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The effects of chronic administration of *Aloe vera* gel on some haematological, hemostasis and electrolytes indicies on wistar albino rats.

Maduka Stephen Ozoemena[1], Ugwu Chidiebere Emmanuel[2*] and Iloka Emeka Paul[1].
1. *Department of Human Physiology, Faculty of Basic Medical Sciences Nnamdi Azikiwe University, Nnewi Campus.*
2. *Department of Human Biochemistry, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus*

ABSTRACT

There are folkloric claims that *Aloe vera* has many pharmacological properties which have not been conclusively investigated. The aim of the study is to investigate the protective/toxic effects of chronic administration of *Aloe vera* gel extract on the haemostasis, haematology and renal handling of electrolytes, urea and creatinine in Wistar rats. The experimental animals were grouped into four: group 1(control) received vehicle and the test groups (2,3,4) received different doses of the extract (200, 300, and 500mg bwt/day) respectively for 21 days. The bleeding time significantly decreased as the concentration of the extract increased while the clotting time increased relative to the control ($P<0.05$). The packed cell volume was only significantly decreased in group four ($P<0.05$) while the control group had a higher white blood cell count compared to the test groups ($P>0.05$). The serum electrolytes, urea and creatinine were not altered significantly ($P>0.05$). From the results of this study, it can be concluded that the chronic administration of *Aloe vera* gel did not disrupt the renal function of the wistar rats.

Keywords: Aloe vera, haematology, haemostasis, electrolyte.

*Corresponding Author Email: ugwuchidiksu@yahoo.com
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INTRODUCTION

Aloe vera (*Liliaceae*) is a perennial cactus like plant. The leaves are fleshy, spiny and filled with viscous gel [1,2]. Aloe *barbadensis* is the commonest Aloes referred to as *Aloe vera* [2] . The latex yellow juice and the gel are the two products obtained from the leaves [3]. The gel is majorly made up of water while the other components have been reported [4,5]. The *Aloe vera* plant has been employed for medicinal purposes over the centuries with more than 75 active components identified from the gel but the therapeutic effects have not been adequately correlated with the individual constituent [6].

The plant has commercial values ranging from health care to cosmetic products which have elicited research on its active components as well as their biological properties [7]. As different groups of chemicals are contained in *Aloe vera*, it has exhibited different pharmaceutical activities[8]. The reported pharmacological potentials of the plant include abortifacient[9], antimutagenic[10], antidiabetic[11], antimicrobial[12], antifungal[13], antiviral [14], anti-inflammatory [15],anti-ulcer[16], anxiolytic[17], antioxidant[18], as well as hepatoprotective activity[19].

Despite the acclaimed folkloric use of the plant, the effect of the chronic administration of the gel on haematological, haemostasis and electrolyte parameters have not been conclusively investigated. The present study is a complementary study aimed at investigating the protective or toxic effects of chronic administration of *Aloe vera* gel extract on the haemostasis, haematology and renal handling of electrolytes, urea and creatinine in Wistar rats.

MATERIALS AND METHOD

Collection and identification of plant material.

Fresh leaves of *Aloe vera* (*A. vera*) were obtained from a garden in Agulu village Anaocha, Anambra State, Nigeria. The leaves were identified at the herbarium section of the Department of Botany, Nnamdi Azikiwe University Awka, as *Aloe vera barbendensis Miller.*

Extract preparation.

The fresh leaves of *A. vera* with length between 30 and 50cm were used for the aqueous gel extraction. They were washed with distilled water to remove dirt and exudate from their surfaces. The epidermis was carefully separated from the parenchyma by a scalpel-shaped knife. The lower one inch of the leaf base and the tapering 2-4 inch of the leaf top and the spines around the leaves were removed using a neat knife. Then the knife was introduced into the mucilage layer below the green rind and the mucilage was collected into a sterilized beaker with the help of a spatula. The gel extract was blended in a Warring commercial blender for 30 minutes for proper

homogenization. The blended gel was filtered through a Whartman filter paper and stored at 0^{o}C in a refrigerator till use. This method was as described by Femenia et al. [20].

