Effect of *Aloe vera* (*Aloe barbadensis* Miller) gel on doxorubicin-induced myocardial oxidative stress and calcium overload in albino rats

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Administration of a single dose of doxorubicin (DOX) (7.5 mg/kg, iv) produces cardiotoxicity, manifested biochemically by significant decrease in blood glutathione (GSH) and tissue GSH along with elevated levels of serum lactate dehydrogenase (LDH) and serum creatine phosphokinase (CPK). In addition, cardiotoxicity was further confirmed by significant increase in lipid peroxides expressed as malondialdehyde (MDA, secondary indicator of lipid peroxidation), tissue catalase and tissue superoxide dismutase (SOD). Administration of *A. vera* gel (100 and 200 mg/kg) orally for 10 days produced a significant protection against cardiotoxicity induced by DOX evidenced by significant reductions in serum LDH, serum CPK, cardiac lipid peroxides, tissue catalase and tissue SOD along with increased levels of blood and tissue GSH. The results revealed that *A. vera* gel produced a dose dependent protection against DOX induced cardiotoxiaty.

Keywords: *Aloe vera* gel, Antioxidants, Free Radicals, Magnesium, Polysaccharides

Aloe vera (L.) Burm. f (*Aloe barbadensis* Miller) belongs to Lileacea family, of which there are about 360 species. Aloe vera has been promoted for large variety of conditions like diabetes mellitus, hyperlipidemia, inflammation, treatment of acne and has come to play a prominent role as a contemporary folk remedy[1]. The fresh leaves of *A. vera* are used to obtain two components, firstly bitter yellow latex from peripheral bundle sheath of aloe, called *A. vera* sap, *A. vera* juice or aloes. Aloes contain anthraquinone derivatives (aloe emodin) and their glycosides (aloin) which are known for their cathartic effect[2]. Aloes also contains amino acids, auxins, gibberellins, minerals, vitamins, an aspirin like compound, magnesium lactate and various enzymes like superoxide dismutase (SOD) and catalase[1]. Secondly, a mucilaginous gel from the parenchymatous tissue in the leaf pulp of *A. vera* has been used since early times for the topical treatment of burns and wounds[3]. *A. vera* gel can be used in skin diseases, constipation, inflammation, cancer, ulcer, diabetes and as a free radical scavenger[4-6]. Present day's *A. vera* gel has been extensively utilized as functional food in preparing food health drinks and beverages[7]. Despite the extensive history and popular acceptance of dermatological products containing aloe and proven potential as antioxidant, only few studies have discussed role of *A. vera* gel in doxorubicin (DOX) induced oxidative stress.

DOX is an anthracycline anticancer drug, effective against wide range of malignancies like leukemia, lymphomas and several solid tumours. Dose dependent cardiotoxicity limits its use, resulting from the overwhelming production of reactive oxygen species (ROS) with concomitant calcium overload. The present investigation has therefore undertaken to evaluate antioxidant potential of *A. vera* gel (marketed preparation, promoted as health drink) and vitamin E (positive reference)[8] against DOX induced oxidative stress and calcium overload. Magnesium (physiological antagonist of calcium) content in *A. vera* gel was determined with the help of atomic absorption spectroscopy to evaluate its role in calcium overload during DOX toxicity.

Materials and Methods

Plant material—Aloe vera gel was purchased from Nature Forever Living Products International, Arizona, USA.

Drugs and chemicals—Vitamin E was procured from Hi Media, Mumbai, India. DOX was a gift sample from

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Dabur Research Foundation, Ghaziabad, India. Diagnostic kits of lactate dehydrogenase (LDH) and creatine phospokinase (CPK) were purchased from Span Diagnostics Ltd. Surat, India. All other chemicals were of the highest product grade.

