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Protective Effect of *Aloe vera* Against Cadmium Induced Toxicity on Liver of Swiss albino mice

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ABSTRACT

Cadmium is a lethal heavy metal that can cause diverse tissue toxicities and pathologies. Conversely, *Aloe vera* is a natural anti-oxidant that can ameliorate cytotoxic effects of tissue toxicants. In this study, the objective was to assess the protective effect of *Aloe vera* against Cadmium-induced toxicity in liver of Swiss albino mice. Mice were divided into 3 groups of 4 animals each. This current study was performed by the Morphological observations and by the analysis of biochemical parameters in mice as affected by the oral administration of a single dose equivalent to 1/20 from LD50 (100 mg/kg B.W.) of Cadmium individually for 21 days. The results from present study showed a significant ($P < 0.05$) reduction of the body weight total cholesterol and total Protein due to Cadmium exposure. Oral administration of Cd reduced GSH level and activities of antioxidant enzymes were significantly reduced in Cd treated group. The co-treatment of *Aloe* with Cd was very effective and it significantly reversed the Cd induced biochemical alterations almost similar to that of control. *Aloe vera* was able to improve the cellular redox system in mice treated with Cd. It can be concluded that *Aloe vera* has protective effects against Cd-induced oxidative damage.

Keywords: *Aloe vera*, Heavy metals, Cadmium, toxic effect, Liver

INTRODUCTION

In the modern era environmental pollution and increased exposure of heavy metals is one of the major health issue. Heavy metals are naturally occurring elements that have a high atomic weight high malleability, electrical conductivity and a density at least 5 times greater than that of water (Pouls 2005; Igwegbe et al., 2013). The environment is threatened due to their wide uses in industrial, domestic, agricultural, medical and technological applications. Because they are being used at a very large scale they have various side effects on human health and the environment.

Dose, route of exposure, chemical species, age, gender, genetics and nutritional status of exposed individuals are the various factors on which their toxicity depends. Configuration of metals varies among different areas, which result in spatial differences of surrounding concentrations (Monisha Jaishankar et al., 2014).

Observations of earlier studies stated that heavy metals are not biodegradable, have long biological half-life and can accumulate in the different vital organs which results in tissue toxicities leading to tissue dysfunction (Sathawawara et al. 2004; Bagdatlioglu et al. 2010).

Cadmium (Cd) has been accumulated in agricultural due to wide use of phosphate fertilizers and pesticides (Zhai L et al.2008). It has been reported that the administration of Cd in humans results to both acute and chronic tissue injury and adversely affects all essential vital organs including liver (Järup and Åkesson 2009; Ahaskar and Sisodia 2006). According to IARC, 1993 all Cd mixtures have been classified as human carcinogenic fundamentals. The mechanisms of action of Cd toxicity are not well known but several pathways have been identified through which Cd expresses its deleterious effects on the human health. The generation of imbalance of antioxidant status in the living systems is the most common and prominent mechanism followed by Cd.

Cadmium (Cd) stimulates the development of reactive oxygen species (ROS) and metallothionein causing oxidative damage to various tissues consequential to the membrane functions (Eybl et al. 2006). Long-term exposure to Cd leads lipid peroxidation and causes inhibition of SOD (superoxide dismutase) activity, representative oxidative damage in different vital organs like testes, kidney and liver (Valko et al. 2005). Many studies suggested that the formation of reactive oxygen species (ROS) and its interference with the cellular antioxidant system is one central mechanism by which cadmium's toxic effect is mediated (Knoflach et al 2011). Working like efficient chelators the antioxidant can play a significant role in the treatment of metal-induced oxidative stress (Colacino et al. 2014).

Liver is the major target organ for both acute and chronic cadmium exposure. Due to acute exposure it causes swelling in hepatocyte and massive necrosis resulting in marked elevation of enzymatic biomarkers (Naima and Zine 2012).

Aloe barbedensis (Mill.) belongs to family Liliaceae and commonly known as *Aloe vera*. It has been used from centuries for its health, beauty, medicinal and skin care properties. It contains 75 potentially active constituents, including polysaccharides, anthraquinone, lectin, superoxide dismutase, glycoprotein, vitamins C and E, salicylic acids and amino acids (Vogler and Ernst 1999).

