submitted to double skeletal staining with Alizarin red (bone) according to the technique described by Kimmel and Trammel (1981). Fetuses were then stored in glycerol for macroscopic skeletal examination. However, in this work, the focus was mainly on the evaluation of the bone malformations. Findings were classified as malformations, according to currently used nomenclature (Green, 1952).

Statistical analysis

The statistical analysis of the data was performed using the SPSS 20.0 software. Differences between groups were assessed using one-way ANOVA followed by Duncan post hoc test. A p value of less than 0.05 was considered as significant. The results obtained are expressed as mean ± standard deviation.

Ethical consideration

Experimental procedure 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

Table 1. Groups treatments.

Groups	Treatments
Control treatments	
Group 1	Negative control received distilled water for a period of 90 days.
Group 2	Positive control received 12 mg of lead acetate /kg.bw diluted in distilled water for a period of 90 days.
Protective treatment	
Group 3	Were co-treated with 12 mg of lead acetate/kg.bw and 50 mg of hydroethanolic extract of *Spirulina platensis* (HESP) /kg.bw.
Group 4	Received 12 mg of lead acetate/kg/bw + 100 mg of HESP/kg.bw.
Group 5	Co-exposed to 12 mg of lead acetate/kg/bw + 200 mg of HESP/kg.bw.
Curative treatment	
Group 6	Females were given for the first 30 days 12 mg of lead acetate/kg.b.w and then treated with 50 mg /kg/bw of HESP from the 31st to the 90th day.
Group7	Females were treated for the first 30 days with 12 mg of lead acetate/kg.b.w and then received 100 mg/kg/b.w of HESP from the 31st to the 90th day.
Group 8	Females were administered for the first 30 days with 12 mg of lead acetate/kg.b.w and then received 200 mg /kg/b.w of HESP from the 31st to the 90th day.

Table 2. Protective effect of HESP on reproductive toxicity in guinea pigs exposed to lead acetate.

Parameters	Controls		Protective treatment (mg/kg.bw)			p
	0 (T-) (n=6)	Pb (T+) (n=6)	HESP 50 (n = 6)	HESP 100 (n = 6)	HESP 200 (n = 6)	
No.of fetus	1.50±0.54	1.66±0.57	1.60±0.89	1.25±0.5	1.8±0.83	0.12
No.of corpora lutea	1.83±0.75	2.00±0.00	1.60±0.54	2.00±1.41	1.40±0.89	0.36
No.of placental	1.50±0.54	1.66±0.57	1.60±0.89	1.25±0.5	1.8±0.83	0.32
No. of live fetuses	1.50±0.54	1.33±0.57	1.60±0.89	1.25±0.50	1.80±0.83	0.33
No. of dead fetuses	0.00±0.00^{b}	0.33±0.57^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0 ^{b}	0.01
Placental weight (g)	5.78±1.22	4.96±2.84	6.14±2.83	4.46±1.73	6.60±2.67	0.23
No.implantation	1.50±0.54^{ab}	2.33±0.57^{a}	1.80±0.83^{ab}	1±0.00^{b}	1.60±0.54 ^{ab}	0.02
Pre-implantation loss (%)	0.00±0.00^{b}	33.33±57.70^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.03
Post-implantation loss (%)	0.00±0.00^{b}	40.00±54.77^{a}	33.33±57.7^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.03

n: number of animals; a, b: Values with the same letter per row are not significantly different (p>0.05); T0-: negative control; T+: positive control 12 mg lead acetate/kg bw; HESP: hydroethanolic extract of *Spirulina platensis; No: number* P = probability value.

guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986.

