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# Assessment of Chronic Administration of *Aloe Vera* Gel On Haematology, Plasma Biochemistry, Lipid Profiles and Erythrocyte Osmotic Resistance in Wistar Rats

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**Summary:** The study was designed to investigate the effects of chronic administration of *Aloe vera* gel extract on markers of hepatic damage, lipid profiles and erythrocyte osmotic fragility using the Wistar rats. Forty male Wistar rats divided into four groups of ten rats per group were used in the study. Group I which served as the control received 0.9% physiological saline while those in groups II, III and IV received *Aloe vera* gel (100, 250 and 500mg/kg), respectively, for four weeks. There was significant increase in the haemoglobin concentration while the PCV, RBC count, MCH and MCHC though showed some marginal increases but the increases were not significant in all the treated rats. No significant change was also observed in the erythrocyte osmotic fragility. However, there were significant reductions in plasma ALT ($P<0.001$), AST ($P<0.05$) and ALP ($P<0.05$) levels in animals that received the gel compared with the control while the plasma albumin ($P<0.01$) and total protein ($p<0.05$) values were higher than those of the control. All the animals that received the gel also showed significant reduction in plasma total cholesterol ($P<0.001$), triglycerides ($P<0.001$) and LDL-cholesterol ratio ($p<0.01$) compared with the control. In a similar manner, those animals that were administered with 500mg/kg gel had significantly higher ($P<0.001$) HDL-cholesterol ratio than those of the control. This study showed that, chronic administration of *Aloe vera* gel extract had no significant effects on the haematological parameters of the rats and did not affect erythrocyte osmotic resistance. It however showed some cholesterol lowering action.

**Keywords***: Aloe vera*, Haematology, Osmotic fragility, Plasma biochemistry



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## INTRODUCTION

Aloes are members of the *Liliaceae* family and are mostly succulent with a whorl of elongated, pointed leaves (Klein and Pennys, 1988). The commonest Aloes, *A. barbadensis* has been referred to as *A. vera* (Coats and Ahola, 1979). The central bulk of the leaf has been reported to contain colourless mucilaginous pulp, made up of large, thin-walled mesophyll cells containing the *Aloe vera* gel itself (Rajasekaran *et al*., 2006). The mucilaginous gel from parenchymatous cells in the leaf pulp of *Aloe vera* is incorporated in creams, ointment, lotions and other preparations for topical use (Anshoo *et al*., 2005). Conflicting reports concerning the efficacy of such preparations have been reported (Vogler and Ernst, 1999). Chemical constituents of *Aloe vera* have been reported to include water-soluble and fat-soluble vitamins, minerals, enzymes, polysaccharides, phenolic compounds and organic acids (Burn *et al*., 2003; Boudreau and Beland, 2006).

*Aloe vera* has also been reportedly used in the treatment of many disorders such as arthritis, gout, dermatitis, peptic ulcer as well as treatment of burns (Grindlay and Reynolds, 1986). The fresh gel, juice and formulated products have long been used for medical and cosmetic purposes and general health (Chithra et al., 1998; Reynolds and Dweck, 1999). The beneficial properties of *Aloe vera* may be attributed to the polysaccharides present in the inner gel of the leaf, especially acemannan (acetylated mannans), which has also been shown to be an

immune-modulator (Djeraba and Quere, 2000; Liu *et al*., 2006).

Aloe extracts have equally been demonstrated to have antioxidant abilities in humans and animals (Rajasekaran, 2005; Kardosova and Machova, 2006; Loots *et al*., 2007), whilst also protecting the liver, (the major detoxification organ) against injury (Can *et al*., 2004; Chandan *et al*., 2007) and improve liver enzyme functions that are associated with carcinogen metabolism (Singh *et al*., 2003). The antioxidant and anti-inflammatory (Yagi *et al*., 2002; Speranza *et al*., 2005; Yagi and Takeo, 2003), anti-cancer (Lee *et al*., 2000a; Lee *et al*., 2000b; Heggie *et al*., 2002; Chen et al., 2004; Su *et al*., 2004), anti-diabetic (Ayse *et al.*, 2004) and wound healing properties (Choi *et al*., 2001) of *Aloe vera* have also been widely reported.

