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Research Article

Change in Liver Histoarchitecture by Sulphur Dioxide Induced Toxicity and Its Vitalization by *Emblica officinalis* in Albino Rat

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Abstract: Atmosphere is a dynamic entity, which absorbs different pollutants from nature and anthropogenic sources, hence act as sink for those pollutants. Different types of gaseous pollutants are absorbed by the atmosphere, SO_2 is one of them. SO_2 enters in the body through the inhalation and then makes its entry into the blood and affects different body systems. Liver is an important vital which control most of the body activities, any change in liver or liver cell architecture leads to its abnormal functioning, results in deleterious effects. *Emblica officinalis* is a nutrient rich supplement which contains tannins, alkaloids, phenolic compounds, amino acids, carbohydrates and a rich source of vitamin C. *Emblica officinalis* has a potential to cure the histopathological alterations induced by SO_2 in albino rats.

Keywords: Pollutants, Liver, *Emblica officinalis*, Histopathology, Albino Rat.

1. Introduction

Air pollution is a problem that is old as civilization and on a smaller scale must date back to prehistoric cultures. Surely, the black lining of caves inhabited by some of our ancestors are evidence of indoor air pollution with wood or coal smoke, understandably, smoke was the first pollutant to be regulated. The atmosphere is a dynamic system, which absorbs various pollutants from natural as well as man-made sources, thus acting as a natural sink. A lot of gases such as SO_2, CO, CO_2, hydrogen sulphide and oxides of nitrogen as well as particulate matter are discharged in the environment from various sources such as automobiles exhaust, industries, public health activities, non-metal product, agriculture, printing and publishing. Environmental Protection Agency (EPA) regards the air pollutants as among top environmental threat to human health, and well established chronic and acute health effects. Its effects are acute sickness or death, alteration of physiological functions and storage of potentially harmful material in the body.

Sulphur dioxide is considered to be a serious air pollutant. It is released into environment by the combustion of coal, wood, natural gases, transportation, coal based power plants and automobiles exhaust etc. The manufacture of explosives, power plants, anaerobic bacterial breakdown of nitrogenous compounds, industrial installations and nitrogenous fertilizer are the important sources of nitrogenous oxides, while in metallurgical operation such as zinc, copper and lead, sulphur dioxide is evolved. Sulphuric acid plants, paper manufacturing plants and open burning of refuse and municipal incinerators also contribute sulphur dioxide in the urban atmosphere. These gaseous pollutants are in the chief constituent of photochemical smog and are detrimental to plants, animals and human beings (Agarwal *et al*., 2009 and Yadav *et al*., 2013[a]).

Toxic gases inhaled through lungs from where it enters the blood, which is an important vital constituent of body. Blood is a pathophysiological reflector of the whole body. All organs and tissues of the body depend on the blood for exchange of gases and nutrients, biological wastes removal and hormonal communication. Any change in its composition disturbs the metabolic activities. Blood perfuses all the organs of body and can carry beneficial substances as well as toxic substances. It may also affect the vital organs of the body. Among these vital organs, lung and liver play an important role in regulating various physiological, biochemical and metabolic process of the body.

Liver is a major organ to be exposed to toxic substance due to its portal blood supply. Although toxic substances are delivered to the liver to metabolized and excreted, this can frequently lead to activation and liver injury. It performs several functions such as

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maintenance of blood glucose concentration, lipid metabolism, cholesterol synthesize and detoxification of various toxic materials circulating in the blood.

Sulphur dioxide is powerful oxidants which produce free radicals in the animal body. These free radicals are harmful to the animals and causing several metabolic and biochemical disorder. Antioxidant acts as free- radical scavenger, may reduce the effect of these oxidants (Qin and Meng, 2006; Bai and Meng, 2005; Meng and Liu, 2007 and Rajaii *et al.*, 2008).

