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Evaluation of Anti-Anemic Activity of Aerial Parts of *Cynodon dactylon* (L.) Pers against Phenyl Hydrazine Induced Anemia in Wistar Albino Rats

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ABSTRACT

Background: Worldwide, anaemia affects more than 2 billion individuals. For the treatment of their anaemia, the majority of people in poor nations rely on herbal remedies and nutritional supplements. **Aim And Objective:** To act in a live setting Ethanolic extract of Cynodon dactylon aerial parts has anti-anemic properties against rat models of phenyl hydrazine-induced anaemia. **Materials And Methods:** A total of thirty adult albino rats were chosen, six of which were regarded as typical controls. The remaining 24 rats received PHZ intraperitoneally, which caused anaemia. After two days of PHZ administration, 24 rats were determined to be anaemic and divided into four groups at random based on haemoglobin (Hb) estimation. Groups IV and V received the ethanolic extract of Cynodon dactylon aerial parts for a period of 14 days, whilst Groups II and III functioned as positive and negative controls. **Result:** Following PHZ injection, rats experienced haemolytic anaemia, as evidenced by a noteworthy reduction in the number of red blood cells (RBCs), haemoglobin concentration, and haematocrit percentage. Remarkably, the deteriorating effects on PHZ on RBCs, HGB, and HCT were considerably reversed by therapy using an ethanolic extract of aerial portions of Cynodon dactylon. Furthermore, the reductions in serum levels of total glutathione and superoxide dismutase activities were substantially halted by Cynodon dactylon therapy. **Conclusion:** The result of this study thus indicated that *Cynodon dactylon* is effective remedy to manage anemia in humans.

KEYWORDS: Anaemia, Cynodon dactylon, Phenyl hydrazine, Hematological and anti-oxidant parameters.

INTRODUCTION:

A disease known as anaemia occurs when the body's needs for oxygen are not being met by the amount of red blood cells (RBCs) or their ability to carry oxygen. Anaemia comes in more than 400 varieties, many of which are uncommon, but all of them involve circulating red blood cells that are insufficient in comparison to normal. There are numerous varieties of anaemia, including megaloblastic anaemia, sickle cell anaemia, thalassaemia, sideroblastic anaemia, aplastic anaemia, haemolytic anaemia, pernicious anaemia, and iron deficient anaemia. Anaemia is believed to be most commonly caused by iron deficiency worldwide.[1]

Both genetic and acquired factors can contribute to anaemia. Anaemia can also result from other conditions such genetic illnesses, chronic inflammation, deficiencies in folate, vitamin B12, vitamin A, and chronic inflammation as well as a lack of access to a balanced food.[2]

When anaemia sets in, the body either produces insufficient red blood cells or kills a large number of red blood cells. The health, well-being, and social and economic effect of India are all significantly impacted by iron deficiency anaemia.[3]

Blood lacking sufficient healthy red blood cells to supply oxygen to tissues and the developing foetus is called anaemia during pregnancy. Birth malformations such as spina bifida, which are anomalies of the neural tube, and low birth weight can be directly linked to a folate shortage. Dietary adjustments along with iron, folic acid, and vitamin supplements can be used to treat iron, folic acid, and vitamin deficient anaemia.

Humans have been using plants or their herbs as medicine for thousands of years to treat ailments including fever, pain, and colds. The use of herbal remedies is heavily relied upon by the vast majority of people living in rural areas, and using these medications has numerous positive effects. Herbal therapy can be safely utilised as an alternative therapy or in conjunction with conventional therapies because it is non-invasive and non-toxic. A variety of plants, including dates, grapes, beetroot, broccoli, honey, and pomegranates, are traditionally used as therapeutic remedies for anaemia.[4]

It is known that several compounds have haematinic action, including phenols and flavonoids.[5]The Poaceae family member Cynodon dactylon is used as an appetiser, an anthelminthic, for skin-related conditions such as psoriasis, herpes, allergies, rashes, haemorrhoids, and to restore normal skin colour. It also aids in wound healing, reduces itching, menorrhagia, irregular menstrual cycles, habitual abortion, strengthens the uterus, and has a cooling effect when used for urinary tract infections.[6,7]

Thus, the purpose of this study is to identify the haematinic and antioxidant properties of Cynodon dactylon aerial parts.

