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Phytochemicals of *Cynodon dactylon* and the Toxicological Effect of its Aqueous Extract on Wistar Rats

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ABSTRACT

Cynodon dactylon is a perennial grass used in the treatment of diseases such as diabetes, dropsy and syphilis. Phytochemicals of *Cynodon dactylon* and effect of its aqueous extract on some biochemical parameters of Wistar rats was evaluated. Sixteen (16) adult male Wistar rats were randomly divided into four groups (1 - 4) of four rats each. Group 1 (control) was administered water only. Groups 2 - 4 were administered 100, 200 and 400 mg/kg body weight of aqueous extract of *C. dactylon* respectively. Phytochemicals detected were: saponin (1.25 ± 0.01%), flavonoid (4.15 ± 0.15%), steroid (0.87 ± 0.02%), alkaloid (5.00 ± 0.01%) and tannin (1.62 ± 0.05%). After 21 days' daily administration of the extract, there was no significant ($p>0.05$) difference in the relative liver weight, relative-kidney weights, aspartate amino transferase (AST), alanine amino transferase activity (ALT), total bilirubin, conjugated bilirubin, creatinine and bicarbonate levels in all the groups compared to their controls. Sodium and potassium concentrations were significantly ($p<0.05$) higher compared to their controls. Liver and kidney histology did not show visible disruption of their architecture. Aqueous extract of *C. dactylon* showed minimal signs of toxicity at the administered doses.

Keywords: Biochemical, *Cynodon dactylon*, Histology, Phytochemical, Toxicological.

Introduction

Plants have remained the primary source of medicine for management and treatment of diseases. Majority (80%) of the world population source their medicine from plants.[1] More than 20% of drugs used globally are sourced from herbal plants.[2,3] Medicinal plants contain important bioactive compounds (essential oils, phytochemicals and so on) that are very effective for managing various ailments[4]; hence, their wide exploitation by pharmaceutical industries.[5] These bioactive compounds have been reported to possess different pharmaceutical properties.[6] Plant-derived medicines are believed to be less toxic than synthetic drugs.[4]

Cynodon dactylon Pers is a very important medicinal plant used in traditional healthcare. It belongs to the family of *'Poaceae"* and commonly known as Bermuda grass, bahama grass, dhub; chiaawar sarki (Hausa), kooko igba (Yoruba). It is a perennial grass that grows as commonly as weed in Africa, Asia, Australia and Europe.[7] In additional to its use as medicine, it is planted on fields to check erosion and also to feed live stocks. The plant is used by herbalists to treat wounds,[8] snake bits, gout and rheumatism.[9] Its root decoction is used as medicine to treat syphilis and urinary tract disorders.[10] The antimicrobial activity, [11] Antidiabetic and antidiarrhoeal activity[12] has been reported. However, its toxicological effect has not been reported despite its varied uses in ethnomedicine. The study assessed the phytochemical content *C. dactylon* and the toxicological effect of its aqueous extract using some biochemical parameters of Wistar rats.

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Materials and Methods

Plant material

Fresh matured leaves of *C. dactylon* were harvested around evening hours from a natural population at Umuariaga, Oboro, Ikwuano LGA, Abia State in August, 2015. The plant was identified by Dr Flora Mukah in the Taxonomic unit of Plant Science and Biotechnology Department, Michael Okpara University of Agriculture, Umudike (MOUAU) and assigned a voucher specimen number of MOH 0156.

Sample preparation

The leaves were sorted and cleaned to remove dust particles and debris. The plant sample was air-dried under shed in the Biochemistry Laboratory, Michael okpara University of agriculture, Umudike. The dried samples were ground with the aid of a Thomas milling machine. One hundred grams of the powdered sample was weighed out using a top loading balance (Scout^{Tm}-pro spu402), soaked in 700 mL of distilled water for 72 hours and thereafter, filtered with a muslin cloth. The filtrate was then evaporated slowly at 45˚C in a water bath to get a solid extract which was stored in a refrigerator until use.

Phytochemical analysis

Determination of alkaloid, saponin and flavonoid contents were determined according to the method described by Harbourne.[13] Phenol and tannin were determined by the method of AOAC[14] and AOAC[15] respectively.