RESULTS

Protective effect of HESP on reproductive toxicity in female guinea pigs exposed to lead acetate

The oral administration of lead at 12 mg/kg bw for 90 consecutive days caused a non-significant (p>0.05) decreased in placental weigh and number of live fetuses. Inversely, lead increased the number of dead fetuses, the pre and post-implantation loss (Table 2). The placental weigh and the number of viable fetuses were increased non-significantly (p>0.05) in spirulina treated pregnant females at doses of 50 and 200 mg/kg.bw as compared with the positive control groups. The co-administration of lead and Spirulina extract whatever the dose significantly (p<0.05) decreased the number of dead fetuses, the pre and post-implantation loss as shown in Table 2.

Protective effects of HESP on fetuses' skeletal anomalies of pregnant guinea pig exposed to lead acetate

As shown in Table 3, the exposure of pregnant guinea pig to lead reduced the fetal weight compared with negative

Table 3. Protective effects of HESP on fetuses' skeletal anomalies in guinea pigs.

Incidence of anomalies	Controls		Protective treatment (mg/kg.bw)			p
	0 (T-) (n=6)	Pb (T+) (n=6)	HESP 50 (n = 6)	HESP 100 (n = 6)	HESP 200 (n = 6)	
Fetal weight (g)	27.82±10.70^{ab}	23.66±11.60^{b}	39±20.65 ^{ab}	40.75±36.5 ^{a}	46±32.13^{a}	0.01
Total length (mm)	5.66±1.71^{a}	6.16±1.25^{a}	6.68±2.69^{a}	5.57±2.88^{a}	6.72±1.97^{a}	0.47
Head Length (mm)	1.45±0.26^{a}	1.36±0.3^{a}	1.88±0.71^{a}	1.45±0.42	1.60±0.42^{a}	0.13
Stern Length (mm)	1.06±0.23^{a}	1.13±0.23^{a}	1.14±0.61^{a}	1.17±0.56^{a}	1.28±0.56^{a}	0.44
limb Hyperflexion (%)	0.00±0 ^{b}	33.33±7.77 ^{a}	25.00±50.0 ^{a}	20.00±4.72 ^{a}	20.00±44.72 ^{a}	0.05
Limbs Anomalies (%)	0.00±0.00 ^{b}	33.33±7.7 7^{a}	0.00±0.00 ^{b}	0.00±0.00 ^{b}	20±4.72 ^{a}	0.03
Incomplète skull fusion (%)	0.00±0,00^{b}	66.66±7.77^{a}	20.00±4.72 ^{b}	0.00±0.00 ^{b}	0.00±0.00 ^{b}	0.02
Incomplète ossification of sternebrae (%)	0.00±0.00 ^{b}	33.33±7.77 ^{a}	0.00±0.00^{b}	0.00±0.00 ^{b}	20±4.72 ^{a}	0.03

n: number of animals; a, b: Values with the same letter per row are not significantly different (p> 0.05); T0: negative control; T+: positive control 12 mg lead acetate/kg bw; HESP: hydroethanolic extract of *Spirulina platensis;* No: number; P = probability value.

control. The co-exposure of guinea pigs to lead and *S. plantesis* at doses of 100 and 200 mg/kg bw increased their fetal weight with reference to the positive control (T+). The fetal anomalies (limb hyperflexion, limb anomalies, incomplete skull fusion and incomplete ossification of sternebrae) increased in lead treated guinea pig compared to the negative control. The co-administration of lead with 100 mg/kg.bw of HESP led to a significant ($p<0.05$) decreased of the values of these anomalies and mitigates skeletal damages.

Curative effect of HESP on reproductive function of pregnant guinea pig exposed to lead acetate

The numbers of fetus, corpora lutea, placenta, viable fetuses and dead fetuses, the placenta weight in group of guinea pig submitted to lead only and those exposed to lead and *S. plantesis* were comparable. In the other hand, the number of implantation sites, the pre and post implantation loss were significantly ($p < 0.05$) reduced in pregnant females exposed to lead compared with the negative control group. However, Spirulina-extract at doses of 50 and 100 mg/kg.bw increased significantly ($p<0.05$) the values of these parameters with reference to the positive controls (T+) as show in Table 4.