MATERIALS AND METHODS

Madras Medical College's animal house housed healthy rats for three months prior to the study's commencement.

COLLECTION OF PLANT MATERIAL

The plant material aerial portions of Cynodon dactylon Pers were obtained in Ammankovilpathy village, Krishnagiri district, Tamil Nadu, during the month of May 2024.

PREPARATION OF ETHANOLIC EXTRACT

Via Soxhlet extraction, the ethanolic extract was made available. The extraction process required roughly 450g of powdered plant components. Ethanol was used for the chemical ingredient extraction process after petroleum ether was used for the defatting process. To be utilised in the experiment, the products were gathered and dried.

EXPERIMENTAL ANIMALS

For this investigation, thirty adult albino rats weighing between 100 and 200 g and of either sex were employed. Before the trial started, the animals were given a week to get used to the research lab. The animals were housed in conventional settings, with a temperature of 25°C±3, a humidity range of 35–60%, and a light and dark period of 12/12 hours. Ad libitum food and water were provided to all animals. The Institutional Animal Ethics Committee of Madras Medical College in Chennai officially authorised the study protocols. The CPCSEA guidelines were followed for conducting the study.

ACUTE TOXICITY STUDIES

Anthelmintic effects and toxicity of Cynodon dactylon (L.) Pers. in rodent models are research that the author, Arun K. Yadav et al., has already completed on acute toxicity; the animals did not exhibit any toxic effects up to a dose of 2000 mg/kg. For this reason, the current study's dosages of 1/10th and 1/5th of the maximum amount given—200 mg/kg and 400 mg/kg—respectively.[8]

EXPERIMENTAL DESIGN

Table 1: Grouping of animals

GROUP NO.	NAME OF THE GROUP	INDUCING PERIOD	TREATMENT PERIOD	NO. OF ANIMALS
Group I	Vehicle Control	Treatment with vehicle (Normal saline)	Treatment with vehicle (Normal saline) from day 3 to day 14	6
Group II	Disease control	40 mg/kg phenyl hydrazine from day 1 to day 2	Treatment with vehicle (Normal saline) from day 3 to day 14	6
Group III	Standard control	40 mg/kg phenyl hydrazine from day 1 to day 2	Treatment with Iron supplements from day 3 to day 14	6
Group IV	Test group 1 - Low dose	40 mg/kg phenyl hydrazine from day 1 to day 2	Treatment with 200 mg/kg of extract P.O from day 3 to day 14 (test dose 1)	6
Group V	Test group 2 - High dose	40 mg/kg phenyl hydrazine from day 1 to day 2	Treatment with 400 mg/kg of extract P.O from day 3 to day 14 (test dose 2)	6
			Total no. of animals	30

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ANALYSIS OF HEMATOLOGICAL PARAMETERS

Following a 14-day course of treatment, anaesthesia was used to remove a full blood sample from the retro-orbital plexus. The quality of blood is indicated by the quantity, form, volume, and colour of the red blood cells. After two weeks of therapy, samples were put to a tube containing ethylenediaminetetraacetic acid. At day 14, an automatic blood cell counter was used to measure the RBC number, Hb, haematocrit (PCV), MVC, mean corpuscular Hb (MCH), and MCH concentration (MCHC). The conventional approach was also utilised to test antioxidant potential, including glutathione (GSH) and superoxide dismutase (SOD).[9,10]

STATISTICAL ANALYSIS

Results were expressed as mean ± standard error of mean (SEM). All data will be analysed by one-way analysis of variance test (ANOVA), followed by Dunnett's multiple comparison test using Graph Pad Prism, version (10.3.1), with level of significance set as $P < 0.05$, $P < 0.01$ and $P < 0.001$ versus control.

RESULT

Effect of ethanolic extract of aerial parts of Cynodon dactylon *Pers* (200 mg/kg and 400 mg/kg) on the haematological parameters compared with that of the standard in following tables and graphs.

Table 2: Haematological and antioxidant parameters after the treatment with ethanolic extract of Cynodon dactylon Pers., at the end of 14 days.

