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EVALUATION OF THE PROTECTIVE ACTIVITY OF AQUEOUS LEAF EXTRACT OF *CISSUS POPULNEA* AGAINST LEAD-INDUCED TESTICULAR TOXICITY IN WISTAR RATS.

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ABSTRACT

Cissus populnea, a plant native to West Tropical Africa, has garnered attention, particularly for its use in traditional medicine to improve genital erection in males. Accordingly, this study investigated the possible protective effects of aqueous leaf extract of Cissus populnea (ACPE) against lead acetate-induced testicular toxicity in Wistar rats. Twenty adult Wistar rats were divided into four groups and administered various treatments, including 50mg/kg body weight (BW) of lead acetate, 1000 mg/kg BW of ACPE, or a combination of both, for 28 days via an orogastric tube. Thereafter, animals were sacrificed and various parameters, including body weight changes, sperm quality, and histology of testes were evaluated. Lead acetate exposure led to significant (P<0.05) weight loss, reduction in sperm count and motility as well as histological alterations of the testes. However, ACPE treatment significantly (P<0.05) mitigated the adverse effects induced by lead in the testes of exposed rats. Taken together, these findings underscore the potential of Cissus populnea as a possible protective agent against lead-induced testicular toxicity, thus offering important insights for future treatment options derived from natural sources.

Keywords: Lead toxicity, Cissus *populnea*, Testes, Infertility, Wistar rats.

INTRODUCTION

Lead can be found in our food, water, air, and soil. It is emitted by smelters and boilers that burn motor oil and are frequently deposited in the soil, where it is taken up by crops (Chiras, 2013). It is considered an enzymatic toxicant, neurotoxic, cardiotoxic, nephrotoxic, immunotoxic, carcinogenic, teratogenic, and mutagenic (Moreira and Moreira, 2004). Lead interferes with the environment because of its wide range of applications such as for making pipes, paints, enamels, and glazes (O'Connor *et al*., 2018). Lead does not have any detectable beneficial biological role, however, its detrimental effect on physiological, biochemical, and behavioural dysfunctions have been documented in animals and humans (Kumar and Devi, 2018). Lead is a male reproductive toxicant and is deposited in the testes, epididymis, vas deferens, seminal vesicles, and seminal ejaculate of experimental animals (Haouas et al., 2015). Reports indicate that lead causes a significant reduction in sperm count and motility and inhibits spermatogenesis in experimental animals (Elgawish and Abdelrazek, 2014; Awadi, 2017).

Cissus populnea belongs to the family Vitaceae/Ampelidaceae and the genus *Cissus* which comprises about 350 species. Studies from herbarium collections indicate that it is confined to the savannah zones of Nigeria and is more abundant in the northern region where it is used by the Fulanis to feed their cattle, ostensibly to increase milk production (Soladoye and Chukwuma, 2012). All parts are mucilaginous, yielding a viscid sap, which is occasionally drunk from freshly cut stems (Oyedemi, 2012). The plant is also used in Niger, Kogi, Plateau, Adamawa, Kwara, and Benue states of Nigeria for making vegetable soup for post-natal stoppage of bleeding (Soladoye and Chukwuma, 2012). The roots are also used in the western part of Nigeria to cure sore breasts of women during childbirth and as a male coital adjunct (Van, 2015). Other uses of the plant in traditional medicine include the treatment of venereal diseases, indigestion, catharsis, urinary tract infections, sexual dysfunction, wounds, boils, skin disease, oedema, and intestinal parasites. Particularly, it is used as a medicinal plant to improve genital erection in males and enhance spermatogenesis (Rotimi*et al*., 2023). The extracts of *Cissus populnea* are reported to possess pharmacological activities such as antidiabetic, antihyperlipidemic, larvicidal, hepatoprotective, hypoglycemic, anti-lipogenic, antiparasitic, antimalarial, anthelmintic, antibacterial, and antioxidant effects (Rotimi *et al*., 2023). These activities are associated with a range of secondary metabolites such as phytosterols, terpenoids, cardiac glycosides, alkaloids, steroids, iron, tannins, vitamins, flavonoids, saponins, and phenolics (Rotimi *et al*., 2023). There has been a continual revival of interest in the use of medicinal plants in many developing countries, particularly for novel drug discovery, since herbal medicines have been reported to be safe and with minimal side effects when compared to synthetic drugs (Enogieru and Momodu, 2021; Omoruyi *et al*., 2019; Enogieru *et al*., 2018). Accordingly, this study was designed to investigate the possible protective effects of aqueous leaf extract of *Cissus populnea* against lead acetate-induced testicular toxicity in Wistar rats.

