Effects of Oral Administration of Aloe Vera Plus on the Heart and Kidney: A Subacute Toxicity Study in Rat Models

Type of Article: Original

O.C. Koroye* I.M. Siminialayi ** E.N. Etebu *

***Federal Medical Center, Yenagoa, Bayelsa State and **Department of Pharmacology, College of Health Sciences, University of Port Harcourt.**

ABSTRACT

Background: Aloe vera gel is one of several nutritional supplements gaining widespread popularity in Nigeria and many other countries of the world. Adverse effects of ingestion of low doses of Aloe vera are seldom reported. It has however, been associated with diarrhea, electrolyte imbalance, kidney dysfunction, and hepatitis. The aim of this study is to investigate the effects of oral administration of GLND'S *Aloe vera Plus* on the kidneys and hearts of rats.

Methods: 80 apparently healthy, adult Wistar albino rats were divided into five groups, three of which were given three different doses of *Aloe vera plus* twice a day for 14, 28 and 42 days. The 4th group served as controls while the 5th was given *Aloe vera plus* for 28 days, following which the drug was withdrawn for another 28 days. The animals were sacrificed at the end of the experiment and the hearts and kidneys harvested for histopathological analysis.

Results: The results indicate that Aloe vera plus caused nephrotoxic changes, including chronic inflammatory cell infiltration, hyalinization, thickening of renal capillaries, tubular collapse and necrosis, and glomerular and interstitial fibrosis. It also caused cardiotoxic effects but not in a significant number of rats.

Conclusion: The nephrotoxic effects of aloe vera occurred more in the dose range recommended by the manufacturers and least with the highest dose and longest duration of treatment. We recommend that this nutritional supplement be subjected to the same regulatory standards as pharmacotherapeutic agents.

Keywords: GNLD, Aloe vera Plus, Kidney, heart, rats.

Correspondence: I.M. Siminalayi

INTRODUCTION

The use of *Aloe vera* and other nutritional supplements including herbal (natural) remedies for the self-treatment of a wide range of diseases has been increasing progressively all over the world[1]. The transparent gel derived from the pulp of the meaty leaves of *Aloe vera* has been used both topically and orally for thousands of years for the treatment of wounds, burns, skin infection, constipation, cancer, diabetes, inflammation, ulcer and hepatitis[2-4]. Its antioxidant effect was recently reported as being of value in the treatment of diabetes[5-7]. However, the adverse effects of ingestion of low doses of *Aloe vera* are seldom reported and include diarrhea, electrolyte imbalance, kidney dysfunction, hepatitis, contact dermatitis, photosensitivity and a number of drug interactions[8,9].

Aloe vera contains antioxidants such as APS-1 and dihydrocoumarin derivatives and it also assists the absorption of some antioxidants such as Vitamins A and C and increases the half-lives of this vitamins[10-12]. It has been reported that the leaf gel extract of *Aloe vera* is effective in restoration of altered fatty acid compositions in the liver and kidney of diabetic rats[13]. Given at a dose of 300mg/kg per day to streptozotocin-induced diabetic rats, for a period of 21 days, *Aloe vera* caused a significant reduction in fasting blood glucose, hepatic transaminases, plasma and tissue (liver and kidney) cholesterol, triglycerides and a significant improvement in plasma insulin[13].

Aloe vera is also a known oxidant, as it has been reported to induce oxidative stress[14]. *Aloe barbadensis Miller* (*Aloe vera*) juice administered to the salt water crustacean *Artenia franciscana* showed a consistent reduction in overall activity of redox related enzymes. Exposure of *Artenia franciscana* to sublethal levels of Aloe vera juice resulted in a decreased activity of thioredoxin reductase, glutathione reductase, and glutathione peroxidase by 34% (66% enzymatic activity), 79% (21% enzymatic activity) and 90% (10% enzymatic activity), respectively. Coexposure of Aloe vera with vitamin E overcame/blocked this effect[16].

We could find no dosing study that has been done to establish the optimum daily intake of Aloe vera as a food supplement. This paper reports the effects of three different oral doses of Aloe vera plus administered for varying periods of time, on the kidneys and hearts of albino Wistar rats.