S. No.	Parameters	Normal control	Disease control	Standard	Treatment 1 (Low dose)	Treatment 2 (High dose)
1.	RBC Count (million/cu.mm)	8.40 ± 0.61	4.39 ± 0.87	8.09 ± 1.10	6.87 ± 0.98	7.62 ± 0.51
2.	Haemoglobin (g/dl)	15.82 ± 0.96	8.40 ± 0.59	14.83 ± 0.90	11.04 ± 1.50	13.19 ± 1.96
3.	Packed Cell Volume (%)	41.69 ± 3.12	30.18 ± 2.91	43.98 ± 3.12	37.79 ± 2.58	39.99 ± 2.64
4.	Mean Cell Volume (fl)	61.01 ± 1.63	56.96 ± 6.98	58.61 ± 6.91	57.13 ± 8.3	57.91 ± 1.32
5.	Mean Cell Haemoglobin (pg)	18.99 ± 2.13	17.02± 1.12	17.61 ± 1.23	17.29 ± 2.11	17.58 ± 1.01
6.	Mean Cell Haemoglobin Concentration (g/dL)	33.01 ± 3.4	29.91 ± 1.26	30.39 ± 1.39	30.09 ± 1.63	30.93 ± 3.03
7.	Superoxide Dismutase	9.48 ± 0.57	5.71 ± 0.61	7.68 ± 0.47	6.51 ± 0.85	7.29 ± 0.91
8.	Glutathione	15.31 ± 0.31	10.96 ± 0.38	13.33± 0.63	11.98 ± 0.37	13.09 ± 0.67

Values are expressed as Mean ± Standard deviation (n=6).

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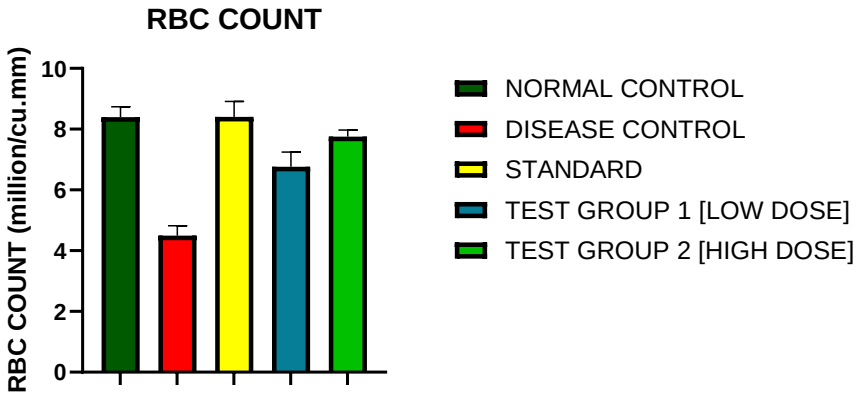

Fig 1: Graphical representation of changes in RBC count

The values are expressed as mean ± SD; N=6; P <0.05 (control group vs extract); P <0.005 (control group vs standard)**.** The data was analyzed using One way Analysis of Variance (ANOVA) with Graph pad prism 10.3.1 software.

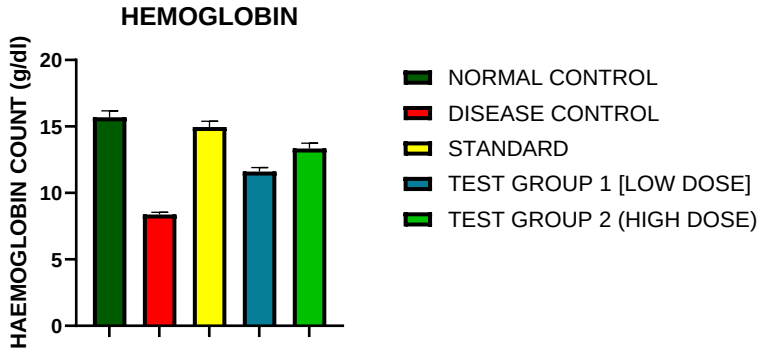

Fig 2: Graphical representation of changes in Haemoglobin

The values are expressed as mean ± SD; N=6; P <0.05 (control group vs extract); P <0.005 (control group vs standard)**.** The data was analyzed using One way Analysis of Variance (ANOVA) with Graph pad prism 10.3.1 software.

