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Effects of Bermuda Grass (Cynodon dactylon) Extracts on Some Haematological Parameters of Streptozotocin-Induced Wistar Albino Rats

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Abstract
Effects of Aqueous and Ethanolic extracts of *Cynodon dactylon* on some haematological parameters (Fasting blood glucose, packed cell volume, heamoglobin, red blood cell, white blood cell and Fe^{2+}/Fe^{3+} ratio) of streptozotocin – induced diabetic wistar albino rats were investigated. Diabetes was induced by treatment with streptozotocin. Aqueous and ethanolic extracts of *Cynodon dactylon* at varying concentrations (300mg, 500mg and 700mg/kg body weight) were administered to the diabetic rats, and the findings revealed that aqueous and ethanolic extract of herbal preparation decreased the fasting blood glucose. The decrease was time and concentration dependent with 500mg/kg b/wt of aqueous and 300mg/kg b/wt of ethanolic extracts as the most effective. Also there was a significantly decrease ($p<0.05$) in RBC concentration. The aqueous and ethanolic extracts of the grass was observed to have no effect on the PCV, Hb, WBC, and Fe^{2+}/Fe^{3+} ratio with respect to duration of treatment at different concentrations. The results of this study strongly suggest that *Cynodon dactylon* possess hypoglycemic properties, with no adverse significant ($p>0.05$) effect on other parameters studied and as such could be beneficial in the management of diabetes.
Keywords: Aqueous, Bermuda, blood, cell, fasting, glucose, ethanolic, extracts, grass.
Abbreviation:HDL- High density lipoprotein, LDL- Low density lipoprotein, STZ- Streptozotocin, TG- triglycerides.

Introduction

The mode of action of some hypoglycemic plants varies. Some stimulate the pancreas to secrete insulin, others slow the digestion and absorption of starches, some act by decreasing the production of glucose by the liver and increasing glucose utilization by the tissues, while another increase the sensitivity of the tissues to insulin. A patient may receive treatment from several plants to provide the best effect.

Cynodon dactylon has been studied at the University of Allahabad in India, and is reported to have antimicrobial and antiviral properties, and has been suggested for treatment of urinary track infections, syphilis, and dysentery (Santosh, *et al*, 2007). Additional research is being conducted on *C. dactylon* involving its glycemic potential, which is involved in the treatment of diabetes. In laboratory rats treated with the ethanol extract of defatted *C. dactylon*, hypoglycemic and anti-diabetic results were observed on the blood glucose levels of the tested population (Santosh *et al*, 2007). Test populations showed nearly a 50% drop in blood glucose levels when the proper dosage was administered. This suggests the potential for *Cynodon dactylon* to become an alternative to current diabetes medications used in traditional cultures for toothaches and dysentery.

This research work is aimed at determining the effects of Bermuda grass (*Cynodon dactylon*) on blood glucose level and some haematological parameters of Streptozotocin-induced Wistar Albino rates.

MATERIALS AND METHODS

Materials

Animals

Wistar Albino rats weighing between 100-200g were obtained from the animal house of the Department of Biochemistry, University of Port Harcourt, Choba campus, Port Harcourt.

Plants

The Bermuda grass (*Cynodon dactylon*) was identified by Dr. Edwin Nwosu of the plant science department in university of Port Harcourt and obtained from University of Port Harcourt, Abuja Campus, Rivers State, Nigeria.

Chemicals

a) Streptozotocin (ZANOSAR) STZ, were obtained from Buhzor chemical at No. 96 Rumuola road, Port Harcourt.
b) Daonil and Glucophage was a product of Swiss Pharmacy. Nigeria Ltd, 5 Dopemu Road, Agege, Lagos State, Nigeria.

All drugs, chemicals and reagents used were of analytical standard.

Instruments

Accu-Chek glucometer and Strips – Roche, GmbH, Mannheim, Germany.
Analytical balance – Sartorius, TE153S, Gottingen, Germany.

Microscope – Biocular, XSZ107BN, B. BRAN SCI. COY. England.
Rotary extractor – Uniscope, SM801A, England.
Thermostatic water bath – HH-6, Techmel and Techmel, USA.
Microhaematocrit centrifuge – Sorvall Instruments, GLC-4, USA.
Refrigerator – Daewoo, Refrigerator FR-142, Korea.
Gas chromatography – HRGC, DB-5MS, England.
Spectrophotometer 22D^{+}, USA.

