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Performance Characteristics of Wistar Rats Gavaged with Aqueous Extract of *Alchornea cordifolia* Leaf Meal

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Authors' contributions

This work was carried out in collaboration between all authors. Authors PCNA and PJA designed the study, wrote the protocol, carried out the experiment performed the statistical analysis, and wrote the first draft of the manuscript. Author EIO proof-read and improved the quality of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

A 42 day feeding trial of graded administration of aqueous extract of *Alchornea cordifolia* leaf meal (ACLM) infusion on the body weights and internal organs of forty eight male albino rats (Wistar strain) weighing 131.60±6.79-139.20±6.03g, divided into 4 groups and three replicates of 3rats/replicate in a completely randomized design (CRD) experiment. Treatment I which served as the control received 0.2ml physiological saline solution while the second, third and fourth groups were gastro-gavaged with 200, 400 and 600 mg/kg of ACLM extract orally on daily basis respectively, for six weeks. Rat chow and water intakes were measured twice a week. The weight of each rat was recorded on day 0 and at weekly intervals throughout the course of the study and no mortality nor abnormal behavior of treated rats were observed. The rats were fasted for 24hrs and then sacrificed. Liver, kidney, heart, lung and spleen in the rats were removed. No pathological changes such as organ swelling, atrophy, hypertrophy or remarkable differences in weights of liver, kidney, heart, lung and spleen were observed on the main visceral organs in all treated

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rats during the study. This indicated that organ relative weights were not affected by administration of ACLM infusion. Weight gains were recorded up to the third week after that those under 400-600gm/kg began to lose weight and the losses were significant when compared with 200mg/kg and the control which were similar. The results indicated a positive health status for 200mg/kg gavaged rats but significant weight depreciation for those gavaged with 400-600mg/kg from the fourth to the sixth week. Therefore, ACLM should not be fed to animals for more than 3weeks on continuous basis to avoid weight losses.

Keywords: Alchornea cordifolia leaf meal; visceral organs; weight gain; Wistar rats.

1. INTRODUCTION

Alcornea cordifolia belongs to the family of *Euphorbiacea* and it is erect or straggling perennial shrub to a small tree. It is a common West African tropical flora. The plant which has heart-shaped leaves with brown stem and green hanging fruits is widely distributed in West Africa, mostly Nigeria [1-3] and Zaire republic. In Cross River State of Nigeria it is called 'Mbom' by the Efiks and 'Ashenshen' by the Bekwarra people, Epain (Ijaw), Ewe Pepe (Yoruba) Christmas bush (English), Bambani (Hausa), Ubebe (Igbo), Ebe-uhosa (Edo) [4,5]. The result of proximate composition showed that the leaf meal contained moisture (9.96%), crude protein (17.94%), carbohydrate (39.53%), crude fat (4.34%), energy (3.37 kcal/g), ash (11.38%) and crude fibre (16.85%). Elemental analysis revealed that the minerals detected in the leaf meal and their concentrations were calcium (2.88%), magnesium (0.22%), potassium (7.25 mg/kg),copper (32.5 mg/kg), iron (192.5 mg/kg) manganese (58.35mg/kg) and cobalt (40mg/kg) and Phytochemical screening revealed the presence of alkaloids, saponins, tannins (naturally occurring polyphenol), flavonoids, terpenes and glycosides with steroidal rings [6-8]. It is reasonably safe with an LD50 of the methanolic extract at 1131.4mg/kg. The leaf infusion or decoction is used to treat colds, bronchial problems, stomach ache, dysmenorrhea in mensural women, fever and eye problems. The powdered and pulverized leaf is used for treatment of cuts, burns, bruises, ulcers and piles [9-11]. In Nigeria, the decoction of the plant leaf is used against gonorrhoea [12] and is taken orally for urinary tract infection in Zaire [13]. The decoction of the leaf is used for conjunctivitis in Senegal [10]. *Alchornea* occurs extensively in the wild in Wilberforce Island, hence the need to examine its usefulness in animal production. This study aims at investigating the effects of aqueous extract of *Alchornea cordifolia* leaf meal (ACLM) infusion on the body weights and internal organs of Wistar rats.