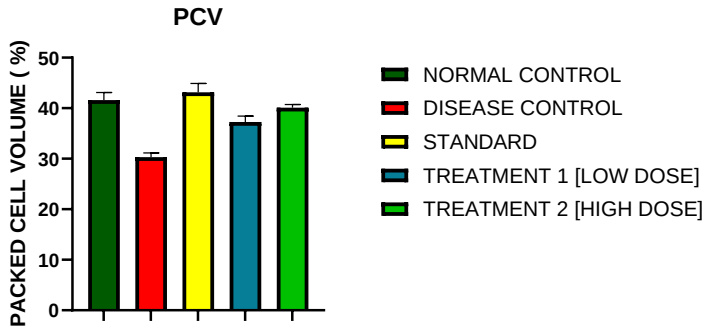

Fig 3: Graphical representation of changes in Packed Cell Volume (PCV)

The values are expressed as mean ± SD; N=6; P <0.05 (control group vs extract); P <0.005 (control group vs standard)**.** The data was analyzed using One way Analysis of Variance (ANOVA) with Graph pad prism 10.3.1 software.

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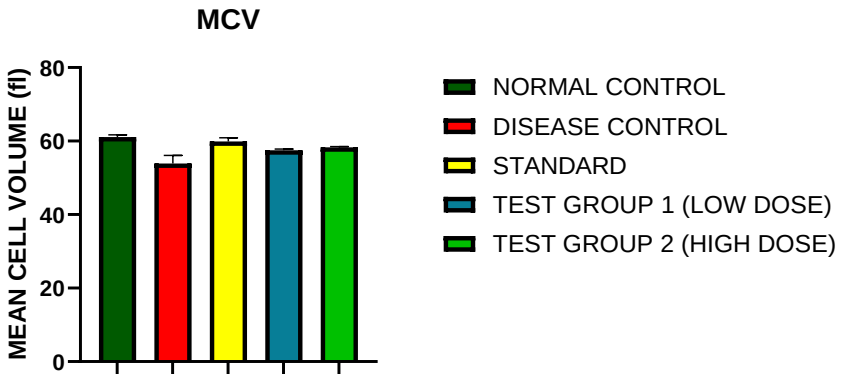

Fig 4: Graphical representation of changes in Mean Cell Volume (MCV)

The values are expressed as mean ± SD; N=6; P <0.05 (control group vs extract); P <0.005 (control group vs standard)**.** The data was analyzed using One way Analysis of Variance (ANOVA) with Graph pad prism 10.3.1 software.

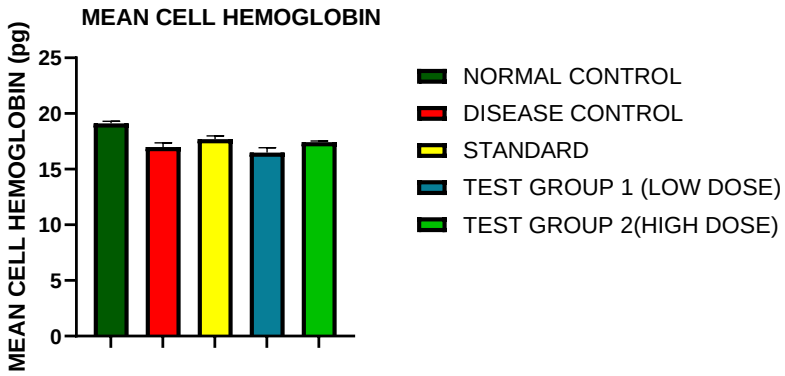

Fig 5: Graphical representation of changes in Mean Cell Haemoglobin

The values are expressed as mean ± SD; N=6; P <0.05 (control group vs extract); P <0.005 (control group vs standard)**.** The data was analyzed using One way Analysis of Variance (ANOVA) with Graph pad prism 10.3.1 software.