Animals and treatment

A total of twenty eight laboratory male Wistar albino rats weighing between 140-180g and aged 7 weeks were used in the study. They were kept in animal cages for two weeks to acclimatize in the animal house of the Faculty of Basic Medical Sciences Nnamdi Azikiwe University Nnewi Campus, Nigeria. They were allowed free access to water and food *ad libitum*. The protocol for the experiment was approved by the Faculty of Basic Medical Sciences Experimental Ethics Committee in line with the guideline of the National Institute of Health (NIH). The experimental animals were randomly distributed into four groups of seven rats each. The animals in group A were placed on normal rat feed and served as control while the animals in groups B, C, and D were orally administered with 200, 350 and 500mg/kg/bwt of extract respectively. The experiment lasted for 21 days.

Collection of samples

Overnight prior to sample collection, the animals were starved of food. Blood were collected from the ocular median-cantus vein of the rats with the aid of capillary tubes, transferred to test tubes, allowed to clot and subsequently centrifuged to obtain the serum component used for biochemical analysis.

Determination of the bleeding time

Bleeding time was performed prior to the sacrifice of the animals. The bleeding time was as reported by Zoja et al. [21] and Aurora et al. [22]. The tail of the rats were warmed for one minute in water at about 40^{o}C and then dried. A small incision was made in the middle of the tail with a scalpel. Bleeding time started when the first drop of blood touched the circular filter paper. The bleeding from the cut was cleansed every 15 seconds intervals to determine the time of arrest.

Determination of the clotting time

The clotting time was determined using the Lee-White method as adopted by Ochei and Kolhalktar [23].

Determination of haematological parameters

The red blood cell (RBC) count (million /mm^3) and white blood cell (WBC) count (thousand/mm^3) were estimated according to Dacie and Lewis[24].

Haematocrit value (Hct) or packed cell volume (PCV) was estimated by using microhaematocrit method as described by Alexander and Griffiths [25].

Biochemical parameters

The serum electrolytes were assayed based on ion exchange selective electrode principle using ISE 4000 SFRI auto analyser (France). The serum urea and creatinine concentrations were analysed using Roche/Hitachi 917 automatic analyser CAN 700 (Roche Diagnostic-Indianapolis).

Statistical analysis

All results are expressed as mean ± SD. The data were analysed by one-way analysis of variance (ANOVA) followed by Fischers LSD post- hoc test using SPSS version 20 software (SPSS Inc. Chicago, IL, USA). Statistical significance was considered at $P<0.05$.

RESULTS AND DISCUSSION

The results of the bleeding and clothing time are presented in table 1. The results show a decrease in the bleeding time as the concentration of the extract is increased. However, the decrease in bleeding time was statistically significant in groups 3 and 4 compared to the control group ($p<0.05$). The clotting time increased as the concentration of the extract increased. As indicated on the bleeding time result, the clotting time of group 3 and 4 significantly increased compared to the control group ($p<0.05$). The effect of the gel extract on the haematological parameters is represented in Table 2. From the results, there was a decrease on the PCV as the concentration of the extract was increased. The PCV was only significantly decreased in group 4 relative to the control ($p<0.05$). The control group (group 1) had a higher value of white blood cells compared to the test groups. The results showed that only the test group 4 had a significantly lower white blood cell count relative to group 1 ($p<0.05$) indicating a downturn in the extract negative impact on the white blood cell count. There was a progressive increase in the platelet count but these increase were not significant ($p>0.05$). The effect of the extract on the serum electrolytes and renal parameters is shown in table 3. The aqueous A. vera gel extract did not significantly alter any of the serum electrolytes concentrations. Also, the marginal increases in the mean serum creatinine and urea concentrations were not statistically significant ($p>0.05$).

Table 1: The effect of *Aloe vera* gel extract on the bleeding and clotting time.

Group.	Bleeding time (Seconds)	Clotting time (Seconds)
1	306.60 ±27.67	91.00± 05.08
2	268.00± 09.30	98.60 ±03.38
3	213.00± 05.61*	161.60 ±15.16*
4	183.00 ±11.02*	220.80 ±21.87*

Results are mean ± SEM of triple determinations. Values with superscript in a column are statistically significant to the control ($p<0.05$).