Administration of *Aloe* to mice probably helped in maintaining the balance upto some extent between free radicals and antioxidant level and thereby provided protection to mice liver.The present study tries to study the against Cd-induced toxicity. Therefore, the objective of this study was to assess the protective effect of *Aloe vera* after exposure to cadmium-induced damage and *Aloe vera* supplementation.

MATERIAL AND METHOD

Chemicals:

Cadmium and all the chemicals used in the experiments were of analytical mark and purchased from Himedia Laboratories Private Limited. (Mumbai, India).

Animals

For the present study, male Swiss albino mice of 6-7 weeks old, weighing 24-26 gm was selected from an inbred colony. The selected animals were maintained under controlled conditions of temperature and light during the experimental period. The animals were provided standard mice feed (procured from Ashirwad Industries, Chandigarh, India) and water ad libitum. Tetracycline was also given along with drinking water to them once fortnight as a preventive measure against infection. All experimental processes were completed following the recommendations found in the Guide for the Care and Use of Laboratory Animals (Refer) and permitted by committee Institutional Animal Ethics Committee (IAEC) (R. No. 1402/a/10/CPCSEA) of the Jayoti Vidyapeeth Women's University of Jaipur. Established ethical strategies were also followed in all Experiments.

Experimental Design:

The Swiss albino mice were divided into three groups comprising of 4 animals in each group as follow:-

Group I: - Control (normal) swiss albino mice, fed with standard pellet diet and water.

Group II: - Were given 100mg/kg body weight of Cadmium chloride.

Group III: - Was given 100mg/kg body weight of the *Aloe vera* extract after exposure to Cadmium.

The dose was given for 21 days and a study was taken on day 21, 28, 35 and 42 respectively. Following parameters were studied:-

1. Morphological changes
2. Effects on Body Weight
3. Biochemical analysis (Cholesterol, Total Protein, LPO, GSH)

RESULT

MORPHOLOGICAL CHANGES

Signs of cadmium induced toxicity (Loss of food and water intake, diarrhoea, facial oedema, excessive lacrimation, ruffling of hair and lethargic condition) were not observed in the animal of Group I. Whereas, animals II showed loss of food and water intake, weight loss, eye patches and lethargines. There was a recovery observed in animals of group III. Mortality was not observed in any of the control as well as experimental sets upto the last day of this study.