Emblica officinalis (Amla) enjoys a hallowed position in Ayurveda – an Indian indigenous system of medicine. According to believe in ancient Indian mythology, it is the first tree to be created in the universe. The species is native to India and also grows in tropical and subtropical regions including Pakistan, Uzbekistan, Sri Lanka, South-East Asia, China and Malaysia. The fruit of this plant is round shaped with vertical stripes. Fruit is acrid, cooling, refrigerant, diuretic and laxatives. It is greenish yellow in colour and tastes sour. The fruit is fibrous in nature. It is often used in the form of "Triphala" formulation. *Emblica officinalis* primarily contains tannins, alkaloids phenolic compounds, amino acids and carbohydrates. Its fruit juice contains the highest vitamin C (478.56mg/100ml) (Zhang *et al.*, 2006). The fruit when blended with other fruits boosted their nutritional quality in terms of vitamin C content.

With this in view, present study is carried out to investigate the protective role of *Emblica officinalis* on sulphur dioxide-induced histopathological changes in liver of albino rat, *Rattus norvegicus* (Berkenhout).

2. Material and Methods

Adult healthy male Wistar albino rats of equal weight ranging from 150-195g were kept in polypropylene cages. Inbred colony of albino rats were maintained at animal house of Zoology Department in standard condition. The rats were fed on standard laboratory animal diet commercial food pellets, golden feed, New Delhi and water ad libitum. The experimental protocol used in this study was approved (Reg.-1608/CPCSEA) by the Institution Animal Ethical Committee (IAEC) for the purpose of control and supervision on experimental animals of Dr. B. R. Ambedkar University, Agra.

80ppm SO_2 gas was generated by controlled action of 5% sulphuric acid on sodium sulphite in a sulphur dioxide generator.[21] Rats were exposed in fumigation chamber (AP 07 model SFC 120), Standard Appliances, Varanasi.

The fruits of *Emblica officinalis* were purchased from local market. After pulling of the cuticle the fruit (1kg) was cut into small pieces and macerated in the electric mixer. This macerated pulp was socked on 1 litre of distilled water and stirrer intermittently and then left overnight. The macerated pulp was then filtered through muslin cloth (22). The dose of *Emblica officinalis* extract (200mg/kg b.wt./day) was administered in rats by oral gavage. The dose was selected by as guideline as per traditional medicinal system.

The rats were randomly divided into control set (1) and experimental sets (2 and 3) of ten rats each.

Control set (1): Without SO_2 Exposure.

Experimental set (2): Exposed to SO_2 gas (80ppm/hr./day) for 30 and 60 days.

Experimental set (3): Exposed to SO_2 gas (80ppm/hr./day) along with oral administration of freshly prepared aqueous extract of *Emblica officinalis* (200mg/kg b.wt./day) for 30 and 60 days.

2.1 Histoarchitectural studies of Liver

The animals were etherized and liver was dissected out to observe changes at histopathological level. The liver of each of the rat was then washed in physiological saline (pH 7.4). It was fixed in 10% formalin, dehydrated and embedded in paraffin wax (M.P. 60^{o}C). 5µm thick sections were cut and stained with hematoxylin and eosin and mounted in Canada balsam (Humanson, 1979). The stained sections were then examined under Motic high power trinocular research microscope. The photographs were taken at different magnifications to observe the changes.

3. Results

Liver is almost a solid organ consisting of four major lobes – median, right lateral, left and caudate. Each lobe is made up of numerous lobules, under the microscope, each lobule is found to be composed of tightly packed, plates of epithelial cells termed hepatocytes. Hepatocytes are large polyhedral cells with round nuclei. These cells are arranged as thin plates separated by fine vascular sinusoids through which blood flows. The centre is occupied by the central vein and binucleated cells of hepatocytes are common in control sections of liver (Plate Ia and Ib).

3.1 After 30 Days Exposure to 80ppm Sulphur Dioxide Gas

After 30 days exposure to 80ppm SO_2 gas, moderate hepatocytes necrosis and degeneration of hepatocytes have observed in most of the places in the photomicrographs of the liver section. Hepatocytes take a swollen, edematous appearance (ballooning degeneration). Degeneration of hepatocytes showing debris within the focal necrotic region (Plate IIa and IIb).