Table 2: The effect of *Aloe vera* gel extract on haematological parameters.

Group	Packed cell volume	White blood cell count	Platelet count

	(%)	(x 10^3/µl)	(x 10^6/µl)
1	38.40± 0.92	8.18± 0.86	95.20± 11.75
2	35.25 ±1.88	5.00± 2.00	123.75± 08.57
3	36.20 ±3.23	5.18 ±1.33	106.20± 08.61
4	27.20 ±3.97*	3.16 ±0.87*	126.80± 16.40

Results are mean ± SEM of triple determinations. Values with superscript in a column are statistically significant to the control ($p<0.05$).

Table 3: The effect of *Aloe vera gel* extract on the serum electrolytes and some renal indices.

Group	Na^+ (mEq/L)	K^+(mEq/L)	Cl^-(mEq/L)	HCO^-_3 (mEq/L)	Urea (mEq/L)	Creatinine (mEq/L)
1	139.60±1.32	4.00± 0.07	101.00±1.30	16.00± 1.00	4.46 ±0.48	52.60± 3.07
2	140.00±0.94	4.14 ±0.05	100.42±0.74	17.00± 1.00	4.50± 0.28	50.60± 2.15
3	140.00±2.65	4.46± 0.30	101.20±2.35	16.80± 1.88	4.52± 0.13	53.80± 3.83
4	143.00±0.89	4.96± 0.50*	104.00±0.54	17.60± 2.27	5.20± 0.49	61.60± 8.33

Results are mean ± SEM of triple determinations. Values with superscript in a column are statistically significant to the control ($p<0.05$).

DISCUSSION

Coagulation is an important part of haemostasis. Disorders of coagulation can lead to an increased risk of bleeding or obstructive clotting [26]. Haemostasis involves the spontaneous arrest of bleeding from damaged blood vessels necessary for initiation of tissue repair processes and prevention of tissue death as a result of haemorrhage [22,28]. Haemostasis occurs in four stages thus: vasoconstriction, platelet response, blood coagulation while the fourth stage is when the clot is dissolved following repair of the blood vessel [29]. Because haemostasis is a lifesaving mechanism it is important to search for medicinal plants that facilitate this process.

The results from this study showed that the *A. vera* gel exhibited haemostatic potential by decrease in the mean bleeding time with groups 3 and 4 rats being statistically significant to the control ($P<0.05$). The bleeding time is one of the indices of measuring blood coagulation and is used to study the vascular and platelet responses associated with haemostasis [29,30]. The significant decrease in the bleeding time as shown in this study is an indication that the *A. vera* gel has a positive effect on haemostasis probably by acting on the integrity of the blood vessel or involvement of platelets forming the haemostatic plug or both. The extract may have inhibited the formation of prostaglandin by the vessel walls during injury. The release of prostaglandins at the site of injury are responsible for vessel relaxation that leads to increase in bleeding time [28,31]. Previous reports have suggested that the polysaccharides in *A. vera* gel have therapeutic properties such as immunostimulation, anti-inflammatory effects, wound healing and promotion of radiation damage repair[32,33].

The clotting time measures the intrinsic pathways of blood coagulation and is indicative of the functions of clotting factors I, II,V,VIII,IX and XII [23]. Previous reports on the effect of Aloe vera gel on clotting and bleeding time are scarce. The results in this study on clotting time is in line with that of Dapper et al.[30] that reported that treatment with aloe vera extract increased the clotting time significantly in dose dependent manner. The report of Weremfo et al. [28] showed that the stem juice of *M. paradisiaca* exhibited haemostatic effect by reducing clotting time. The significant increase in the clotting time seen in this study may be an indication that there was a decrease in one or more of the clotting factors involved in the intrinsic pathway. The report of Esua and Rauwald[34] suggests that if *A. vera* gel is tested in a wound healing experiment and it contains high amount of veracylglucan B and is perhaps also contaminated with anthraquinones from the exudate, it will most probably result in retardation of wound healing. Also the report indicated that if the gel was obtained from a plant with higher concentrations of veracylglucan C, it would probably end in a positive wound healing effect[34]. The plant has been previously reported to improve pain management by inhibiting the production of bradykinin and thromboxane and to enhance blood coagulation through vasodilator effect [35,36]. Different studies have explained the haemostatic effect of *A. vera* gel on wound and burns by different mechanisms such as enhancing the production of a wound-healing advancement factor[36], enhancing the collagen matrix of wounds [37], enhancement of early epithelisation of burn wounds[38], among other mechanisms [39,40]. It is not clear how the reported effects of *A. vera* on bleeding and clotting time on this study could contribute to its effect in enhancing wound healing process.