Phytochemical screening—*A. vera* gel was concentrated by boiling and solidifying. A solution of solidified aloe (1%) was prepared in water and subjected to various chemical tests for identification of its variety[9]. The chemical constituents in the *A. vera* gel were screened qualitatively using standard phytochemical reagents and procedures[10]. In general, tests for the presence or absence of phytochemical compound using the above methods involve the addition of an appropriate chemical agent to the crude material in a test tube. The mixture was then shaken vigorously or gently as the case may be. The presence or absences of various chemical constituents (glycosides/carbohydrates/vitamins/enzymes/proteins etc.) were observed.

Proximate analysis—The recommended methods of the Association of Official Analytical Chemist[11] were employed in the determination of the levels of ash, crude fat, crude protein and crude fibre. Ash content was determined by incinerating the sample in a muffle furnace at $550^{\circ}C$. Crude fat was estimated gravimetrically following soxhlet extraction. Fibre content was estimated by acid/alkali hydrolysis of the insoluble residue and crude protein was estimated by Kjeldahl method using a conversion factor of 6.25. The moisture content was estimated by the AOAC method no. 934. Percentage carbohydrate was given by difference, according to the formula [carbohydrate = 100 – (% ash + % moisture + % fat + % protein)] and energy content was finally determined by the formula [energy content = 4% protein + 9% fat + 4% carbohydrate]. All the experiments were done in triplicate.

Mineral analysis—Magnesium analysis was done in triplicate using an Atomic Absorption Spectrophotometer (AAS)[12]. A 1.0 g sample was ashed in a muffle furnace at 550°C for 5 h until a white residue of constant weight was obtained. The minerals from the residue were extracted by adding 20 ml of 2.5% HCl. Mixture was heated on a steam bath to reduce the volume to 7 ml, and this was transferred quantitatively to a 50 ml volumetric flask. It was diluted to 50 ml volume with deionised water, stored in clean polyethylene bottles and mineral contents determined using an AAS (Perkin-Elmer, Analyst 400, Massachusetts, USA).

Toxicity studies

Sub-acute toxicity study—Albino rats (20, 150-200 g) of either sex were divided into two groups. The first group received vehicle normal saline (control) and the second group received *A. vera* gel in a dose of 200 mg/kg (po) daily. Both groups of rats were kept under similar laboratory conditions and were allowed to take usual pellet diet and water *ad libitum*. The animals were observed for their general condition, gross behaviour, body weight etc. All the animals were sacrificed on the 15th day; the viscera were removed and examined for the histopathological changes including gastric erosion in stomach.

Acute toxicity studies—Mean lethal dose or LD_{50} was calculated for the *A. vera* gel after administration through oral route. Swiss albino mice (25-40 g) of either sex were subjected to fasting overnight but the animals had free access to water. A pilot study was carried out before the main experiment and based on these findings, doses of 1, 2, 3, 4, 5, 6 and 7 g/kg were selected for the final study. Animals were divided into 7 groups of 10 each. *A. vera* gel in the above mentioned doses were given orally, as a single dose. Mortality was observed after a 24 h period. The LD_{50} was calculated by the arithmetic method of Parmar and Ghosh[13].

Experimental protocol—Albino rats (200-250 g) from the Central Animal House facility (Reg. 173/2005/CPCSEA) of Jamia Hamdard were kept in polypropylene cages under standard conditions of temperature (25° ± 1°C) with 12:12 h L:D cycle and had a free access to a commercial pellet diet (Amrut Labortary Rat and Mice Feed, Navmaharashtra Chakan Oil Mills Ltd., Pune, India) and water *ad libitum*. Animals were randomized and divided into following 9 groups of 6 animals each. All test drugs were given every day at the dose levels mentioned for 10 days and DOX was given on fifth day after starting the treatment. Gr. I: normal control (0.9 % saline, 1 ml/kg, po), Gr. II: vehicle (10 % acacia) of test drugs, Gr. III: *A. vera* gel (100 mg/kg, po), Gr. IV: *A. vera* gel (200 mg/kg, po), Gr. V: vitamin E (100 mg/kg, po), Gr. VI: toxic control (DOX 7.5 mg/kg, iv), Gr. VII: *A. vera* gel+ DOX (100 mg/kg, po + 7.5 mg/kg, iv), Gr. VIIII: *A. vera* gel+ DOX (200 mg/kg, po + 7.5 mg/kg, iv), Gr. IX: vitamin E + DOX (100 mg/kg, po + 7.5 mg /kg, iv). Animals were subjected to biochemical and histopathological estimations on eleventh day.