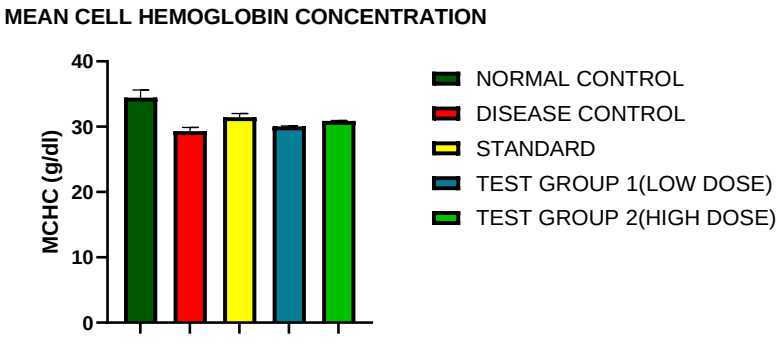

Fig 6: Graphical representation of changes in Mean Cell Haemoglobin Concentration (MCHC)

The values are expressed as mean ± SD; N=6; P <0.05 (control group vs extract); P <0.005 (control group vs standard)**.** The data was analyzed using One way Analysis of Variance (ANOVA) with Graph pad prism 10.3.1 software.

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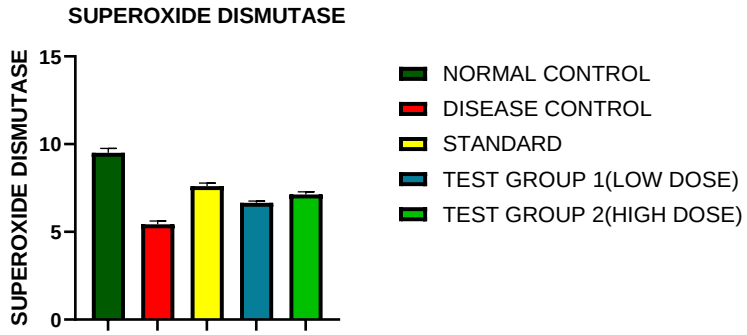

Fig 7: Graphical representation of changes in Superoxide Dismutase (SOD)

The values are expressed as mean ± SD; N=6; P <0.05 (control group vs extract); P <0.005 (control group vs standard)**.** The data was analyzed using One way Analysis of Variance (ANOVA) with Graph pad prism 10.3.1 software.

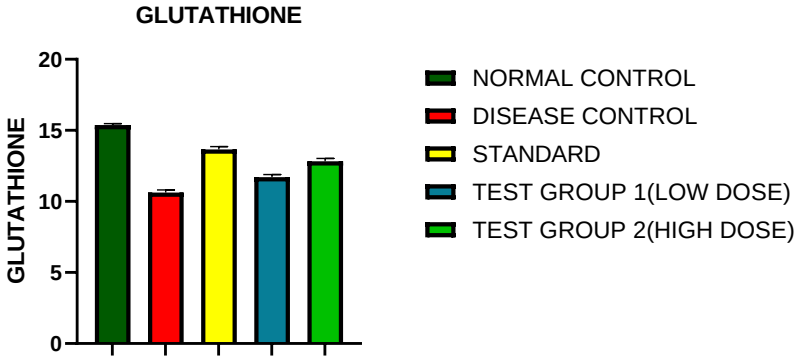

Fig 8: Graphical representation of changes in Glutathione (GTH)

The values are expressed as mean ± SD; N=6; P <0.05 (control group vs extract); P <0.005 (control group vs standard)**.** The data was analyzed using One way Analysis of Variance (ANOVA) with Graph pad prism 10.3.1 software.

Thirty mature, healthy albino rats were used throughout the entire study. Six rats were deemed normal controls, while the remaining 24 rats had anaemia brought on by intraperitoneal phenylhydrazine (PHZ) injection. After receiving PHZ for two days, 24 rats were determined to be anaemic based on Hb estimate. Tests were compared to control groups in terms of their haematological and antioxidant potential.

Rats from anaemic control, standard, test Group I, and test Group II had significantly lower haemoglobin rates ($P < 0.05$) after receiving PHZ for two days.The results demonstrate an improvement in haemoglobin levels in the groups treated with iron supplements and those given an ethanolic extract of Cynodon dactylon aerial parts after 14 days of treatment with the relevant treatments.A speedier recovery is possible using an ethanolic extract of Cynodon dactylon aerial parts, 400 mg per day. For example, the Hb grew gradually and naturally in the negative control rats.

On day two following PHZ injection, rats' red blood cells decreased. After treatment for 14 days, there were notable increases in RBCs. As of the second week, the results indicated that the rats in Groups III–V had nearly fully recovered.