Method

Preparations of Streptozotocin (STZ)

The range of diabetogenic dose of STZ is quite narrow and a light overdose may cause the death of many animals (Lenzen *et al,* 1996).

5g of STZ was dissolved in 100ml of distilled water to give a 5% stock solution of which a single dose of 70mg/kg body weight was injected intraperitoneally on the rats.

Preparation of plant extract

Aqueous extract;

Fresh Bermuda grass *(Cynodon dactylon*) was washed with distilled water to remove debris and contaminants after which they were air dried. The grass was homogenized into fine powder and the aqueous extract was prepared by weighing out 200g of pulverized grass into 1.5litres of distilled water. The resultant mixture was allowed to stand for 24hours with occasional shaking after which it was filtered. The filtrate was evaporated and dried to paste with the aid of a thermostatic water bath at a temperature of 50^{O}C.

An aliquot of the extract was prepared by dissolving 7g, 5g and 3g in 50ml of distilled water respectively to form three (3) concentrations and stored at 4^{O}C, which served as stock crude drug.

Ethanol extract

Fresh Bermuda grass (*Cynodon dactylon*) was washed with distilled water to remove debris and contaminants, after which they were dried. The dried grass was homogenized into fine powder. 200g of powdered grass was soaked in one (1) litre of absolute ethanol and the resultant mixture was allowed to stand for 24hours with occasional shaking, after which it was filtered. The filtrate was evaporated, first with a rotary evaporator and dried to paste with the aid of a thermostatic water bath at 45^{O}C.

An aliquot of the extract was prepared by dissolving 7g, 5g and 3g in 50ml of distilled water respectively to form three (3) concentrations and stored at 4^{O}C, which served as stock crude drug.

Blood collection

Blood samples were collected at each phase of the experimental period (day 7, 14 and 21). The rats were fasted overnight and sacrificed under chloroform anesthesia. Then blood samples were collected from the jugular vein into EDTA (ethylene diamine tetra acetic acid) containers, for haematology (Hb, PCV, RBC, WBC T & D, and F^{2+}/ Fe^{3+} ratio).

At the point of sacrifice, the fasting blood glucose was analyzed immediately with the blood using an automated ACCU-CHEK glucometer and strips.

ASSAY METHOD

Fasting Blood Glucose (FBG)

Glucose concentration was determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed, reacts under catalysis of peroxidase with phenol and 4-aminophenazone to form a red violet quinoneimine dye as an indicator which was measured glucometrically in mmol/l.

Reaction principle:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GoD}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{PoD}} \text{quinonemine} + 4H_2O$$

Packed Cell Volume (PCV)

Microheamatocrit method was used. The sample was collected into a heparinized capillary tube and spun at 3,000rpm for 5minutes. The resultant product consisting of packed cell, buffy coat and plasma was read with the reader and the value expressed in percentage volume.

White Blood Cell (WBC)

Turk's solution containing 1.0% glacial acetic acid was used as the diluents. The 1:20ml dilution was then charged on an improved Neuber chamber and counted under microscope (X10). Values are expressed in mg/dl.

Formula for counting:-

$$\frac{N \times DF \times 10^{6}}{A \times D}$$

Where;

N = No of cell counted

DF = Dilution factor

A= Area counted (n=4)
D = Depth of chamber (0.1)

Red Blood Cell (RBC)

Turk's solution containing 1.0% glacial acetic acid was used as the diluents. The 1:200ml dilution was then charged on an improved Neuber chamber and counted under a binocular microscope (X 10) expressed in mg/dl.

Haemoglobin (Hb)

Haemoglobin level was assayed using the method of Baker et al; (1985). Drabkin solution (4ml) was introduced into a test-tube and 0.02ml of blood sample added. The test-tube was stopped with a rubber cock and inverted severally for proper mixing. This was allowed to stand for 10minutes at room temperature (25^{OC}) for complete conversion of cyanomethaemoglobin. This was read at 540nm against blank (4ml of Drabkin's reagent only). The absorbance of low standard was read alongside those of the sample.

