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ORIGINAL ARTICLE

Role of *Emblica officinalis* against Gaseous Air Pollutants Induced Hepatotoxicity in Albino Rats

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ABSTRACT

Air pollution is the most serious environmental threat to all over the world. The gaseous air pollutants includes $SO_2$and NO_2 which are considered as the most poisonous and irritant gases as they alters the physiology, biochemistry and behavior of the living individuals. The present study has been conducted to demonstrate the curative effect of Emblicaofficinalis against $SO_2$and NO_2 induced hepatotoxicity in albino rats. The twenty five albino rats were grouped in five sets- control set (1) were kept in control conditions, while, experimental set (2) exposed to 80ppm of NO_2 gas 1h/d for 30 and 60 days, while experimental set (3) was exposed to 80ppm of SO_2 gas 1h/d for 30 and 60 days, experimental set (4) were exposed to 80ppm of SO_2 gas 1h/d with oral administration of Emblica officinalis fruits extracts (200mg/kg b.wt.) for 30 and 60 days respectively ,experimental set (5) were exposed to 80ppm of NO_2 gas 1h/d with oral administration of Emblica officinalis fruits extracts (200mg/kg b.wt.) for 30 and 60 days respectively. The results of the present findings indicate that these gases causes significant increase in serum enzymes AST, ALT, and ALP ($p<0.01$) in liver of albino rats. Oral administration of Emblica officinalis recovers all these alterations at their normal level which is an indication of remedial effect of E. officinsalis on these gaseous air pollutants induced hepatotoxicity in rat.

***Key words**: Albino rats, gaseous air pollutants, E. officinalis, serum enzymes*

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INTRODUCTION

The atmosphere is a dynamic system, which absorbs various pollutants from natural as well as anthropogenic 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.

Nitrogen dioxide and sulphur dioxide are considered to be serious air pollutants. These pollutants are 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 breakdown of nitrogenous compounds by bacteria, 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 to 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.Toxic gases inhaled through lungs and enter the blood, which is an important vital constituent of body. Blood is a patho-physiological 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 vitals of the body. Among these vital organs 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 maintenance of blood glucose concentration, lipid metabolism, cholesterol synthesize and detoxification of various toxic materials circulating in the blood.

Emblica officinalis (Amla) enjoys a hollowed 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 sub-tropical regions including Pakistan, Uzbekistan, Srilanka, 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 "Triphla" formulation. *Emblica officinalis* primarily contains tannins, alkaloids phenolic compounds, amino acids and carbohydrates. Its fruit juice contains the highest vitamin C (478.56 mg/100ml). The fruit when blended with other fruits, boosted their nutritional quality in terms of vitamin C content.

As with any toxic challenge the obvious solution is to remove, or at least decrease to an acceptable level, the source of trouble. Toxicity may rise from an imbalance of biological pro-oxidant and antioxidant processes linked to increased exposure to oxidants or the presence of impaired antioxidant defences. Oxidative stress is one of the main mechanism underlying the toxic effect of air pollutants, which trigger a number of sensitive signaling pathways, such as inflammatory response. Nitrogen dioxide and sulphur dioxide constitutes a well define and ducible model of oxidative stress. When nitrogen dioxide and sulphur dioxide are present in combination this may have a synergistic effect increasing the damage more than the sum of the individual effects of nitrogen dioxide and sulphur dioxide alone. Since activated inflammatory cells also generate and release large quantities of free radicals, which attack local tissue components and cause cell injury. Antioxidants are molecules that slow or prevent the oxidation of toxic chemicals. The aim of present study is to investigate the protective role of *Emblica officinalis*, a strong antioxidant on toxic effects of nitrogen dioxide and sulphur dioxide, major oxidant air pollutants in mammals.

MATERIALS AND METHODS

EXPERIMENTAL ANIMAL:

Adult and healthy wistar albino rats were selected for present study. The colony of wistar albino rat was bred at the animal house of Zoology Department, Dr. B.R. Ambedkar

University, Agra.Albino rats of weight ranges from 180 to 200g were kept in polypropylene cages, measuring 45x27x15cms at the temperature 25±2^{0}C, relative humidity 50±5% and 12hr light cycle and 12 hr dark cycle. The roof of cages was made up of galvanized steel mesh. A sliding removable tray was placed below the cage to hold excreta which was cleaned daily. Each and every cage was equipped with a metal food plate and water bottle. To avoid undesirable microbial contamination, cages were sanitized three time per week. The rats were fed on standard laboratory animal diet (commercial food pellet Golden feed, New Delhi) and water *ad libitum*. The rats were kept for one month to adapt to their new environment. 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.

EXPERIMENTAL GASES:

Nitrogen dioxide and sulphur dioxide were selected for the present study.Nitrogen dioxide gas was prepared by latest modified method by Levaggi *et al*. (1972).Sulphur dioxide gas was prepared by the method described by Singh and Rao (1979).

