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# MODULATORY EFFECT OF *ALOE VERA* LEAF EXTRACT AGAINST SULPHUR DIOXIDE INDUCED LIVER INJURY IN ALBINO RAT

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## ABSTRACT

The present study was carried out to evaluate the antioxidative effect of *Aloe vera* (*A. vera*) aqueous leaf extract on hepatotoxicity induced by $SO_2$ inhalation in male albino rats. Hepatotoxicity was assessed by estimating the Serum Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Gamma Glutamyl Transferase (GGT) and histopathological changes in liver of rats. Experimental rats were exposed to 80ppm hrd-1, $SO_2$ gas per day for 30 and 60 days. A significant increase in Serum ALP ($P<0.001$), AST ($P<0.01$), ALT ($P<0.001$), GGT ($P<0.05$) and histopathological changes include hepatocytes necrosis, degeneration of hepatocytes, centrilobular necrosis, ballooning degeneration and cellular debris have observed after $SO_2$ exposure in comparison to control rats. A marked reduction in liver injury is reported after exposure with oral administration of *A. vera* leaf extract (200mgkg-1 body weight d-1) in comparison to $SO_2$ exposed rats. Antioxidative and anti-inflammatory properties of *A. vera* shows potential to modulate the hepatocellular damage induced by $SO_2$ gas in rats.

**KEY WORDS**: **Male albino rat, $SO_2$, *A. vera* aqueous leaf extract, Liver injury**

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# INTRODUCTION

Sulphur dioxide ($SO_2$) is the most toxic air pollutant and detrimental to many organs[1]. The major sources of $SO_2$ emission are combustion of fossil fuels, burning of coal in thermal power plants, petroleum refineries, fertilizers and paper and pulp industries etc. Sulphur dioxide inhalation causes production of reactive oxygen species (ROS) in the tissues. An imbalance between the production of ROS and biological system's ability to readily detoxify the reactive intermediate causes oxidative stress[2]. The oxidative stress caused by $SO_2$ leads to damage or death of tissue, causing the leakage of enzymes in the affected tissue(s) in to the blood stream[3]. Hepatotoxic effects of any toxic substance is mainly diagnosed by markers of liver enzymes such as ALP, ALT, AST, and GGT are employed in assessing liver injury[4].*Aloe vera* (L.) belongs to the family Liliaceae commonly known as "Gheekunwar". It has therapeutic properties such as – anti-oxidative, anti-inflammatory effects, wound healing, anti-bacterial, anti-viral, anti-fungal, anti-diabetic and stimulation of hematopoiesis[5]. The aloesin derivatives of *Aloe vera* posses strong OPPH radical and superoxide anion scavenging activities[6,7].With this in view, present study is carried out to investigate the protective role of *A. vera* leaf extract on $SO_2$ induced serum enzymes and histopathological change in liver of albino rat.

# MATERIALS AND METHODS

### *Animals*

Thirty male wistar albino rats (2 months old, body weight 120-135g) were used in present study. The animals were maintained as per the norms of institutional Animal Ethics committee. Experimental rats were kept in polypropylene cages, and maintained at temperature (25±2$^0$C), humidity (60±5%) and photoperiod of 12 hrs $d^{-1}$. The rats were acclimated for two weeks prior to experiment. During this period rats were fed on commercial food pellets (Golden feed, New Delhi) and the water *ad libitum*.

### *Generation Of $SO_2$*

$SO_2$ gas (80ppm) was generated by controlled action of 5% sulphuric acid and 22% sodium sulphite solution in $SO_2$ generator[8]. To produce 1ppm of Sulphur dioxide/ $m^3$ air, the strength of sodium sulphite in the vessel was maintained to 2.7 mgml-1 and 5% sulphuric acid was used in the reaction.

### *Exposure of $SO_2$ gas*

Rats were exposed in fumigation chamber (AP 07 model SFC 120), Standard Appliances, Varanasi.