MATERIALS AND METHODS

80 apparently healthy, adult Wistar albino rats of both sexes obtained from the Department of Pharmacology Animal House, University of Port-Harcourt were used for the experiment which was carried out between February and April, 2009. The rats weighed between 150g 250g (mean weight = 189.7g). All animals were kept under normal laboratory conditions of temperatures between 28°c 32°c, with a 12 hour day-night cycle. The animals were fed with animal feeds (Pfizer Nig Ltd) and drank clean water *ad libitum*.

Aloe vera plus manufactured by the GNLD company was

obtained from an authorized GNLD vendor. The product is stated to contain *Aloe vera* juice with ginseng. Other substances present include chamomile, fructose, trace amounts of naturally occurring aloe-sugars, potassium sorbate, magnesium citrate, vitamin C, citric acid, natural lemon flavour and sodium benzoate.

1ml syringes were used to measure the dose of *Aloe vera plus* and the drug administered orally by means of an inflexible oral cannula. The doses to be administered were calculated using the recommended dose of the manufacturers (60 120mls) daily in two divided doses for adult humans. The presumptive human adult weight was taken as 50kg and the doses to be administered to the rats were calculated using this weight and the mean weight of the rats. The 3 doses used were the equivalents of the minimum recommended dose, maximum recommended dose and double the maximum recommended dose. Thus the doses arrived at were 0.2ml, 0.4ml and 0.8ml to be given twice daily.

The rats were divided into four groups of 15 rats each. Groups A, B and C received 0.2ml, 0.4ml and 0.8ml of *Aloe vera plus* twice daily, respectively, for 14, 28 and 42 days. Five animals from each group were sacrificed on the 15th, 29th, and 43rd days of the experiment. Group D (15 animals) served as the control group and did not receive *Aloe vera plus*, but took only distilled water. Five rats from this group were also sacrificed on day 15, 29 and 43. Group E rats (n=20) served as the reversal group. These rats received *Aloe vera plus* for 28 days after which the drug was withdrawn for another 28 days. This group was subdivided into 4 subgroups of 5 rats each that received 0.2ml, 0.4ml and 0.8ml of *Aloe vera plus* twice daily respectively, while the 4th subgroup received distilled water only. All animals in the group were sacrificed on the 57th day of the study, to determine if the effects of the drug were reversible upon withdrawal of the drug.

All animals were sacrificed by anaesthesizing them with chloroform in a closed container. They were then dissected and the kidneys and hearts harvested for histopathological analysis. Immediately after harvesting, the organs were placed into 10% formaldehyde solution. The tissue specimens were processed for microscopic examination in the histopathology laboratory of the University of Port Harcourt Teaching Hospital, Port-Harcourt. They were dehydrated in alcohol series, processed in xylene and then embedded in paraffin. Sections from each specimen were stained with haematoxylin-eosin. All sections were evaluated with Olympus light microscope at a magnification of 40x and photomicrographs taken.

RESULTS

Results of the histopathological analysis of the hearts and kidneys were obtained for animals sacrificed on days 15, 29, 43 and 57 for all the 80 rats used for the experiment. We define mild to moderate nephrotoxicity as chronic inflammatory cell infiltration of renal tissues, thickening of the renal vascular wall and hyalinization of tubules, and severe nephrotoxicty as fibrosis of interstitium or glomeruli and dystrophic calcification or renal tubular necrosis. In the case of the heart, severe toxicity is defined as myocardial necrosis.

Kidneys

As shown in the tables1 and 2, after 14 days of administration of *Aloe vera plus*, 3 out of 5 rats (60%) that received 0.2ml twice daily had evidence of nephrotoxicity as indicated by chronic inflammatory cell infiltration of the interstitium and tubules. Of the 5 rats that received 0.4ml twice daily, 3 (60%) had evidence of nephrotoxicity, while 2 (40%) did not. Out of 3 rats with toxic effects, 2 had chronic inflammatory cell infiltration, while one had collapse of tubules, tubular damage and parenchymal haemorrhage. Of the 5 rats that received 0.8ml twice daily, 3 had nephrotoxicity manifesting on histology as chronic inflammatory cell infiltration (1 animal), and tubular necrosis, collapse, hyalinization and intra-parenchymal haemarrhage (2 animals).

