International Journal of Research In Pharmaceutical sciences and Technology

Preclinical toxicological evaluation of Aloe vera health drinks in wistar rats

P. Sudhakar*, V. Vinoth Prabhu, B. Jamuna, R.S. Adithya, Lakshmi soman, Anu Joy, R. Anand

Department of Pharmacology, Swamy Vivekanandha College of Pharmacy, Tiruchengode, Tamil Nadu, India

ABSTRACT

Human consumption of Aloe vera as a beverage has recently increased in popularity. These benefits are controversial with some sources pointing out that the putative effects of aloe are unsupported by clinical studies; it is important that marketed products be tested for toxicities following oral consumption. Hence this study was designed to evaluate the toxicological effect of marketed aloe health drinks. Thirty either sex Wistar rats (200-300gm) were enrolled in this study and are divided into 5 groups. Group I receives Normal saline serves as vehicle control, Group II and III receives Product A- Low dose (0.5 ml twice daily, p.o) and High dose (1.0 ml twice daily, p.o) respectively. Group IV and V receives Product B- Low dose (0.5 ml twice daily, p.o) and High dose (1.0 ml twice daily, p.o) respectively. Weekly body weight and daily feed intake were measured. On 28th day total urine output volume, faecal consistency, Haematological, biochemical, and organ weight were measured to assess the toxicity of aloe health drinks. The result of this study shows that continuous usage of aloe health drinks showed milder weight reduction, significant improvement in erythropoiesis also it increases the WBC count and increases the weight of spleen it may confirm the immune modulatory effect of aloe health drink. At the higher doses, it increased the SGOT, SGPT, serum urea and creatinine it may lead to the hepatotoxicity and nephrotoxicity. In gastrointestinal tract on prolonged uses, it produced few lesions and diarrhoea. It might be concluded that prolonged consumption of unprocessed aloe health drink contains latex, an ingredient which has many health risks associated with it. So it can aggravate health problems.

Keywords: Aloe drinks; Haematological; Biochemical; Gastrointestinal toxicity.

ISSN: 2581-9143
Research Article

Corresponding Author

Name: Mr. P. Sudhakar
Email: sudhakar00pharma@gmail.com
Contact: + 91-9600616196

Article Info

Received on: 06-07-2018
Revised on: 21-08-2018
Accepted on: 30-09-2018

INTRODUCTION

Herbal medicines are obtained from plant or herb sources, but the active ingredients can still be potent chemicals. Because of this, herbal supplements can have an adverse effect and drug interactions, even with each other or with food or alcohol. Not all of the plants reported to be useful are harmless. Herbal products contain pharmacologically active ingredients, some of which have been associated with adverse effects [1].

However, available herbal products have no clear statement of content or medically related information on the package labels, and have not been validated or certified by any recognized body. This concerns consumers and medical practitioners who may unknowingly counter-prescribe these herbal products. Herbal drugs associated toxicity can occur because of various factors *viz.* presence of contaminants, incomplete processing, drug-herb interactions, coexisting diseases, direct toxicity (dose/duration), improper herb identification, and preparation method[2].

Among this Aloe vera juice as a beverage of human consumption has recently increased in popularity. Because of its numerous pharmacological properties like skin care medicine, dyspepsia, amenorrhea, burns, colic, hyper adenosis, hepatopathy, splenopathy, constipation, abdominal tumors, dropsy carbuncles, sciatica, lumbago, flatulence, anti-inflammatory, antidiabetic, and immunity enhancement. Apart from that anticancer, antibacterial and antiviral properties

also reported in aloe vera preparations[3]. These potential properties are may be due to the presence of natural source which has a lot of nutritional and medicinal properties. The Aloe vera plant can be utilized in three basic forms: Aloe gel, aloe latex, and the whole plant extract[4].