Increased serum ALT and AST values have been indicated for hepatic damage. ALT is a known hepato-specific enzyme that is principally found in the cytoplasm of hepatocytes (Benjamin, 1978; Ringler and Dabich, 1979). Similarly, AST, an enzyme that is present in high quantities in the cytoplasm and mitochondria of liver cells, is also present in the heart, skeletal muscle, kidney and brain (Benjamin, 1978; Ringler and Dabich, 1979). It has been shown that increase in the enzymatic activity of ALT and AST in the serum directly reflects a major permeability or cell rupture (Wittwer and Bohmwald, 1986).

Despite its wide use in folk medicine for the treatment of various diseases for decades, chronic administration of this wonder plant on markers of liver damage, lipid profiles and erythrocyte osmotic fragility have not been investigated. The present study was therefore carried out to evaluate whether *Aloe vera* leaf gel extracts have any protective or harmful effect on the liver and erythrocyte membrane after prolonged administration in rats.

We hypothesized that chronic administration of *Aloe vera* gel extract might cause hepatic damage, alter lipid profiles and induce erythrocyte membrane fragility using the plasma aminotransferases and erythrocyte membrane stability in hypotonic solution as yardsticks for assessment of hepatic damage and membrane fragility, respectively. The effect of the *Aloe vera* on the haematological parameters was also assessed.

## MATERIALS AND METHODS

### Plant Material

Specimens of *Aloe vera* (L.) were collected from the Institute of Animal Health, Research and Training, Moore Plantation, Ibadan, Nigeria. Fresh leaves of this cultivated plant were used in this study. The study was carried in 2009 at the Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

### Preparation of the Samples

*A. vera* leaves (10 big leaves) were weighed, washed and cut in the middle, the gel was separated by scratching with a spatula into a clean beaker. The Aloe vera leaf gel extract was prepared according to the method of Ayse *et al.* (2004).

### Experimental animal models

Male albino rats (Wistar strain) weighing between 180-200g, purchased from the Central Animal House, University of Ibadan were used for the study. They were bred and housed in the Experimental Animal unit of the Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. The animals were kept in wire mesh cages under controlled light cycle (12h light/12h dark) and fed with commercial rat chow (Vital Feeds Nigeria Limited) *ad libitum* and liberally supplied with water. All of the animals received humane care according to the criteria outlined in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy Science and published by the National Institute of Health. The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animals' welfare during experiments (PHS, 1996). Forty male rats divided into four groups consisting of ten rats per group were used in this study. Group I, (Control) animals received 0.9% physiological saline orally while those in groups II, III and IV were administered with *Aloe vera* leaf gel extract orally in doses of (100, 250 and 500mg/kg), respectively for four weeks.

### Blood sample collection

Blood sample (5ml) was collected from all the rats through the retro-orbital venous plexus into lithium heparinised tubes for determination of the haematological parameters. The blood was centrifuged afterward at 4,000 rpm for 10 minutes to obtain the plasma for the biochemical assays.

### Haematology

From the blood samples, the packed cell volume (PCV) was determined by microhaematocrit method. Red blood cells (RBC) and white blood cells (WBC) were counted using the haemocytometer method. Haemoglobin concentration (Hb) was determined by the cyanmethaemoglobin method. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated

from the PCV, RBC and Hb values. Erythrocyte osmotic fragility was determined by adding 0.02 ml of blood to tubes containing increasing concentration of phosphate-buffered sodium chloride (NaCl) solution at pH 7.4 (0, 0.1, 0.3, 0.5, 0.7, 0.8, and 0.9%) as described by Oyewale (1992). The tubes were gently mixed and incubated at room temperature ($29^oC$) for 30 minutes. The content of each tube was then centrifuged at 3500 rev/ min for 10 minutes and the supernatant decanted. Optical density of the supernatant was determined spectrophotometrically at 540nm using SM22PC Spectrophotometer (Surgienfield Instruments, England). Haemolysis in each tube was expressed as a percentage, taking haemolysis in distilled water (0% NaCl) as 100%.