Animal grouping

Sixteen male Wistar rats (52-74 g) were acquired from the Animal Breeding Unit of the College of Veterinary Medicine, University of Nigeria Nsukka (UNN). After two weeks of acclimatization, they were weighed and divided into four groups of four rats each. An oral gavage was used to administer different doses of the aqueous extract to the groups as follows: -

Group 1: Control fed with the normal rat feed and water.
Group 2: 100 mg/kg body weight of *C. dactylon*
Group 3: 200 mg/kg body weight of *C. dactylon*

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Group 4**:** 400 mg/kg body weight of *C. dactylon*
The rats were weighed at the beginning and before sacrifice. After 21 days daily administration of aqueous extract of *C. dactylon*, blood samples were collected from the rats by cardiac puncture. Blood was collected into plain and EDTA bottles for biochemical parameters. Portions of organs (liver and kidney) were also excised and stored in 10% formalin for histology. Ethical approval for this study was obtained from the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike (MOUAU/CVM/REC/202202).

Determination of Relative organ weights
The relative organ weights (liver and kidney) of the animals in each group were calculated using the formula:

$$\text{Relative organ weight} = \frac{Weight\ of\ organ}{Weight\ of\ rat} \times 100$$

Biochemical analysis
Method described by Reitman and Frankle[16] was employed for determination of aspartate amino transferase (AST) and alanine amino transferase activity (ALT). Alkaline phosphatase (ALP) activity was determined using Randox commercial enzyme kit, based on the methods of Rec GSCC.[17] Serum urea was determined using the Urease-Berthelot method described by Weatherburn.[18] Serum creatinine was determined using the method described by Henry.[19] Determination of serum sodium was determined using the method first described by Maruna[20] and Trinder.[21] Potassium was determined using the method described by Terri and Sesin.[22] Determination of serum chloride was carried out using the colorimetric method described by Schoenfeld and Lewellen[23] while determination of serum bicarbonate was carried out using the method described by Forrester *et al.* [24]

Histology
The method described by Bancroft and Steven[25] was employed. Sections of the different organs from each group were collected in a sterile universal container containing 10% neutral buffered formalin for 16-24 hours. The tissues were dehydrated through graded solutions of ethanol (50%, 70%, 90% and two times of 100% alcohol) respectively to ensure complete dehydration, cleared in xylene to render the tissue transparent by removing alcohol from dehydrated sections and embedded in paraffin wax to provide a hard support for sectioning. The blocks were sectioned in transverse plane at 5-7 µm using LEICA microtome. Every third section was mounted on glass slide and stained with haematoxyline and eosin. A drop of Depex (DPX) mountant devoid of air bubbles was place on the slide and cover slips were carefully placed over the slide. Photomicrograph of selected sections were captured using motic 2001 camera (motican Uk) attached to a microscope.

Statistical analysis
Descriptive statistics were carried out on the data generated. Results were expressed as mean ± SEM (Standard Error of Mean). One-way analysis of variance (ANOVA) was used to separate means with LSD multiple range test. All statistical analysis was done using IBM SPSS version 20. Data from the test groups were compared with their respective controls and differences at $p < 0.05$ were considered to be significant.

Results and Discussion

Phytochemical composition of Cynodon dactylon
The results of the phytochemical composition of *C. dactylon* are shown in Table 1. The result showed the presence of appreciable quantities of saponins, flavonoids, steroid, alkaloid, phenol and tannins. Alkaloid was the most abundant phytochemical detected while phenol was the least. These phytochemicals are known to perform vital functions in plants. Alkaloids possess analgesic, hypotensive, and sedative potentials;[26] Flavonoids, phenols and tannins are phenolic compounds[27] with antioxidant properties.[28] Saponins have hypotensive; antifungal;[26] and antihypercholesterolemic[29] properties. Flavonoids are well known for their antihyperglycaemic activity;[30] antioxidant properties and ability to suppress tumor progression.[26] Steroids have anti-inflammatory properties, Tannins have anti-inflammatory and wound healing property.[26] The presence of these phytochemicals lend support to the use of *C. dactylon* for treatment of various health disorders in ethnomedicine.