The haematological indices of blood provide useful information for the diagnosis of different blood diseases. A profoundly decreased values of the these indices could point to different forms of anaemia caused by loss of blood through haemorrhage, bone marrow defects, iron and vitamin B12 deficiency etc [41]. The haematological indices indicate a reduction in the mean serum concentrations as the concentration of the gel extract is increased except for the platelets values which increased. The mean PCV and WBC was only significantly decreased ($P<0.05$) at 500mg/kg/bwt of the extract administration. This decrease in the mean PCV values are in contrast to the results of Iji et al.[2] that reported *A. vera* gel slightly increased the PCV and WBC count in wistar abino rats. The results of Ahmed et al. [42] showed that the administration of the *A. vera* juice to rabbits concurrently with malathion decreased the percentage of increase in WBC counts shown in malathion treated rabbits. The increase in the platelet count was in agreement with the report of Iji et al.[2]. Because of the absence of any significant changes in the blood haematology as shown in

this study, it can be inferred that the gel extract has no deleterious effect on the haematological system.

The results from this study demonstrated that the *A. vera* gel treatment did not induce significant electrolyte imbalance in the experimental rats. Some previous studies have reported electrolyte imbalance [43,44]. This study reported non- significant increase in the sodium levels while Saka et al.[44] reported a significant decrease in the sodium levels. The plasma levels of urea and creatinine could be a useful tool for renal toxicity and renal function test [44]. This study reported a marginal non-significant increase in the mean serum urea and creatinine levels in the test groups. The report of Iji et al. [2] is in line with the results from this study were there is no significant changes in the urea and creatinine levels. It should be noted from this study that the administration of the gel at 500mg/kg/bwt was the only concentration that induced significant effect on some of the studied parameters. Our results showed that the *A. vera* gel extract did not induce nephrotoxic effects seen on the urea, creatinine and electrolytes levels. The study of Saka et al.[44] reported a significant increase in the creatinine level of test groups relative to the control in line with the study of Avila et al.[45] that reported cytotoxic effect of aloe.

CONCLUSION

The differences in the plant composition due to geographic area, gel extraction procedure adopted may have contributed in some of the differences obtained from this study and other reports. The results from this study indicate that the chronic administration of Aloe vera gel did not disrupt the renal function of the wistar rats.

REFERENCES

1. Klein A D, Penneys N. Aloe vera. J Am Acad Dermatol 1988; 18: 714–20.
2. Iji OT, Oyagbemi AA, Azeez OI. Assessment of chronic administration of aloe vera gel on haematology, plasma biochemistry, lipid profiles and erythrocyte osmotic resistance in wistar rats. Nig J Physiol Sci 2010;25:107–113.
3. Agarry OO, Olaleye MT, Michael BCO. Comparative antimicrobial activities of *Aloe vera* gel and leaf. Afr J Biotechnol 2005; 4(12): 1413-1414.
4. Anderson FA. Final report on the safety assessment of Aloe andongensis extract, Aloe andongensis leaf juice, Aloe arborescens leaf extract. (Cosmetic Ingredient Review Panel Expert panel, Washington, DC). Int J Toxicol 2007; 26:1-50.
5. Can A. Aloe barbadensis extracts. J Invest Dermato. 1999; 110:811-817.