### *Preparation of Aloe vera leaf extract*

Fresh leaves of *A. vera* were collected from the Diwan Farm, Barara, Agra. The thick epidermis was selectively removed and inner colourless mucilaginous pulp was taken. The fresh pulp (1kg) of *A. vera* grinded in spice grinder and extracted with one litre of distilled water and kept with magnetic stirrer in cold room temperature for 48hours. The suspension was filtered by Whatman's No. 1 filter paper and filtrate was used for experimentation. The dose of *A. vera* leaf extract (200mgkg$^{-1}$ body weight $d^{-1}$) was administered in rats by gavaging. The dose was selected as guidelines as per Traditional Medicine System[9].

### *Experimental design*

The rats were randomly divided into six groups ($C_1$,$C_2$,$E_1$ , $E_2$ , $E_3$ and $E_4$ ) of five rats each- two control group ($C_1$&$C_2$) were exposed to ambient air and four experimental groups ($E_1$ & $E_2$ and $E_3$ & $E_4$). Experimental groups ($E_1$ & $E_2$) were exposed to 80ppm $SO_2$ gas and Experimental groups ($E_3$ & $E_4$) were exposed to 80ppm $SO_2$ with oral administration of *A. vera* (200mgkg-1 body weight d-1) for one hour per day for 30 and 60 days respectively. The rats of control and experimental groups were dissected carefully under light anaesthesia and blood samples (4-5ml) were collected from the ventricle of heart in sterilized plain centrifuge tubes for separation of serum. Blood samples were centrifuged at 2500rpm for 30min and serum was separated for the estimation of enzyme

activities. Liver tissues were taken to assess the histopathological study.

### *Biochemical study*

Serum enzymatic activities (ALP, ALT AST and GGT) were measured by the International Federation of Clinical Chemistry (IFCC) kit methods described by Bradley[10]; Tietz[11].

### *Histopathological study*

The small pieces of liver tissue of each rat was fixed in 10% formalin after washing in saline. Dehydrated tissue through a series of ethanol solution and paraffin blocks were prepared and sections at 5 micron were cut and stained with haematoxylene and eosin[12]. The stained sections were examined and photomicrographs were taken.

### *Statistical analysis*

The results were expressed as Mean ± S.Em. were signified by using Student's 't' test. Statistical calculation was carried out by using one way ANOVA with the help of computer statistical program KpKy plot (version- 3.0).

## RESULTS

Results of present study show a significant increase ($P<0.05$) in Serum ALP, AST, ALT and GGT activities in $SO_2$ exposed rats in comparison to control group, while a significant decrease ($P<0.05$) in $SO_2$ exposed rats with *A. vera* in comparison to 80ppm $SO_2$ gas exposed rats after 30days (Table-1).A highly significant increase ($P<0.01$) in Serum ALP, AST, ALT and GGT activities in $SO_2$ exposed rats in comparison to control group, while a very highly significant decrease ($P<0.001$) in Serum ALP, ALT activities, highly significant decrease ($P<0.01$) in AST and significant decrease ($P<0.05$) in GGT activity in $SO_2$ exposed rats with *A. vera* in comparison to 80ppm $SO_2$ gas exposed rats after 60days (Table-2).Histopathological changes were observed in liver tissue of experimental rats in comparison to control rats include moderate hepatocytes necrosis, degeneration of hepatocytes, centrilobular necrosis, ballooning degeneration and cellular debris (Fig. 2a and 2b) after 30 days exposure to 80ppm $SO_2$ gas, while after 60days exposure to 80ppm $SO_2$ gas, severity in hepatocyte necrosis and degeneration of hepatocytes have been observed in liver section. Ballooning degeneration, cellular debris and centrilobular necrosis are more pronounced in comparison to 30 days (Fig. 3a and 3b).Photomicrographs of liver section shows an improvement in hepatotoxicity after 30days exposure to $SO_2$ with administration of *A. vera* in rats. Mild hepatocyte necrosis and degeneration of hepatocytes have been observed at some places. Occasional debris, cellular boundaries are slightly disintegrate and cells appear in their normal shape and architecture. Binucleated cells of hepatocytes are also seen (Fig. 4a and 4b).Photomicrographs of liver section after 60days exposure to $SO_2$ with administration of *A. vera* shows modulation of hepatic injury in rats, which is evidenced by the appearance of central vein in their normal shape and architecture and cellular boundaries are clearly identify. Binucleated cells of hepatocytes are also seen (Fig. 5a and 5b).

