WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 4, 91-100. Research Article ISSN 2277– 7105

ALOE VERA GEL REVERSES HAEMOSTATIC DERANGEMENT IN RATS FOLLOWING SALT LOADING.

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Article Received on
24 Dec. 2017,

Revised on 14 Jan. 2018,
Accepted on 04 Feb. 2018

DOI: 10.20959/wjpr20184-10839

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ABSTRACT

This research investigated the effect of *Aloe vera* (gel) on haemostatic status of high salt loaded rats. Twenty wistar rats (200-250g) were randomly assigned into 4 groups (n=5) thus: control, Aloe (600mg/kg of *Aloe vera*), Salt-fed [SF] (high salt diet- 8% NaCl in feed and 1% NaCl in H_2O) and salt treated [SF+Aloe] (high salt diet+600mg/kg of *Aloe vera*) groups. All groups took rat feed and water throughout the duration (six weeks) of treatment. Blood samples were collected via cardiac puncture and tail pricking for analysis. Platelet count was significantly decreased in Aloe ($p<0.001$) and SF ($p<0.01$) groups compared with control. But it was significantly ($p<0.001$) increased in SF+Aloe group compared with other groups. MPV and P-LCR were significantly increased in SF+Aloe group compared with Aloe ($p<0.05$) and SF ($p<0.01$) groups. Clotting, bleeding and prothrombin times were significantly ($p<0.01$) increased in SF group compared with control and Aloe groups. They were however significantly ($p<0.01$) decreased in SF+Aloe group compared with SF group. High salt intake caused deleterious haemostatic effects by reducing platelet count and increasing clotting, bleeding and prothrombin times but *Aloe vera* however ameliorated these effects and can therefore be effective in treating bleeding disorders.

KEYWORDS: Aloe Vera; Bleeding; Clotting; Salt; Platelet.

INTRODUCTION

Dietary salt is an ingredient used in food and several other areas.[1] It is composed chemically of sodium and chlorine. The sodium component is important in the maintenance of body fluid

volume which makes intake of salt essential for sustenance of life. Salt intake should be controlled because excessive intake is dangerous to health due to the cellular oxidative damage it causes.[2,3] High salt intake is associated with various health complications. It causes hypertension[4], renal stones[5], apoptosis of hepatocytes[6], osteoporosis[5,7], damage to sperm cells[8,9] and gastric cancer.[10]

Medicinal plants have been used for centuries in the treatment of several ailments and by pharmaceutical industries as components for drug production. *Aloe vera* is one of such plants. *Aloe vera* belongs to the family Asphodelaceae. It is native to North Africa but grown in the drier tropic and subtropic regions of the world as an ornamental and medicinal plant.[11] Several reports have shown the medicinal properties of this plant. *Aloe vera* has anti-ulcer[12], anti-atherosclerotic[13], immune-stimulatory[14], anti-inflammatory, anti-arthritic, anti-bacterial and hypoglycaemic effects[15] and is effective in wound healing.[16] It is presumed that *Aloe vera* reduces lipid peroxidation and increases levels of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione.[17]

Some studies have shown various effects of high salt intake and *Aloe vera* on haemostatic function.[11,18,19] Salt loading has been reported to increase PDW, MPV and P-LCR with no significant effect on platelet count.[18] Dapper *et al.*[11] reported that *Aloe vera* increased clotting, bleeding and prothrombin times in rats. Another study[19] on the other hand reported that *Aloe vera* was able to decrease clotting, bleeding and prothrombin times after being raised by thermally oxidized palm oil diet. It is presumable that high salt diet will impact negatively on haemostasis. With the increasing use of *Aloe vera* for medicinal purposes and its availability, it is important to investigate if *Aloe vera* can prevent the deleterious haemostatic effects that could possibly be associated with high salt loading. Presently, there are no reports on the effects of high salt diet on clotting, bleeding and prothrombin times and whether or not *Aloe vera* can ameliorate such effect. The present study was therefore carried out to investigate the effect of *Aloe vera* on haemostatic function of high salt loaded wistar rat.