2. MATERIALS AND METHODS

2.1 Experimental Animal Models

Forty male albino rats (Wistar strain) weighing between 131.60±6.79-139.20±6.03g, divided into 4 groups of 10 rats/group. The rats were collected from the animal house of the Nigeria Natural Medicine Research Council (NNMRC). The animals were kept in wire mesh cages under controlled light cycle (12h light/12h dark) and fed with commercial rat chow (Vital Feeds Nigeria Limited) ad *libitum* and liberally supplied with water. The animals were weighed, sorted into four treatment groups (Control, (0.2ml saline solution) while the second, third and fourth group were gastro-gavaged with 200, 400 and 600 mg/kg of ACLM extract orally on daily basis. The chosen dose of ACLM extract was based on the active

pharmacological dose range obtained from the orientation study earlier conducted. The animals were equally given equivalent amount of distilled water via the same route. They were housed in galvanized aluminium cages with wire mesh floor (to prevent caprophagia), and appropriate compartments to enable the collection of feces and determination of feed intake. All of the animals received humane care according to the criteria outlined in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institute of Health. The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animals' welfare during experiments [14].

2.2 Preparation of the *Alchornia cordifolia* Leaf Meal (ACLM)

Fresh dirt free leaf samples harvested from Niger Delta University Campus were air and sundried and ground in a hand mill and sieved through an 80 mm mesh screen. The resultant powder here after referred to as *Alchornia cordifolia* leaf meal (ACLM) was stored in air tight bottle prior to analysis. About 100 g of the powder was soaked in 400 mL distilled water and allowed to stand for 3 days with occasional stirring, using a magnetic stirrer to ensure proper mixing. The content was then filtered, using a sintered funnel and the filtrate concentrated, using a rotary evaporator. The proximate composition and mineral content of the leaf is presented in Table 1, while Table 2 shows the phytochemicals and anti-nutrient of the leaf powder. The composition of the basal diet used to feed the animals is presented in Table 3.

Table 1. Proximate Composition (%) mineral contents of ACLM (mg/kg)

Proximate composition	%	Mineral content	Mg/kg
Moisture	9.96±0.40	Calcium	288
Total fat	4.340±0.23	Magnesium	22
Fatty acid	3.47±0.24	Potassium	7.25
Crude proteins	17.94±0.40	Cobalt	40
NFE	43.53±0.21	Manganese	58.35
Total ash	11.38±0.26	Iron	192.5
Crude Fibre	12.85±0.16	Copper	32.5
Energy (Kcal/g)	3.367		

Each value represents means ± standard deviation of three replicate determinations
Alikwe and Owens [6]

2.3 Body and Organ Weight Measurements

After 42 days of the feeding trial, the rats were fasted overnight (for at least 15-16 h), then anesthetized with ethyl ether. Their blood samples were collected, approximately 2 ml, from the posterior vena cava and they were then sacrificed for organ samples. Gross examination on the external surfaces of all visceral organs, thoracic and abdominal cavities were carried out. The weight of each rat was recorded on day 0 and at weekly intervals throughout the course of the study. Feed and water intakes were measured twice a week. Following blood collection, the rats were sacrificed. Liver, kidney, heart, lung and spleen in the rats were removed. The macroscopic appearance of the organs was noted and their weights were recorded.

Table 2. ACLM phytochemical and anti-nutrients

Phytochemicals	(Water extract) Qualitative	Quantitative (% composition)
Alkaloids	-	
Anthraquinones	-	
Flavonoids	-	
Cardiac glycosides	+	0.11
Saponins	+	2.04
Steroids	-	
Tannins	-	
Triterpenoids	-	
Phenols	+	1.16
Anti-Nutrients		
Phytate	+	1.21
Oxalate	+	0.86
HCN	+	22.3mg/kg

+ = Present, - = Absent Alikwe and Owens [6]

Table 3. Basal diet

Composition	(g/kg)
Casein	200.00
Corn starch	150.00
DL Methionine	3.00
L Cysteine	3.00
Fiber (Cellulose)	50.00
Dextrose	250.00
Sucrose	250.00
Choline bicarbonate	2.00
Soybean oil	50.00
Mineral premix	42.00
Total	**1000**

2.4 Statistical Analysis

The experiment was arranged in a completely randomized design with ten rats per treatment. Analysis of variance was conducted on the data collected and significant differences between the control and treatment means were determined using Duncan multiple range tests (SPSS version 17). A probability level of p <0.05 was considered as significant