After 28 days of administration of *Aloe vera* plus, 3 out of 5 rats that received 0.2ml twice daily (60%), had nephrotoxicity typified by interstitial fibrosis in 1 and chronic inflammatory cell infiltration and thickening of the renal vascular wall, in the other 2. Two rats (40%) had no evidence of nephrotoxicity. Of the 5 rats that received 0.4ml twice daily, 3 (60%) had evidence of severe nephrotoxicity (renal damage) typified by extensive glomerular and interstitial fibrosis, tubular necrosis and hyalinization of tubules. 2 (40%) of the rats did not show any evidence of nephrotoxicity. Of the 5 rats that received 0.8ml twice daily, 2 (40%) had evidence of nephrotoxicity, as shown by tubular necrosis and chronic inflammatory cell infiltration. 3 rats (60%) did not have evidence of nephrotoxicity.

Tables 1 and 2 also show that after 42 days of *Aloe vera* plus administration, 3 rats (60%) of the 5 that received 0.2ml twice daily had evidence of nephrotoxicity, while 2 did not show nephrotoxicity. Of the 5 rats that received 0.4ml twice daily, 2 (40%) had nephrotoxicity while 3 rats (60%) did not show nephrotoxicity. 5 rats received 0.8ml twice daily for 42 days out of which 3 (60%) had mild nephrotoxicity as shown by chronic inflammatory cell infiltration. There was no organ damage. Results obtained from the reversal group on the 57th day (i.e.28 days after withdrawing *Aloe vera* plus) revealed a partial reversal of the nephrotoxic effects except for glomerular fibrosis. In this group, of the 5 rats that received 0.2ml twice daily, no nephrotoxic effect was seen. Five rats received 0.4ml twice daily out of which 3 (60%) had evidence of nephrotoxicity, while 2 (40%) did not have any evidence of toxicity. The severity of toxicity was however less, the kidneys showing only mild chronic inflammatory cell infiltration. Of the rats that received 0.8ml twice daily, one (20%) had evidence of nephrotoxicity, while 4 rats (80%) did not have evidence of nephrotoxicity.

There appears to be an increase in the incidence of severe toxicity as the dose of Aloe vera was increased from 0.2ml to 0.4ml and then to 0.8ml over 14 days of administration (Table 1). Over 28 days however, the increase in the incidence of severe renal toxicity does not extend to 0.8mls (Table 1). Over 42 days, the incidence of severe renal toxicity is nil for the 3 doses administered. Similarly, none of the 3 doses of Aloe vera induced severe nephrotoxicity over 42 days. The incidence of severe renal toxicity thus appears to be time- and

dose-dependent, the lower doses and shorter durations of treatment, causing more nephrotoxicity than the highest dose and longest duration of treatment.

Heart

The histopathological findings in the heart were interstitial oedema, infiltration of cardiac tissue by chronic inflammatory cells, congestion of cardiac vessels and myocardial necrosis.

Table 3 summarizes the cardiac effects induced by *Aloe vera plus*. 5 rats received 0.2ml twice daily for 14 days out of which one rat (20%) had evidence of mild cardiotoxicity as indicated by infiltration of cardiac interstitium by chronic inflammatory cells. The other 4 rats (80%) did not have any evidence of cardiotoxicity. Of those that received 0.4ml twice daily, one rat (20%) had mild cardiotoxicity as shown by focal interstitial oedema, the other four (80%) did not have any evidence of cardiotoxicity. Of the 5 rats that received 0.8ml twice daily for 14 days, one (20%) had evidence of cardiotoxicity (myocardial oedema) and four (80%) did not.

After 28 days of *Aloe vera* plus administration, the following results were obtained: of the 5 rats that received 0.2ml twice daily, two (40%) had evidence of mild cardiotoxicity while 3 rats (60%) did not have any cardiotoxic effects. Amongst the 5 rats that received 0.4ml twice daily, one (20%) had mild cardiotoxicity, while the other 4 (80%) did not have any evidence of cardiotoxicity. Of the 5 rats that received 0.8ml twice daily, 2 (40%) had evidence of cardiotoxicity. Out of these two, one was of the severe type involving necrosis of the myocardium. Three rats (60%) did not have any evidence of cardiotoxicity.

After 42 days of drug administration all the 5 rats that received 0.2ml twice daily did not have any evidence of cardiotoxicity. This was the same for those that received 0.4ml and 0.8ml twice daily.