Aloe vera is known to have 75 nutrients that are documented: 20 minerals, 20 amino acids, and 12 vitamins. Minerals found are calcium, chromium, copper, iron, magnesium, manganese, potassium, phosphorus, sodium, zinc, and selenium these trace elements are essential for growth. Amino acids are the building blocks of proteins and muscle tissues. There are 12 essential amino acids present in Aloe vera; they are alanine, arginine, asparagine, cysteine, glutamic acid, glycine, histidine, proline, serine, tyrosine, glutamine, and aspartic Acid. Vitamins are defined as a group of organic micronutrients that are essential for normal metabolism. Vitamins present in Aloe vera are vitamin A, B (Thiamine), B_2 (Riboflavin), B_3 (Niacin), B_5, B_6 (Pyridoxine), B_{12}, vitamin C, E, choline and folic Acid. Vitamin B complex and C are considered to play an important role in reducing stress and inflammation. Vitamin B_{12} is required for the production of blood cells. Enzymes present in Aloe vera are ligase, amylase, catalase, bradykinase, lipases, and proteases. Among these constituents, anthraquinones are the most important active ingredient of high medical values. The biological activities of Aloe vera are due to the synergistic action of a variety of compounds, rather than a single defined compound[5]. Chronic abuse of anthraquinone stimulant laxatives can lead to hepatitis and electrolyte disturbances (hypokalemia, hypocalcaemia), metabolic acidosis, malabsorption, weight loss, albuminuria, and haematuria. Weakness and orthostatic hypotension may be exacerbated in elderly patients when stimulant laxatives are repeatedly used[6-7].

Applying aloe vera gel is considered safe, but consuming unprocessed juice extracted from the latex can cause several side effects leading to major health risks. Like Aloe vera juice contains a substance called anthraquinone, a laxative, which can cause diarrhoea when consumed in large amounts. Severe diarrhea can cause pain, cramps, and dehydration. So this screening is mainly focused on the subacute toxicity of marketed aloe health drinks in Wistar rats.

MATERIALS AND METHODS

Drugs and Chemicals

The two different manufacturer marketed product of aloe health drinks were purchased and included for toxicological screening. These are labelled as Product A and Product B. All the chemicals used in this study were of analytical grade.

Selection of Animals

The colony inbred adult both male and female Albino Wistar Rats, weighing 200-300gm were used in this study. The animals were kept under standard environmental conditions of 12/12 light/dark rhythm, maintained at controlled room temperature (23±2^{0}C) and a relative humidity of 60%±10%, in polypropylene cages. They were fed with standard pellet diet and water *ad libitum.* Each cage contained 3 rats of the same sex with a bedding of husk. The immature animals were acclimatized under laboratory conditions three days prior to initiation of the experiment. The cages were cleaned daily by changing the husk bedding. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Swamy Vivekanandha College of Pharmacy, Elayampalayam, Namakkal-637205, care and use of laboratory animals were confirmed to CPCSCEA guidelines (Approval no: IAEC Reference No: SVCP/IAEC/UG/1/05/2016).

Experimental design

Thirty Wistar rats weighing 200 to 300gm were randomly divided into five groups of six per each. Group I, vehicle control receives normal saline; Group II, receives 0.5 ml of product A at low dose twice daily; Group III, receives 1ml of product A at high dose 1.0 ml twice daily; Group IV, receives 0.5 ml of product B at low dose twice daily, Group V, receives 1 ml of product B at high dose twice daily.

Body Weight

Body weight of each rat in all groups was measured weekly till continuation of the treatment using a weighing balance and the changes was recorded.

Urine output

Urine output volume was measured in all the treatment on the 28th day, each animal was placed in an individual metabolic cage 24 hr prior to the commencement of the study for adaptation. Animals are placed in the metabolic cages, specially designed to separate urine and faeces and kept at 21°C±0.5°C. The total volume of urine collected for 5 hr was measured at the end. During this period free assess of food and water was made available to animals[8].

Examination of faecal consistency

Throughout the study period, the faecal consistency and frequency were the monitor. On 28th day after administration of health drinks animals were placed in cages, where the floor was lined with non-wetting paper sheets of uniform weight. Non-wetting paper sheets were changed every hour up to 6 hours. Characteristic diarrhoeal droppings of every hour up to the 6th hour were recorded after draining the urine by gravity[9].

Haematology

On the 28th day of the study, the animals were anesthetized with diethyl ether. The blood was drawn through retro-orbital plexus and collected into Ethylene diamine tetraacetic acid (EDTA) anticoagulant

tubes. The blood was analyzed following haematological parameters which included red blood cells (RBC) count, white blood cells (WBC) counts, haemoglobin (Hb) content, and packed cell volume (PCV) using standard laboratory methods as described by Ghai[10].

Biochemical parameters

On 28th day biochemical analyses were performed in serum obtained after centrifugation of blood without anticoagulant at 3000 rpm for 10 min. The analysis of serum glutamate pyruvate (SGPT) and glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP)[11], total bilirubin, Urea and creatinine[12-13] were estimated in serum by standard laboratory techniques.