MATERIALS AND METHODS

Experimental Animals

Twenty (20) male wistar rats weighing 200-250g were used for the study. The rats were bought from Department of Agriculture, University of Calabar, Nigeria and kept in properly ventilated metabolic cages in the animal house of Physiology Department, University of

Calabar. The animals were handled according to Helsinki's[20] laid down principles. They were allowed to acclimatize for seven days before treatment began. They were given rat feed and water *ad libitum* and exposed to 12/12 hours light/dark cycle. Ethical approval was obtained from the Faculty of Basic Medical Sciences Ethics Committee. The ethical standards of experiments were in accordance with the guidelines provided by the CPCSEA.

Preparation of *Aloe vera* gel extract

Aloe vera plant was obtained from a garden in the University of Calabar and was identified by a botanist in the herbarium of the Department of Botany, University of Calabar. The fresh leaves were thoroughly washed with tap water to remove dirt. The base and apex of the leaves were cut with surgical blades and were sliced open along the margin to reveal the transparent mucilage. With the aid of a spatula, the transparent mucilage was carefully scooped into a beaker and further processed by blending in an electric blender for 20 minutes. A greenish but gel-like liquid was obtained. This was allowed to settle for 20 minutes and sieved afterwards using Whatman filter paper to obtain a particulate-free gel.[21] The *Aloe vera* extract was refrigerated (4-6°C) for 3 days after use each day.

Preparation of High Salt Diet and Drinking Water

According to the method of Obiefuna and Obiefuna[22] high salt diet containing 8% of sodium chloride was prepared using a standard diet containing 0.3% sodium chloride.

Experimental Design and Extract Administration

Twenty (20) male wistar rats were randomly assigned into four (4) groups (n=5) thus: Group 1 (control): received normal rat feed and water. Group 2 (aloe vera group [Aloe]): received 600mg/kg of aloe vera, orally once daily. Group 3 (salt-fed[SF]): received high salt diet (8% NaCl feed + 1% NaCl drinking water). Group 4 (Salt-fed + Aloe vera [SF+Aloe]): received high salt diet + 600mg/kg of aloe vera. All groups had access to rat feed and water throughout the six-week duration of the experiment.

Collection of Blood Samples

At the end of the 6 weeks, the rats were sacrificed under chloroform anaesthesia (3.5%) and blood samples collected via cardiac puncture using 5mL syringes with 21G needles into pre-labelled ethylenediaminetetracetate (EDTA) vials to obtain serum for analysis. The vials were gently agitated to ensure uniform spread of EDTA. Thereafter, the samples were immediately used for determination of platelet indices.

Determination of Platelet Indices

Platelet indices (Platelet count, PDW, MPV and P-LCR) were determined using automated cell counter (Coulter Electronics, Luton, Bedfordshine, UK) having standard calibrations in line with the instructions of the manufacturer.

Determination of Clotting Time

Clotting time was determined using the Wright's Capillary Method.[23] The tail of the wistar rat was cleaned using methylated spirit and allowed to dry. A lancet was used to make a quick deep prick which resulted in the outflow of blood. The blood was immediately drained into the capillary tube (10cm) by placing one end of the capillary tube on the drop of the blood and the tube tilted downward to ensure easy flow of blood into the tube. The capillary tube was removed after getting filled. After about two minutes, small lengths of the tube were snapped off at 30 seconds interval. The blood column broke easily and cleanly initially. At the end point, a thick strand or coagulated blood column was seen stretching between the broken ends. The time was noted and the tube was gently broken without jerking the ends apart. This was to prevent the strand from snapping by the movement before it was observed. This method gives a normal clotting time of about three to eight minutes.

Determination of Bleeding Time

Bleeding Time was determined using Duke's method.[24] Methylated spirit was used to clean the tail of the wistar rat and allowed to dry. A lancet was used to make a quick deep prick on the tail. This resulted in outflow of blood and the time was noted. The blood was dabbed every 30 seconds using a filter paper. A fresh part of the filter paper was used for each touch. This continues until bleeding stops. The blood spot on the filter paper got smaller till in disappears on cessation of bleeding. The blood spots were counted and the number divided by two to give the bleeding time in minutes.