6. Habeeb F, Shakir E, Bradbury F, Cameron P, Taravati MR, Drummond AJ, et al. Screening methods used to determine the anti-microbial properties of Aloe vera inner gel. Methods 2007; 42: 315-320.
7. Rajasekaran S, Sivagnaman K, Subramanian S. Beneficial effects of Aloe vera leaf gel extract on oxidative stress in rats treated with streptozotocin. J Pharm Pharmacol 2005; 57:241-246.
8. Sadique H, Anzar A, Nasreen J, Shamim A, Heena KS. Sibr (Aloe vera) and its therapeutic efficacy described in Unani Medicine: a review. J Sci Innovat Res 2014; 3(5): 545-551.
9. Kosif R, Akta G, Öztekin A. Microscopic Examination of Placenta of Rats Prenatally Exposed to *Aloe barbadensis*: A Preliminary Study. Int J Morphol 2008.; 26(2): p. 275-281.
10. Stanic S. Anti-genotoxic effect of *Aloe vera* gel on the mutagenic action of ethyle methanesulfonate Arch Biol Sci Belgrade. 2007; 59(3): 223-226.
11. Ali E, Mohamed K. Antidiabetic, antihypercholestermic and antioxidative effect of *Aloe vera* Gel extract in alloxan induced diabetic rats. Austr J Basic Appl Sci 2011; 5(11): 1321-1327.
12. Mariappan V, Shanthi G. Antimicrobial and phytochemical analysis of *Aloe vera* L. Inter Res J Pharm 2012; 3(10): 158-161.
13. Sitara U, Hassan N, Naseem J. Antifungal activity of *Aloe vera* gel against plant pathogenic fungi. Pak J Bot 2011; 43(4): 2231-2233.
14. Chatterjee P, Chakraborty B, Nandy S. Aloe vera Plant: Review with significant Pharmacological Activities. Mintage J Pharm Med Sci 2013; 2(3):21-24.
15. Bautista-Pérez R, Segura-Cobos D, Vázquez-Cruz B. *In vitro* antibradykinin activity of *Aloe barbadensis* gel. J Ethnopharmacol 2004; 93:89–92.
16. Borra SK, Lagisetty RK, Mallela GR. Anti-ulcer effect of *Aloe vera* in nonsteroidal anti-inflammatory drug induced peptic ulcers in rats. Afri J Pharm Pharmacol. 2011; 5(16): p. 1867-1871. 39.
17. Sultana N, Najam R. Anxiolytic Activity of *Aloe vera* (L.) burm.f tested in rodents. Pak J Pharmacol 2012; 29(1): 7-15.
18. Nwanjo HU. Antioxidant activity of the exudate from Aloe barbadensis leaves in diabetic rats. BIOKEMISTRI 2006; 18(2): p. 77-81.

19. Ebenyi LN, Ibiam UA, Inya Agha RO, Ogbanshi ME, Uhuo CA. A comparison of the effects of *Aloe varbadensis* and *Allium sativum* extracts on paracetamol induced hepatotoxicity in albino rats. IOSR J Pharm Biol Sci 2012; 4(5): p. 28-31.
20. Famenia A, Garcia-Pascual P, Simal S, Rossello C. Effects of heat treatment and dehydration on bioactive polysaccharide acemannan and cell wall polymers from Aloe barbadensis Miller. Carbohydr Polym 2003; 51(4):397-405.
21. Zoja C, Noris M, Corna D, Vigano G, Perico N, de Gaetano G, et al. L-arginine, the precusor of nitric oxide, abolishes the effect of estrogen on bleeding time in experimental uremia. Lab. Invest. 1991; 65:479-483.
22. Aurora G M, Gonzalez-Iiavan J, Christiana L, Consula RP. Standardization of rat blood clotting tests with reagents used for humans. Proc West Pharmacol Soc 2001;44:153-155.
23. Ochei J, Kolhatkar A. Medical Laboratory. Theory and Practice. 2nd., New Delhi:Tata Mcgraw-Hill Publishing Company Limited; 2000: 331-349.
24. Dacie JV, Lewis SM. Practical haematology. Churchill Living Stone, Edinburgh, 1991; 41-57.
25. Alexander R R, Griffiths J M. Haematocrit in: Basic Biochemical methods, 2nd ed., John Wiley and Sons Inc. New York, 1993b; 186-187.
26. David L, Nigel K, Michael M, Denise O. 2009. Practical hemostasis and Thrombosis. Wiley-Blackwell. Pp. 1-5. ISBN 1-4051-8460-4.
27. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo and delta. States of Nigeria. Global J Pure Appl Sci 2002; 8: 203-208.
28. Weremfo A, Adinortey MB, Pappoe ANM. Haemostatic effect of the stem juice of Musa paradisiaca L. (Musaceae) in Guinea pigs. Advan Biol Res 2011; 5(4):190-192.
29. Tortora G, Grabowski S. Principles of Anatomy and Physiology. 9th ed. New York NY, John Wiley; 2000.
30. Dapper DV, Achinike PN, Gwotmut MD. The effects of Aloe vera (gel) on Clotting time, Prothrombin time and Plasma fibrinogen concentration in albino wistar rats. Port Harcourt Med J 2007; 2:56-60.
31. Bunting S, Gryglewski R, Moncada S, Vane JR. Arterial wall generates from prostaglandins endoperoxides a substance prostaglandin X which relaxes strips of mesenteric and celiac arteries and inhibits platelets aggregation. Prostaglandins 1976; 12: 897-915.

