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Biometric Effects of Ethanolic and Methanolic Earthworm (Eudrilus eugeniae) Cast Extracts in Wistar Rat

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Abstract

Earthworm cast is an earthworm byproduct with an untapped therapeutic potential. This study evaluated the morphological effect of ethanolic and methanolic cast extracts on Wistar rats. Fifteen (15) adult female rats (197±46 g) were randomly assigned into three groups (n = 5) after two weeks of acclimatization. The control was subjected to distilled water (DW), treatment (T1) to 15% Ethanolic cast extract (ECE) and treatment (T2) to 15% Methanolic cast extract (MCE). Administration was done via free drinking with feeding ad libitum for four weeks. Morphological indices were evaluated using standard methods, while PCA was used to determine the association between parameters. Results showed that the control, gradual and steady increases in measured parameters, ECE exhibited consistently higher values while the MCE demonstrated early but fluctuating responses. Body weight revealed rapid early gains in the MCE group at day 7 (18%) and delayed but increases in the ECE at day 28 (11%), whereas the control showed typical gradual growth at day 28 (7%). Organ–body weight ratios were largely stable, with notable variations in liver and stomach, reflecting organ-specific responses to extract administration. PCA identified three major patterns of organ variability: general organ enlargement, a liver–gut metabolic axis, and independent heart variation. In conclusion, the findings indicate that ECE exerts the most pronounced and sustained biometric effects, whereas MCE induces early but less stable responses. Therefore, earthworm cast extracts can serve as bioactive agents for growth promotion and organ-specific modulation, providing a foundation for further mechanistic and safety studies.

Keywords: earthworm cast, ethanolic extract, methanolic extract, body weight

Introduction

Earthworms, particularly the species Eudrilus eugeniae (African nightcrawler), have gained recognition for their ecological and medicinal significance. They are known for their ability to decompose organic matter and improve soil fertility, also possess bioactive compounds with therapeutic potential [1]. Recent studies have shown that extracts from earthworms, derived from various solvents contain numerous biologically active components which demonstrates a wide range of effects including anti-inflammatory, antioxidant, and antimicrobial properties [2].

Animal model such as wistar rats are frequently used due to their physiological and genetic similarities to humans [3]. They serve as an ideal species for evaluating parameters such as biometrics and biochemical markers which are crucial indicators of an organism's overall health and can be used to assess the effects of substances on metabolism, organ function, and immune response [4].

Methanol and ethanol are known to dissolve different polar and non-polar compounds, making them suitable solvents for the extraction of pharmacologically relevant molecules [5].

Numerous studies have reported on the various benefits of earthworm extracts. For example, Balakrishnan et al. [6] showed that methanolic extracts of Eudrilus eugeniae exhibited significant hepatoprotective effects in rats exposed to hepatotoxins. Similarly, Kumar et al. [4] demonstrated that ethanolic earthworm extracts

improved antioxidant status and reduced oxidative stress markers in rats subjected to environmental toxins.

Biometric studies involving natural extracts such as those derived from Eudrilus eugeniae have the potential to uncover therapeutic benefits and guide the development of novel treatments for various diseases which can be applied in human and veterinary medicine.

However, despite the growing body of evidence on the medicinal properties of earthworm extracts, limited or no research has been conducted on the comparative biometric effects of ethanolic and methanolic earthworm cast extracts from Eudrilus eugeniae on Wistar rats. Therefore, this study aimed to bridge that gap by evaluating the impact of this cast extracts on biometric parameters in Wistar rats.

Materials and Methods

Experimental Animals and Housing

Fifteen (15) adults female Wistar rats (197 ± 46 g), were obtained from a certified animal facility in Abeokuta, Ogun State, Nigeria. Animals were housed in standard polypropylene cages under controlled laboratory conditions with a 12-hour light/dark cycle, ambient temperature of 25 ± 2°C, and relative humidity of 50–60%. Rats were allowed a two-week acclimatization period to minimize stress and ensure adaptation to the laboratory environment. During this period, animals had free access to standard laboratory chow and distilled water.

Ethical Approval

All experimental procedures were conducted in accordance with the guidelines of the National Open University of Nigeria (NOUN) Ethics Research Committee, and approval was obtained prior to the study (Approval No. ETC/2022/NOUN/12/032). All efforts were made to minimize animal suffering and reduce the number of animals used.

Earthworm Cast Extract (ECE) Preparation

Earthworm (Eudrilus eugeniae) cast were collected from a controlled vermiculture [7], air-dried, and finely pulverized using wooden mortar and pestle. Two types of extracts were prepared as follows;

Ethanolic Extract (ECE) and Methanolic Extract (MCE): 100 g of pulverized cast were macerated in 1000 mL of 70% ethanol and methanol for 72 hours respectively with intermittent shaking, and then filtered using Whatman No 1.