MATERIALS AND METHODS

Plant Material

Aqueous extraction of *Cissus populnea* was done using the Freeze-drying method as previously described (Enogieru and Omoruyi, 2022). Briefly, the leaves were allowed to dry at room temperature. The dried leaves were milled into fine powder in an electric blender and five hundred grams (500 g) of the powder were soaked in 2 L for 24 h. The mixture was filtered with white filter paper and the residue was separated from the filtrate. The filtrate was concentrated using Freeze dried technique in the National Centre for Energy and Environment at the University of Benin, Benin City.

Care of Experimental Animals

Twenty (20) adult Wistar rats weighing between 150 and 180 g were used for this experiment. Care and management of animals were carried out following the guidelines for the care and use of laboratory animals (NRC, 2010). The animals were allowed to acclimatize for two weeks before the commencement of the experiment.

Treatment Regimen

The rats were randomly assigned into a Control group (A) and three treatment groups (B-D) containing five (5) animals each. Rats in Group B were administered 50 mg/kg body weight of lead acetate only, daily, as previously reported (Alotaibi *et al*., 2023); Group C received 50 mg/kg body weight of lead acetate and 1000 mg/kg body weight of aqueous leaf extract of *Cissus populnea* extract daily; Group D received 1000 mg/kg of aqueous leaf extract of *Cissus populnea* extract only, daily. All administrations were via an orogastric tube for 28 days. The dose of *Cissus populnea* extract was selected based on previous studies demonstrating that it is non-toxic and has a safety index greater than 5000 mg/ kg body weight (Kasar, 2021).

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Animal Sacrifice and Sperm Analysis

At the end of the experimental period, rats were sacrificed by cervical dislocation. The testes were excised and sperm characteristics analysis was performed on spermatozoa samples collected from the caudal epididymis as previously described (Obembe and Ige, 2016). Progressive motility was assessed immediately. Briefly, a drop of diluted sperm suspension (5µl) was placed on a prewarmed slide and two drops of warm 2.9% sodium citrate were added and covered with a cover slip. Progressive forward motility was examined and scored to the nearest 10. Sperm count was done under the microscope with the aid of the improved Neubauer hemocytometer. Counting was done in five Thoma chambers. The epididymis was immersed in 5 ml of normal saline in a measuring cylinder and the volume displaced was taken as the volume of the epididymis. The sperm cells were counted and the percentage was calculated as previously described (Obembe and Ige, 2016).

Histological Assessment

Following appropriate fixation in Bouin's fluid for seventy-two hours, the testes were processed through the paraffin wax embedding, and the hematoxylin and eosin staining methods were carried out as previously described (Drury and Wallington, 1980).

Statistical Analysis

Statistical analysis was done using SPSS version 16. All values are presented in Mean ± standard error of the mean for all groups, significance was determined using one-way ANOVA and a value of $P<0.05$ was taken as statistically significant.

RESULTS

Effect of Treatment on Weights

Table 1 shows the weight results obtained across experimental groups. There was a significant ($P<0.0001$) decrease in final body weight and weight change in group B (lead acetate) and group C (*Cissus populnea* + lead) rats when compared to control. There was no significant ($P>0.05$) difference in final body weight and weight change in group D (*Cissus populnea*) rats when compared to control.

Table 1: Weight differences across experimental groups

Groups	Initial Body Weight(g)	Final Body Weight(g)	Change in Weight (g)
Control	255.00 ± 5.00	233.80 ± 4.05	25.75 ± 4.25
Lead acetate only	260.00 ± 8.49	183.80 ± 2.38*	-39.25 ± 5.22*
Cissus populnea + Lead	204.30 ± 5.89	188.00 ± 5.78*	-16.25 ± 5.36*
Cissus populnea	188.80 ± 4.73*	212.50 ± 2.86	23.75 ± 2.98

Values are represented as Mean ± SEM for each group; superscript * indicates a significant difference at $P<0.05$ compared with control.

Effect of Treatment on Sperm Parameters

Table 2 shows the sperm parameters obtained across experimental groups. There was a significant decrease ($P=0.0276$) in total sperm count and progressive motility in group B rats (lead acetate) when compared to control. Also, there was a significant increase ($P=0.0068$) in non-progressive motility and abnormal sperm in group B rats (lead acetate) when compared to control. Conversely, there was a significant increase ($P=0.0068$) in total sperm count, progressive motility, and immotility as well as a significant decrease ($P<0.0001$) in non-progressive motility and abnormal sperm in group C (*Cissus populnea* + lead) rats when compared to lead acetate-treated rats. There were no significant differences ($P>0.05$) in sperm parameters between rats in group D (*Cissus populnea*) and control.

Table 2: Comparison of sperm parameters across experimental groups.