Determination of Prothrombin Time

Prothrombin Time was determined using Quick's[25] One Stage Method as described by Ochei and Kolhatkar.[26] 0.1 ml of plasma was introduced into the bottom of a 75×10 mm tube in a water bath at 37°C and 0.1 ml of thromboplastin added to it. After a minute, 0.1 ml of warmed 0.025 M calcium chloride was added and the contents of the tube were carefully mixed. A stop watch was started and the tube held with its lower end submerged, was continuously but gently inclined from the vertical to just short off the horizontal. This was to enable the contents of the tube to be observed for the first clotting signs. A fibrin clot

developing within a second marked the end point. The test was repeated three times and the mean reading taken.

Statistical Analysis

Results are presented as mean ± standard error of mean (SEM). Computer software, SPSS (version 21) was used for data analysis. Statistical measures used were one way analysis of variance (ANOVA) along with post hoc multiple comparison test (least square difference). $p<0.05$ was considered statistically significant.

RESULTS

Comparison of Platelet Indices in the Different Experimental Groups

Table 1 shows platelet count ($x10^3$ cell/µL), PDW (fL) MPV (fL) and P-LCR (%) for control, Aloe, SF and SF+Aloe groups. Platelet count was significantly decreased in Aloe ($p<0.001$) and SF ($p<0.01$) groups compared with control. It was significantly increased ($p<0.001$) in SF+Aloe group compared with control, Aloe and SF groups. PDW was not significantly different between the groups. MPV and P-LCR were not significantly different between control, Aloe and SF groups. MPV and P-LCR were significantly increased in SF+Aloe group compared with Aloe ($p<0.05$) and SF ($p<0.01$) groups.

Table 1: Comparison of platelet indices in the different experimental groups

Parameter	Control	Aloe	SF	SF+Aloe
Platelet count ($x10^3$ cell/µL)	250.40 ± 4.83	232.60 ± 4.07***	231.80 ± 3.92**	283.00 ± 4.06^{***,c,z}
PDW(fL)	7.16±0.34	6.36±0.32^{ns}	7.30±0.34^{ns}	7.20±0.67^{ns}
MPV(fL)	5.80±0.18	5.74±0.12*	5.50±0.09	5.94±0.06^{a,y}
P-LCR (%)	9.04 ± 1.38	7.02 ± 0.48	8.16 ± 0.68	12.32 ± 1.72^{a,y}

Values are expressed as mean ± SEM, n = 5.

ns = not significant

**p<0.01, **p<0.01, ***p<0.001 vs control*

a= p<0.05, b= p<0.01, c= p<0.001 vs Aloe

y= p<0.01, z= p<0.001 vs SF

Comparison of Clotting Time, bleeding Time and Prothrombin Time in the Different Experimental Groups

Table 2 shows clotting time (sec), bleeding time (sec) and prothrombin time (sec) for control, Aloe, SF and SF+Aloe groups. Clotting time was significantly ($p<0.01$) increased in SF group compared with other groups. Bleeding time was significantly ($p<0.01$) increased in SF

group compared with other experimental groups. Prothrombin time was significantly increased in SF group compared with control ($p<0.001$) and Aloe ($p<0.01$) groups and significantly decreased in SF+Aloe group compared with control ($p<0.05$) and SF ($p<0.001$) groups.

Table 2: Comparison of Clotting Time, bleeding Time and Prothrombin Time in the Different Experimental Groups

Parameter	Control	Aloe	SF	SF+Aloe
Clotting time (sec)	82.60±3.91	72.00±5.92	98.60±3.08^{**,b}	85.00±0.89^{y}
Bleeding time (sec)	28.70±2.15	22.24±0.68	34.06±4.86^{**,b}	27.26±0.12^{y}
Prothrombin time (sec)	30.42±2.70	25.40±2.91	52.45±2.46^{***,b}	20.47±1.96^{*,z}

Values are expressed as mean ± SEM, n = 5.

