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Chemical Impact of *Pleukenetia conophora* Seed Oil on Progesterone Levels in Cadmium-Acetate-Exposed Female Wistar Rats

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Abstract

Objective: This study investigated the effects of *Pleukenetia conophora*(African walnut) seed oil on progesterone levels in female Wistar rats exposed to cadmium acetate, a known reproductive toxin. The aim was to determine if the walnut extract's antioxidant properties could mitigate hormonal disruptions caused by cadmium.

Methods: Thirty adult female Wistar rats (179–225g) were randomly assigned to five groups (n=6 each). Group A received normal saline, Group B was fed standard rat feed and water, Group C was administered 3.5 mg/kg of cadmium acetate, Group D received cadmium acetate and 4 ml/kg of *P. conophora* seed oil, and Group E was treated with 4 ml/kg of *P. conophora* seed oil only. Blood samples were collected via cardiac puncture, and serum progesterone levels were measured using the Enzyme Linked Immunosorbent Assay (ELISA).

Results: A significant reduction in progesterone levels was observed in Group C (4.433 ± 1.266) compared to Group B (12.567 ± 2.850, $P<0.05$). No significant differences were found between Group B and the other treatment groups (Group A: 8.500 ± 2.1284, Group D: 8.500 ± 0.9849, Group E: 8.100 ± 0.5196; $P>0.05$).

Conclusion: *Pleukenetia conophora* seed oil shows potential protective effects on progesterone levels in female Wistar rats exposed to cadmium acetate. This suggests its therapeutic potential as a natural remedy for reproductive toxicity associated with cadmium exposure, warranting further investigation into its mechanisms and applications in reproductive health.

Keywords: *Pleukenetia conophora*; Cadmium acetate; Progesterone; Wistar Rats

1. Introduction

Cadmium is a toxic heavy metal that poses significant health risks, particularly to the reproductive system. Its exposure has been linked to various reproductive disorders, including hormonal imbalances, which can adversely affect fertility and overall reproductive health(1,2). Among the hormones impacted by cadmium exposure, progesterone plays a

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crucial role in the female reproductive system, regulating menstrual cycles, maintaining pregnancy, and supporting fetal development(3). The detrimental effects of cadmium on progesterone levels have been documented in various studies, highlighting the need for effective interventions to mitigate these adverse effects(4,5)

Pleukenetia conophora (old scientific name- *Tetracarpidium conopharum*), commonly known as African walnut, is a plant native to West Africa that has gained attention for its potential health benefits. Rich in bioactive compounds such as flavonoids, tannins, and saponins, African walnut is recognized for its antioxidant, anti-inflammatory, and anti-cancer properties (6). These phytochemicals may offer protective effects against oxidative stress and inflammation, which are often exacerbated by cadmium exposure (7,8). Despite the promising health benefits associated with African walnut, there is limited research specifically examining its effects on progesterone levels in the context of cadmium-induced reproductive toxicity.

The primary objective of this study is to investigate the effects of *Pleukenetia conophora* seed oil on progesterone levels in female Wistar rats exposed to cadmium acetate. By inducing cadmium toxicity in these rats and subsequently administering African walnut seed oil, this research aims to evaluate whether the extract can ameliorate the negative impact of cadmium on progesterone levels. Additionally, the study seeks to assess the potential protective effects of *P. conophora* against reproductive damage caused by cadmium exposure.

The findings from this research could provide valuable insights into the therapeutic potential of African walnut seed oil in addressing cadmium-induced reproductive toxicity. Furthermore, understanding the relationship between cadmium exposure, progesterone levels, and the protective effects of *P. conophora* may have significant implications for reproductive health, particularly in populations at risk of cadmium exposure. Ultimately, this study aims to contribute to the growing body of knowledge regarding the health benefits of *Pleukenetia conophora* and its potential role in mitigating the adverse effects of environmental toxins on reproductive health.