Erythrocyte Fe^{2+}/Fe^{3+} ratios

Principle: This is based on the fact that a peak absorbance of haemoglobin occurs at 540nm, while that of methaemoglobin occur at about 630nm (Davidson and Henry, 1974; George; 1976). The Fe^{2+}/Fe^{3+} ratio was determined after an initial determination of haemoglobin and methaemoglobin. Haemoglobin was determined as percentage of total methaemoglobin. Similarly, methaemoglobin was determined as percentage of total hemoglobin.

Procedure: Exactly 5mls of distilled H_2O was added to all the test-tubes; Groups 1-10. Then 0.1ml of blood sample was added to the distilled water in all the test-tubes. Then the mixtures were incubated for 5minutes, the solution properly mixed and absorbance read at the different wavelengths (540nm and 630nm) using a spectrophotometer (i.e. Fe^{2+} read at 540nm and Fe^{3+} read at 630nm).

The relationship for calculating haemoglobin and methaemoglobin are as follows:

Percentage haemoglobin (%Hb) = $A\ (540)^2 \times 100 / A\ (540)^2 + A\ (630)^2$

Percentage methaemoglobin (%MHb) = $A\ (630^2) \times 100 / A\ (630)^2 + A\ (540)^2$.

RESULTS

The assay values for the different haematological parameters across the groups are shown in the below.

TABLE 1: FBG Values (mmol/L) of Normal and Diabetic Controls/Diabetic test Rats treated with standard drugs, Aqueous and Ethanolic extracts of Bermuda grass.

GROUPS	WEEK 1	WEEK 2	WEEK 3
NCR	6.9 ± 1.27^a	7.0 ± 3.25^a	4.8 ± 0.92^a
DCR	10.1 ± 0.71^c	12.1 ± 0.00^f	12.3 ± 0.21^f
DTR on Daonil	8.3 ± 0.14^b	11.1 ± 0.21^e	7.7 ± 0.00^a
DTR on Glucophage	9.8 ± 0.91^d	9.2 ± 0.71^c	10.2 ± 0.14^d
DTR on 300mg Aq. Extract	10.9 ± 0.07^d	9.1 ± 2.90^c	10.2 ± 3.46^d
DTR on 500mg Aq. Extract	11.3 ± 0.35^e	10.9 ± 0.85^d	8.8 ± 1.41^b
DTR on 700mg Aq. Extract	10.9 ± 0.21^d	10.2 ± 1.13^c	10.8 ± 1.34^d
DTR on 300mg Et. Extract	9.8 ± 0.57^c	9.4 ± 0.71^c	9.2 ± 0.42^c
DTR on 500mg Et. Extract	11.1 ± 0.49^e	8.8 ± 0.85^b	9.4 ± 1.34^c
DTR on 700mg Et. Extract	11.9 ± 0.71^f	8.3 ± 0.71^b	9.3 ± 1.41^c

Results are mean ± S.D of triplicate determination.

Values in the same column with different superscript letter are statistically significant at 95% confident level ($p \leq 0.05$).

TABLE 2: PCV Values (in % vol.) of Normal and Diabetic Control/ Diabetic test Rat treated with standard drug as well as Aqueous and Ethanolic extracts of Bermuda grass.

GROUPS	WEEK 1	WEEK 2	WEEK 3
NCR	38.0 ± 2.83^a	38.0 ± 14.14^a	28.0 ± 1.41^a
DCR	34.5 ± 0.71^a	37.5 ± 0.71^d	30.1 ± 7.01^c
DTR on Daonil	37.0 ± 7.01^a	33.0 ± 1.41^b	32.5 ± 2.12^{ab}
DTR on Glucophage	33.5 ± 0.71^c	29.5 ± 0.71^a	33.0 ± 5.66^b
DTR on 300mg Aq. Extract	39.5 ± 3.54^c	28.5 ± 9.19^a	30.0 ± 4.24^b
DTR on 500mg Aq. Extract	38.0 ± 2.12^a	37.0 ± 0.00^b	36.0 ± 5.66^c
DTR on 700mg Aq. Extract	39.5 ± 6.36^d	30.5 ± 6.36^c	29.0 ± 4.24^b
DTR on 300mg Et. Extract	37.5 ± 3.54^a	34.5 ± 0.71^b	32.5 ± 3.54^c
DTR on 500mg Et. Extract	35.0 ± 1.41^a	32.0 ± 5.66^b	25.5 ± 0.71^c
DTR on 700mg Et. Extract	37.0 ± 1.41^a	30.0 ± 0.00^b	28.0 ± 9.90^c

Results are mean ± S.D of triplicate determination.