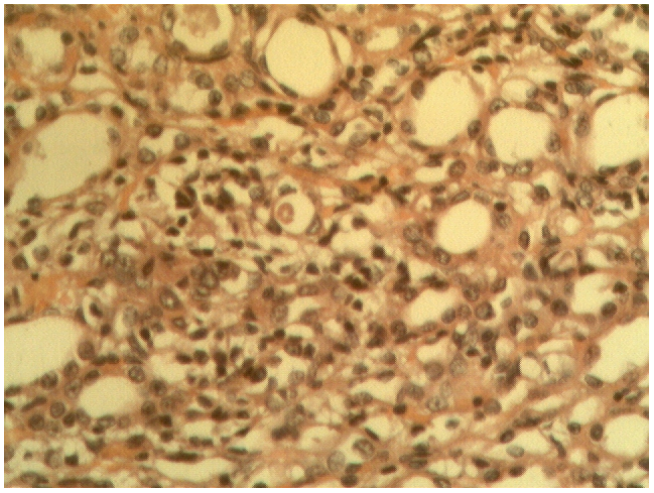

Figure 1: Photomicrograph of kidney tissue of control

Showing normal renal structure

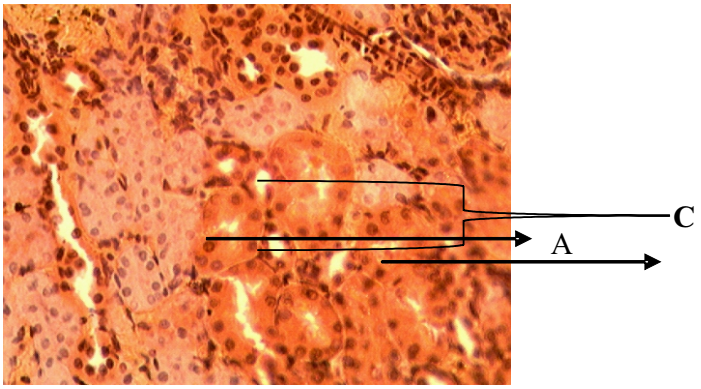

Figure 2: Photomicrogragh of
Kidney tissue after receiving 0.8ml of aloe vera plus for 28days showing (A) glomerular fibrosis, (B) diffuse renal tubular necrosis, hyalinization of tubules, (C) multiple foci of dystrophic calcification and infiltration by Lymphocytes.

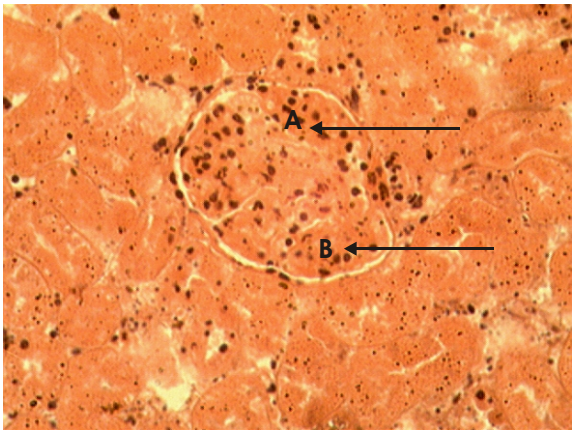

Figure 3: Photomicrogragh of Kidney tissue after 28days of administration of aloe vera plus for 28days showing (A) extensive glomerular and interstitial fibrosis with loss of glomeruli. Also (B) tubular necrosis, collapse and fibrosis

Table 1: Effect of duration of treatment with Aloe vera plus on kidney toxicity

Duration of Treatment	NUMBER OF RATS								
	No Toxicity			Mild to Moderate Toxicity			Severe Toxicity		
	0.2ml	0.4ml	0.8ml	0.2ml	0.4ml	0.8ml	0.2ml	0.4ml	0.8ml
14 days	2	2	2	3	2	1	0	1	2
28 days	2	2	3	2	0	1	1	3	1
42 days	2	3	2	3	2	3	0	0	0

Table 2: Effect of Increasing Dose of Aloe vera plus on Kidney Toxicity

Dose of Aloe vera Plus twice daily	NUMBER OF RATS								
	No Toxicity			Mild to Moderate Toxicity			Severe Toxicity		
	14days	28days	42days	14days	28days	42days	14days	28days	42days
0.2ml	2	2	2	3	2	3	0	1	0
0.4ml	2	2	3	2	0	2	1	3	0
0.8ml	2	3	2	1	1	3	2	1	0