3.2 After 60 Days Exposure to 80ppm Sulphur Dioxide Gas

After 60 days exposure to 80ppm SO_2 gas, severe hepatocytes necrosis and degeneration of hepatocytes have been observed in almost places in liver section.

Ballooning degeneration, cellular debris and centrilobular necrosis are more pronounced in comparison to 30 days exposure to SO_2 gas. Cellular boundaries are disintegrated and cells have lost their normal shape and architecture almost places (Plate IIIa and IIIb).

3.3 After 30 Days Exposure to 80ppm Sulphur Dioxide Gas with *Emblica officinalis*

After 30 days of exposure to 80ppm sulphur dioxide gas with *Emblica officinalis*, mild hepatocytes necrosis and degeneration of hepatocytes have been observed in some places in the photomicrographs of the liver section. Occasional debris is also seen in some places (Plate IVa and IVb).

Centrilobular necrosis is observed at some places. Cellular boundaries are slightly disintegrated and cells appear in their normal shape and architecture in comparison to 80ppm SO_2 gas exposed rats (Plate IVa).

3.4 After 60 Days Exposure to 80ppm Sulphur Dioxide Gas with *Emblica officinalis*

After 60 days of exposure to 80ppm SO_2 gas with *Emblica officinalis*, minimal hepatocytes necrosis and degeneration of hepatocytes have observed in the photomicrographs of the liver section. Occasional debris is also seen in some places (Plate Va).

Centrilobular necrosis is almost disappeared, cellular boundaries are slightly disintegrated and cells appear in their normal shape and architecture. Binucleated cells of hepatocytes are also observed (Plate Va and Vb).

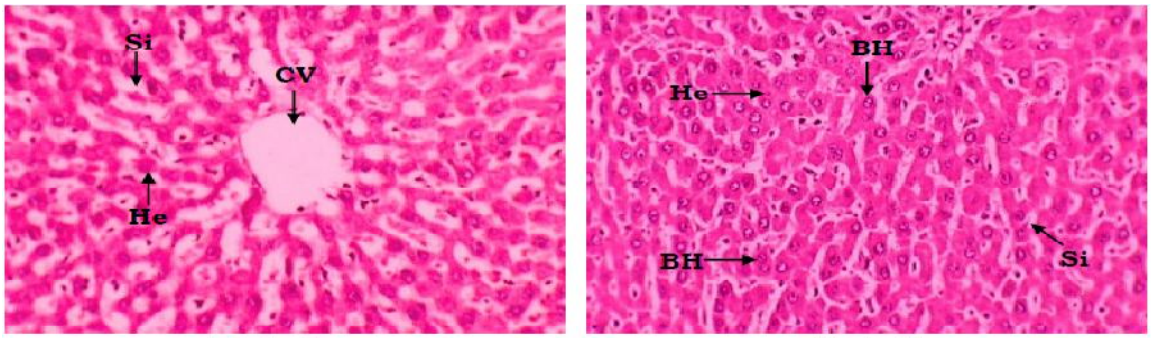

Plate-Ia Plate-Ib

Plate Ia and Ib. Photomicrograph of liver section of albino rat after exposure to ambient air (x400).