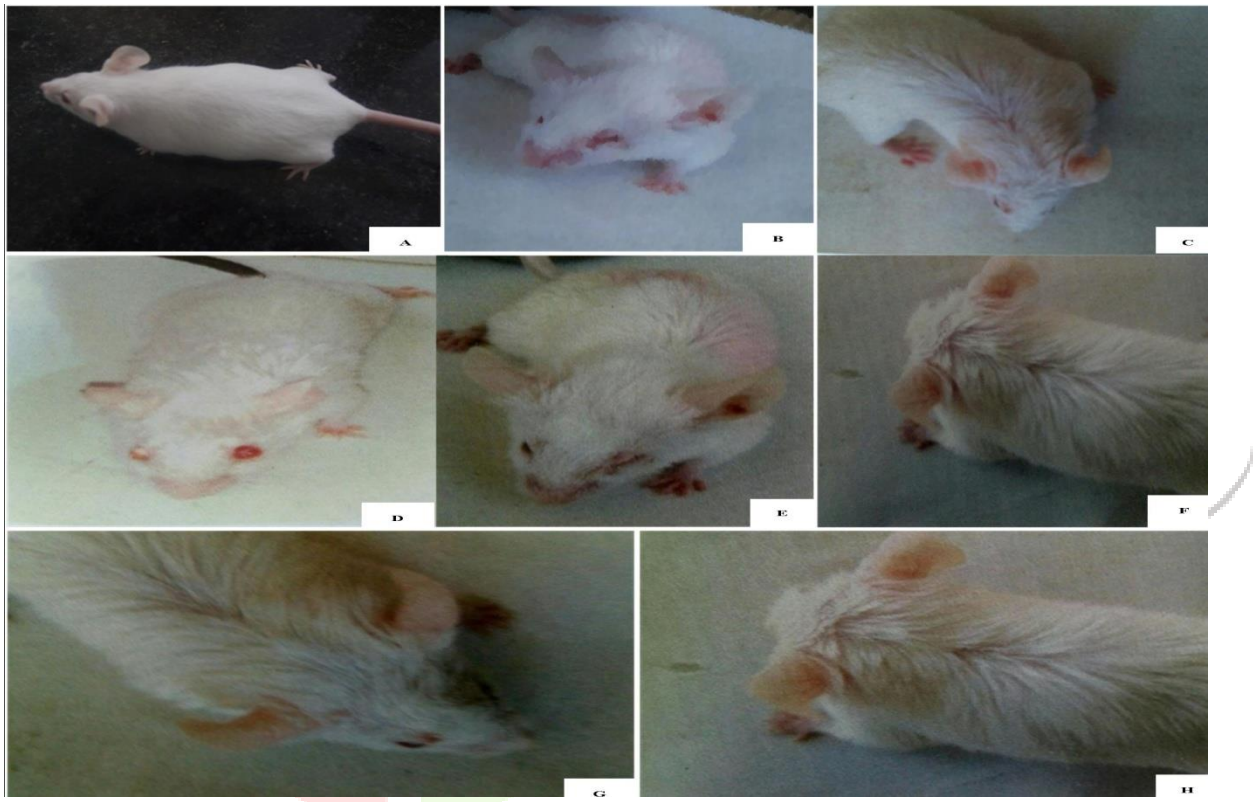

Fig.1: Morphological changes in mice during experiment.

Day 21

A. Mice of group I was healthy with smooth, shiny and puffy hair.

B. Cadmium treated mice showed changes in eye opening (Group II)

C. Cadmium and *Aloe* treated mice showed changes in hair increase. (Group III)

Day 28

D&E. Cadmium treated mice has lost its weight and eye patches & opening (Group II)

F. Cadmium and *Aloe* treated mice showed less damage as compare to control (Group III)

Day 35

G. Cadmium treated mice got decreased in size (Group II)

H. Cadmium and *Aloe* treated mice were with dispersed hair with patchy coats. (Group III)

EFFECTS ON BODY WEIGHT

All the animals were weighed every week until the end of the experimental protocol. The effect of Cadmium and cadmium+*Aloe* on body weight of mice during the experimental period is represented. (**Fig 2)** The results showed a significant ($p < 0.05$) decrease in body weight of Cd treated mice compared to normal mice. However, treatment with *Aloe vera* body weight gain significantly ($p < 0.05$) than in albino mice exposed to cadmium.

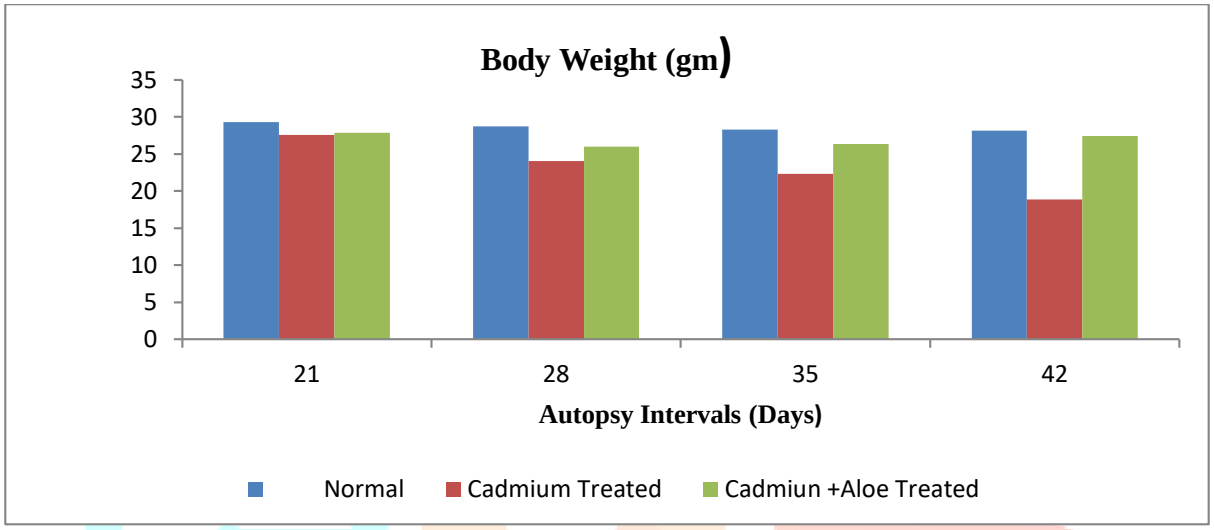

Fig. 2: The effect of Cadmium and Cadmium +*Aloe vera* on Body Weight

BIOCHEMICAL ANALYSIS:

Treatment with Cd caused a significant decrease of cholesterol (4.16±0.04) when ($p<0.05$) compared to the control mice (6.42±0.19). (**Fig. 3)** The treatment of *Aloe vera* as cadmium-treated mice increased cholesterol level in compared to cadmium treated mice.