Biochemical estimations—Blood samples were collected from retro orbital plexus and subjected for the blood glutathione (GSH)[14], serum LDH[15] and serum CPK[16] estimations. Rats were sacrificed by decapitation under light ether anaesthesia. Hearts were removed and weighed. Small sections from the hearts were fixed in 10% buffered formalin (neutral, buffered 10%w/v in phosphate buffer) for histopathological studies and remaining heart samples were subjected to tissue thiobarbituric acid reactive substances (TBARS)[17], tissue GSH[18], tissue catalase[19], tissue SOD[20] estimations.

Histopathological observation—Heart sections (5 µm) fixed in 10% neutral buffered formalin and were subjected to haematoxylin and eosin staining[21].

Statistical analysis—All data were presented as mean ± SD and analyzed by one way ANOVA followed by Dunnett's test's for the possible significance identification between the various groups. $P<0.05$ was considered statistically significant. Statistical analysis was carried out using Graph pad prism 3.0 (Graph pad software, San Diago, CA)

Results

Phytochemical and proximate analysis—Chemical test confirmed that the *A. vera* gel belongs to *Aloe barbadensis* and preliminary phytochemical analysis demonstrated the presence of glycosides (prominently C-glycosides/anthraquinone glycosides), carbohydrates (both monosaccharide and polysaccharides), proteins, amino acids, tannins and phenolic compounds, vitamins (vitamin C and D) and

enzymes (peroxidase and catalase). The proximate analysis of the *A. Vera* gel (*p*H 4.8±0.31) revealed the high percentage of moisture (64.57±3.24%) and carbohydrate by difference (13.51±0.58%) along with ash (13.59±0.78%), crude fat (3.87±0.54%), proteins (4.46±0.32%) and crude fiber (14.01±1.58%). The energy content of the *A. Vera* gel was calculated to be 1.9±0.14 cal/100 g.

Toxicity studies—Sub-acute toxicity studies did not reveal any untoward effect of the *A. vera* gel on behavioural, body weight, normal reflexes and visceral appearance in rats. The gel was well tolerated up to 3 g, while 100% mortality was observed at 6 g when given by oral route. The LD_{50} of *A. Vera* gel was found to be 4.8 g.

Mineral analysis—Results from the atomic absorption study revealed 2.12×10^3 mg of Mg/ 100g of *A. vera* gel.

Anthropometric and biochemical estimations—All rats injected with DOX (7.5 mg/kg, iv) showed a significant ($P < 0.001$) decrease in food and water intake along with decrease in body weight as compared to control animals (Table 1). Significant reduction in the ratio of heart weight/body weight in toxic control was observed ($P < 0.01$) in comparison to vehicle control. Concomitant administration of the *A. vera* gel significantly increased the ratio of heart weight/body weight. Significant increase in serum LDH and CPK levels was observed in the animals treated with DOX in comparison to normal control. Blood and tissue GSH levels in the normal control animals were 2.42±0.45 mg% and 11.90±1.13 µmol/g wt of tissue, respectively and treatment with DOX significantly decreased the blood and tissue GSH

Table 1—Effect of *A. vera* gel, vitamin E and DOX on anthropometric measurements in different groups

[Values are mean ± SD for 6 animals in each group]