***p<0.01, ***p<0.001 vs control*

b= p<0.01 vs Aloe

y= p<0.01, z= p<0.001 vs SF

DISCUSSION

High salt intake has impacted negatively on various tissues and organs of the body. *Aloe vera* has been reported to exhibit various therapeutic effects. This study was carried out to investigate the effect of *Aloe vera* gel on platelet indices, clotting, bleeding and prothrombin times in high salt loaded rats.

Platelet count was significantly decreased in all treatment groups except SF+Aloe group compared with control. PDW was not significantly different between the groups. MPV and P-LCR were not significantly different between the control, Aloe and SF groups but they were significantly increased in SF+Aloe group compared with Aloe and SF groups. These results indicate that aloe vera and high salt diet have inhibitory effect on thrombopoiesis during the hematopoietic process and as a result affect the blood clotting system. However, co-administration of aloe vera and salt diet seems to cause opposite effect by stimulating platelet production. It is likely that there are certain constituents in *Aloe vera* which help to ameliorate the deleterious effect associated with salt loading. *Aloe vera* richly contains antioxidants that could help mob up free radicals caused by salt loading.[19] MPV decreases when the body's production of platelet decreases and vice versa. The increase in MPV observed in the SF+Aloe group is in line with the increase in platelet count associated with this group. Results for MPV in other groups are also in line with platelet count in those groups. The increase in P-LCR in SF+Aloe group indicates that co-administration of high salt

diet and aloe vera enhanced platelet aggregation. Contrary to the present study, Ofem *et al.*[18] reported that salt loading increased MPV, PDW and P-LCR.

Clotting, bleeding and prothrombin times increased significantly in salt-fed group compared with control and Aloe groups. This indicates that high salt loading affected the blood clotting process, delaying the time taken for blood to clot and prolonging bleeding. This effect could be due to the decreased platelet count caused by salt loading since platelets and other clotting factors are needed for blood to clot and to facilitate stoppage of bleeding. Clotting factors are important in haemostasis and clotting, bleeding and prothrombin times are markers of haemostatic function. Clotting time is a measure of the intrinsic pathway.[27] This implies that clotting time measures the functions of factors I, II, V, VIII, IX, X, XI and XII.[27] Prothrombin time on the other hand is a measure of the extrinsic pathway[27] implying that it measures the functions of factors III, V, VII and X.[19] Since these clotting factors are synthesized by the liver, it is possible that the salt loading caused oxidative damage to hepatocytes which affected the synthesis of these factors thereby reducing their effect and prolonging bleeding and clotting and activation of prothrombin. Damage to hepatocytes is marked by increases in serum Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and alkaline phosphatase (ALP) concentrations.[28] A previous study shows significant increase in serum concentrations of these enzymes following salt loading.[29] Bleeding time increased in SF group probably because of the decreased platelet count associated with salt loading.

However, *Aloe vera* demonstrated a protective effect on platelet indices as platelet count, MPV and P-LCR were significantly increased in SF+Aloe group compared with SF group. This is seen in our result where clotting, bleeding, and prothrombin times were significantly decreased in SF+Aloe group compared with SF group. Clotting, bleeding and prothrombine times also decreased although not significant in Aloe group compared with control demonstrating the ability of *Aloe vera* to maintain haemostasis. This is contrary to Dapper *et al.*[11] who reported significant decrease in clotting and prothrombin times following administration of *Aloe vera.* The reduction of clotting and bleeding time by *Aloe vera* is probably via its ability to increase fibrinogen level. Fibrinogen is critical to the formation of a stable fibrin clot. *Aloe vera* has been reported to increase fibrinogen levels in rats whose fibrinogen levels were reduced by thermally oxidized palm oil.[19] *Aloe vera* has been reported to enhance wound healing.[16]

CONCLUSION

High salt intake exhibited deleterious effects on haemostatic function of wistar rats by decreasing platelet count and increasing clotting, bleeding and prothrombin times. However, *Aloe vera* ameliorated the haemostatic derangement caused by high salt intake and can therefore be effective in the treatment of bleeding disorders.

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