Safety evaluation of *Aloe vera* pulp aqueous extract based on histoarchitectural and biochemical alterations in mice

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Received 26 February 2015; revised 19 January 2016

*Aloe vera* has a long history as a popular herbal remedy. A scrutiny of literature reveals that it possesses beneficial as well as harmful effects, thus making it essential to assess its toxicity profile before employing it as a medicinal agent. Various authors have reported conflicting observations on different applications of *Aloe vera* in experimental animals. Qualitative phytochemical analysis of *Aloe vera* extract used in the present study revealed the presence of tannins, carbohydrates and anthraquinone glycosides. For assessing the toxicity profile, Balb/c mice were divided into four groups—control and *Aloe vera* treated @ 250, 100 and 50 mg/kg body weight, and were administered with respective doses for 30 days. Histopathological examination of various tissues revealed significant structural alterations on administration of higher doses of *Aloe vera* indicating the toxicity, which was also associated with enhanced peroxidative damage and serum LDH activity. Low dose of *Aloe vera* revealed normal histoarchitecture of various tissues along with biochemical parameters studied. Considering these results 50 mg/kg body weight *Aloe vera* extract is considered safe and shall be employed for further studies.

**Keywords:** Cosmetics, Herbal, Histopathology, Lactate dehydrogenase, Lipid peroxidation, Nutraceuticals

*Aloe vera* (syn. *Aloe barbadensis* Miller) is a stemless, long, thick, succulent plant with turgid green fleshy leaves joined in a rosette pattern, belonging to the family Liliaceae or Asphodelaceae1. *Aloe vera* has been used as traditional medicine in India, Egypt, China, and different European countries since time memorial2. It has multiple medicinal uses as laxative, anti-helmintic, anti-hemorrhoid, uterine stimulant and antiseptic3. Various authors describe *Aloe vera*’s skin and the inner lining of its leaves as a cold, bitter remedy which is downward draining and is used to cure constipation due to accumulation of heat4. *Aloe vera* is also widely used in the cosmetics, nutraceuticals and pharmaceutical products5. *Aloe vera* gel contains glucomannan, acemannan, anthraquinone, glycoproteins, glucose, linolic acid, saponins, lignin, sterols, cholesterol, amino acids, vitamins; minerals, etc.6 and several of these have significant biological activities. The bioactivities of various constituents of *Aloe vera* have been reported as immunomodulatory, antimicrobial, anti-inflammatory, antidiabetic and wound-healing, etc.5,7,8.

Anthraquinones and polysaccharides present in *Aloe vera* exhibit antioxidant and free radical scavenging activity9. Acemannan has been reported to reduce skin reactions induced by radiation in mice10. Yu et al.11 have demonstrated antioxidant activities of polysaccharides which rendered beneficial effects against oral ulcers in animals. Lupeol, campesterol and sitosterol are aspirin like compounds present in *Aloe vera* that exhibit anti-inflammatory property12. Haque et al.13 revealed that this miraculous plant also contains minerals such as Ca, Mg, Mn, Zn, etc. which play important roles in several bodily functions. *Aloe*-emodin has been reported to inhibit cell growth in several tumors cell lines14 and showed high specificity for neuroectodermal tumor cells15. *Aloe*-emodin has been also shown to inhibit lipid peroxidation16 indicating its antioxidant potential. *Aloe vera* has also been shown to be a potential therapeutic agent for the treatment of sepsis and hepatotoxicity17. *Aloe vera* leaf extract was found to have damage-resistant properties against whole body radiation-induced deleterious effects as evident by the alterations induced in oxidative stress markers and antioxidant defense system in various tissues18.
Even though Aloe vera has been used for medicinal purposes since long time, its beneficial effects are contentious considering the ambiguous reports available in literature. Literature survey reveals that use of Aloe vera is associated with both beneficial and detrimental effects. Various studies have reported both antioxidant and pro-oxidant potential of Aloe vera extract, their action being dependent on their concentration\(^1\). Both low dose and high dose of Aloe vera have been reported to cause side effects but severity is more pronounced with high dose\(^6\). Rabe et al.\(^20\) reported incidence of diarrhea, electrolyte imbalance, kidney dysfunction, hepatitis, contact dermatitis, photosensitivity and a number of drug interactions as side effects on administration of low dose of Aloe vera extract. Consumption of Aloe whole-leaf powder resulted in pigmentation in renal tubular, mesenteric lymph nodes and lamina propria of the colonic mucosa, and proliferation in mesenteric lymph nodes\(^21\). Acute renal failure and acute hepatitis has been reported with 500 mg/kg body wt. Aloe vera consumption\(^22\). There are a number of discrepancies about the dose of Aloe vera and the effects observed thereof. Negative effects of Aloe vera (100 and 300 mg/kg body wt.) against paracetamol induced toxicity showed deleterious histopathological alterations in liver\(^23\). Carcinogenic potential of Aloe vera has also been reported\(^6\). Aloe vera gel has also shown chemomodulatory action on carcinogen metabolizing enzymes and antioxidant defense system in animal model\(^24\).