**Biochemical assays**

Total protein (T.P) was determined by Biuret method according to Lowry et al. (1951). Serum alanine transaminase (ALT) activity was determined by the method of Reitman and Frankel (1957), Aspartate aminotransferase (AST) was assayed according to the principle described by International Federation of Clinical Chemistry, Committee on Standards (1980), alkaline phosphatase (ALP) activity by the method of Walter and Schütt (1977). Albumin which quantitatively binds (bromocresol green) BCG to form an albumin-BCG complex was measured as an endpoint reaction at 596 nm according to method of (Doumas and Biggs, 1972). Plasma was analyzed for total cholesterol (TC), triglycerides (TAG), and HDL cholesterol (HDL-C) were determined by enzymatic method coupled with spectrophotometry using assay kit (Randox Lab. Ltd., Co. Antrim, UK). LDL-cholesterol was estimated with the use of Friedewald's formula (Friedwald *et al*., 1972). The creatinine and blood urea nitrogen concentration were determined according to Harrison (Harrison, 1947).

**Statistical Analysis**

All data are expressed as means ± SD and analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison tests. Differences between means were considered significantly different when values of $P \leq 0.05$, using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

## RESULTS

Table 1 shows the effects of chronic administration of *Aloe vera* gel on the haematological parameters of the Wistar rats. Although, oral administration of the gel increased the PCV, RBC, Hb, MCH and MCHC values, only the haemoglobin concentration was statistically significant. The Hb values of the rats administered 100mg/kg and 500mg/kg *Aloe vera* gel were higher (P<0.05) than that of the control. But no significant change was observed on the erythrocyte membrane fragility in hypotonic solution (Fig. 1).

Results from Table 2 show that those animals that received 100, 250 and 500mg/kg of the gel extract had significantly lower (P<0.001) plasma ALT levels than that of the control. While those animals in group IV that received 500mg/kg gel extract had significantly lower (P<0.05) plasma AST values than the control. Those rats that received 250 and 500mg/kg extract (groups III and IV) also had significantly lower (p<0.05) ALP levels than the control. Plasma albumin of the animals in groups III and IV was also significantly higher (P<0.05) with attendant significant increase (P<0.05) in the total plasma protein in rats administered with 100 and 500mg/kg gel extract in comparison with control.

**Table 1:**
Haematological parameters of male Wistar rats after prolonged oral administration of *Aloe vera* gel. Values are means ± SD. Number of animals in parenthesis.

| Haematological Parameters | Group I Control (10) | Group II 100mg/kg (10) | Group III 250mg/kg (10) | Group IV 500mg/kg (10) |
|---|---|---|---|---|
| **PCV (%)** | 40.4±3.78 | 43.2±3.49 | 41.2±3.70 | 44.2±3.42 |
| **Hb (g/dl)** | 12.74±0.45 | 14.6±1.16* | 13.8±1.44 | 14.82±0.99* |
| **RBC (x $10^6$/µl)** | 6.89±0.42 | 7.28±0.43 | 7.01±0.54 | 7.43±0.67 |
| **WBC (x $10^3$/µl)** | 7.25±0.78 | 6.64±2.06 | 7.24±0.72 | 6.84±0.82 |
| **Platelets (x $10^6$/µl)** | 118.6±10.57 | 112.5±12.89 | 125.0±13.4 | 125.0±10.71 |
| **MCV (fl)** | 58.6±2.43 | 59.43±1.06 | 58.77±2.21 | 59.49±3.0 |
| **MCH (ρg)** | 18.49±0.45 | 20.05±0.36 | 19.69±0.97 | 19.94±1.65 |
| **MCHC (g/dl)** | 31.53±1.10 | 33.80±0.67 | 33.50±1.31 | 33.53±2.13 |

Asterisk indicate significant difference from the control at P<0.05.