Group	Treatment	Food intake (g)	Water intake (ml)	$\frac{\text{Heart wt.}}{\text{Body wt.}} \times 100$ (g)
I	Normal (normal saline, 1ml/kg, po)	123.30 ± 5.21 [b]	207.50 ± 8.52[c]	0.33 ± 0.03[b]
II	Vehicle control (10% acacia, 1ml/kg, po)	121.70 ± 3.21 [b]	210.50 ± 11.25[c]	0.34 ± 0.03[b]
III	*A. vera* gel (100 mg/kg, po)	127.58 ± 6.52 [a]	209.58 ± 10.25[c]	0.34 ± 0.03[b]
IV	*A. vera* gel (200 mg/kg, po)	116.67 ± 7.24 [b]	208.33 ± 9.26[c]	0.31 ± 0.03[b]
V	Vitamin E (100 mg/kg, po)	148.09 ± 4.44 [a]	234.58 ± 11.85[a]	0.36 ± 0.03[a]
VI	DOX (7.5 mg/kg, iv)	70.00 ± 13.52	165.00 ± 19.11	0.23 ± 0.03
VII	*A. vera* gel (100 mg/kg, po)+ DOX (7.5 mg/kg, iv)	107.92 ± 11.28 [b]	185.25 ± 8.24[c]	0.31 ± 0.03[c]
VIII	*A. vera* gel (200 mg/kg, po)+ DOX (7.5 mg/kg, iv)	120.08 ± 9.52 [b]	218.18 ± 7.55[c]	0.33 ± 0.03[b]
IX	Vitamin E (100 mg/kg, po)+ DOX (7.5 mg/kg, iv)	123.64 ± 17.25 [b]	215.00 ± 19.41[c]	0.34 ± 0.03[b]

Statistical significance compared to toxic control (group-VI) by Dunnett test (*P* values: [a]< 0.001, [b]< 0.01, [c] < 0.05)

levels (Table 2). In the hearts of the DOX treated rats, there was a significant increase in the TBARS generation in comparison to the control indicating ROS generation and oxidative stress (Table 3). SOD and catalase activity in the DOX treated animals was increased (Table 3).

Histopathology—Heart section of the normal rats (Gr. I), show normal myocardial fibre (Fig. 1a). Focal areas of degeneration in myocardium with scanty infiltration and vacuoles were seen in animals treated with DOX only (Fig. 1b). Mild to moderate degenerative changes were observed in animals treated with *A. vera* gel (100 mg/kg) along with DOX (Fig. 1c). Minimal degenerative changes were found in animals of toxic groups (Gr.-VIII and IX) treated with gel (200 mg/kg) and vitamin E showing normal myocardial muscle cells with minimal vacuoles seen in isolated cells (Fig. 1c and d).

Discussion

Processing of *A. Vera* gel from leaf pulp has grown as a huge industry worldwide and improper processing techniques may lead products containing either very less or virtually no active ingredients, particularly polysaccharides. Preliminary chemical studies confirmed that it is suitable for further studies.

High moisture content with carbohydrate is responsible for imparting the moisturizing effect to the gel. Phytochemical analysis showed that carbohydrates present were mainly polysaccharides and together with water, polysaccharide forms a jelly like consistency that holds the water, providing a sustained moist environment. This property accounts for the skin hydrating effect of the gel and so, its use in various cosmetic preparations. Results from the toxicity studies suggest that it is also well tolerated.

Table 2—Effect of *A. vera* gel, vitamin E and DOX on serum LDH, CPK, Blood GSH and tissue GSH levels in different groups.

[Values are mean ± SD for 6 animals in each group]