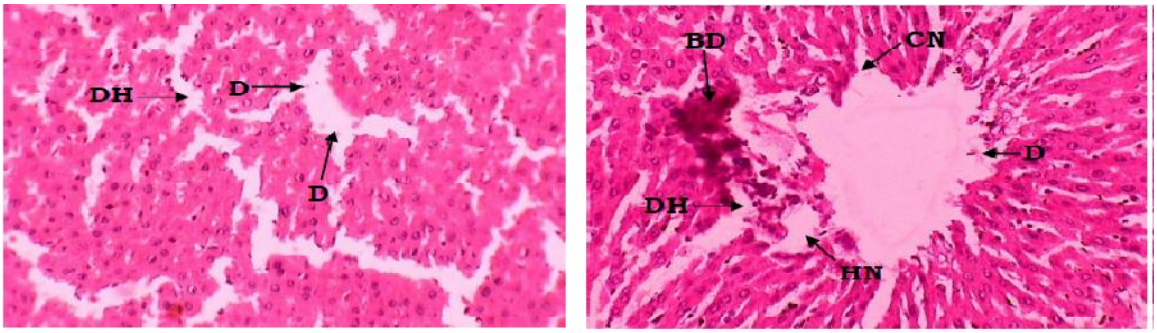

Plate-IIa Plate-IIb

Plate IIa and IIb. Photomicrograph of liver section of albino rat after 30 days exposure to 80ppm SO_2 gas (x400).

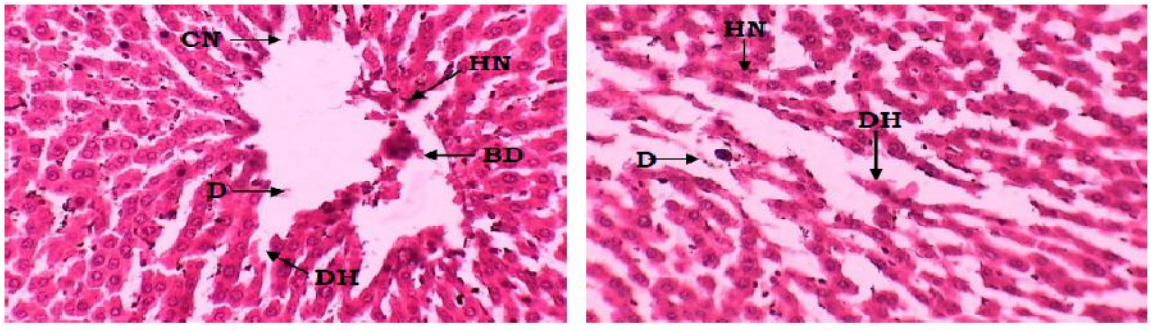

Plate-IIIa Plate-IIIb

Plate IIIa and IIIb. Photomicrograph of liver section of albino rat after 60 days exposure to 80ppm SO_2 gas (x400).

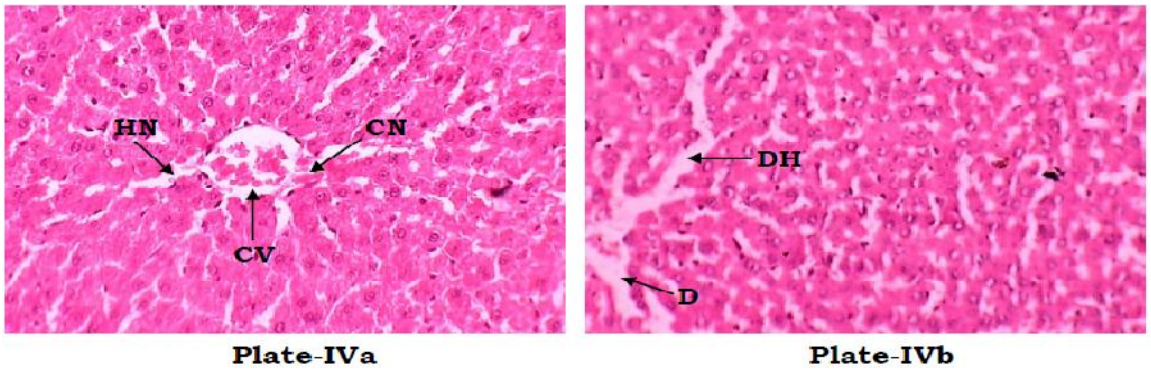

Plate IVa and IVb. Photomicrograph of liver section of albino rat after 30 days exposure to 80ppm SO_2 gas with oral administration of *Emblica officinalis* (x400).