Considering these dual reports of detrimental as well as beneficial effects, it becomes essential to standardize the safe dose of Aloe vera in order to explore its medicinal potential against various diseased conditions with minimal ill effects. Therefore, the present study was undertaken to standardize the safe dose of Aloe vera for possible intervention against various diseases.

**Materials and Methods**

**Aloe vera preparation**

Aloe vera gel extract was prepared by a modified method of Gehlot et al.\(^25\). Fresh leaves of Aloe vera were collected from botanical garden of Panjab University, Chandigarh. The colourless gel was separated from the thick outer green cuticle. The gel was dried in oven at 60°C and powder was obtained. The powder was dissolved in double distilled water and centrifuged at 5000 \(\times g\) for 10 min. The supernatant was collected and filtered. The filtrate was dried and powdered form of Aloe vera was obtained. The extract was reconstituted in double distilled water immediately before oral administration to the experimental animals as described earlier\(^26\).

**Phytochemical screening of Aloe vera extract**

Aloe vera gel extract was subjected to screening for phytochemical compounds as per the method described by Koul et al.\(^27\).

**Experimental design**

Healthy male Balb/c mice in the weight range of 25-30 g procured from Central Animal House, Panjab University, Chandigarh, India were housed in polypropylene cages bedded with sterilized rice husk. Mice in all the groups were maintained on standard animal pellet diet (Ashirwad Industries Ltd., Ropar, Panjab, India and water ad libitum throughout the experiment. The temperature of the animal room was maintained at 21±1°C, humidity 50-60% and a 12 h dark and light cycle. All the experimental protocols were approved by the Institutional Ethics Committee (Panjab University, Chandigarh, India) and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals. Mice were randomly divided into four groups depending upon the treatment they received (n=5). Group I animals served as control and received no special treatment. Groups II-IV animals were given oral administration of Aloe vera extract at a dose level 250, 100 and 50 mg/kg body wt., respectively for 30 days. After completion of respective treatments, animals were autopsied by cervical dislocation after 24 h of the last day of treatment. Various tissues were excised out and analyzed for lipid peroxidation (LPO) levels, lactate dehydrogenase (LDH) activity and histopathological alterations.

**Histopathological studies**

Briefly, after the completion of various treatments, liver, spleen, kidney and testes were excised out and fixed in 10% formalin and embedded in paraffin, sectioned (5μm) and then stained with hematoxylin and eosin to investigate various histopathological alterations.

**Lactate dehydrogenase (LDH)**

The LDH activity was estimated according to the method described by Bergmeyer and Bernt\(^28\). LDH catalyses the reduction of pyruvate with NADH to form NAD+ which was measured as a
decrease in absorbance at 340 nm. The enzyme activity was defined as nanomoles of NADH consumed/min/mg protein using an extinction coefficient of 6.22 mM$^{-1}$ cm$^{-1}$.

**Lipid peroxidation (LPO)**

The assay for LPO was performed according to the method as described earlier$^{29}$. This method involved the measurement of the pink colour of TBA-MDA chromophore produced by the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA) whose absorbance was read at 532 nm spectrophotometrically. The amount of MDA formed was calculated by using an extinction coefficient of 1.56 × 10$^5$ M$^{-1}$ cm$^{-1}$. The results were expressed as nanomoles of MDA-TBA chromophore formed/mg protein/30 min.

**Results**

**Phytochemical screening of Aloe vera extract**

The qualitative phytochemical examination of aqueous Aloe vera gel extract used in the present study revealed the presence of anthraquinone, carbohydrates and tannins (Table 1). The phytochemicals were qualitatively analyzed based on the presence or absence of colour change indicated as positive or negative results.

**Analysis of histoarchitectural alterations**

Histopathological analysis of liver from control animal revealed normal histoarchitecture comprising of innumerable lobules consisting of a vast inter anastomosing network with single cell thick plates of hepatocytes separated by vascular sinusoids along with vascular channels radiating out from central veins Fig. 1A. Our investigation showed Aloe vera extract treated animals (250 and 100 mg/kg body wt.) (Fig. 1 B and C) revealed, cell degeneration, enlargement of central vein with congested sinusoids, while low dose of Aloe vera 50 mg/kg body wt. exhibited normal histoarchitecture of liver of mice. Spleen from control animal revealed normal tissue architecture (Fig. 2A), which included normal areas consisting of white pulp with germinial center surrounding lighter marginal zone, and red pulp. White pulp contained lymphoid aggregations, mostly lymphocytes, and macrophages which were arranged around the arteries. Red pulp had plenty of vascular sinuses. There were connective tissues termed as trabeculae. Aloe vera treatment, groups I and II (250 and 100 mg/kg body wt.) (Fig. 2 B and C)
revealed widespread infiltration in white pulp, decreased white pulp, increased red pulp, dilated sinusoids, while group III animals (50 mg/kg body wt.) (Fig. 2D) revealed normal histoarchitecture with normal areas of white pulp with germinal center and surrounding lighter marginal zone, and red pulp.