Curative effects of HESP on fetuses' skeletal anomalies of pregnant guinea pig exposed to lead acetate

All fetuses in the negative control and positive groups showed a comparable fetal weight, total length, head and stem length. Meanwhile, the oral administration of spirulina at dose of 100 mg/kg.bw for 90 days significantly ($p<0.05$) increased the fetal weight and the total length of fetuses. The fetal anomalies included limb hyperflexion, limb anomalies, incomplete skull fusion and incomplete ossification of sternebrae reduced in lead treated guinea pig compared to the negative control. The administration of HESP whatever the dose significantly ($p<0.05$) decreased the values of these anomalies.

DISCUSSION

The protective and curative effects of hydro-ethanolic extract of *Spirulina platensis* on fetal toxicity induced by lead acetate were investigated in female guinea pig. The results of this study showed that lead acetate reduced the number of implantation sites and the number of viable fetuses, the weights of placenta, fetuses, and gravid uterus in pregnant females. There was also malformation of the skull, lack of ossifications of the vertebral column and thoracic skeleton in lead exposed animals. Lead that has accumulated in maternal body before pregnancy or exposed during pregnancy may be released in conjunction with calcium due to increased bone turnover during pregnancy and may be transferred to fetus via trans-placental route (Gulson et al., 1997). The lead administration was performed during the organogenesis phase which was the most critical period during gestation and teratogenic effect due to external influences may occur in this time frames. In addition, intrauterine exposure to lead adversely affects cellular proliferation and differen-

Table 4. Curative effects of HESP on reproductive toxicity in guinea pigs exposed to lead acetate.

Parameters	Controls		Curative treatments (mg/kg. bw)			p
	T0- (n=6)	T0+ (Pb) (n=6)	HESP 50 (n = 6)	HESP100 (n = 6)	HESP 200 (n = 6)	
No.of fetus	1.50±0.54 [a]	1.66±0.57 [a]	1.40±0.54 [a]	1.80±0.83 [a]	1.33±0.57 [a]	0.37
No.of corpora lutea	1.83±0.75[a]	2.00±0.00 [a]	1.4±0.89 [a]	2.00±1.00 [a]	1.00±0.00 [a]	0.10
No.of placental	1.5±0.54 [a]	1.66±0.57 [a]	1.4±0.54 [a]	1.8±0.83 [a]	1.33±0.57 [a]	0.37
No. of live fetuse	1.5±0.54 [a]	1.33±0.57 [a]	1.4±0.54 [a]	1.6±0.89 [a]	1.33±0.57 [a]	0.61
No. of dead fetuses	0.00±0.00 [a]	0.33±0.57[a]	0.00±0.00 [a]	0.2±0.44 [a]	0.00±0.00 [a]	0.15
Placental weight (g)	5.78±1.22[a]	4.96±2.84 [a]	5.36±2.96[a]	6.31±4.68[a]	5.52±0.84 [a]	0.57
No.implantation	1.50±0.54 [a]	2.33±0.57 [a]	1.4±0.54 [a]	1.8±0.83 [a]	1.66±0.57 [a]	0.02
Pre-implantation loss (%)	0.00±0.00[a]	33.33±7.73[b]	20.0±4.72[ab]	40±4.18 [a]	0.00±0.00 [b]	0.04
Post-implantation loss (%)	0.00±0.00[a]	40±4.77 [b]	0.00±0.00[a]	0.00±0.00[a]	33.33±7.77[b]	0.02

n: number of animals; a, b: Values with the same letter per row are not significantly different (p > 0.05); T0: negative control; T+: positive control 12 mg lead acetate/kg bw; HESP: hydroethanolic extract of *Spirulina platensis;* No: number; P =probability value.

Table 5. Curative effects of HESP on fetuses' skeletal anomalies in female exposed to lead acetate.