Values in the same column with different superscript letter are not statistically significant at 95% confident level

($p \geq 0.05$).

TABEL 3: Hb Values (in mg/dl) of Normal and Diabetic Control/Diabetic test Rats treated with standard drugs as well as Aqueous and Ethanolic extracts of Bermuda grass.

GROUPS	WEEK 1	WEEK 2	WEEK 3
NCR	12.7 ± 0.92^{a}	12.7 ± 4.74^{a}	9.3 ± 0.50^{a}
DCR	11.50 ± 0.49^{a}	12.5 ± 0.21^{b}	10.0 ± 2.21^{c}
DTR on Daonil	12.3 ± 2.33^{a}	11.0 ± 0.42^{b}	10.8 ± 0.71^{a}
DTR on Glucophage	11.2 ± 0.21^{c}	9.8 ± 0.21^{a}	11.0 ± 2.12^{b}
DTR on 300mg Aq. Extract	13.0 ± 1.20^{e}	9.6 ± 3.11^{c}	10.0 ± 1.41^{b}
DTR on 500mg Aq. Extract	12.7 ± 0.92^{a}	12.3 ± 0.00^{b}	12.0 ± 1.63^{c}
DTR on 700mg Aq. Extract	13.0 ± 2.12^{d}	10.2 ± 2.12^{c}	9.7 ± 1.41^{b}
DTR on 300mg Et. Extract	12.5 ± 1.41^{a}	11.5 ± 0.28^{a}	10.8 ± 1.28^{d}
DTR on 500mg Et. Extract	11.7 ± 0.49^{a}	10.7 ± 1.91^{b}	8.5 ± 0.28^{c}
DTR on 700mg Et. Extract	12.3 ± 0.49^{a}	10.0 ± 0.00^{b}	9.5 ± 3.32^{c}

Results are mean ± S.D of triplicate determination.

Values in the same column with different superscript letter are not statistically significant at 95% confident level ($p \geq 0.05$).

Table 4: RBC Values (in $x10^{12}$/L) of Normal and Diabetic Controls/ Diabetic test Rats treated with standard drugs as well as Aqueous and Ethanolic extracts of Bermuda grass.

GROUPS	WEEK 1	WEEK 2	WEEK 3
NCR	5.5 ± 0.71^{a}	5.0 ± 1.41^{a}	3.9 ± 0.14^{a}
DCR	4.5 ± 0.71^{a}	5.4 ± 0.21^{b}	4.2 ± 0.20^{a}
DTR on Daonil	5.7 ± 1.31^{c}	4.9 ± 0.00^{c}	4.4 ± 0.21^{b}
DTR on Glucophage	4.6 ± 0.14^{a}	4.5 ± 0.00^{a}	4.2 ± 0.28^{a}
DTR on 300mg Aq. Extract	5.8 ± 0.35^{c}	4.0 ± 1.41^{b}	3.9 ± 0.92^{a}
DTR on 500mg Aq. Extract	6.3 ± 1.06^{e}	5.5 ± 0.00^{c}	5.3 ± 1.06^{c}
DTR on 700mg Aq. Extract	6.3 ± 1.06^{e}	5.4 ± 0.57^{a}	4.0 ± 0.71^{a}
DTR on 300mg Et. Extract	5.3 ± 1.06^{b}	5.4 ± 0.71^{b}	4.6 ± 0.57^{b}
DTR on 500mg Et. Extract	4.7 ± 0.14^{a}	4.3 ± 1.06^{a}	3.4 ± 1.41^{a}
DTR on 700mg Et. Extract	5.2 ± 0.28^{a}	4.6 ± 0.14^{a}	4.0 ± 1.41^{b}

Results are mean ± S.D of triplicate determination.

Values in the same column with different superscript letter are statistically significant at 95% confident level ($p \leq 0.05$).