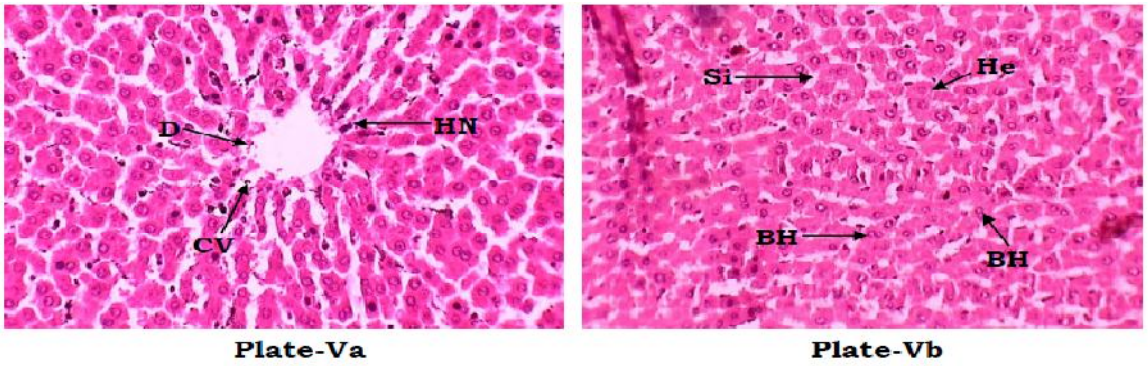

Plate Va and Vb. Photomicrograph of liver section of albino rat after 60 days exposure to 80ppm SO_2 gas with oral administration of *Emblica officinalis* (x400).

(CV-Central vein; **DH-**Degeneration of hepatocytes; **He-**Hepatocyte; **BD-**Ballooning degeneration; **Si-**Sinusoids; **HN-**Hepatocyte necrosis; **BH-**Binucleated hepatocyte cell; **D-**Debris; **CN-**Centrilobular necrosis).

4. Discussion

In the present study, the potentiating effect of SO_2 gas shows hepatocytes necrosis, degeneration of hepatocytes, centrilobular necrosis, ballooning degeneration and cellular debris in albino rat. These histological alterations in the liver are the indication of hepatocellular injury accompanied with inflammation. Histological changes in liver are due to dramatic secondary changes in the tissues as some degree of tissue injury occurs in almost every pathological condition. Hepatocyte necrosis may precede the onset of inflammation. Virtually any significant toxic effect to the liver may cause hepatocyte necrosis. The most obvious with necrosis of hepatocytes immediately around the central vein causes centrilobular necrosis accompanied by ballooning degeneration. Further, the degeneration of endothelial cells leading to tissue necrosis and influx of debris of these cells into the focal region. Hepatic necrosis, centrilobular necrosis and ballooning degeneration is due to inflammation in liver (Carton *et al.*, 1999). Similar to the present findings, Bai *et al*., (2004) have observed sulphur dioxide induces hepatocytes necrosis and degeneration of hepatocytes in mice liver after sulphur dioxide inhalation. Meng *et al.*, (2004) have also observed damaging effects of SO_2 in liver cells of mice. Similarly, the results of Bai and Meng (2005) showed that exposure to SO_2 gas cause changes in liver of rats. Further, Meng and Liu (2007) reported that inhaling of SO_2 gas caused pathological changes due to inflammation in liver cells of mice. Rajaii *et al*., (2008) have also reported morphologic changes in hepatocyte and cellular necrosis due to inflammatory reaction in mouse liver after exposure to SO_2. Similarly, Zhao *et al*., (2008) have reported sulphur dioxide-induced oxidative stress in liver of mouse.

The present findings gain support by Harkonen *et al*., (1983) who reported long-term effect of sulphur dioxide on different organs. Gairola and Wagner (1991) have also noted the liver tissue injury in mice and rats chronically exposed to cigarette smoke. Supporting findings are also observed by Czekaj *et al*., (2002) who observed morphological changes in the liver of pregnant rats exposed to cigarette smoke.