Relative organ weight
Results of effect of aqueous extract of *C. dactylon* on relative organ weight are presented in Table 2. The result obtained indicated a non-significant effect of the extract on these parameters. Relative organ weight differences are key pointers to the effect of an extract in animal models even when there are no morphology changes.[31] It is therefore used to assess toxicity. [32] Increase in organ/body weight ratio is usually attributed to the presence of inflammation while a decrease signals cellular constriction. [33] The non-significant increases in liver and kidney body weight ratios in this study could be an indication that the extract was not toxic. [34] Result of this study corroborated that of Kaid *et al.* [35]

Liver function
In Table 3 are the results of the effect of *C. dactylon* on some liver function parameters. Significant changes were not observed for all the parameters studied. Liver enzyme assays and bilirubin concentrations tells a lot of the functionality and cellular integrity of the liver.[36] Cytosolic enzymes like AST, ALT and ALP leak into the blood following injury to the hepatic cells.[37]

Aspartate amino transferase (AST) and ALT are intracellular liver enzymes that serve as biomarkers of toxicity.[38] Alkaline phosphatase is located in the biliary duct of the liver.[39] When the biliary duct is obstructed, ALP levels increase in the serum. Bilirubin is a by-product of heme catabolism whose accumulation results in jaundice. The non-significant ($p<0.05$) difference in AST and ALT in this study could be an indication of low toxicity of the extract. However, the significant ($p<0.05$) increase in ALP activity observed in the 200 mg/kg extract could be an indication of infiltrative diseases of liver and bone or biliary obstruction. [40]

Kidney function
Results of effect of *C. dactylon* aqueous extract on urea and creatinine concentration is depicted in Table 4.

Table 1: Phytochemical composition of *Cynodon dactylon*

Phytochemical	Composition (%)
Saponin	1.25 ± 0.01
Flavonoid	4.15 ± 0.15
Steroid	0.87 ± 0.02
Alkaloid	5.00 ± 0.01
Phenol	0.46 ± 0.02
Tannin	1.62 ± 0.05

Values are expressed as mean ±standard error of mean (SEM) of duplicate determinations.

Table 2: Effect of *Cynodon dactylon* aqueous extract on relative organ weights of Wistar rats

Group	Dose (mg/kg)	Liver	Kidney
1 **(**Control)	---	4.65 ± 0.11	0.84 ± 0.01
2	100	4.78 ± 0.26	0.99 ± 0.20
3	200	4.30 ± 0.38	0.93 ± 0.03
4	400	5.00 ± 0.11	0.81 ± 0.02

Values are expressed as mean ± standard error of mean (SEM) of duplicate determinations.

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Table 3: Effect of *Cynodon dactylon* aqueous extract on some liver function parameters of Wistar rats

Dose mg/kg)	AST (U/I)	ALT (U/I)	ALP (U/I)	Total Bilirubin (mg dl^{-1})	Conjugated Bilirubin (mg dl^{-1})
Control	11.25 ± 3.35	16.00 ± 3.34	144.90 ± 12.76	1.11 ± 0.39	0.88 ± 0.37
100	8.75 ± 1.18	18.50 ± 5.70	156.55 ± 1.59	1.22 ± 0.36	0.90 ± 0.26
200	8.50 ± 1.19	9.50 ± 0.96	185.30 ± 14.35*	1.99 ± 0.53	1.30 ± 0.25
400	12.00 ± 2.35	16.25 ± 5.31	139.18 ± 2.38	0.81 ± 0.11	0.44 ± 0.17

Values are expressed as mean ± standard error of mean (SEM) of duplicate determinations. * = Significantly different

Table 4: Effect of *Cynodon dactylon* aqueous extract on urea and creatinine concentration of Wistar rats

Group	Urea (mg/dl)	Creatinine (mg/dl)
Control	49.34 ± 6.75	1.91 ± 0.53
100	27.81 ± 7.97*	1.91 ± 0.55
200	66.73 ± 3.68	0.91 ± 0.25
400	66.63 ± 5.45	1.25 ± 0.31

Values are expressed as mean ±standard error of mean (SEM) of triplicate determinations. * = significantly different from control.

Urea concentration increased as the dose increased from 100 to 400 mg/kg extract. However, the urea concentration obtained for rats administered 100 mg/kg body weight of extract was significantly ($p < 0.05$) lower than the control. Creatinine concentrations were statistically similar ($p > 0.05$). Urea, creatinine and electrolytes (sodium, potassium, chloride and bicarbonate) concentrations were measured to assess the functionality of the kidney.

The kidney excretes toxic metabolites, produces renal enzymes and main normal acid-balance.[38] Abnormal functioning of the kidney leads to accumulation of nitrogen breakdown products that are easily measured in the form of blood urea and creatinine.[38] Urea and creatinine are excreted in the kidney as end products of protein metabolism in the liver[41] and non-enzymatic breakdown of phosphocreatinine in the muscle[42] respectively. Results from this study indicated that the urea and creatinine values were statistically similar. However, the significant decrease in serum urea concentration observed in this study for 100 mg/kg extract group could be a result of alterations in the secretory and excretory functions of the kidney.