**Table 2:**
Effects of chronic administration of *Aloe vera* gel on plasma biochemical parameters. Values are means ± SD. Number of animals in parenthesis.

| Groups | ALT (U/I) (10) | AST(U/I) (10) | ALP(U/I) (10) | Albumin g/dL (10) | Total protein g/dL (10) |
|---|---|---|---|---|---|
| Group I | 73.67±6.76 | 98.00±5.06 | 116.30±9.14 | 2.12±0.12 | 5.03±0.23 |
| Group II | 35.00±5.34*** | 104.00±8.00 | 108.30±6.84 | 2.22±0.18 | 4.50±0.10* |
| Group III | 34.80±4.57*** | 105.50±4.50 | 106.30±1.11* | 2.46±0.21** | 4.75±0.41 |
| Group IV | 25.75±2.10*** | 88.86±3.57* | 84.20±4.64* | 3.13±0.44** | 5.98±0.64* |

Asterisks indicate significant difference from the control group *=P<0.05, **=P<0.01, ***=P<0.001

**Table 3:**
Effects of chronic administration of *Aloe vera* gel extract on lipid profiles and metabolites. Values are means ± SD. Number of animals in parenthesis.

| Groups | Creatinine (mg/100ml) | Urea (mg/100ml) | T. cholesterol (mg/dl) | TAG (mg/dl) | HDL-cholesterol (mg/dl) | LDL-cholesterol (mg/dl) |
|---|---|---|---|---|---|---|
| Group I (10) | 0.24±0.02 | 10.80±0.58 | 182.30±7.33 | 271.00± 4.00 | 39.00± 3.46 | 60.00± 5.00 |
| Group II (10) | 0.26±0.04 | 10.75±0.75 | 79.67±6.89*** | 115.00± 6.00*** | 40.00± 4.36 | 34.67± 1.76** |
| Group III (10) | 0.25±0.03 | 10.50±0.50 | 73.33±5.36*** | 105.00 ± 7.1*** | 43.00± 2.00 | 27.00± 2.00** |
| Group IV (10) | 0.24±0.02 | 10.25±0.25 | 52.33±5.36*** | 82.00± 5.69*** | 57.00± 7.55*** | 21.00± 2.00** |

Asterisks indicate significant difference from the control group *=P<0.05, **=P<0.01, ***=P<0.001

There was a significant reduction (P<0.001) in the total plasma cholesterol and triglycerides in animals that were administered 100, 250 and 500mg/kg, respectively compared with control animals (Table 3). Similarly, LDL-cholesterol levels in the animals administered 100, 250 and 500mg/kg of the gel were significantly lower (P<0.05) than those of the control while HDL-cholesterol level in the group IV animals (which received 500mg/kg of the gel) was higher (P<0.001) than in the control.

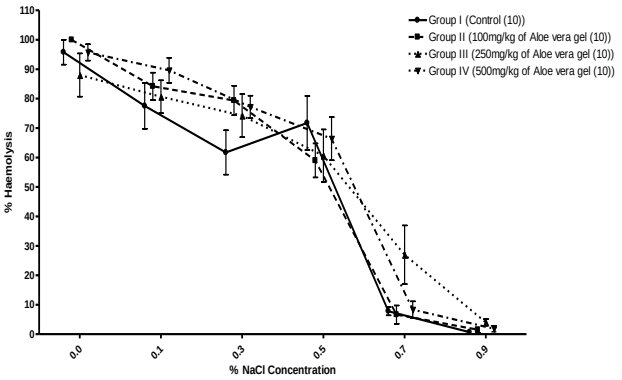


Fig. 1
Erythrocyte osmotic fragility of adult male rats after prolonged oral administration of *Aloe vera* gel. Values are means± SEM of the number of animals in parenthesis

## DISCUSSION

Contrary to our initial speculation, the result of this study showed that *Aloe vera* gel slightly increased the PCV and RBC count while haemoglobin concentration was increased significantly. It also significantly reduced the plasma ALT, AST and ALP of the treated animals. Higher Hb as well as PCV and RBC values in the rats that received the *Aloe vera* gel is an indication that the gel stimulated increased erythropoiesis in those rats, although its potency as a haematinic is low, considering the fact that the effect on RBC count and PCV was not pronounced. This attribute observed in the *Aloe vera* gel may be due to the presence of thiamine, riboflavin, folic acid and other essential and non essential amino acids in the mucilaginous gel (Hamman, 2008).