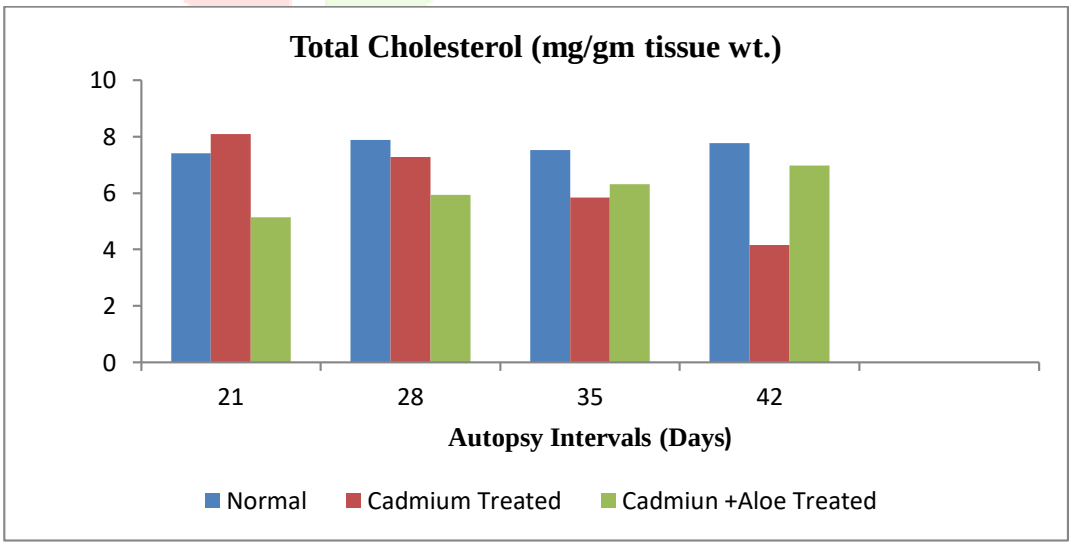

Fig. 3: The effect of *Aloe vera* on Total Cholesterol (mg/gm tissue wt.) in Cadmium treated Mice.

Meanwhile, the concentation of total protein was reduced in Cd treated (2.98±0.05) ($p < 0.05$) as compared to the control. The group of mice co-treated with *Aloe* and Cd showed a marked increase (50%) in total protein when compared to Cd intoxicated mice. **(Fig. 4)**