Histopathological examination of kidney from control animal revealed normal histoarchitecture (Fig. 3 A and B). A gross section of kidney corpuscle revealed two distinguished regions i.e. outer cortex and inner medulla. Outer cortex consist of a tuft of capillaries lined with epithelial cells, proximal and distal convoluted tubule (PCT and DCT) which are enclosed within a fibrous capsule called Bowman’s capsule known as Renal corpuscle. The thick and thin parts of loop of Henle and greater part of collecting ducts are present in inner medulla comprised of renal pyramids as explained. Group I and II animals treated with Aloe vera extract @250 and 100 mg/kg body wt. (Fig. 3 C-F) resulted in alterations in renal tissue architecture which included atrophy of renal corpuscle, decrease in glomerular cellularity, reduction in number of Bowman’s capsule, glomerular congestion with increased Bowman’s spaces, inflammatory cell infiltration/glomeruli infiltration in cortex region and crowding of nuclei with hyperplastic response in inner medulla was observed. Group III animals were treated with 50 mg/kg body wt. Aloe vera extract (Fig. 3 G and H) showed normal cortical labyrinth and medullary region.

Histopathological analysis of testes from control animal revealed normal architecture (Fig. 4A) which included lumen of seminiferous tubules with normal spermatogonia which are in direct contact with epithelial basal lamina, diploid primary spermatocytes, spermatids with sperms in the lumen, testosterone producing leydig cells, sertoli cells enclosed in round, thick and fibrous capsule called tunica albuginea. Group I and II animals (Fig. 4 B and C) disturbed the normal histological features resulting in wide spectrum of damage in testes like shrunken tubules, disorganized and distorted seminiferous tubules, depletion in cell population, cellular inflammation, abnormal widening of interstitial spaces were observed, while testes of animals treated with (50 mg/kg body wt.) Aloe vera extract (Fig. 4D) revealed normal histoarchitecture.

Serum lactate dehydrogenase
A significant increase was observed in serum LDH activity upon administration of Aloe vera (250 and 100 mg/kg body wt.) animals when compared to control group. On the other hand, at the concentration of Aloe vera (50 mg/kg body wt.) LDH activities remained unaltered when compared with control group (Table 2).
Administration of Aloe vera showed significantly increased level of LPO in liver, spleen, kidneys, testes and blood as compared to control group, while at the concentration of (100 mg/kg body weight) resulted significant increase in LPO levels in kidneys and blood, when compared with the control group. Aloe vera (50mg/kg body weight) did not alter the LPO levels in all the tissues when compared with the control group (Table 3).

**Discussion**

Numerous studies have been conducted to determine the potential of Aloe vera in ameliorating cancer, diabetes, wounds and burns, indigestion, ulcers, radiation damage, age related changes etc. These varied biological activities exhibited by Aloe vera may be attributed to the synergistic action of various components present in it rather than by single isolated substance. The results indicate that it possesses beneficial, detrimental and no effects in several conditions. Several studies have reported contrasting results, with some suggesting that Aloe vera possesses toxic potential and others reporting no adverse effects which may be an effect of the dose of Aloe vera employed. Thus, keeping in view, the complexities inherent in Aloe vera pharmacology and the inconsistencies reported in literature regarding its safety and effectiveness, the present study was undertaken to standardize the dose of Aloe vera for possible medicinal effects.

The phytochemical examination of aqueous Aloe vera gel extract in this study showed the presence of anthraquinone, carbohydrates and tannins contradicting Mariappan and Shanthi who had observed absence of tannins and anthraquinones. Anthraquinones and tannins may act as antioxidant and free radical scavengers. The phytochemicals investigated, as shown in this study, have been reported to possess strong antioxidant activities due to their ability to absorb, quench free radicals and decompose peroxides generated in the system. Carbohydrates present in Aloe vera have exhibited immunomodulatory activity and anthraquinones glycosides are powerful purgative and potent antimicrobial agent. Tannins are phenolic compounds known to exhibit analgesic and antioxidant, anti-inflammatory, anti-microbial activities.