**Table-1**

***Protective effects of A. vera on Serum ALP, AST, ALT and GGT activities after 30 days exposure to $SO_2$ in albino rats***

| Groups | Exposure and pre-exposure supplementation | ALP (U/L) Mean±S.Em | AST (U/L) Mean±S.Em | ALT (U/L) Mean±S.Em | GGT (U/L) Mean±S.Em |
|---|---|---|---|---|---|
| Control group-$C_1$ (5) | Ambient air | 160.6±2.18 | 112.6±2.66 | 48.2±3.02 | 15.18±0.87 |
| Experimental group-$E_1$ (5) | 80ppm $SO_2$ gas | 181.6±6.86* | 123.2±2.89* | 58.6±2.34* | 19.86±1.38* |
| Experimental group-$E_3$ (5) | 80ppm $SO_2$ gas + *A. vera* | 159.6±2.56$^{\bullet S}$ | 113.2±1.85$^{\bullet S}$ | 47.2±2.63$^{\bullet S}$ | 15.32±0.87$^{\bullet S}$ |

***5 = Number of rat***
***S.Em.= Standard Error of Mean***
***Significantly different from control $^{\bullet}$ = ($p<0.05$) * = ($p<0.01$)***
***Significantly different from $SO_2$ exposed group S= ($p<0.05$)***

**Table-2**

***Protective effects of A. vera on Serum ALP, AST, ALT and GGT Activities after 60 days exposure to $SO_2$ in albino rats***

| Groups | Exposure and pre-exposure supplementation | ALP (U/L) | AST (U/L) | ALT (U/L) | GGT (U/L) |
|---|---|---|---|---|---|
| | | Mean±S.Em | Mean±S.Em | Mean±S.Em | Mean±S.Em |
| Control group-$C_2$ (5) | Ambient air | 161.2±2.50 | 113.8±3.12 | 49.0±3.21 | 14.85±0.80 |
| Experimental group-$E_2$ (5) | 80ppm $SO_2$ gas | 196.4±7.05** | 130.6±3.67** | 62.6±1.56** | 21.90±1.86** |
| Experimental group-$E_4$ (5) | 80ppm $SO_2$ gas + *A. vera* | 159.8±3.56● VHS | 112.6±1.69● HS | 47.0±1.87● VHS | 16.07±0.41● S |

*5 = Number of rat*
*S.Em.= Standard Error of Mean*

*Significantly different from control*
*● = (p<0.05)*
*** = (p<0.001)*

*Significantly different from $SO_2$ exposed group*
*S= (p<0.05)*
*HS= (p<0.01)*
*VHS= (p<0.00)*

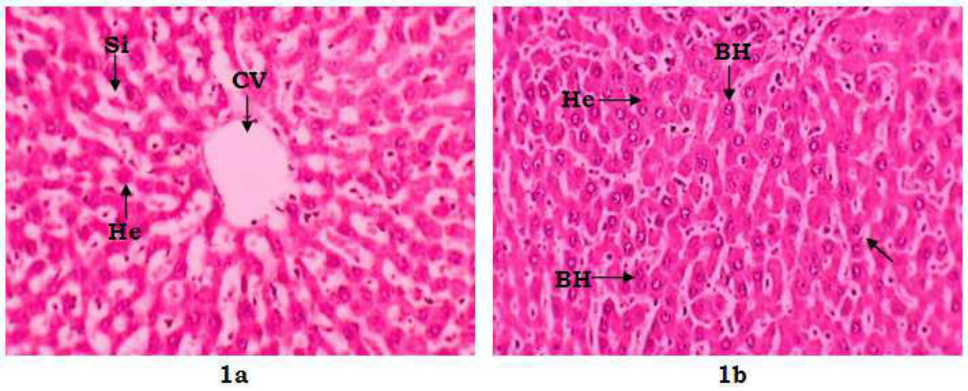


**Figure. 1a and 1b**

***Liver section in control rats. Central Vein (CV), Hepatocytes (He), Sinusoids (Si) and Binucleated Hepatocyte (BH) (x400)***