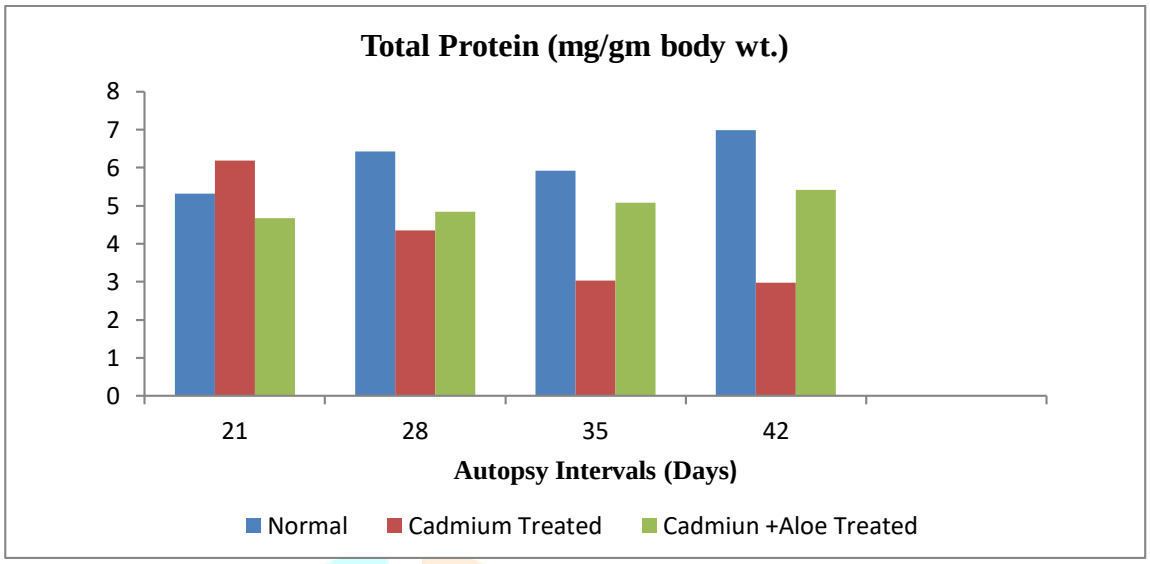

Fig. 4: The effect of *Aloe vera* on Total Protein (mg/gm tissue wt.) in Cadmium treated Mice.

Lipid peroxidation was measured by evaluating the MDA levels in liver tissue homogenates. The results indicated that administration of Cd caused significant ($p < 0.05$) increase in MDA content in liver tissues as compared to the control group. The group of mice co-treated with *aloe* and Cd showed a marked decrease in MDA level when compared to Cd intoxicated mice. LPO level increased in Cd treated mice (4.38±0.03) as compared to control (2.87±0.02). At every autopsy interval it showed a significant decrease. In *Aloe vera* treated with Cd mice; LPO level decrease and at last autopsy interval it almost to control level. **(Fig. 5)**

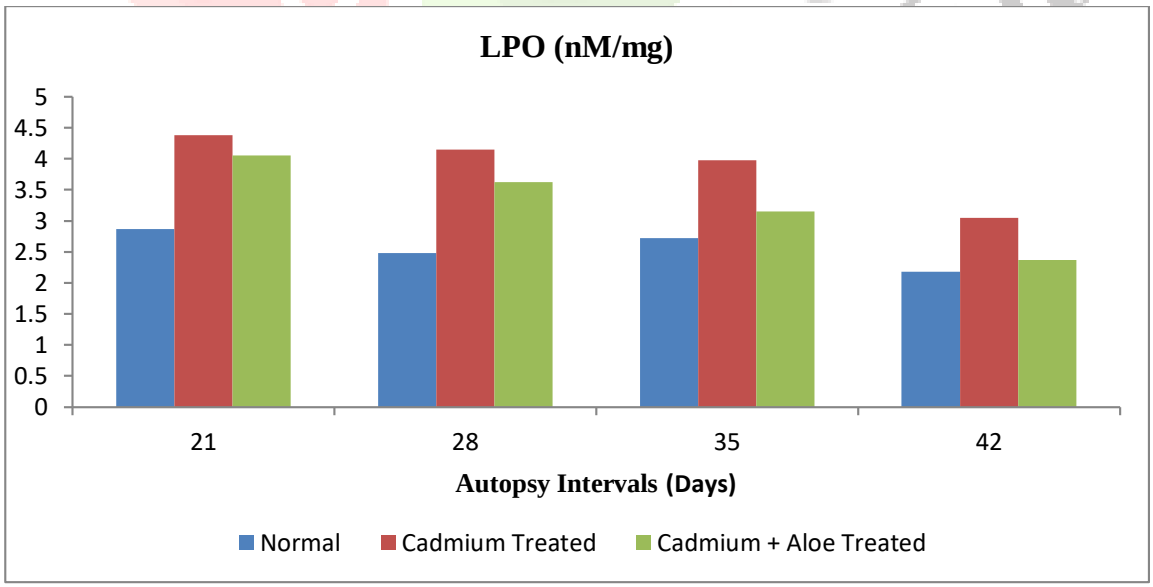

Fig 5: The effect of *Aloe vera* on LPO (nM/mg) in Cadmium treated Mice

A statistically significant decrease was observed in the level of GSH (45.12±0.03) in liver of Cd intoxicated mice as compared to control (63.53±09). *Aloe* treatement significantly restored the

level of GSH in hepatic tissues when compared to Cd only group due to scavenging properties. **(Fig. 6)**