Extracts were diluted to 15% (w/v) in distilled water for administration and prepared fresh weekly to maintain stability.

Experimental Design and Treatment

Following acclimatization, the study adopted a completely randomized design (CRD). The rats were randomly assigned into three groups (n = 5 per group): Control (C) received distilled water only, Treatment 1 (T1) received 15% ethanolic earthworm cast extract (ECE) and Treatment 2 (T2) received 15% methanolic earthworm cast extract (MCE). Exactly, 200 mL of each treatment were administered via ad libitum free drinking for four weeks. Standard laboratory feed was provided ad libitum throughout the study.

Morphological Measurements

Body weight was recorded weekly using a digital balance. At the end of the study, rats were anesthesia by suffocation using chloroform ether [8]. Internal organs (liver, kidney, heart, spleen, lung, and stomach) were harvested, blotted to remove excess fluid, weighed, and expressed relative to body weight to calculate organ–body weight ratios. Organ lengths were measured using meter rule and digital caliper where appropriate.

Data Analysis

Data were expressed as mean ± standard deviation (SD). Biometric parameters were analyzed using Principal Component Analysis (PCA) to evaluate correlations and clustering among variables. Statistical analyses were performed using SPSS v21, mean separation was performed using Tukey Post Hoc test with significance set at $p < 0.05$. Graphical representations were produced using Microsoft Excel, 2016.

Results and Discussion

Body weight

The mean body weight (g) of the experimental animal across the study duration is presented in Table 1; the results show clear differences in the mean body weight across the treatment groups over the study duration. In the control group, the mean body weight increased gradually from the baseline (148.1 ± 1.56 g) to Day 28 (159.1 ± 2.22 g). In contrast, the ECE group recorded the highest values throughout the study. Ranging from baseline (172.9 ± 22.06 g) to Day 28 (192.00 ± 9.19) while the MCE group presented a different trend. Although the baseline (127.37 ± 17.80) was the lowest among all groups, there was an initial increase at Day 7 (150.67 ± 26.94 g). Mean body weight was significant ($p<0.05$).

Table 1: Mean Body weight (n=5)

abcdefgMean±SD with different superscript is significantly different at $p<0.05$

Duration	Control	Ethanolic CE	Methanolic CE
Baseline	148.1±1.56abc	172.9±22.06abcde	127.37±17.80a
Day 7	146.1±3.20ab	176.65±11.24abcdef	150.67±26.94abc
Day 14	151.0±2.35abc	176.45±10.54abcdef	148.00±23.42abc
Day 21	155.5±2.21abcd	177.05±10.68abcdef	142.70±16.52ab
Day 28	159.1±2.22abcd	192.00±9.19bcdef	146.13±17.14ab

Percentage Body Weight Changes

The percentage body weight change (%) of the animals in the study is visualized using the line graph in Figure 1. The

MEC group showed a sharp body weight change increase at Day 7 (18%). Although there was a slight decrease at Days 14 and 21 with a consistently higher change at Day 28 (15%). Furthermore, the EEC group showed a slight rise at Day 7 (2%) and an increase at day 28 (11%) while the control group displayed a steady, gradual increase from day 7 (-1%) to day 28 (7%).