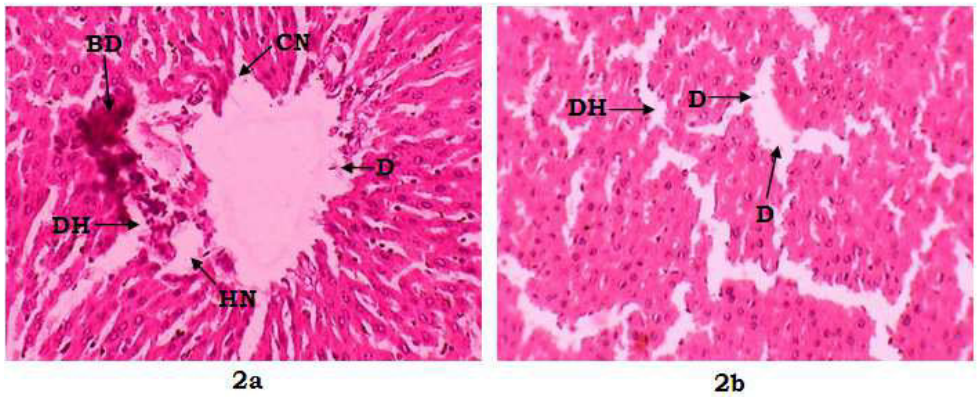


**Figure. 2a and 2b**

***Liver section of $SO_2$ exposed rats after 30 days. Centrilobular Necrosis (CN), Degeneration of Hepatocyte (DH), Ballooning Degeneration (BD), Hepatocyte Necrosis (HN) and Cellular Debris (D) (x400)***

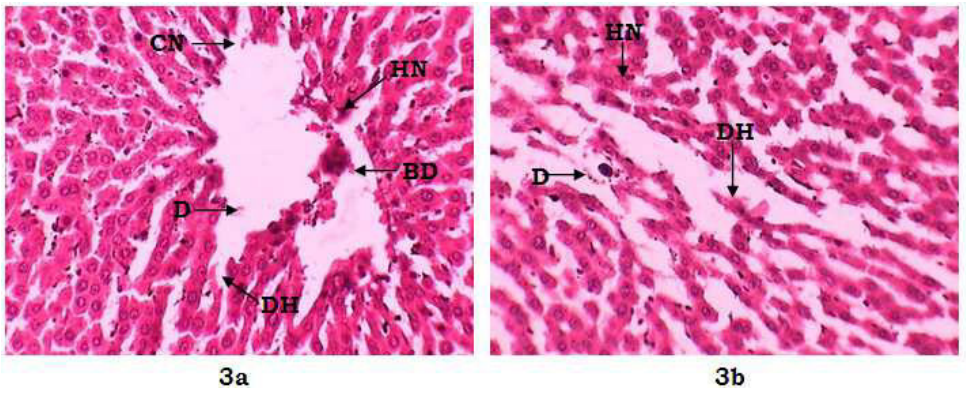


**Figure. 3a and 3b**
***Liver section of $SO_2$ exposed rats after 60 days. Centrilobular Necrosis (CN), Degeneration of Hepatocyte (DH) Ballooning Degeneration (BD), Hepatocyte Necrosis (HN) and Cellular Debris (D) (x400)***