On day 2, haematocrit, MCV, MCH, and MCHC were all reduced with PHZ treatment. After giving extract to Group III–V of anaemic rats for a week, the effect of PHZ was reversed, leading to a considerable rise in PCV, MCV, MCH, and MCHC.

On day two, the PHZ treatment also reduced the levels of antioxidant markers like SOD and GSH. The effect of PHZ was reversed in anaemic rats after two weeks of therapy, leading to a substantial rise in GSH and SOD.

DISCUSSION

The objective of the current investigation was to assess the anti-anemic properties of an ethanolic extract of *Cynodon dactylon* aerial parts produced in albino rats by PHZ. Over 30% of the world's population suffers from iron deficiency anaemia, which has major economic repercussions and impedes national growth.[11,12]

Therefore, it was vital to conduct this study in order to identify a cost-effective medication for managing the anaemia condition. Comparing the Hb, RBC, PCV, MCV, MCH, and MCHC levels to the negative control group, the study demonstrates that *Cynodon dactylon* successfully improves these levels. The ability of PHZ to produce aryl and hydroxyl radicals, which have been linked to its interaction with erythrocytes, to induce haemolysis both in vitro and in vivo is well known. It has been shown that intraperitoneal PHZ treatment reduced PCV, RBC, and Hb levels. According to Peter et al., intraperitoneal injection of 40 mg/kg PHZ for two days lowers haematological parameters.[13]

One key haemolysis process thought to be present in erythrocytes is oxidative stress. Flimsiness, dehydration, and elevated reactive oxygen species generation are the causes of broken membrane integrity. Hb is lost due to chronic haemolysis. Some critical components of the cell, such as membrane phospholipids, may be oxidised due to the buildup of hydrogen peroxide in addition to the red cell's detoxifying ability. The ultimate haemolysis of impacted cells is likely influenced by these changes.

The study's findings suggested that the plant's vitamin and mineral contents may be the cause of the increase in haematological indices that the *Cynodon dactylon* extract displayed. The administration of *Cynodon dactylon* may suggest that the plant extract has the capacity to activate erythropoietic factors, which directly impact bone marrow blood production. A maturation factor for red blood cell production, erythropoietin raises the proportion of committed, erythropoietin-sensitive stem cells in the bone marrow that eventually mature into mature erythrocytes and RBCs. The blood parameter recovery time was shortened when the lowest dosage of 200 mg/kg was administered. Additionally, after two weeks of nonstop therapy, the treated groups' PCV and Hb concentration were higher than those of the control groups due to the recovery's progressive nature. Furthermore, it was shown that the recovery of the treated groups was dose-dependent, with the highest dose—400 mg/day—causing the biggest change in recovery.

RBC, Hb, PCV, and red cell indices (MCV, MCH, and MCHC) have all been shown to significantly correlate with diagnostic results in both humans and rats[14]. Similar to people, animals too experience reductions in Hb, RBC, and PCV, which are suggestive of anaemia.[15].

In cases of haemolytic anaemia, MCHC (the amount of haemoglobin per unit erythrocyte volume) is frequently reduced; in cases of extensive intravascular haemolysis, it is typically raised. Because of reticulocytosis, haemolytic anaemia frequently results in an increase in MCV (average volume of the erythrocyte). Haemolytic anaemia frequently causes an increase in MCH (MCH, or the average quantity of Hb per cell).

In the current investigation, rats that were intoxicated with PHZ showed a significant drop in GSH content in comparison to control rats. Rats intoxicated with PHZ exhibit a considerable rise in GSH levels following the administration of Cynodon dactylon L. extract. When comparing the negative control to the normal control, a significant drop in SOD is also seen. Furthermore, treated groups have higher SOD levels than negative control.

CONCLUSION

Rats given PHZ injections experienced haemolytic anaemia, which was characterised by a decrease in haematological markers. The Hb level was markedly elevated upon oral administration of an ethanolic extract derived from the aerial portions of Cynodon dactylon at doses of 200 mg and 400 mg. Furthermore, the outcomes showed that Cynodon dactylon suppresses ROS production in rats, potentially having an antioxidant impact. This finding demonstrates the anti-anemic activity of the aerial portions of Cynodon dactylon Pers. To comprehend the mechanism underlying Cynodon dactylon Pers.'s antianemic activity, more research is required.

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