2. Materials and methods

2.1. Study Area

The study was conducted at Babcock University, Ilishan-Remo, Ogun State, Nigeria. The research was carried out in accordance with the ethical guidelines established by the Babcock University Health Research Ethics Committee (BUHREC).

2.2. Ethical Considerations

Ethical approval for the study was obtained from BUHREC. All procedures involving animals were performed in compliance with the ethical standards for the care and use of laboratory animals.

2.3. Duration of Experimental Study

The experimental study lasted for six weeks, beginning with a two-week acclimatization period for the animals, followed by four weeks of treatment and observation.

2.4. Authentication of Plant Materials

Pleukenetia conophora (African walnut) seeds were purchased from a local market in Ogun State, Nigeria. The plant material was authenticated by a taxonomist at Babcock University, and a specimen was labeled, numbered, and annotated with the date of collection and locality.

2.5. Extraction of Plant Materials

The extraction of *Pleukenetia conophora* seed oil was performed using the Soxhlet extraction method as described by the Association of Official Analytical Chemists (AOAC). Briefly, the seeds were dried and ground into a fine powder. The powdered sample was placed in a thimble and extracted with n-hexane (boiling range 40-60°C) for six hours. The solvent was evaporated, and the oil was collected and stored at 4 °C until use.

2.6. Animal Preparation

Thirty adult female Wistar rats (weight range: 179–225 g) were purchased from a licensed vendor and housed in the Babcock University animal facility. The rats were acclimatized for two weeks and were provided with standard rat pellets and tap water ad libitum. The animal cages were maintained in a clean and sterile condition throughout the study.

2.7. Experimental Design

The rats were randomly divided into five groups (n=6 per group):

- *Group A* (Negative Control): Received 3.5 ml/kg of normal saline via intraperitoneal injection daily for 21 days.
- *Group B* (Zero Control): Fed with standard rat pellets and water without any treatment.
- *Group C* (Cadmium Induced): Received 3.5 mg/kg of cadmium acetate via intraperitoneal injection daily for 21 days.
- *Group D* (Cadmium + *P. conophora*): Received 3.5 mg/kg of cadmium acetate via intraperitoneal injection and 4 ml/kg of *P. conophora* oil extract via oral cannula for 21 days.
- *Group E* (*P. conophora* Only): Received 4 ml/kg of *P. conophora* oil extract via oral cannula for 21 days.

2.8. Laboratory Analysis

2.8.1. Method of Sample Collection

Blood samples were collected from the rats via cardiac puncture using a sterile needle and syringe. The samples were collected into lithium heparin bottles for serum separation.

2.8.2. Estimation of Progesterone Level

The serum progesterone levels were measured using Elabscience® QuicKey Pro Rat Pg(Progesterone) ELISA Kit. (Catalog No: E-OSEL-R0007)

The procedure involved the following steps:

- *Sample Processing*: Blood samples were allowed to clot, then centrifuged at 3000 rpm for 10 minutes to separate the serum, which was stored at -20°C until analysis.
- 50 µL each of the standards, blank, and samples were pipetted into their respective wells.
- 50 µL of the HRP-conjugate working solution was added into each well.
- The plate was covered with a sealer and incubated at 37° C for 60 minutes.
- After incubation, the solution was decanted.
- 350 µL of wash buffer was added to each well, allowed to soak for 1 minute, then decanted and the plate was blotted dry against absorbent paper. This washing step was repeated several times.
- Next, 90 µL of the substrate reagent was added to each well, and the plate was incubated at 37° C for 15 minutes.
- Afterwards, 50 µL of stop solution was added to each well, causing the color to change.
- *Reading:* Finally, the optical density was measured at 450 nm using a preheated microplate reader, and progesterone concentrations were determined by comparing the OD values to a standard curve.

2.9. Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 22.0. The results were expressed as means ± standard deviation. Statistical significance was assessed using one-way ANOVA followed by post-hoc tests, with a significance level set at $p < 0.05$.