Organ weight

At the end of the study all the animals were sacrificed and vital organs viz. liver, kidney, spleen, heart, brain, uterus, and testes were removed and subjected to gross examination and later weighed. Intestinal tract was removed and subjected to gross examination.

Statistical analysis

The data represents as mean ± SEM of six replicated determinations. Results were analyzed statistically by one-way ANOVA followed by post hoc Dunnet's test by using SPSS V.17 (Student trail version). The difference was considered significant when $p<0.05$.

RESULTS AND DISCUSSION

Aloe vera is a succulent cactus plant, belonging to the family Liliaceae. It is widely known as "the Miracle plant" for its various medical, cosmetic and nutraceutical purposes. Aloe vera gel is a slick substance that is extracted from the interior of the aloe vera leaf, while latex refers to the yellow part which lies beneath the leaf skin. Using aloe vera gel is considered safe, but consuming unprocessed juice extracted from the latex can cause several side effects leading to major health risks. Aloe vera juice contains a substance called anthraquinone, a laxative, which can cause diarrhea if taken in large amounts. Severe diarrhea can cause pain, cramps, and dehydration. However, because of increased consumption of aloe vera drinks, it is important that marketed products have to be tested for toxicities following oral consumption.

In this study, we have selected two marketed product of Aloe health drink from two different manufacturers for oral toxicological screening. At end of the study body weight of Group-II and Group-III (product A-low dose and high dose), Group-V (product B-high dose) exhibited a reduction in body weight when compared to the initial body weight. It presented in Table 1.

On long term usage of aloe health drinks show slight weight reduction. Urine output volume was significantly ($P<0.001$) decreased in the Aloe heath drink treatment when compared to the control group it represented in Table 2.

It might be due to dehydration because of the laxative effect of the anthraquinone glycoside content. It increases intestinal water content by reducing the absorption of water from intestinal lumen by inhibiting the activity of Na^+, K^+ adenosine triphosphatase and stimulate water secretion by increasing the paracellular permeability across the colonic mucosa[14]. The net result is a reduction in water absorption and the formation of softer stools[15] and also the loss of fecal consistency (diarrhoea) is may be due to the unprocessed juice extracted contain anthraquinone, Aloe emodin-9-anthrone and Aloe emodin. Intestinal lesions in aloe health drink fed treatment group also may due to the long-term consumption of unprocessed latex contain anthraquinone in aloe juice. Diarrhoea was seen in the treatment groups III, IV, and V when compared to the vehicle control whereas faecal consistency was normal. Milder liquefied stools are seen in group II. It was represented in Figure 1.

Aloe health drink slightly increased the RBC count, WBC count, haemoglobin concentration and PCV was increased significantly. Higher Hb, PCV and RBC values in the rats that received the Aloe drinks is an indication that it stimulated erythropoiesis in those rats, although its potency as a haematinic is low. It presented in Table 3.

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[16]. In this study, serum enzymes SGOT and SGPT in aloe health drink higher dose treated groups of rats exhibited a significant change in comparison with control groups. Liver cell damage is characterized by rise in these serum enzymes[17]. It might be due to long-term usage of aloe health drink may lead to liver toxicity.

This study revealed that prolonged use of a higher dose of aloe induces a significant increase in serum Urea and creatinine. This is in consonance with the study of Avila *et al*[18]. reported that the cytotoxic effect of aloe. This study suggests that aloe promotes nephrotoxicity, thus causing impaired renal function evident by an increase in serum urea and creatinine concentration Table 4.

The relative weight of Liver, heart, brain, uterus and testes did not show any evidence of toxicity and also no significant changes in weight as compared to control rats. Kidney weight was significantly increased in aloe drink treatment which may reveals that prolong usage of aloe health drink, may lead to nephrotoxicity. Spleen weight also shows increases it might be due to the immunomodulatory effect of aloe health drinks. Table 5

Table 1-5: Values were expressed as mean±SEM, n=6. Symbols represent statistical significance: ***p<0.001, **p <0.01, *p<0.05

Table 1: Changes in body weight

Treatment	Initial body weight (g)	Final body weight (g)
Group-I (Vehicle control)	251.67±17.01	258.33±12.49
Group-II (Product A- Low dose)	253.30±12.56	243.33±4.21
Group-III (Product A- High dose)	253.33±10.85	238.33±8.33
Group-IV (Product B- Low dose)	248.33±12.22	261.67±10.14
Group-V (Product B- High dose)	258.33±20.40	248.40±14.00