The polysaccharides, which are the major component of the gel, have also been reported to stimulate erythropoiesis (Choi and Chun, 2003 and Ni *et al.*, 2004). Of all the components of *Aloe vera* gel, thiamine alone can account for the haemopoietic properties of the gel because thiamine as a vitamin has been known to be responsible for glucose uptake in erythrocytes through the formation of pyruvate dehydrogenase complex and α-ketoglutarate dehydrogenase in the Krebb's cycle. Deficiency of thiamine therefore results in thiamine deficiency megaloblastic anaemia syndrome (Oishi et *al.*, 2002).

Plasma ALT, AST and ALP levels in the rats treated with the *Aloe vera* gel was lower than those of the control, despite the prolonged administration. This might not be unconnected with the hepato-protective and antioxidant properties of the gel extract. For example, Chadan *et al.* (2007) reported that aqueous extract of dried aerial parts of *A. vera* significantly reduced hepatic damage induced by carbon tetrachloride in mice and reversed certain biochemical parameters. Histopathological studies also confirmed the curative efficacy of the water extract of *A. vera* against carbon tetrachloride induced liver damage as indicated by reversal of centrilobular necrosis, macro-vascular fatty changes and scattered lymphomononuclear cell infiltrate in hepatic parenchyma. The hepatoprotective action of the plant was attributed to the preservation of the liver enzymes through the antioxidant properties of the gel. In actual facts superoxide dismutase, catalase, β carotene, α tocopherol and other antioxidants have been isolated from the *Aloe vera* gel by several authors (Hammann, 2008). The hepatoprotective activities against arsenic poisoning also had been reported (Gupta and Flora, 2005). This was attributed to the anti-oxidative activities of *Aloe vera*. Similarly, there was no significant increase in the erythrocyte osmotic fragility of the rats even after the prolonged exposure to *Aloe vera* gel. This shows that consumption or oral administration of the gel did not increase the erythrocyte membrane fragility in hypotonic solution. Zhang *et al.* (2006) also reported the antioxidant activities of *Aloe vera* with which it is expected to reduce erythrocyte fragility by mopping up free radicals that are usually involved in the destruction of membrane protein and lipid peroxidation. Findings from this study also indicated that the gel extract increased plasma albumin and reduced that of bilirubin, confirming that chronic administration of *Aloe* for up to one month might not be toxic to the body.

Possible alteration in lipid profiles was also assessed in this work. Findings from our results showed significant reduction in plasma total cholesterol, triglycerides, and low density lipoprotein (LDL) with concomitant significant increase in high density lipoproteins (HDL)-cholesterol after prolonged oral administration of *Aloe vera* gel extract. Fatty acids, an important component of cell membranes are required for both the structure and function of every cell in the body (Rajasekaran *et al*., 2006). Phytosterols, one of the major constituents of *Aloe vera* has been found to reduce visceral fat accumulation, improve hyperlipidemia and hyperglycemia (Misawa *et al*., 2008). This reduction may be attributed to increased clearance and decreased production of the major transporters of endogenously synthesized cholesterol and triglycerides (Subbiah *et al*., 2006). Research on immune stimulation in mice has indicated that acemannan, a polysaccharide within aloe, to demonstrate dose-dependent macrophage activation. When given orally to animals, mannans have also been shown to inhibit cholesterol absorption and lower cholesterol (Sikarwar *et al*., 2010).

Liver tissue participates in the uptake, oxidation and metabolic conversion of fatty acids, the synthesis of cholesterol and phospholipids and the secretion of specific classes of lipoproteins (Rajasekaran *et al*., 2006). The hepatoprotective action was also attributed to preserving the metabolizing enzymes of the liver through an antioxidant activity (Chandan *et al.*, 2007). *Aloe vera* has also been reported to increase bile flow and bile solids thereby stimulating the secretary activity of the liver cells (Hamman, 2008).

In conclusion, prolonged administration of *A. vera* for up to one month increased the haemoglobin concentration with no significant change in the erythrocyte osmotic resistance. Furthermore, no negative effects were observed on the markers of hepatic damage and also positively improved cholesterol status, which was presumably mediated by a control of lipid metabolism. Hence, this study therefore confirms the cholesterol lowering effect of *Aloe vera* gel.

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