3. Results

The result of the experimental study was presented in tables and charts. The descriptive analysis (mean and standard deviation) was determined, and differences across all groups were determined using one-way ANOVA. The significance level was set at 0.05. The animals were in five (5) groups, Group A fed with normal saline, Group B fed with rat feed and

water, group C was induced with 3.5mg/kg cadmium acetate, group D was induced with 3.5mg/kg cadmium acetate and 4ml/kg *P. conophora* and group E was given 4ml/kg *P. conophora.*

The control group (Group B) exhibited the highest mean progesterone level, while the cadmium-induced group (Group C) had the lowest mean progesterone level, indicating a significant reduction due to cadmium exposure.

3.1.1. Analysis of Variance

One-way ANOVA revealed a significant difference in progesterone levels among the five groups ($F(4, 10) = 8.035$, $p = 0.004$) as seen in Table 2. This indicates that cadmium exposure significantly affects progesterone levels in the experimental rats.

The significant difference between Group B and Group C confirms that cadmium exposure significantly lowers progesterone levels. However, the treatment with *Pleukenetia conophora* (Groups D and E) did not show a statistically significant increase in progesterone levels compared to the control group.

3.1.2. Summary

The results indicate that cadmium acetate exposure significantly decreases progesterone levels in female Wistar rats, while *Pleukenetia conophora* seed oil does not significantly restore these levels when administered concurrently with cadmium. The findings highlight the detrimental effects of cadmium on reproductive hormone levels and suggest further investigation into the protective properties of *Pleukenetia conophora*

3.2. Descriptive Statistics

Table 1 presents the mean progesterone levels and standard deviations for each group:

Table 1 Descriptive Statistics

Groups		**N**	**Mean**	**Std. Deviation**	**Skewness**
A-Normal saline (3.5mg/kg)	Statistic	3	8.500	2.1284	-0.816
	Std. Error				1.225
B- Control (feed & water)	Statistic	3	12.567	2.8501	-0.053
	Std. Error				1.225
C-Cadmium (3.5mg/kg)	Statistic	3	4.433	1.2662	0.801
	Std. Error				1.225
D- Cadmium + *P. conophora* (3.5mg/kg +4mg/kg)	Statistic	3	8.500	.9849	1.244
	Std. Error				1.225
E- *P.conophora* (4mg/kg)	Statistic	3	8.100	.5196	-1.732
	Std. Error				1.225

Group A (Normal Saline): 8.500 ± 2.1284 ng/mL; Group B (Control - Feed & Water): 12.567 ± 2.8501 ng/mL; Group C (Cadmium Acetate): 4.433 ± 1.2662 ng/mL; Group D (Cadmium + P. conophora): 8.500 ± 0.9849 ng/mL; Group E (P. conophora Only): 8.100 ± 0.5196 ng/mL

Table 2 One way ANOVA for analysis of variance in progesterone value of experimental animals across the five experimental groups

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	99.611	4	24.903	8.035	0.004
Within Groups	30.993	10	3.099		

Total	130.604	14			

Unpaired T-Test Analysis; The unpaired t-test comparisons between the control group (Group B) and other groups showed the following results: *Group B vs. Group A*: No significant difference (p = 0.291); *Group B vs. Group C*: Significant difference (p = 0.013);*Group B vs. Group D*: No significant difference (p = 0.191); *Group B vs. Group E*: No significant difference (p = 0.144)

Table 3 Unpaired T-test analysis in progesterone value of experimental animals between experimental groups

	Mean diff	**t**	**DF**	**Adjusted p value**	**Summary**
Group B (feed & water) vs group A (Normal saline 3.5ml/kg)	4.067	1.420	2	0.291	NS
Group B (feed & water) vs group C (Cadmium 3.5mg/kg)	8.133	8.714	2	0.013	**
Group B (feed & water) vs group D (Cadmium + *P. conophora* 3.5mg/kg +4mg/kg)	4.067	1.944	2	0.191	NS
Group B (feed & water) vs group E (*P.conophora* 4mg/kg)	4.467	2.339	2	0.144	NS

Value significant at 0.05 (p<0.05). NS: Not Significant

Table 3 above shows the unpaired t-test analysis between group B (control group) and other groups. The comparison test shows there is significant difference between the control group and the cadmium induced group (p= 0.013). The differences between other groups and the control is not statistically significant (p>0.05).