	Total Sperm Count (x 106 cells/ml)	Progressive motility (%)	Non-progressive motility (%)	Immotility (%)	Abnormal Sperm (%)
Control	400.00 ± 11.55	76.67 ± 3.33	8.33 ± 1.67	16.67 ± 4.41	13.33 ± 3.33
Lead acetate only	216.70 ± 32.83*	53.33 ± 6.67*	23.33 ± 3.33*	13.33 ± 3.33	50.00 ± 5.77*
Cissus populnea + Lead	366.70 ± 24.04$^{\#}$	63.33 ± 3.33$^{\#}$	16.67 ± 3.33$^{\#}$	20.00 ± 2.50$^{\#}$	16.67 ± 3.33$^{\#}$
Cissus populnea	393.30 ± 40.58	63.33 ± 3.33	10.00 ± 0.00	16.67 ± 3.33	13.33 ± 3.33

Values are represented as Mean ± SEM for each group; superscript * indicates a significant difference at $P<0.05$ compared with control. $^{\#}$ indicates a significant difference at $P<0.05$ compared with lead acetate only.

Effect of Treatment on Histology

Figure 1 represents the histology of the testes across experimental groups. Testes micrograph from the control group showed a basal region containing early germ cells of the spermatogenic series, clumps of tufty tails of spermatozoa in the lumen, and an adluminal region containing late germ cells of the spermatogenic series. The histology of the rats´ testes in group B displayed distorted germinal epithelium; vacuoles and empty lumen of the seminiferous tubules. The histology of the rat testes in groups C and D displayed a normal basal region containing early germ cells of the spermatogenic series, clumps of tufty tails of spermatozoa in the lumen, and an adluminal region containing late germ cells of the spermatogenic series.