Table 2: Effect of Aloe health drinks on urine output volume

Treatment	Total Urine Volume (ml/kg b.wt./5 h)
Group-I (Vehicle control)	6.6±0.64
Group-II (Product A- Low dose)	2.0±0.39***
Group-III (Product A- High dose)	1.7±0.30***
Group-IV (Product B- Low dose)	2.9±0.30***
Group-V (Product B- High dose)	1.7±0.48***

Table 3: Effect of Aloe health drinks on haematological parameters

Treatment	RBC (10^6/mm^3)	WBC (10^3/mm^3)	Hb (g %)	PCV (%)
Group-I (Vehicle control)	6.52±0.15	5.55±0.3	11.4±0.17	38.75±1.39
Group-II (Product A -Low dose)	7.01±0.41	7.31±0.36***	13.1±0.49***	41.17±2.38
Group-III (Product A- High dose)	7.82±0.34*	8.30±0.58***	14.2±0.32***	55.35±2.91***
Group-IV (Product B- Low dose)	7.22±0.45	7.49±0.50***	12.7±0.43**	45.25±2.00
Group-V (Product B- High dose)	7.75±0.61*	9.18±0.36***	14.5±1.31***	53.02±2.56***

Table 4: Effect of Aloe health drinks on Biochemical parameters

Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Bilirubin (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Group-I (Vehicle control)	85.28±3.32	41.87±2.60	140.15±12.89	0.13±0.01	27.97±1.19	0.50±0.04
Group-II (Product A-Low dose)	92.36±2.38	50.57±1.56	150.23±10.74	0.14±0.01	32.16±2.19	0.50±0.04
Group-III (Product A-High dose)	97.88±4.53*	51.15±3.64*	147.47±13.49	0.14±0.01	47.78±2.14***	0.71±0.04**
Group-IV (Product B-Low dose)	92.55± 3.20	51.97±3.29**	142.33±13.34	0.14±0.01	27.63±1.83	0.61±0.05
Group-V (Product B-High dose)	129.35±13.27***	62.47±4.07***	150.20±10.89	0.13±0.01	48.21±2.79***	0.73±0.04***

Table 5: Effect of Aloe health drinks on Organ weight (g/100 gm body weight)

Treatment	Liver (g/100)	Kidney (g/100)	Heart (g/100)	Brain (g/100)	Spleen (g/100)	Uterus (g/100)	Testes (g/100)
Group-I (Vehicle control)	4.31±0.10	0.77±0.02	0.31±0.01	1.08±0.03	0.22±0.01	0.50±0.01	3.00±0.09
Group-II (Product A-Low dose)	4.34±0.13	0.82±0.01*	0.31±0.01	1.09±0.04	0.32±0.01***	0.50±0.01	3.10±0.11
Group-III (Product A-High dose)	4.37±0.13	0.84±0.02 **	0.31 ±0.01	1.17±0.06	0.39±0.03***	0.49±0.01	3.17±0.08
Group-IV (Product B-Low dose)	4.40±0.12	0.82±0.02*	0.32±0.02	1.13±0.06	0.22±0.01	0.50±0.04	3.04±0.06
Group-V (Product B-High dose)	4.35±0.16	0.85±0.01***	0.32±0.02	1.06±0.04	0.47±0.03***	0.48±0.02	3.07±0.06

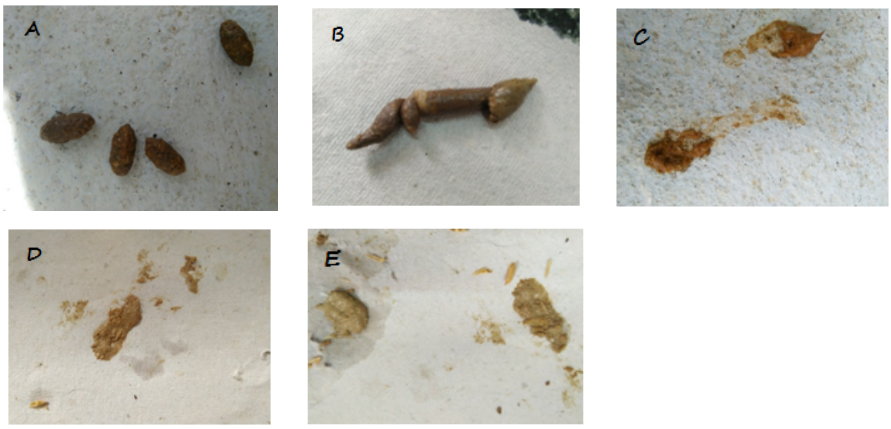

Figure 1: Effect of Aloe health drink on faecal consistency-Group-I (vehicle control); Group-II (product A-low dose); Group-III (product A-high dose); Group-IV (product B-low dose); Group-V (product B-high dose)