After the completion of treatment with various doses of Aloe vera, the tissues were analyzed for histoarchitectural alterations. Control animals exhibited normal histoarchitecture of liver, spleen, kidney and testes. Treatment with higher dose of Aloe vera extract (250 and 100 mg/kg body wt.) caused detrimental changes in the histoarchitecture of various tissues when compared to control animals and animals.
treated with low dose (50 mg/kg body wt.) of *Aloe vera* extract. Liver section of animals treated with high dose of *Aloe vera* extract revealed enlargement of central vein with congested sinusoids as compared to control animals and animals treated with low dose of *Aloe vera* extract. Decreased white pulp with infiltration, increased red pulp, dilated sinusoids were prominent in spleen of animals treated with high dose of *Aloe vera* extract. The animals treated with higher concentration of *Aloe vera* extract showed atrophy of renal corpuscle, decrease in number of Bowman’s capsule, increase in shrinkage of Bowman’s capsule, inflammatory cells infiltration in cortex region, attenuation of glomeruli, glomerular congestion with increased Bowman’s spaces. A wide spectrum of damage in testes like shrunken tubules, disorganized and distorted seminiferous tubules, depletion in cell population, cellular inflammation, abnormal widening of interstitial spaces were observed in animals treated with high concentration of *Aloe vera* extract as compared to the control animals and animals treated with lower dose of the extract. Similar kind of histoarchitectural alterations indicating negative effects have been reported with metal and drug induced toxicities.

Serum lactate dehydrogenase (LDH) activity is a marker of cell damage and has been employed in various toxicity studies. Subsequent to tissue or cell damage causes leakage of LDH enzyme out of the cell, and thus serum LDH activity may be elevated. Rise in serum LDH activity, has been associated with lung tissue injury. Increased activity of LDH was observed in patients with acute myocardial infarction (AMI). LDH activity was significantly elevated in rat liver when treated with paracetamol. Doxorubicin administered to rats resulted in significant increase in LDH activity in testes and was used as a tissue injury marker. Increase in LDH activity has been associated with NDEA induced hepatotoxicity. In the present study, increased serum LDH activity was observed upon treatment with high dose of *Aloe vera* extract compared to the control group. No significant alteration was observed in serum LDH activity of low dose group (Gr. III) compared to its control counterparts. The increase in LDH activity in animals of high dose *Aloe vera* group may be related to the histoarchitecture damage observed in the various tissues studies.

Lipid peroxidation is a process which involves the formation and propagation of lipid radicals which causes a rearrangement of the double bonds in unsaturated lipids and the eventual disruption of the intracellular membranes and cellular damage. It is a process generated naturally in small amounts in the body, mainly by the effect of several reactive oxygen species (ROS). These ROS readily attack the polyunsaturated fatty acids of the fatty acid membrane, initiating a self-propagating chain reaction. The destruction of membrane lipids and the end-products of such lipid peroxidation reactions alter the viability of cells, even tissues. *Aloe vera* (250 mg/kg body wt.) treated animal revealed significantly increased lipid peroxidation levels in liver, testes and blood as compared to the control and animals administered with low dose of *Aloe vera* extract.

Aloin and aloe-emodin may act as either pro-oxidant and antioxidants dependent on their concentration. It has been reported that *Aloe vera* extract @ 100 and 70 mg/kg body wt. resulted in decreased sperm motility and sperm counts while 30 mg/kg body wt. caused no change in sperm count and motility in male rats. Adverse effects of *Aloe* whole-leaf powder have been reported at concentrations of 2 g/kg body wt. Pregnant women are advised not to take *Aloe* latex because of its cathartic action, which may cause severe uterine contractions and increase the risk of miscarriage. It should also not be ingested by nursing mothers because of the possibility of causing severe cramps and diarrhea in the infant. Excessive consumption of *Aloe vera* cause adverse effects in human. *Aloe vera* acts as a diuretic and it can cause cardiovascular and kidney problems. The side effect of *Aloe vera* has been reported because of chronic exposure of high levels of *Aloe latex*. In high doses there was a decrease in red cell count and significant sperm damage in mice. The present study was undertaken to establish the toxicity profile of *Aloe vera* in experimental animals which render strong evidence for its safety against various diseases.

**Conclusion**

It is evident that administration of high dose (250 and 100 mg/kg body wt.) of *Aloe vera* extract to mice caused histoarchitectural damage in liver, testes, spleen and kidney which was associated with enhanced LDH activities and LPO levels. The low dose (50 mg/kg body wt.) of *Aloe vera* extract appeared to be safe and did not exhibit any drastic alterations when compared to the control counterparts, and may thus be employed for further studies. It may be inferred from the present study as well as other
reports available in literature that the potential efficacy of Aloe vera in the treatment of particular disorders is complicated by differences in dosage and duration of consumption.

Acknowledgment

The authors gratefully acknowledge the financial support provided by University Grant Commission—Rajiv Gandhi National Fellowship, New Delhi, India (RGNF-SC-CHA-3794 2011-12) for carrying out the present work.

References