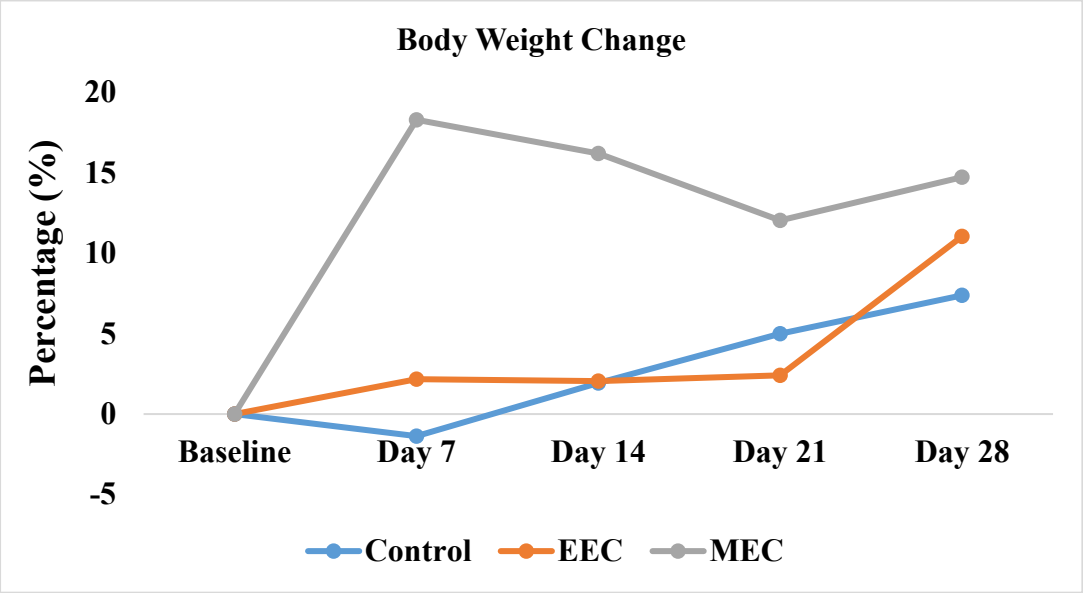

Figure 1: Trend of body weight change in rat

Organ-Body Weight Ratio

The organ–body weight ratio data from the study is presented in Table 2, the result shows how different treatments affected the relative size of several internal organs. The heart weight was relatively similar across all groups, with the ECE group showing a slightly higher mean ratio (0.34) compared to the control (0.32) and MCE (0.30). Lung weight ratios were also comparable, with both ECE and MCE showing slightly lower means (0.81 each) than the control (0.86). Gut length remained consistent across groups. The control and MCE groups had similar average lengths (55.73 and 55.42), while the ECE group showed a slightly shorter gut (54.69).

The ECE group had a higher liver–body weight ratio (3.35) compared with the control (2.97) and MCE (3.29). Kidney weight ratios were similar between the control and ECE groups (0.57 each), but slightly higher in the MCE group (0.64). Spleen weight showed minimal variation. The control and MCE groups had similar means (0.34 and 0.35), while the ECE group had a slightly lower value (0.31). Spleen length, however, was highest in the MCE group (2.097) and lowest in the ECE group (1.98). Stomach weight displayed the most pronounced treatment effect. The ECE group showed a noticeably reduced stomach–body weight ratio (0.65) compared with the control (0.83) and MCE (0.88).

Table 2: Organ –Body Weight Ratio

Parameters	Treatments	Mean (n=5)	Std. Deviation
	Control	0.32	0.10
Heart Weight	Ethanolic Cast Extract	0.34	0.01
	Methanolic Cast Extract	0.30	0.05
	Control	0.86	0.08
Lung Weight	Ethanolic Cast Extract	0.81	0.14
	Methanolic Cast Extract	0.81	0.07
	Control	55.73	4.63
Gut Length	Ethanolic Cast Extract	54.69	2.21
	Methanolic Cast Extract	55.42	11.27
	Control	2.97	0.15
Liver Weight	Ethanolic Cast Extract	3.35	0.10
	Methanolic Cast Extract	3.29	0.22
	Control	0.57	0.06
Kidney Weight	Ethanolic Cast Extract	0.57	0.04
	Methanolic Cast Extract	0.64	0.06
	Control	0.34	0.05
Spleen Weight	Ethanolic Cast Extract	0.31	0.01
	Methanolic Cast Extract	0.35	0.06
	Control	2.07b	0.13
Spleen Length	Ethanolic Cast Extract	1.98ab	0.00
	Methanolic Cast Extract	2.097b	0.08
	Control	0.83	0.02
Stomach Weight	Ethanolic Cast Extract	0.65	0.11
	Methanolic Cast Extract	0.88	0.15

Ab Mean±SD with different superscript on the same row are significantly different at p≤0.05

Principal Component Analysis (PCA)

The principal component analysis of biometric parameters is shown in Table 3 and the three-dimensional plot in rotated space (Figure 2). The Principal Component Analysis (PCA) reveals how the measured biometric parameters group together and contribute to overall variability in the dataset. Three principal components (PC1, PC2, and PC3) collectively accounted for 77.68% of the total variance. The PC1, accounted for the largest proportion of variance (40.41%), is heavily influenced by variables related to spleen and gastrointestinal morphology. Spleen weight (0.907) and spleen length (0.865) load most strongly on this component, followed by stomach weight (0.747), lung weight (0.716), and kidney weight (0.673).