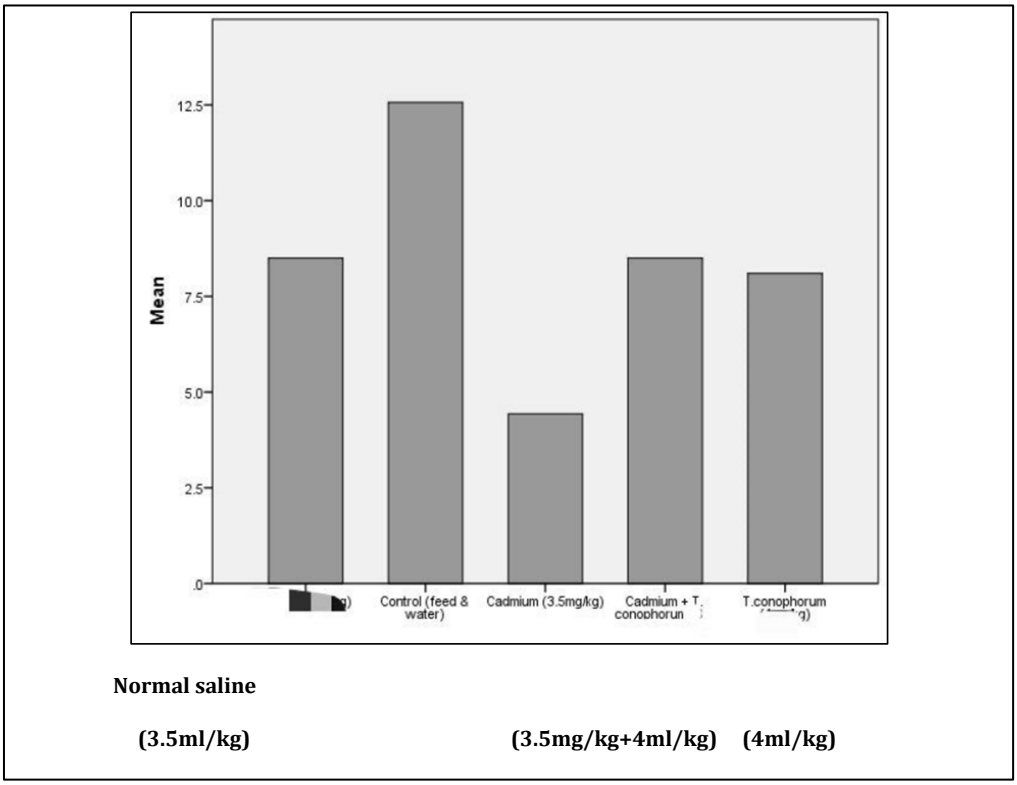

Figure 1 Bar chart showing the mean value of progesterone across all groups. Values are mean SEM of data obtained; p values (p<0.05)