Table 3: Effect of Aloe vera plus on the heart

Duration of Treatment	No Toxicity			Cardiotoxicity		
	0.2ml	0.4ml	0.8ml	0.2ml	0.4ml	0.8ml
14 days	4	4	2	1	1	1
28 days	3	2	2	2	0	2*
42 days	5	5	5	0	0	0

Cardiotoxicty manifested as focal interstitial oedema, myocardial oedema and necrosis of the liver. *One of these animals had necrosis of the myocardium

The pattern of cardiotoxicity seems to mirror that of kidney toxicity i.e., smaller doses causing more toxicity that higher doses and longer durations of treatment causing less toxicity than shorter durations of treatment.

DISCUSSION

Aloe vera plus induced nephrotoxicity, but only an insignificant number of rats had cardiotoxic changes as seen on histopathological slides. Nephrotoxicity ranged from mild to moderate e.g. chronic inflammatory cell infiltration and hyalinization of tubules, to severe nephrotoxicity characterised by tubular necrosis, interstitial fibrosis and glomerular fibrosis. These findings appear to be dose- and time-dependent.

There was an increase in the incidence of severe renal toxicity when the dose of Aloe vera plus was increased from 0.2ml twice daily for 14 days to 0.4ml twice daily for 14 days and then to 0.8ml twice daily for 14 days (Table 2). A similar increase occurred when the dose was raised from 0.2ml twice daily for 28 days to 0.4ml twice daily for 28 days. There was no corresponding increase however, when the dose was raised further to 0.8ml for 28 days. None of the doses administered, caused severe renal toxicity when the drug was given for 42 days.

These findings are in consonance with earlier reports that low doses of *Aloe vera* are associated with diarrhea, electrolyte imbalance, kidney dysfunction, hepatitis, contact dermatitis and conventional drug interactions[2,8,9]. Luyckx et al[15] have also reported a case of a man who developed acute oliguric renal failure and liver dysfunction following ingestion of a herbal remedy containing Cape aloes.

Infiltration by chronic inflammatory cells was the most common finding in the kidneys. This occurred equally at all doses and for all durations of drug administration. This chronic inflammatory response may have led to the glomerular/interstitial fibrosis seen on histopathological examination of the slides and which occurred in a time-dependent manner (no fibrosis being seen after 14 days of drug administration). *Aloe vera* is known to induce oxidative stress in aerobic cell systems[14]. This study demonstrated that sublethal doses of *Aloe vera* induced a decreased activity of thioredoxin reductase, glutathione reductase and glutathione peroxidase, resulting in a reduction in overall activity of redox enzymes[14]. We think the reduced protection from oxidative activity of free radicals may have induced the inflammation and subsequent infiltration of the tissues by chronic inflammatory cells and renal damage. *Aloe vera plus* thus appears to have antioxidant activity at high doses or following accumulation of the drug over time as there was less or no severe toxic changes at high doses and this antioxidant activity of aloe vera has been widely reported[10-12,16]. *Aloe vera* contains antioxidants such as APS-1 and dihydrocoumarin derivatives and it also assists the absorption of some antioxidants such as vitamins C and E[10-12]. The ethanolic leaf extract has also been reported to cause a significant lowering of blood sugar, thiobarbituric acid reactive substances, hydroperoxides and alphatocopherol, as well as a significant improvement in ascorbic acid, reduced glutathione and insulin in plasma of diabetic rats[7]. It was also associated with a significant reduction in superoxide dismutase, catalase and glutathione peroxidase with a resultant increase in reduced glutathione[7].

Animal studies have shown that Aloe vera gel extract, administered at a dose of 300mg/kg per day to streptozotocin-induced diabetic rats for a period of 21 days, effectively restored fatty acid compositions in the liver and kidney of diabetic rats[13]. It was observed that a significant reduction in hepatic transaminases, and tissue (liver and kidney) cholesterol, triglycerides, free fatty acids and phospholipids[13].

In the slides of the animals sacrificed on the 57th day, following the withdrawal of *Aloe vera plus* for the preceding 28 days indicated that most of the nephrotoxic changes were reversed except when insterstitial and/or glomerular fibrosis had occurred (Fig2,3).