The PC2 accounting for 21.88% of the total variance is dominated by liver weight (0.826), followed by negative contributions from gut length (–0.719) and lung weight (–0.475). Furthermore, the PC3, accounting for 15.39% of the total variance, is driven almost entirely by heart weight, which loads very strongly on this component (0.922). Stomach weight also contributes moderately (0.439). Together, these components show that variation in organ morphology clusters into three distinct biological patterns

Table 3: Principal Component Analysis

Biometric-Parameters	Component PC1	PC2	PC3
Spleen weight	0.907	0.120	-0.172
Spleen Length	0.865	-0.149	-0.240
Stomach weight	0.747	0.181	0.439
Lung weight	0.716	-0.475	0.296
Kidney weight	0.673	0.412	0.169
Liver weight	0.264	0.826	-0.103
Gut Length	0.169	-0.719	-0.311
Heart weight	-	-	0.922
Eigen value	3.233	1.750	1.231
% variance	40.41	21.88	15.39
Cumulative %	40.41	62.29	77.68

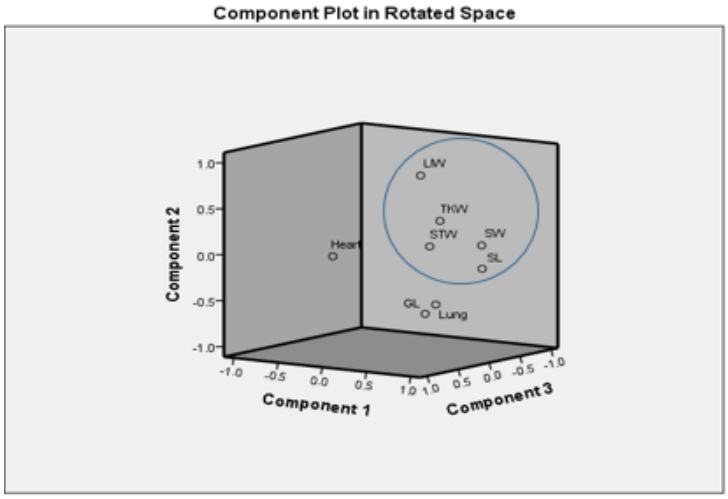

Figure 2: Three-dimension PCA plot in rotated space

Discussion

The present study demonstrated clear differences in morphometric parameters across the treatment groups. The control group exhibited a gradual increase in the measured parameter from reflecting normal growth progression consistent with baseline growth in untreated laboratory animals [9]. In contrast, the ECE group consistently recorded the highest body weight throughout the study. The significant differences compared to both the control and MCE groups indicate a strong and sustained effect of the ethanolic earthworm cast extract, corroborating previous studies showing that bioactive compounds in extracts can enhance metabolic and physiological performance [10]

The MCE group exhibited a distinct trend, with a low baseline value that increased sharply by at Day 7 but fluctuated thereafter. Variability in methanolic earthworm cast extract responses may be due to differences in extract solubility, bioavailability, or phytochemical composition [11].

The percentage body weight changes further illustrated extract-specific effects. The MCE group displayed the most rapid early weight gain, maintaining higher percentage changes throughout the study, indicative of a potent weight-inducing effect. Furthermore, the ECE group showed delayed but significant increases, whereas the control group followed a steady growth trajectory. These trends are consistent with studies showing that methanolic extracts can induce early but transient physiological responses, whereas ethanolic extracts produce more gradual and sustained effects on growth and metabolism [12,13].

The organ–body weight ratio revealed that most organs remained relatively stable, with notable variations in liver and stomach weights. The ECE group exhibited mild hepatomegaly and reduced stomach weight, whereas the MCE group showed modest increases in kidney and stomach ratios. These findings suggest organ-specific responses to earthworm cast extract administration, likely reflecting differential metabolic or detoxification demands [14]. Prior studies have reported mild liver enlargement associated with bioactive compounds in medical plant extracts, emphasizing their hepatotropic effects [15].

Principal Component Analysis (PCA) clarified patterns of organ variability. The PC1 was dominated by spleen, stomach, lung, and kidney weights, representing general organ-size variation. PC2 highlighted a liver–gut functional axis, reflecting metabolic adaptations, while PC3 isolated heart-related variation, indicating independent cardiac responses. These results align with studies using PCA to summarize complex organ-specific responses to bioactive extracts [16,17].

Conclusion

The ethanolic earthworm cast extract (ECE) produced the most pronounced and sustained effects on biometric parameters, particularly liver enlargement and delayed but meaningful body weight gain. The methanolic earthworm cast extract (MCE) induced early but less consistent effects, especially on body weight and kidney size. Organ–body weight ratios indicated overall stability, with liver and stomach showing the clearest treatment-related differences. PCA revealed distinct organ response patterns, highlighting general organ enlargement, a liver–gut metabolic axis, and independent heart variation.

Recommendations

Ethanolic earthworm cast extract is recommended for interventions requiring sustained growth promotion and enhancement due to their consistent effects. Furthermore, methanolic extract may be useful for early responses but may require optimization of dosage or formulation to achieve stable long-term effects. However, future studies should incorporate histopathological analyses to assess organ-specific effects and safety of earthworm cast extracts. More so, investigations into the bioactive compounds, bioavailability, and metabolic mechanisms of these extracts are recommended to better understand their differential physiological impacts.

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