The fruit of amla, *Emblica officinalis* is an antioxidant with free radical scavenging properties which may be due to the presence of low molecular weight hydrolysable tannins- Emblicanin A and Emblicanin B, along with pedunculagin and

punigluconin. Antioxidant activity of amla against oxidative stress leading to decrease in hepatotoxicity in SO_2 exposed rats. Similar findings have been reported by Ghosal *et al*., (1996) and Khan (2009) who have evaluated strong free radical scavenging activity of amla extract against oxidative stress due to presence of tannins- Emblicanin A and Emblicanin B. Similarly, Bhattacharya *et al*., (1999 and 2000[a&b]) have reported antioxidative activity of tannoid of *Emblica officinalis* in chronic stress-induced rats.

Further, the fruit of amla have also been considered rich in vitamin C (ascorbic acid) content and high levels of superoxide dismutase, accounts of antioxidant activity and leads to cytoprotection. In support of present findings, Scartezzini *et al.,* (2006) and Raghu *et al*., (2007) have observed that the fruit of amla is rich in vitamin C (ascorbic acid) content, accounts of approximately 45-70% of antioxidant activity. Ellagic acid and low level of β-Glucogallin and other mucic acid gallates also present in aqueous extract of *Emblica officinalis* have also powerful antioxidant property (Zhang *et al*., 2001). Present findings are agreement with the findings of Tasduq *et al*., (2005[b]) who have reported that amla is used in Indian system of medicine for the treatment of hepatotoxicity. Asmowi *et al*., (1993) and Yokozawa *et al*., (2007) have also reported that fruit extract of amla have anti-inflammatory activity in rats.

In the present study, a significant reduction in histopathological alterations viz- hepatocytes necrosis, degeneration of hepatocytes, ballooning degeneration and centrilobular necrosis after oral administration of *Emblica officinalis*. Hepatoprotective effect of *Emblica officinalis* against SO_2 induced hepatic injury is due to its anti-inflammatory, anti-oxidative and rejuvenating properties, which protect the tissues and enhance cellular regeneration by suppressing the oxidative damage resulting reduction in histopathological changes in liver of rats. In support of present findings, De and Bhavsar (1993) have reported that fruit pulp of *Emblica officinalis* is used against a variety of liver injury. Similar to the present findings, Jose and Kuttan (2000) and Sultana *et al*., (2004) have reported that histopathological analysis – acute cell swelling and liver necrosis were significantly minimized by *Emblica officinalis* in rats. Similarly, Banu *et al*., (2004) have observed the protection afforded by amla is associated with its antioxidant capacity and modulatory effect on hepatic activation and detoxifying enzymes against 7,12-dimethylbenz(a)anthracene induced hepatotoxicity in Swiss albino mice. Similar to the present findings, Mir *et al.,* (2007) have noted that *Emblica officinalis* significantly reversed the abnormal histopathological alterations in rat liver and provided protection to antioxidant system of liver. Similarly, Gopumadhavan *et al.,* (2008) have reported that histopathological evaluation showed vacuolar degeneration, necrosis and prominent vacuolated to cells in the hepatic sinusoids were ameliorated by partysmart (amla) treatment against alcohol-induced hepatic injury in rats. Prakash *et al.,* (2008) have also observed that the hepatic cells, central vein and portal triad become normal in amla treated rats. Sharma *et al.,* (2009) have also reported that *Emblica officinalis* fruit extract significantly reduced oxidative stress, liver necrosis and cytoplasmic vacuolization against arsenic-induced toxicity in Swiss albino mice.

5. Conclusion

In the light of present study we can conclude that, the toxicity of sulphur dioxide gas increases with increase in time period of exposure in albino rats, while after oral administration of *Emblica officinalis*, the toxic effects of sulphur dioxide gas have mitigated to a greater extent and altered values reach upto control values in albino rats.

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