In this study, the ability of *Aloe vera* to cause cardiotoxicity was minimal and insignificant but the mechanism(s) responsible appear to be similar to those responsible for renal toxicity.

CONCLUSION

This study demonstrated that GNLD"s *Aloe vera* plus is nephrotoxic, but was found not be significantly cardiotoxic.

Aloe vera plus appear to be significantly nephrotoxic causing severe renal damage (tubular necrosis and glomerular fibrosis) in a dose dependent manner. However, the more common mild to moderate nephrotoxicity such as chronic inflammatory cell infiltration appears to be neither dose nor time dependent. The nephrotoxic effects in this study appear to be reversible upon withdrawal of the drug except glomerular fibrosis which appears not to be reversible. The identity of the toxic components in the juice is not known.

RECOMMENDATIONS

We recommend that this product and all other so called nutritional supplements should be brought under more stringent regulation by food and drug regulatory authorities in various countries in order to ascertain both their effectiveness and safety. Secondly, we also recommend that the pharmacokinetic properties of *Aloe vera* be studied in detail in order to increase our understanding of the drug.

REFERENCES

1. Satish S, Raveesha KA, Janardhana GR. Antibacterial activity of plant extracts on phytopathogenic *xanthomonas campestris pathovars*. Lett Appl Microbiol, 1999; 28 (2): 145-147.
2. Boudreau MD, Beland FA . An evaluation of the biological and toxicological properties of *Aloe barbadensis miller* (*Aloe vera"*). J Environ Sci Health C Environ Carcinog Ecotoxicol Rev, 2006; 24 (1): 103-154.
3. Kahlon J, kemp MCX, Yawei N, Carpenter RH, McAnalley HR, Shannon WM, McDaniel BH. An evaluation of the synergistic antiviral effects of acemannan in combination with azidothymidine and acyclovir. Mol Biother, 1991; 3:214-223.
4. Hu Q, Hu Y, Xu J. Free radical-scavenging activity of *Aloe vera (Aloe barbadensis Miller)* extracts by supercritical carbon dioxide extraction. Food Chemistry, 2005; 91, 85-90.
5. Rajasekaran SK, Sivagnanam K and Subramanian S. Antioxidant effect of Aloe vera gel extract in streptozotocin-induced diabetes in rats. Pharmacol. Rep, 57:90-96.
6. Bezakova L, Oblozinsky M, Sykorova M, Paulikova I and Kostalova D. Antilipoxygenase activity and the trace elements content of Aloe vera in relation to the therapeutical effect. Ceska Slov Farm, 2005; 54(1): 43-46.
7. Rajasekaran SK, Sivagnanam K and Subramanian S. Modulatory effects of aloe vera leaf gel extract on oxidative stress in rats treated with streptozotocin. J Pharm Pharmacol, 2005; 57: 241-246.
8. Ernst E. Adverse effects of herbal drugs in dermatology. BJD, 2000; 143 (5): 923-9.
9. Rabe C, Musch A, Schirmacher P, Kruis W, and Hoffmann R. Acute hepatitis induced by an aloe vera preparation: a case report. World J Gastroenterol, 2005; 11(2): 303-304
10. Vinson JA, Al Kharrat H and Andreoli L. Effect of Aloe vera preparations on the human bioavailability of vitamins C and E. Phytomedicine, 2005; 12(10): 760-765.
11. Zhang XF. Isolation, structure elucidation, antioxidative and immunomodulatory properties of two novel dihydrocoumarins from Aloe vera. Bioorg Med Chem Lett, 2006; 16(4): 949-953.
12. Wu JH, Xu C, Shan CY and Tan RX. Antioxidant properties and PC12 cell protective effects of APS-1, a polysaccharide from Aloe vera var clinensis. Life Sci 2006; 78(6): 622-630.
13. Rajasekaran S, Sivagnanam S, Ravi K and Subramanian S. Beneficial effects of aloe vera leaf gel extract on lipid profile status in rats with streptozotocin diabetes
14. Sirdaata J and Clark IE. Effect of Aloe barbadensis Miller juice on oxidative stress biomarkers in aerobic cells using artenia franciscana as a model. Phytotherapy Research, 2010; 24(3): 360-364.