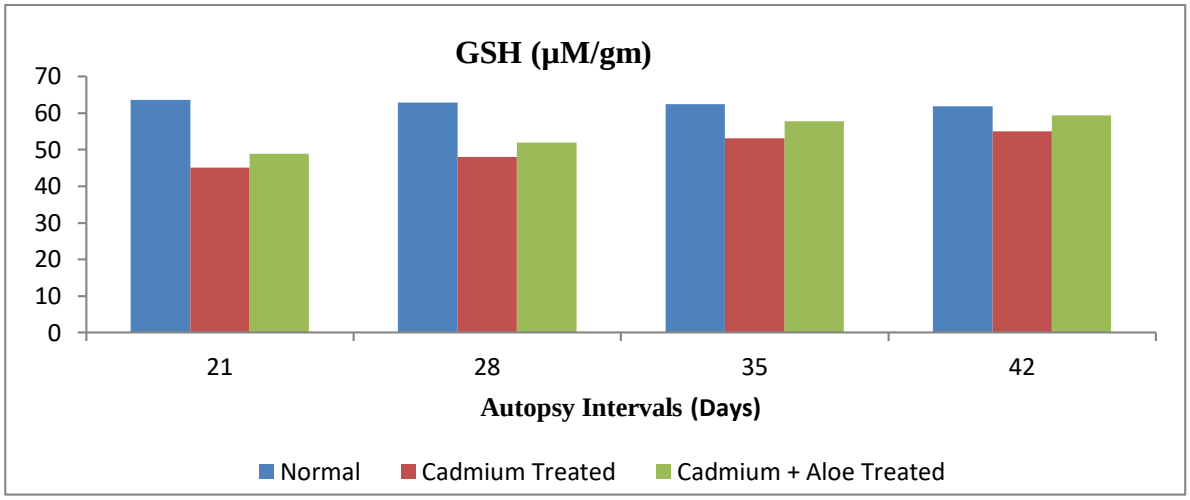

Fig 6: The effect of *Aloe vera* on GSH (µM/gm) in Cadmium treated Mice

(Values (In Gram) are shown as the Mean±SEM. Values are statistically significant at * p<0.05 when compared to normal group)

DISCUSSION

Cd has been reported as one of the most toxic metals which distress all organisms including humans via formation of free radicals and induction of cellular oxidative stress (Tchounwou et al. 2012; Rahimzade et al. 2017; Purohit et al. 2007). Cd has the capacity to accumulate and metabolize in heapatic tissues. The hepatic tissues are reported as to be the primary reservoir and a potential target for cadmium toxicity. Earlier studies suggest that the accumulation of Cd in liver is possible due to presence of high content of metal binding protein, metallothionein. Metallothionein have high affinity for Cd ions. If there is any deficiency in metallothionein it results in hepatic injury. Several studies have been carried out to understand the mechanism of cadmium induced hepatotoxicity which includes contact with membrane components and lipid peroxidation (Bagchi et al. 1996). By inhibition of antioxidant enzymes or by reducing GSH (Glutathione), Cd induces oxidative stress and lipid peroxidation (Bagchi et al. 1996). Oxidative stress and Cadmium induced toxicity can be restored by treatment with various antioxidants cadmium induced toxicity (Eriyamremu et al. 2006; Karbownik et al. 2001). Liver is an important metabolic organ which synthesized the total protein and thus it could be useful as a marker to evaluate the status of hepatic damage by toxicants (Akanji et al. 2008).

In present study, Cd treated mice showed to a substantial and constant decrease in body weight at different intervals over the control group's experiment period. An earlier finding has been support in significantly reduction in body weight of swiss albino mice (Ramaswany and Priya 2011). The reduction in body weight content in the Cd-treated mice group correlates with other studies (Kumari and Sharma 2020; Dadupanthi and Bhargava 2021). In present study, mice treated with *Aloe vera* showed increase in the body weight as compared to the cadmium mice group. These results sugeest that because of the antioxidant properties of *Aloe vera*; it prohibited the free radicals caused oxidative impairment of the cell membrane (Natelson et al.

1951). The treatment with *Aloe vera* to the Cadmium-treated animals enhanced body weights. Several other studies support the result of present study (Afifi and Embaby 2016; Dadupanthi and Bhargava 2021).