Group	Treatment	Serum LDH (IU/L)	Serum CPK (IU/L)	Blood GSH (mg%)	Tissue GSH (μmol/g wt of tissue
I	Normal (normal saline, 1ml/kg, po)	209.82 ± 5.21[a]	37.76 ± 5.47[a]	2.42± 0.45[a]	11.90 ± 1.13[a]
II	Vehicle control (10% acacia, 1ml/kg , po)	208.65 ± 10.21[a]	37.18 ± 3.28[a]	2.06 ± 0.21[a]	12.39 ± 1.54[a]
III	*A. vera* gel (100 mg/kg, po)	205.66 ± 8.52[a]	37.57 ± 3.56[a]	2.61 ± 0.21[a]	13.50 ± 1.87[a]
IV	*A. vera* gel (200 mg/kg, po)	198.52 ± 6.32[a]	37.18 ± 4.98[a]	2.29 ± 0.49[a]	13.73 ± 0.39[a]
V	Vitamin E (100 mg/kg, po)	207.56 ± 6.58[a]	39.08 ± 6.58[a]	2.33 ± 0.48[a]	12.76 ± 0.99[a]
VI	DOX (7.5 mg/kg, iv)	561.85 ± 15.48	102.43 ± 9.54	1.16 ± 0.24	7.12 ± 0.94
VII	*A. vera* gel (100 mg/kg, po)+ DOX (7.5 mg/kg, iv)	322.98 ± 9.88[a]	52.11 ± 7.59[a]	1.47 ± 0.39	8.43 ± 0.1.85
VIII	*A. vera* gel (200 mg/kg, po)+ DOX (7.5 mg/kg, iv)	248.77 ± 13.24[a]	47.40 ± 8.57[a]	2.37 ± 0.57[a]	13.01 ± 1.59[a]
IX	Vitamin E (100 mg/kg, po)+ DOX (7.5 mg/kg, iv)	203.04 ± 9.99[a]	41.98 ± 5.46[a,]	2.11 ± 0.49[a]	12.40 ± 1.26[a]

Statistical significance compared to toxic control (group-VI) by Dunnett test (P values: [a]< 0.001, [b]< 0.01, [c] < 0.05)

Table 3—Effect of *A. vera* gel, vitamin E and DOX on myocardial TBARS, catalase, SOD levels in different groups.

[Values are mean ± SD for 6 animals in each group]

Group	Treatment	TBARS (Nanomoles of MDA/mg of protein)	SOD (units of SOD/mg of protein)	Catalase (Nanomoles of H_2O_2 /min/mg of protein)
I	Normal (normal saline, 1 ml/kg, po)	3.36 ± 0.41[a]	24.26 ± 0.78[a]	51.66 ± 4.25[a]
II	Vehicle control (10% acacia, 1 ml/kg, po)	3.41 ± 0.12[a]	24.05 ± 0.95[a]	50.63 ± 7.52[a]
III	*A. vera* gel (100 mg/kg, po)	3.78 ± 0.38[a]	23.58 ± 1.08[a]	53.38 ± 6.59[a]
IV	*A. vera* gel (200 mg/kg, po)	3.44 ± 0.26[a]	22.55 ± 0.66[a]	51.50 ± 7.55[a]
V	Vitamin E (100 mg/kg, po)	3.30 ± 0.34[a]	22.36 ± 0.87[a]	51.12 ± 5.29[a]
VI	DOX (7.5 mg/kg, iv)	20.38 ± 1.11	29.19 ± 1.88	178.09 ± 8.17
VII	*A. vera* gel (100 mg/kg, po)+ DOX (7.5 mg/kg, iv)	8.31 ± 0.98[a]	26.24 ± 0.98[b]	85.97 ± 11.28[a]
VIII	*A. vera* gel (200 mg/kg, po)+ DOX (7.5 mg/kg, iv)	4.36 ± 0.45[a]	25.42 ± 0.99[b]	61.94 ± 5.11[a]
IX	Vitamin E (100 mg/kg, po)+ DOX (7.5 mg/kg, iv)	3.89 ± 0.33[a]	24.93 ± 0.89[a]	49.94 ± 5.34[a]