CONCLUSION

It might be concluded that prolong consuming of unprocessed aloe health drink contains latex, an ingredient which has many health risks associated with it. It can aggravate health problems like colitis, Crohn's disease, appendicitis, diverticulosis, intestinal obstruction, hemorrhoid, stomach pains, and ulcers. At the higher doses, it increases the SGOT, SGPT, serum urea and creatinine it may lead to the hepatotoxicity and nephrotoxicity. In a gastrointestinal tract on prolong uses, it produces few lesions and diarrhoea also observed in treatments. So proper standardization of aloe health drink is crucial for achieve its putative benefits

REFERENCES

1. Russmann S, Lauterburg BH, Helbling A. Kava hepatotoxicity. Ann Intern Med. 2001; 135: 68–9.
2. Naveen P, Shankar BG, Srikanth V, Laxmaiah C, Prathapani S. A review: herb-drug interactions. Indo Am J Pharm Res. 2011; 1: 364-372.
3. Pankaj KS, Deen Dayal G, Ritu Singh, Priyanka P, Sharmistha G, Atul Kumar S, et al. Therapeutic and Medicinal Use of Aloe vera: A Review. Pharmacol Pharm. 2013; 4: 599-610.
4. Udo NV, Effiong OO, Out OV, Olusola AE, Oleba OE. Comparative effects of Aloe vera gel and aqueous leaf extract of viscum album on bilirubin extraction in Streptozotocin-induced diabetic rats. Int J Biochem Res Rev. 2014; 4:99-115.
5. Sandeep kumar, Yadav JP. Ethnobotanical and pharmacological properties of Aloe vera: A review. J Med Plant Res. 2014; 8: 1387-1398.
6. Godding, EW. Therapeutics of Laxative Agents with Special Reference to the Anthraquinones. Pharmacol. 1976; 14: 78-101.
7. Muller Lissner SA. Adverse Effects of Laxatives: Facts and Fiction. Pharmacol. 1993; 47: 138-145.
8. Lipschitz WL, Hadidian Z, Kerpcsar A. Bioassay of diuretics. J Pharmacol Exp Ther. 1943; 79: 97–110.
9. Akter R, Hasan S, Hossain MM, Jamila M, Hoque ME, Rahman M. In vitro antioxidant and in-vivo antidiarrhoeal activity of hydromethanolic extract of Xanthium indicum Koenig Leaves. Eur J Sci Res. 2009; 33: 305–312.
10. Ghai CL. A textbook of practical physiology. Jaypee brothers Medical publishers (P) Ltd: New Delhi, 1993.
11. Sallie R, Tredger JM, Williams R. Drugs and the liver Part 1: Testing liver function. Biopharm. Drug Dispos. 1991; 12: 251-259.
12. Henry RJ, Cannon DC, Winkelman JW. Clinical Chemistry Principles and Techniques. Happer and Row Publishers: New York, 1974, p. 1629.
13. Pearlman FC, Lee RTY. Detection and measurement of total bilirubin in serum with use of surfactants as solubilizing agents. Clin Chem. 1974; 20: 447–453.
14. Ishii Y, Tanizawa H, Takino Y. Studies of Aloe III. Mechanism of cathartic effect. Chem Pharm Bull. 1990; 38:197-200.
15. Boudreau MD, Beland FA. An Evaluation of the biological and toxicological properties of Aloe barbadensis (Miller), Aloe vera. J Environ Sci Health. 2006; 24:103-154.
16. Hamman JH. Composition and application of Aloe vera leaf gel. Molecules. 2008; 13:1599-1616.
17. Brautbar N, Williams J. Industrial solvents and liver toxicity: risk assessment, risk factors and mechanisms. Int J Hyg Environ Health. 2002; 205: 479–491.

18. 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.