Serum electrolytes

The result of *C. dactylon* aqueous extract on serum electrolytes concentration is presented in Table 5. Observed increase in sodium and bicarbonate concentrations in the rats were not significant ($p>0.05$). Potassium concentration for all the treated rats was significantly ($p<0.05$) higher compared to the control. Rats administered 100 mg/kg extract, had a lower concentration ($p<0.05$) compared to the control. Serum electrolytes concentrations are part of the assessment used to diagnose disease state in patients. Sodium and potassium are the commonly assessed electrolytes. The significant increase in sodium and potassium concentrations indicated that the extract may have affected the reabsorptive function of the kidney.[43] This has lent credence to its reported diuretic properties.[44]

Histology

The liver and kidney histology in this study showed the absence of clear hepatic or renal damage in all the rats as shown in Figure 1 and 2 respectively.

Table 5: Effect of *C. dactylon* aqueous extract on serum electrolyte concentration of Wistar rats

Dose (mg/kg)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)
Control	142.50 ± 5.26	6.93 ± 1.00	93.50 ± 15.02	29.50 ± 2.08
100	192.53 ± 15.45*	12.05 ± 1.29*	74.00 ± 6.68*	30.75 ± 6.70
200	217.25 ± 17.19*	11.20 ± 2.90*	94.50 ± 20.37	31.00 ± 4.08
400	188.75 ± 43.28*	10.23 ± 1.83*	92.75 ± 19.24	33.75 ± 4.50

Values are expressed as mean ± standard error of mean (SEM) of triplicate determinations. * = significantly different from control.

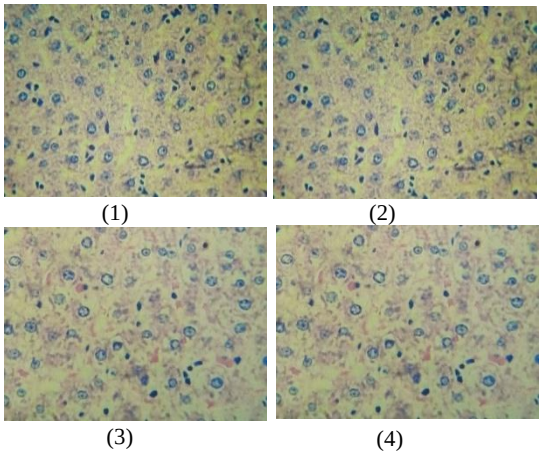

Figure 1: Effect of aqueous extract of *Cynodon dactylon* on liver histology of Experimental rats. (1), (2), (3) and (4) represent liver histotolgy of rats from control, 100 mg/kg, 200 mg/kg and 400 mg/kg groups. (H&E ×400).

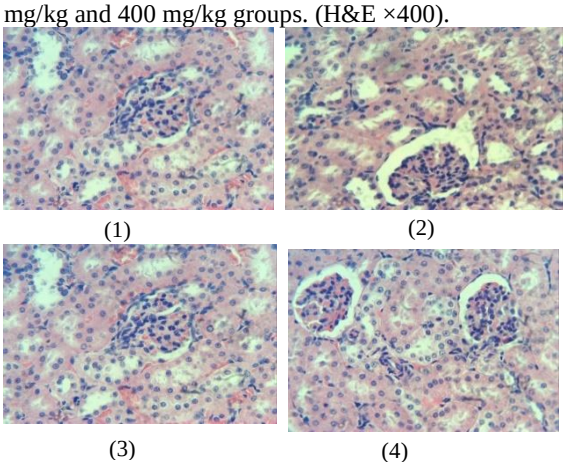

Figure 2: Effect of aqueous extract of *Cynodon dactylon* on kidney histology of Experimental rats. (1), (2), (3) and (4) represent kidney histotolgy of rats from control, 100 mg/kg, 200 mg/kg and 400 mg/kg groups. (H&E ×400).

Conclusion

The result of this study showed that *C. dactylon* contained important phytochemicals and hence contribute to its use in ethnomedicine. The extract has shown some potential for use as a diuretic agent. Though aqueous extract of *C. dactylon* showed minimal toxicity to liver and kidney at the doses administered as obtained in this study, its toxicological studies using other animal models is suggested.

Conflict of interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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