Statistical significance compared to toxic control (group VI) by Dunnett test (P values: [a]<0.001; [b]<0.01; [c]<0.05)
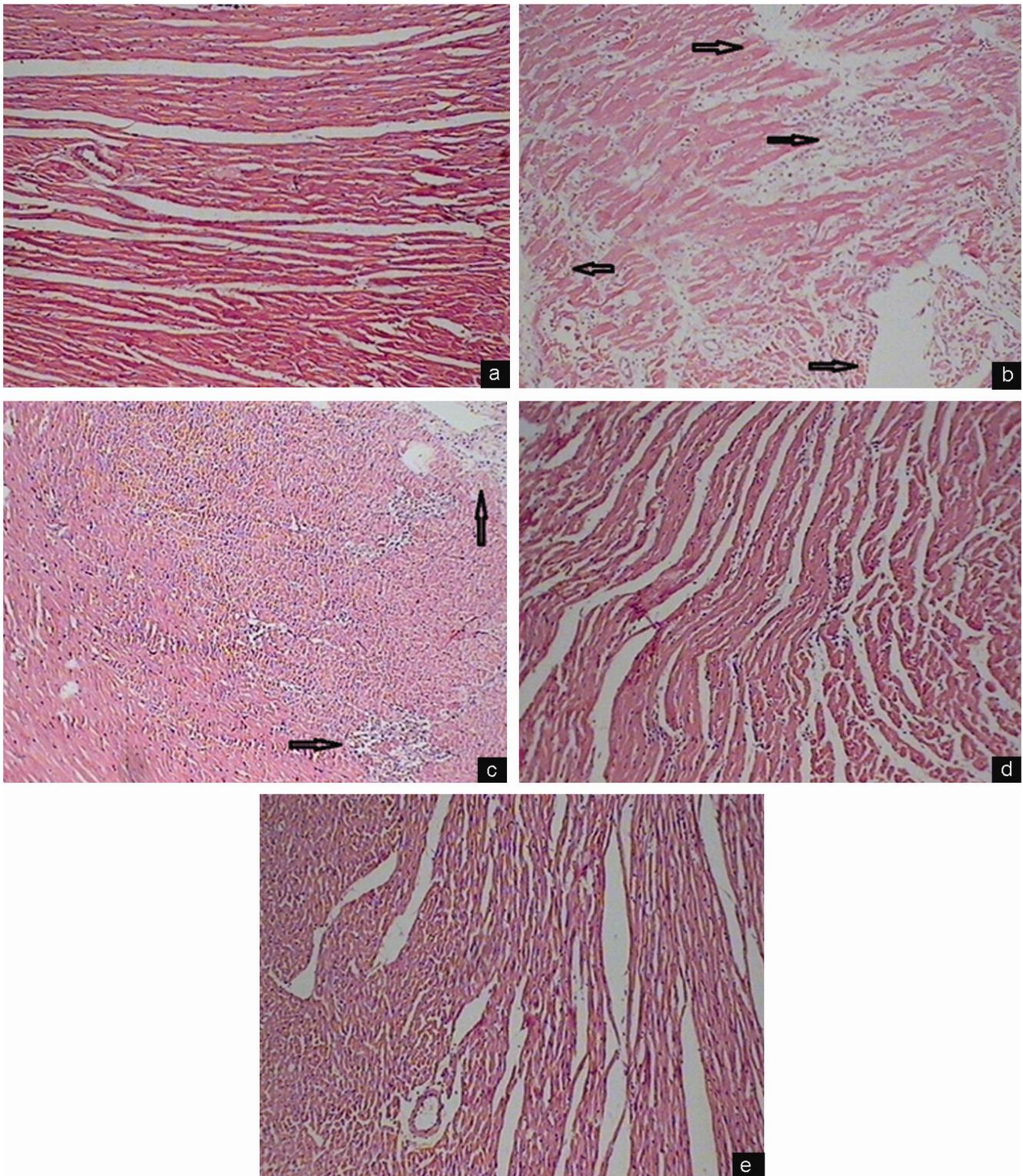
Fig. 1—Effect of *A. vera* gel, vitamin E and DOX on cardiac cell histopathology in different groups [(a) Gr. I: normal control rat's heart section, showing normal myocardial fiber, (b) Gr. VI: Photomicrograph of heart sample from animal treated with DOX (7.5 mg/kg) only showing marked degenerations, (c) Gr. VII: *A. vera* gel (100 mg/kg) + DOX (7.5 mg/kg) treatment showing degeneration, (d) Gr. VIII: *A. vera* gel (200 mg/kg) + DOX (7.5 mg/kg) treatment showing normal myocardial cells, (e). Gr. IX: Vitamin E (100 mg/kg) + DOX (7.5 mg/kg) treatment showing normal myocardial cells.]