Table 5: WBC Values ($\times 10^{9}$/L) of Normal and Diabetic Control/Diabetic test Rats treated with standard drugs as well as Aqueous and Ethanolic extract of Bermuda grass.

GROUPS	WEEK 1	WEEK 2	WEEK 3
NCR	5.1 ± 1.77^{a}	6.9 ± 3.04^{a}	6.0 ± 5.66^{a}
DCR	5.8 ± 0.35^{c}	5.0 ± 2.83	3.8 ± 1.06^{a}
DTR on Daonil	4.8 ± 1.06^{e}	4.5 ± 0.71^{a}	4.6 ± 0.85^{b}
DTR on Glucophage	3.0 ± 0.00^{a}	4.6 ± 0.35^{b}	3.0 ± 0.00^{a}
DTR on 300mg Aq. Extract	3.6 ± 0.07^{b}	4.5 ± 0.71^{e}	3.1 ± 0.14^{a}
DTR on 500mg Aq. Extract	4.5 ± 2.12^{c}	3.8 ± 1.06^{b}	4.1 ± 0.78^{b}
DTR on 700mg Aq. Extract	9.0 ± 1.41^{w}	3.6 ± 0.57^{b}	3.0 ± 0.00^{a}
DTR on 300mg Et. Extract	6.5 ± 2.12^{f}	3.0 ± 0.00^{a}	3.6 ± 0.57^{b}
DTR on 500mg Et. Extract	4.5 ± 0.71^{c}	2.3 ± 0.35^{a}	6.3 ± 1.77^{d}
DTR on 700mg Et. Extract	5.5 ± 0.71^{b}	4.5 ± 0.71^{ab}	3.5 ± 0.71^{a}

Results are mean ± S.D of triplicate determination.

Values in the same column with different superscript letter are not statistically significant at 95% confident level ($p \geq 0.05$).

Table 6: Fe^{2+}/Fe^{3+} Ratio (mg/L) of Normal and Diabetic Controls/ Diabetics tests Rats treated with standard drugs as well as Aqueous and Ethanolic extracts of Bermuda grass.

GROUPS	WEEK 1		WEEK 2		WEEK 3	
	Fe^{2+}	Fe^{3+}	Fe^{2+}	Fe^{3+}	Fe^{2+}	Fe^{3+}
NCR	2.4 ± 0.01^{b}	1.6 ± 0.25^{b}	2.08 ± 0.46^{a}	1.37 ± 0.08^{a}	2.61 ± 0.14^{b}	1.42 ± 0.13^{a}
DCR	2.34 ± 0.21^{b}	1.49 ± 0.12^{b}	2.34 ± 0.25^{a}	1.46 ± 0.11^{b}	2.27 ± 0.40^{a}	1.37 ± 0.02^{a}
DTR on Daonil	2.04 ± 0.41^{a}	1.37 ± 0.12^{a}	2.35 ± 0.04^{a}	1.39 ± 0.01^{a}	2.60 ± 0.12^{b}	1.44 ± 0.05^{a}
DTR on Glucophage	1.94 ± 0.21^{d}	1.39 ± 0.04^{a}	2.47 ± 0.34^{b}	1.46 ± 0.16^{b}	2.39 ± 0.46^{a}	1.42 ± 0.08^{a}
DTR on 300mg Aq. Extract	2.06 ± 0.16^{a}	1.54 ± 0.16^{b}	2.18 ± 0.50^{c}	1.37 ± 0.08^{a}	1.87 ± 0.27^{c}	1.34 ± 0.25^{d}
DTR on 500mg Aq. Extract	1.87 ± 0.28^{w}	1.38 ± 0.64^{e}	2.39 ± 0.12^{b}	1.39 ± 0.04^{c}	2.36 ± 0.18^{d}	1.37 ± 0.01^{e}
DTR on 700mg Aq. Extract	2.11 ± 0.03^{a}	1.45 ± 0.20^{b}	2.06 ± 0.52^{a}	1.34 ± 0.11^{a}	1.80 ± 0.14^{c}	1.33 ± 0.02^{a}
DTR on 300mg Et. Extract	2.25 ± 0.49^{d}	1.55 ± 0.28^{b}	2.16 ± 0.49^{a}	1.35 ± 0.04^{a}	2.02 ± 0.07^{a}	1.31 ± 0.33^{a}
DTR on 500mg Et. Extract	2.15 ± 0.12^{a}	1.45 ± 0.13^{b}	2.01 ± 0.30^{a}	1.3 ± 0.01^{ab}	1.86 ± 0.20^{b}	1.29 ± 0.07^{b}
DTR on 700mg Et. Extract	1.9 ± 0.26^{bc}	1.34 ± 0.4^{a}	2.35 ± 0.45^{b}	1.39 ± 0.03^{c}	1.82 ± 0.35^{a}	1.29 ± 0.5^{cd}