In the present study, the alterations in total protein in the liver of Cd exposed mice suggest the loss of protein synthetic ability of liver. The depletion in total protein on treatment with Cd could be the consequences of mitochondrial and cytosolic dysfunctions (Sushanth et al. 2021; Kumar et al. 2021). The findings of study suggest that treatment with *Aloe vera* may have restored the concentration of total protein through its antioxidant potential. *Aloe* provided protection to plasma membrane against free radical induced alterations in its permeability, which inhibited amino acid transport and ultimately protein synthesis.

Decreased concentration of liver cholesterol may enhance the activity of HMG Co.A reductase, a rate limiting enzyme of cholesterol synthesis, which resulted in an increase of total cholesterol in cd treated mice. Conversely, treatment with *Aloe* probably inhibited HMG Co.A reductase activity and hence, lowered cholesterol concentration. Secondly, nicotinic acid (niacin), a constituent of *Aloe* also decreased the lipolysis in adipocytes and prevented the cholesterol synthesis in this investigation. The decreased activity of HMG Co.A reductase might have been compensated by the decreased metabolism in the liver maintaining liver cholesterol (Pugalendhi et al. 1992).

The increase LPO level under any heavy metal stress is an indicator of damage of the organs of a living system. The observations from the present study showed significant rise in the level of LPO in the hepatic tissues of Cd treated mice. According to the studeis of several researchers the elevation in LPO due to cadmium treatment alters the balance between the levels of antioxidant and oxidant species (Daniel et al. 2004; Eybl et al. 2006; Howlett and Avery 1997; Seif et al. 2019). Lipid peroxidation (LPO) is a free radical chain reaction results in a loss of biochemical and structural architecture of cellular organelles and therefore, it is a highly destructive process (Leyko and Bartosz, 1986). Peroxidation of membrane lipids has a devastating effect on the functional state of the membrane because it alters membrane fluidity and permeability (typically decreasing it) and thereby allowing ions such as Ca 2+ to leak into the cell. Results of present study indicated that treatment with *Aloe* provided protection to cell membranes against free radical induced oxidative damage. Antioxidants like vitamins A (β-carotene), C and E (Atherton, 1998), present in *Aloe* seem to be responsible for inhibiting lipid peroxidation level in liver. These results suggest that the basic cause of lipid peroxidation is not only the free radicals but also the low level of antioxidants in a biological system, which removes them.

The significant reduction in the level of glutathione (GSH) due to Cd treatment noticed in the present study may be associated to the chemical nature of Cd to interact with sulfhydryl (–SH) group of GSH and free thiol groups present in other biomolecules in the organisms. The imbalance in the redox systems is known to trigger induction of oxidative stress in the living systems (Im et al. 2006). Glutathione (GSH), a tripeptide of glutamic acid, cysteine and glycine is the most abundant intracellular thiol compound present in virtually all mammalian tissues (Sen, 1997). GSH depletion does not have direct consequences in the form of acute toxicity but the cells become more susceptible to chemical or oxidative stress. Numerous enzymes like glutathione synthetase, glutathione peroxidase, glutathione S-transferases, leukotriene C4 synthetase 3-iodinase, glutaredoxin and glyoxylase are GSH dependent. The activities of these enzymes may be regulated

by thiosulphide exchanger and thus depend on the GSH status. Therefore, conclusion can be drawn that cells not only become more susceptible to any further challenge, but their basic functions are also perturbed by the extensive GSH depletion (Uhling and Wendel, 1992). In present study the reduction in the level of mice liver GSH due to Cd treatment might lead to the significant rise in LPO. The present study concluded that the administration of *Aloe vera* significantly reduces the toxicities induced by cadmium. Cadmium treatment caused perturbations in the levels of key biomarkers of liver function, transcription factor and also the cytokines responsible for the inflammatory response. However, the co-treatment of *Aloe vera* with cadmium displayed a strong antioxidative, anti-inflammatory and hepatoprotective potential against cadmium induced intoxication.

CONCLUSION

The obsevations from the present study concluded that at low concentration cadmium was able to stimulate free radical production which generated oxidative stress by significantly reducing the levels of enzymatic and non-enzymatic antioxidants in liver of mice. Cadmium treatment affects on liver function, transcription factor. The co-treatment of *Aloe vera* with cadmium showed a strong antioxidative, anti-inflammatory and hepatoprotective potential against cadmium induced intoxication in mice. Thus *Aloe vera* can be used as natural sources of antioxidants and as essential components for reduction of cadmium toxicity in liver.

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