Despite its potential cardiotoxicity, DOX (anthracycline antibiotic) is often used in the treatment of a wide range of human malignancies. However, new and low toxicity anthracycline derivates or liposome encapsulated drugs have influenced the anthracycline induced cardiotoxicity in many ways. Several hypotheses explain the mechanism of anthracycline induced cardiotoxicity and most of them suggested the crucial role of free radicals and calcium ions[22,23]. In biological systems, DOX is known to produce highly reactive oxygen species (ROS) like superoxide and hydroxyl radicals[24], having potential to damage intracellular components. Cardiac muscles are particularly much susceptible to the damage caused by these ROS because they contain very low levels of ROS detoxifying enzymes like SOD, glutathione peroxidase and catalase[25] therefore, antioxidants play a critical role in inactivation of free radicals[26].

DOX induced decrease in food and water intake, weight loss and ratio of heart weight/body weight indicate that the gel can alter the oxidative stress arising due to DOX treatment.

The quinone structure of anthracycline permits these compounds to act as an electron acceptor, the transfer being mediated by various flavoprotein enzymes. Mitochondrial enzymes (e.g. LDH and CPK) have been shown to activate DOX to form the semiquinone radical and superoxide anion. The plasma LDH and CPK enzyme activities are important to measure both early and late phase of cardiac injury, although these enzymes are non specific for measuring cardiac injury but together they could be an indicator of myocardial injury. The DOX induced cardiotoxicity is secondary event following lipid peroxidation of cardiac membranes, leading to increase in leakage of LDH and CPK from cardiac myocytes to plasma. Animals treated with DOX exhibited significant increase in the plasma LDH and CPK activities in comparison to control. Concomitant treatment with the gel and vitamin E produced significant decrease in plasma LDH and CPK levels, which could be possibly explained that the gel exerts antilipid peroxidation activity causing stabilization of cardiac membranes from the peroxidative damage thus preventing the leakage of LDH and CPK.

GSH may play an important role in protecting the heart from peroxidative attack[24]. DOX significantly decreased the level of tissue and blood GSH, which is in accordance with the earlier studies[27,28]. Decrease in the levels of GSH represents its increased utilization by the cardiac cells due to oxidative stress and treatment with the gel and vitamin E significantly helped to restore the levels of GSH, this effect could be attributed either to increased biogenesis of GSH or reduction in the levels of oxidative stress.

DOX has been reported to increase the malondialdehyde (secondary indicator of lipid peroxidation) due to increased free radicals production which reacts[29] with polyunsaturated fatty acids in cell membranes leading to lipid peroxidation. This observation suggests that DOX and its metabolite produce free radical species that attack lipid components, leading to lipid peroxidation and coadministration of the *A. vera* gel significantly prevented the increase in the TBARS levels in DOX treated animals, which was comparable to the standard vitamin E.

The antioxidant enzymes such as SOD and catalase constitute the major supportive team of defense against free radicals. SOD scavenges the superoxide radicals (generated due to redox cycling of DOX) by forming H_2O_2 and molecular oxygen[30]. Most of the past studies have reported the decreased levels of SOD, due to oxidative stress[31] in toxic controls. However, in the present study the activity of the SOD was significantly increased in DOX treated animals. Increase in SOD activity could be due to the physiological compensatory mechanisms to combat oxidative stress. Treatment with the gel significantly normalized the SOD levels in dose dependent manner, comparable to standard vitamin E, suggesting the possible antioxidant potential of the gel in counteracting DOX induced oxidative stress. Catalase is a hemeprotien, which catalyse the reduction of H_2O_2 (produced due to scavenging effect of SOD) and protect the tissue from highly reactive hydroxyl radical. Thus, simultaneous increase in the catalase activity is essential with increase in SOD activity to counteract the deleterious effects of DOX, which was observed in the present study. Concomitant administration of *A. vera* gel, altered the catalase level significantly towards normalization. Above observations were also supported by the histopathological findings.