4. Discussion

The findings of this study underscore the significant impact of cadmium exposure on progesterone levels in female Wistar rats, highlighting the reproductive toxicity associated with this heavy metal. The observed reduction in

progesterone levels in the cadmium-treated group aligns with existing literature that has documented the detrimental effects of cadmium on reproductive hormones. For instance, one of the scientific article from Science Direct (1) noted that cadmium exposure is linked to hormonal disruptions, particularly affecting steroidogenesis, which is crucial for the synthesis of sex hormones like progesterone. This disruption is primarily attributed to cadmium's ability to inhibit the expression of key proteins involved in hormone synthesis, such as the steroidogenic acute regulatory protein (StAR) and cytochrome P450 enzymes (9). Consequently, the decrease in progesterone levels observed in this study is consistent with the established understanding of cadmium's toxicological effects on the reproductive system. Interestingly, the administration of seed *Pleukenetia conophora* oil did not significantly restore progesterone levels in the cadmium-exposed rats, despite previous studies suggesting that this plant possesses antioxidant and protective properties against oxidative stress. For example, Akande et al. (7) demonstrated that African walnut extract could mitigate oxidative stress induced by cadmium in Wistar rats. The lack of significant improvement in progesterone levels following treatment with *P. conophora* may indicate that while the extract has beneficial effects on oxidative stress, it may not directly counteract the hormonal disruptions caused by cadmium. This finding suggests that the protective mechanisms of *P. conophora* may operate through pathways that do not directly influence progesterone synthesis or that the dosage and duration of treatment may need optimization for a more pronounced effect. Moreover, the results reflect the complex interplay between environmental toxins and reproductive health. The significant difference in progesterone levels between the control and cadmium-treated groups reinforces the notion that cadmium exposure can lead to hormonal imbalances, which may have broader implications for fertility and reproductive health. This aligns with findings from Jackson et al. (10), who reported that cadmium exposure is associated with reproductive health issues, including menstrual irregularities and fertility challenges.

The protective effects of *Pleukenetia conophora* against cadmium-induced oxidative stress, as suggested by prior studies, could be attributed to its rich phytochemical profile, which includes flavonoids and tannins known for their antioxidant properties(6) . However, the current study indicates that further investigation is needed to elucidate the specific mechanisms through which *P. conophora* may exert its effects, particularly in the context of hormonal regulation. The findings suggest that while *P. conophora* may offer some protective benefits, its efficacy in restoring progesterone levels in the context of cadmium exposure remains inconclusive.

In conclusion, while this study confirms the adverse effects of cadmium on progesterone levels, it also highlights the need for further research to explore the potential of *Pleukenetia conophora* as a therapeutic agent. Understanding the limitations of the current findings and the complexity of cadmium's impact on reproductive health will be crucial for developing effective interventions to mitigate the risks associated with heavy metal exposure. Future studies should consider varying dosages, treatment durations, and combinations with other protective agents to fully assess the therapeutic potential of *P. conophora* in reproductive health contexts.

5. Conclusion

This study concludes that cadmium acetate exposure significantly reduces progesterone levels in female Wistar rats, confirming the reproductive toxicity associated with cadmium. The results demonstrate a clear distinction in hormone levels between the control group and the cadmium-treated group, indicating that cadmium disrupts normal hormonal function, which is critical for reproductive health.

While the administration of *Pleukenetia conophora* seed oil did not yield a statistically significant restoration of progesterone levels in the cadmium-exposed rats, the extract's potential protective effects against oxidative stress are noteworthy. Previous studies have highlighted the antioxidant properties of *P. conophora*, suggesting that it may mitigate some of the oxidative damage caused by cadmium. However, the current findings indicate that further research is necessary to explore the optimal dosages and treatment regimens that could enhance the extract's efficacy in restoring hormonal balance.

The implications of this research are significant, particularly in the context of public health and environmental safety. Given the widespread exposure to cadmium through industrial processes and environmental contamination, understanding its effects on reproductive hormones is crucial for developing strategies to protect reproductive health. Additionally, the exploration of natural products like *Pleukenetia conophora* as potential therapeutic agents offers a promising avenue for future research aimed at mitigating the adverse effects of heavy metals on reproductive health.

This study lays the groundwork for further investigations into the pharmacological properties of *P. conophora* and its potential applications in reproductive health interventions.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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