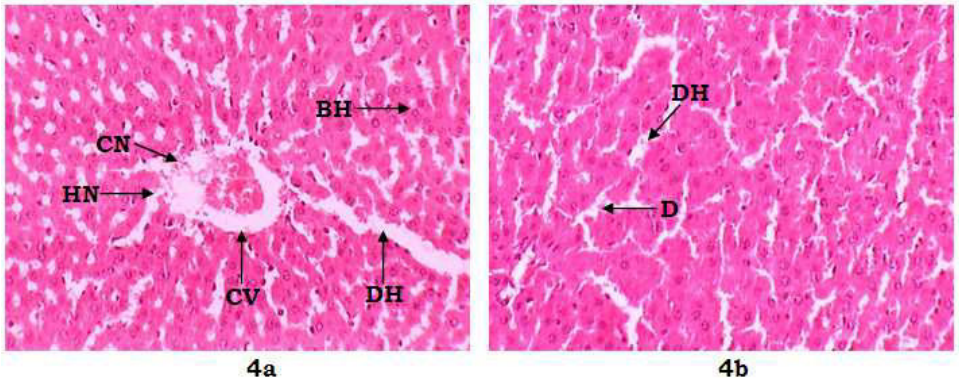


**Figure. 4a and 4b**
***Liver section of $SO_2$ exposed rats with A. vera after 30 days. Centrilobular Necrosis (CN), Degeneration of Hepatocyte (DH), Hepatocyte Necrosis (HN) and Binucleated Hepatocyte (BH) (x400)***

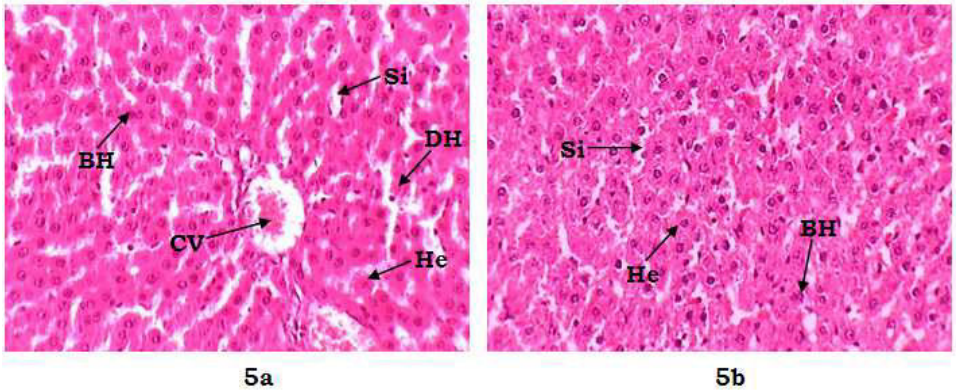


**Figure. 5a and 5b**
***Liver section of $SO_2$ exposed rats with A. vera after 60 days. Central Vein (CV), Polygonal Hepatocyte (He), Sinusoids (Si) and Binucleated Hepatocyte (BH) (x400)***

## DISCUSSION

$SO_2$ gas is an oxidant and produces free radicals that can harm our body including disruption of living cells. The damage or death of the tissue usually the leakage of enzymes in the effective tissue and subsequent release in the blood stream[13]. In the present study, an increase in serum enzyme activity and histological alterations in the rat liver is the indication of hepatocellular injury accompanied with inflammatory responses of $SO_2$ gas [14-18]. Accumulation of free radicals in hepatic cellular component increases cellular degeneration of liver causing elevation in serum enzyme activity[19,20]. The injury to hepatocytes increases the bile acid concentration responsible for leakage of ALP from rat liver[21,22]. The elevated level of serum enzymes is indicative of impaired liver function, which is associated with the hepatic cell damage caused by $SO_2$[3,23-25].In the present study, a significant reduction in hepatotoxicity in albino rat after oral administration of aqueous extract of *A. vera* is the result of antioxidative and anti-inflammatory action of *A. vera* against $SO_2$ toxicity. It contains three malic acid acylated carbohydrates: veracylglucans A,B, and C, which are responsible for anti-inflammatory effects[26.27]. Superoxide dismutase enzymes are responsible for antioxidant effects in reducing hepatic damage by the restoration of lipid peroxidation[5].The administration of *A. vera* exhibits a significant reduction in Serum ALP, AST, ALT and GGT in $SO_2$ exposed rats. *A. vera* protect the tissues and enhance cellular regeneration by suppressing the oxidative damage resulting reduction in hepatocellular changes in rats. Aloe-emodin protects the liver of rats by reduction in the elevated ALT and AST activity[28]. The shrunken nuclei of hepatocytes, granular cytoplasm, dilution of sinusoids and inflammation were reduced after administration of *A. vera* extract due to beneficial and protective effect in rats and mice[29,30]. Present study reveals that *A. vera* aqueous leaf extract can use in modulating the liver injury against toxic effects induced by air pollutants.

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