The existing experimental evidences suggested that the DOX induced oxidative stress was primarily due to the production of free radicals (superoxide and hydroxyl radical) in the cardiac tissue, having potential to damage various intracellular components. Treatment with the gel provides significant protection to the DOX induced oxidative stress, reflecting the

antioxidant potential of the same. Dose dependent antioxidant potential of the gel in variety of animal models[32-34] attributed primarily to the presence of polysaccharides. Kardosova and Machova[35] isolated two neutral polysaccharides HF and A from the *Aloe barbadensis* gel, having high proportion of mannose component (80 and 83 mol%) with low proportions of glucose (11.6 and 2.4 mol%) and galactose (7.1 and 4.7 mol %). The *in vitro* experiments with HF and A prevented the peroxidation of soyabean lecithin liposomes by $^{\bullet}$OH radicals generated in a fenton reaction[35]. In an another study, Chun hui *et al*,[36] isolated compositionally two polysaccharides GAPS-1 and SAPS-1 from the *A. vera* gel irrigated with sea water. Primary composition was found to be mannose, galactose and glucose in a ratio of 120:3:2 and 296:1:36, respectively. Biologically these polysaccharides exhibited significant free radical scavenging and antioxidant activities *in vitro*[36].

Reports also confirm the presence of glutathione peroxidase activity[37], SOD enzyme[38] and phenolic antioxidants (anthraquinone analogues)[39] providing an added advantage to the antioxidant property of the gel. Co-supplementation of the gel significantly changed the altered tissue levels of the enzymatic defence compared to DOX treated rats. This may indicate the usefulness of the gel, as an excellent source of antioxidant in modulating DOX induced cardiotoxicity.

The unexplored area of the DOX induced cardiotoxicity is intracellular calcium overloading[40,41]. However, mechanisms for intracellular calcium accumulation are not clear. It was suggested that DOX impairs the calcium handling of sarcoplasmic reticulum and this contributes to DOX induced late cardiotoxicity[42]. It was also suggested that interference with mitochondrial calcium regulation and irreversible decrease in mitochondrial calcium loading capacity causes calcium accumulation and loss of myocardial function in DOX treated patients[43]. Calcium antagonists effectively inhibit anthracycline-mediated lipid peroxidation[44-46].

Results from atomic absorption study revealed 2.12 × 10^3 mg of Mg/100 g of *A. vera* gel and magnesium being physiological antagonist of calcium, could help in counteracting the calcium overload due to DOX. At the cellular level, magnesium is a cofactor for sodium-potassium ATPase, which is responsible for potassium flux across the myocyte membrane and maintenance of resting membrane potential[47]. Thus an increase in magnesium further increases negative membrane resting potential, which reduces myocardial excitability. Magnesium infusion also increases the absolute refractory period and decreases the relative refractory period and thereby decreases the vulnerable period[48]. Moreover, magnesium interferes with the slow inward calcium current in cardiac myocytes and atrioventricular node and thereby reduces the tendency for transient depolarization or calcium-induced injury caused by calcium overload, especially with reperfusion of ischemic myocardium[49,50].

In conclusion, the treatment with *A. vera* gel reduced the DOX induced cardiotoxicity by multiple mechanisms. Various polysaccharides, antioxidant enzymes, polyphenolic antioxidants and probably magnesium accounts for the cardioprotective effect of the gel. The present is the first report for the gel exhibiting significant cardioprotective activity with previous reports stating antioxidant potential. However, more studies are needed to comment on the exact role of magnesium from *A. vera* gel against DOX induced calcium overload, which are in progress.

Conflict of interest

Authors declare that there is no conflict of interest

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