Results are mean ± S.D of triplicate determination.
Values in the same column with different superscript letter are not statistically significant at 95% confident level ($p \geq 0.05$).

DISCUSSION

On establishment and affirmation of diabetes mellitus on the rats with the use of streptozotocin and Accu check glucometer respectively, treatment commenced and lasted for a total of 21days with 7days analysis for various haematological parameters. The drug Daonil (5mg/70kg body weight), Glucophage (500mg/70kg body weight), and Bermuda grass (300mg/kg, 500mg/kg and 700mg/kg b/wts) of Aqueous and Ethanolic extracts respectively were administered to check their anti- diabetic (hypoglycemic) effect as well as their effects on other parameters such as: packed cell volume (PCV), Haemoglobin (Hb) concentration, Red blood cell (RBC) and White blood cell (WBC) counts and Fe^{2+}/ Fe^{3+} ratio.

From the findings rats on 700mg/kg body weight had about similar results like those of 300mg/kg body weight which were unstable. It could be wise to say that from the findings, the 500mg/kg body weight of the aqueous extract is most effective for the management of diabetes.

The overall values obtained from the groups when compared revealed that the most effective doses are those of 500mg/kg body weight of aqueous extract and 300mg/kg body weight of ethanolic extract. And this gave credence to the work of the Indian researchers Santosh *et al,* (2007). For the fact that a statistically significant decrease ($p<0.05$) in blood glucose level was observed after treatment with the herbal preparation, it shows that it has a potent hypoglycemic property.

The packed cell volume as well as haemoglobin concentrations showed a gradual drop in all groups including the normal and diabetic control rats. With such result affecting both the normal and diabetic non treated rats (groups) given normal feed and water *ad libitum*, the extract (aqueous and ethanolic) from possible adverse effect on the packed cells volume and haemoglobin concentration. It is thus concluded that the grass has no significant ($p>0.05$) effect on packed cell volume and Haemoglobin concentrations, though extracts (aqueous and ethanolic) concentration of 700mg/kg body weight showing remarkably lower levels of PCV and Hb after the first week of administration.

The Red blood cell concentration was observed to drop in all groups including normal and diabetic control rats with a significant difference ($p<0.05$). Although groups six and seven (500mg/kg and 700mg/kg aqueous extracts) respectively showed the highest level of red cells in week 1 ($6.3 \pm 1.06 \times 10^{12}$ /l).

For the white blood cell counts, normal control rats (NCR) and diabetes treated rats (DTR) on 700mg/kg (ethanolic extract) maintain a steady concentration of WBC with the other groups showing an unstable and remarkably low levels of white blood cell count which brings about the need to ascertain the effect of STZ on WBC counts and the possible remedy by higher doses (700mg/kg body weight of both ethanolic and aqueous extracts) of Bermuda grass.

The ratio of Fe^{2+}/Fe^{3+} as observed in the result indicates that Fe^{2+} had higher values compared to Fe^{3+} across all groups with a statistical significant difference ($p<0.05$). It shows that iron pigment of haemoglobin still maintains its normal state Fe^{2+} for oxygen binding indicating that the plant extracts has no adverse negative

effect on the state of Fe^{2+} bound to haemoglobin.

Conclusion

The correlation coefficient done for all test groups shows that the hypoglycemic properties exhibited by the grass (*Cynodon dactylon*) were both concentration and time dependent. Therefore, it is advised that Bermuda grass be administered alongside other hypoglycaemic preparation at controlled dosage (500mg/kg body weight aqueous extract or 300mg/kg body weight ethanolic extract) under medical supervision.

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