3. RESULTS AND DISCUSSION

During the study, no deaths of treated rats were observed. ACLM did not show any abnormal behavior as there were no signs of behavioral changes observed in all treated rats. All rats subjected to different doses of ACLM infusion showed (Table 4) weight gain up to the third week after that those under 400-600gm/kg began to lose weight and the losses were significant (P<0.05) when compared with 200mg/kg and the control which were similar (P>0.05). Initial rat weight at the onset of dosing ranged from 131.60±6.79-139.20±6.03g and increased to 125.40±9.01-171.30±3.28g with weight gain of 19.0±2 to 31.96 g for the

first three weeks after which those in 400-600mg/kg began to lose weight while the control and 200mg/kg were statistically similar (P>0.05). Increment in body weight is important as it determines the health status of the animal groups [15]. Therefore, the results indicated a positive health status for 200mg/kg gavaged rats but significant weight depreciation for those gavaged with 400-600mg/kg from the fourth to the sixth week. Food and water consumption levels were similar in both control and treated animals. ACLM is a good source of medicinal and nutritional substances with low concentration of some antiphysiological factors such as phytates (1.21±%), oxalates (0.86±%) and (HCN 22.3 mg/kg), which could slightly decrease the overall benefits of this plant. A major factor limiting the wide use of many plants is the ubiquitous occurrence in them of this range of natural compounds capable of eliciting deleterious effects in man and animals. These compounds known as antinutrients are of different types and widely distributed in the plant kingdom [16]. The anti-nutritional factors; HCN, oxalates and phytates were present in varying amounts in ACLM. Phytates has been reported to reduce the bioavailability of trace element and minerals [17]. The phytate content of ACLM (1.21mg/100g) is however, below the range reported for most vegetables. Zhou [18] indicated that phytates occur naturally as mixed potassium, magnesium and calcium salt in complex diets. Phytic acid and iron form insoluble complexes that are not available for absorption under the pH conditions of the small intestine. In addition, phytic acid is also known to inhibit the availability of other divalent minerals such as Zn and Mg. Inhibition of iron absorption as a result of dietary phytate can also be partially counteracted by activation of native or the addition of extrinsic phytase to phytate-rich diets or by chemical hydrolysis of the phytate present [19,20]. In contrast to its anti-nutritive effects, phytic acid can be beneficial for the delay of postprandial glucose.

Table 4. Changes in the body weight of Wistar rats fed with aqueous extract of ACLM

Group/treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
0.2ml Saline	139.20	152.80	171.16	182.50	178.30	171.30
(Control)	±6.03a	±10.62a	±16.10a	±7.59b	±3.93b	±3.28b
200 mg/kg ACLM	137.40	151.90	169.50	175.60	174.00	168.80
	±8.43a	±5.18a	±7.26a	±9.77b	±4.46b	±6.72b
400 mg/kg	137.60	146.80	168.901	140.10	131.60	127.20
ACLM	±5.57a	±6.40a	±1.12a	±6.00a	±7.54a	±6.29a
600 mg/kg ACLM	131.60	146.20	150.60	138.20	121.00	125.40
	±6.79a	±4.22a	±5.68a	±8.16a	±8.44a	±9.01a

Each value is expressed as mean ± standard error (n = 5)
Different letters in each column indicate significant differences at P < 0.05 according to the Duncan Statistics

Comparison of organ weights between the treated and untreated groups of animals has been used to predict the toxic effect of the test material [21] Any change in organ weight is an indicator of toxicity, because organ weight will be affected by the suppression of body weight [15,22]. Data on organ weight measurements are presented in Table 5. There were no remarkable differences (P>0.05) in weights of liver, kidney, heart, lung and spleen among the different treatments. This indicated that organ relative weights were not affected by administration of ACLM infusion. No noticeable pathological changes such as organ swelling, atrophy or hypertrophy were observed on the main visceral organs in all treated rats during the study.

Table 5. Changes in the organ weights of Wister rats fed with aqueous extract of ACLM

Group/ treatment (g)	Kidney	Liver	Heart	Lung	Spleen
0.2ml Saline (Control)	0.74±0.05[a]	4.25±0.20[a]	0.39±0.01[a]	1.10±0.26[a]	0.29±0.03[a]
200mg/kg ACLM	0.75±0.04[a]	6.45±1.08[b]	0.40±0.02[a]	0.88±0.07[a]	0.38±0.07[a]
400mg/kg ACLM	0.85±0.02[a]	8.30±0.48[b]	0.50±0.03[b]	0.88±0.07[a]	0.29±0.08[a]
600mg/kg ACLM	0.97±0.04[b]	7.99±0.34[b]	0.55±0.01[b]	1.01±0.04[a]	0.30±0.05[a]

Each value is expressed as mean ± standard error (n = 4)
Different letters in each column indicate significant differences at P < 0.05 according to the Duncan Statistics

4. CONCLUSION

Water extracts of ACLM showed no noticeable gross toxicity in the sub-acute oral test even at a 200mg/kg but weight losses assumed a significant dimension as the quantity of the extracts increased linearly

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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