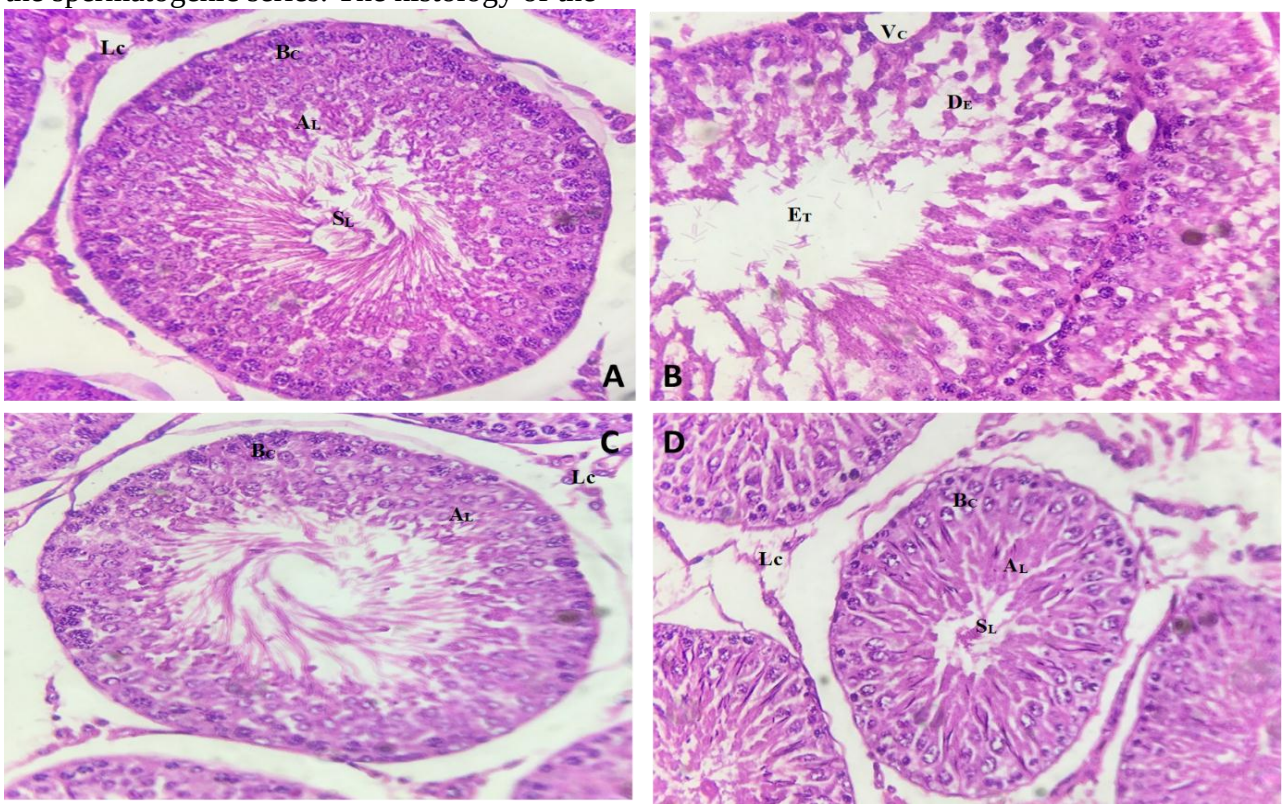

Figure 1: Histology of the testes across experimental groups (A) Control group showing basal region containing early germ cell of the Spermatogenic series (B_C); clumps of tufty tails of spermatozoa in the lumen (S_L); adluminal region containing late germ cell of the Spermatogenic series (A_L) (B) Lead acetate-only group showing Instatitial space (I_s) in interstitial space; distorted germinal epithelium (D_E); Vacoule (V_C) empty lumen of the seminiferous tubule (E_T), Blood vessel (B_V). (C & D) *Cissus populnea* treated groups showing basal region containing early germ cells of the Spermatogenic series (B_C); clumps of tufty tails of spermatozoa in the lumen (S_L); adluminal region containing late germ cells of the Spermatogenic series (A_L). H & E x100.

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DISCUSSION

This study investigated the possible protective effects of aqueous leaf extract of *Cissus populnea* against lead acetate-induced testicular toxicity in Wistar rats. Weight changes serve as a sensitive indication of the general health status of animals (Porwal *et al*., 2017). This means that significant fluctuations in weight can often signal underlying health issues (Porwal *et al*., 2017). These weight changes are particularly useful in assessing the impact of various chemicals and drugs. Lead is reported to have detrimental effects, including significant weight loss (Montoro-Huguet *et al*., 2021). Lead toxicity can disrupt normal growth and development, leading to developmental delays (Vaughan *et al*., 2013). In severe cases, it causes stunted growth (Vaughan *et al*., 2013). Thus, exposure to lead is a serious health concern due to its potential impact on body and organ weight as well as overall development. The significant weight loss in the group that received lead acetate treatment only underscores the already established toxic effect of lead on health. This agrees with the previous study reporting a significant decrease in the body weight of rats treated with lead (Enogieru and Iyoha, 2023; Enogieru and Momodu, 2022). The weight gain observed in the group treated with *Cissus populnea* suggests that this extract protects against lead-induced weight loss and may have beneficial effects on body weight following lead exposure in Wistar rats.

Results from this study show that lead affected sperm quality in the experimental rats. Sperm quality can be identified from the sperm count and viability as well as the amount of spermatogenic layers in the seminiferous tubules. The adverse effects of lead on sperm quality in this study agree with a report by Offor *et al*., (2019) demonstrating that lead acetate-exposed rats displayed decreased sperm quality and fertility disruption as reflected in the decreased testis weight, sperm count, and viability. From this study, the detrimental effects of lead exposure on sperm quality are inhibited by *Cissus populnea*. The findings showed that *Cissus populnea* protects the testis and preserves the sperm quality possibly via its ability to inhibit free radicals and ROS generated by lead.

From the histological findings, the testes of the control rats show a healthy spermatogenesis process. The basal region containing early germ cells of the spermatogenic series indicates active sperm production. The lumen containing clumps of tufty tails of spermatozoa suggests successful maturation and release of sperm. The adluminal region containing late germ cells of the spermatogenic series indicates a continuous process of sperm production. The histology of the testes of rats treated with lead acetate shows several abnormalities. For instance, the increase in the interstitial space indicates potential damage to the seminiferous tubules. The distorted germinal epithelium could negatively impact sperm production and the presence of vacuoles as well as an empty lumen of the seminiferous tubule suggest a decrease in sperm production or release. The histological findings in the testes of lead-exposed rats in this study agree with previous reports (Massányi *et al*., 2020). The histology of the testes of rats treated with both *Cissus populnea* extract and lead acetate shows a similar structure to the control group suggesting that *Cissus populnea* extract may have a protective effect against the harmful effects of lead acetate on the testes and spermatogenesis. *Cissus populnea* has been shown to previously ameliorate cotton seed-induced testicular damage via its potent antioxidant effects (Olooto *et al*., 2022). The protective effects of *Cissus populnea*on the testes could be attributed to its high antioxidant activity which has been earlier reported in in-vitro studies (Olooto *et al*., 2022; Akomolafe*et al*., 2013). Also, the leaf extract of *Cissus populnea* has been shown to contain alkaloids, flavonoids, saponins, and tannins (Soladoye *et al.*, 2012). These phytochemicals have been reported to increase male reproductive ability and fertility (Prabsattroo *et al.*, 2015). The histology of the testes of rats treated with

only *Cissus populnea* extract shows a similar structure to the control group indicating that the extract does not have a detrimental effect on the testicular tissue.

CONCLUSION

Taken together, lead acetate induces a detrimental effect on the testes and sperm quality, however, *Cissus populnea* extract exerts a protective effect. Further studies are necessary to investigate its exact mechanisms of action and these investigations could have important implications for the treatment of lead-induced testicular toxicity.

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