Incidences of anomalies	Controls		Curative treatments (mg/kg. bw)			p
	T0- (n=6)	T0+ (pb) (n=6)	HESP 50 (n = 6)	HESP 100 (n = 6)	HESP 200 (n = 6)	
Fetal weight (g)	24.82±15.70[b]	23.66±21.90[b]	51±45.21 [ab]	66.4±44.86[a]	22.33±9.0[ab]	0.01
Total length (mm)	5.66±1.71[ab]	6.16±1.25[ab]	6.9±3.28[a]	7.82±2.01[a]	4.5±0.53[b]	0.05
Head length (mm)	1.45±0.26 [a]	1.36±0.30[a]	1.56±0.56[a]	1.52±0.41[a]	1.13±0.23[a]	0.18
Stern length (mm)	1.06±0.23[a]	1.13±0.23[a]	1.34±0.73[a]	1.1±0.28[a]	0.9±0.17 [a]	0.65
limb hyperflexion (%)	0.00±0.00 [b]	33.33±7.7 3[a]	33.33±7.73 [a]	0.00±0.00 [b]	0.00±0.00[b]	0.03
Limbs anomalies (%)	0.00±0.00 [b]	33.33±7.73[a]	0.00±0.00 [b]	0.00±0.00[b]	0.00±0.00[b]	0.02
Incomplete skull fusion (%)	0.00±0.00[c]	66.66±7.73[a]	33.33±7.73[ab]	40.00±4.77[b]	33.33±7.7[ab]	0.03
Incomplete ossification of sternebrae (%)	0.00±0.00[b]	33.33±7.73[a]	0.00±0.00[b]	20.00±4.72[a]	0.00±0.00[b]	0.03

n: number of animals; a, b, c: Values with the same letter per row are not significantly different (p> 0.05); T0: neutral control; T-: negative control 12 mg lead acetate/kg bw; T+: positive control lead acetate 12 mg /kg bw; HESP: hydroethanolic extract of *Spirulina platensis;* P = probability value.

tiation, synaptic growth and apoptosis at the time of brain development and causes decreased levels of certain neurotransmitters substances such as acetylcholine, dopamine, and glutamate (Guilarte, 1997; Paponikolaou et al., 2005). In fact, exposure during the gastrulation phase results in widespread damage to the embryo, with multiple malformations, especially structural. In several species such as rodents (hamster, rat, mouse), the kind of alterations range from growth retardation, craniofacial and ocular defects, sternae abnormalities, gut and cardiovascular malformations, to hypopigmentation and skeletal deformities (Yurdakök, 2012).

Treatment of female guinea pigs with hydroethanolic extract of *Spirulina platensis* whether for protective or curative, attenuated lead induced reproductive disrupted, fetal malformation and fetal mortality. These results indicate that Spirulina might have had a beneficial effect in reducing lead toxicity in pregnant female cavies. Protective and curative efficacy of *Spirulina fusiformis* may be due to the presence of several active components. The active component (Phycocyanin) found in Spirulina may provoke the activity of free radical scavenging enzyme systems and render protection against liver damage and oxidative stress (Asadpour et al., 2013; Abdel-Daim et al., 2013). Also, the protective and curative role of *Spirulina platensis* may be attributed to the presence of beta carotene (Prescot, 1978; Seshadri et al., 1991), Vitamin C, E (Mathew et al., 1995), super oxide dismutase enzyme and selenium (Henriksen, 1989). The incidence of resorption shown, can be explained by antioxidant compounds present in the Spirulina during pregnancy, such as vitamin E and vitamin C, which can decrease fetal malformation rate, diminish oxygen radical-related damage, and reduce oxidative stress in the reproductive apparatus, and then contribute to fetus protection.

Conclusion

Lead acetate induced reproductive dysfunction and

developmental of damage function in female guinea pig. Nonetheless, treatment with hydroethanolic extract of *Spirulina platensis* whether preventive or curative at dose of 100 mg/kg.bw reduced the reactive oxygen species attacks. This effect subsequently reduced skeletal anomalies and improved reproductive characteristics.

CONFLICT OF INTEREST

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

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