32. Talmadge J, Chavez J, Jacobs L, Munger C, Chinnah T, Chow JT, et al. Fractionation of Aloe vera L. Inner gel, purification and molecular profiling of activity. Int. Immunopharmacol 2004; 4: 1757-1773.
33. Ni Y, Tizard IR. Analytical methodology: the gel analysis of aloe pulp and its derivatives. In, Reynolds T (Ed). Aloes. The Genus Aloe. Boca Raton:CRC Press ; 2004; 111-126.
34. Esua MF, Rauwald JW. Novel bioactive malonyl glucans from Aloe vera gel:isolation, structure elucidation and in vitro bioassays. Carbohydr Res 2006; 341:355-364.
35. Reynolds T, Dweck AC. Aloe vera leaf gel: a review update. J Ethnopharmacol 1999; 68:3-37.
36. Heggers JP, Elzaim H, Garfield R, Goodheart R, Listergarten D, Zhao J, et al. Effect of the combination of Aloe vera, nitroglycerin, and L-NAMME on wound healing in the rat excisional model. J Atern Complement Med 1997; 3:149-153.
37. Heggers JP, Kucukcelebi A, Listengarten D, Stabnau J, Ko F, Broemeling LD, Robson MC, et al. Beneficial effect of Aloe on wound healing in an excisinal wound model. J Altern Compliment Med 1996; 2: 271-277.
38. Visuthikosol V, Chowchuen B, Sukwanarat Y, Sriurairratana S, Boonpucknavig V. Effect of Aloe vera gel to healing of burn wound: a clinical and histological study. J Med Assoc Thai 1995;78: 403-409.
39. Vogler BK, Ernst E. Aloe vera: a systematic review of its clinical effectiveness. Br J Gen Pract 1999; 49:823-828.
40. Davis RH, Leitner MC, Russo JM, Byrne ME. Wound healing, oral and topical activity of Aloe vera. J Am Podiatr. Med. Assoc 1989; 79:559-562.
41. Channa AA, Qazi IH, Soomro SA, Shah AH, Gandahi JA, Korejo RA, et al. Effect of oral supplementation of *Aloe vera* extract on haematology indices and immune cells of blood in rabbit. Afr J Pharm Pharmacol 2014; 8(19):497-501.
42. Ahmed RH, Mohamed S A, Dalia NI, Mohamed A S. The potential protective and therapeutic effects of aloe vera juice against malathion induced haematological changes in rabbits. Egypt J Hospital Med 2014; 56: 300-310.
43. Kirdpon S, Kirdpon W, Airarat W, Trevanich A, Nanakorn S. Effect of aloe (Aloe vera Linn.) on healthy adult volunteers: Changes in urinary composition. J Med Assoc Thai 2006; 89:S9‑14.

44. Saka WA, Akhigbe RE, Popoola OT, Oyekunle OS. Changes in Serum Electrolytes, Urea, and Creatinine in *Aloe Vera*-treated Rats. J Young Pharmacists 2012; 4(2): 78-81.
45. Avila H, Rivero J, Herrera F, Fraile G. Cytotoxicity of a low molecular weight fraction from Aloe vera (*Aloe barbadensis Miller*) gel. Toxicon 1997; 35:1423‑30.