PROCUREMENT OF PLANT MATERIALS:

In the present study, *Emblica officinalis* was used as antioxidants. Fruits of *Emblica officinalis* were procured from local market of Agra. The plant was taxonomically identified by Department of Botany, School of Life Sciences, Khandari Campus, Dr. B.R.A. University, Agra.Amla is a medium sized deciduous tree, attaining a height of 20 to 25 feet, globose, depressed, about 2cm in diameter. Fruit is 6-prominent lines, greenish when tender and yellow when mature, sour and astringent followed by sweet taste. The fruit is used for culinary and medicinal purpose.

PREPARATION AND DOSAGE OF EXTRACTS:

The fruit of *Emblica officinalis* was procured from local market. After peeling off the cuticle the fruit (1kg) was cut into small pieces and macerated in the electric mixer. This macerated pulp was soaked in 1 liter of distilled water and stirred intermittently and then left overnight. The macerated pulp was then filtered through muslin clothes. This filtrate of fruit served as an aqueous extract of *Emblica officinalis* for experimentation. To increase the shelf life and uniformity this extract was completely lypholized (Mathur *et al*., 1996).The dosage range was employed as guidelines as per Traditional Medicine System (TMS) (WHO, 2001). In the present research study, aqueous extracts of *Emblica officinalis* (200mg/kg b. wt.) was administrated in rats by gavaging.

FUMIGATION CHAMBER:

Fumigation chamber (Model AP-07, SFC-120) manufactured by standard Appliances, Varanasi was used for whole body exposure of sulphur dioxide and Nitrogen dioxide gases. The fumigation chamber measuring 45x45x60cm was made up of a metallic frame with glass walls. In front a glass door was fitted, while a water chamber was fitted on the top of fumigation chamber through which water circulates during the experiment which was connected by a rubber tube for the inlet of water to the vertical pump kept in the bucket containing water, while the outlet rubber tubes were also kept in the same bucket. Various control knobs for air circulation, water circulation, air pressure were located above and below the door of fumigation chamber. Sulphur dioxide and nitrogen dioxide gases with ambient air circulate in the chamber was maintained with the help of speed of blow fan located at the base of the fumigation chamber.

EXPERIMENTAL PROTOCOL:

The experimental albino rats were grouped in six sets (A, B, C, D, E, F). Two control (A and D) and four experimental sets (B, C, E, and F) of ten rats each.

Control Set (A and D): Rats of control set A and D were exposed to ambient air for one hour per day for 15, 30, 60, 90 and 180 days.
Experimental Set (B and E): Rats of experimental set B and E were exposed to 80ppm SO_2 gas and 80ppm NO_2,respectively for one hour per day for 15, 30, 60, 90 and 180 days.
Experimental Set (C and F): Rats of experimental set C and F were exposed to 80ppm SO_2 gas and 80ppm NO_2, respectively with oral administration of *Emblica officinalis* (200mg/kg b. wt.) for 1 hour per day for 15, 30, 60, 90 and 180 days.

EXPOSURE TO SO_2 AND NO_2 GAS:
The rats of experimental sets (B, C, E and F) were kept in fumigation chamber for 15, 30, 60, 90 and 180 days for whole body exposure to 80ppm SO_2 and 80ppm NO_2 gas for one hour per day.

PREPARATION OF SAMPLES:
The blood samples were collected from the ventricles of the dissected albino rats with the help of sterilized syringe fitted with a needle and were poured directly in the sterilized plain glass centrifuge tubes for separation of serum. The blood samples were analyzed individually for each animal.The blood samples collected in plain sterilized centrifuge tubes were allowed to clot for about one hour and were centrifuged at 2000 rpm for 30 minutes. A fine rubber bulb pipette was used to separate the supernatant serum.The serum samples were used for the estimation of biochemical parameters liver function test (Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP).

BIOCHEMICAL ESTIMATIONS:
TheAspartate amino transferase (AST) activity was determined by ERBA AST KIT, International Federation of Clinical Chemistry (IFCC) Method described by Bradley *et al.* (1972).The Alanine amino transferase (ALT) activity was determined by ERBA ALT KIT, International Federation of Clinical Chemistry (IFCC) Method described by Bradley *et al.* (1972). The Alkaline Phosphatase activity was determined by ERBA ALP KIT, International Federation of Clinical Chemistry (IFCC) Method, described by Tietz (1983).

STATISTICAL CALCULATIONS:
For each biochemical parameters a minimum of 5 replicates were done and the results were statistically analyzed by ANOVA. All data evaluated by computer statistical program "Biostat".

RESULTS AND DISCUSSION

In the present study, a significantly increase in the level of serum enzymes such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase, (ALP) after exposure to nitrogen dioxide and sulphur dioxide which is modulated after administration of *Emblica officinalis* in combination (Table 1-6).

Table 1: Effect of *Emblica officinalis* on serum AST conc. (IU/L) in Sulphur dioxideexposed albino rat

Experimental sets	Groups	Mean ±S.Em.				
		15 days	30 days	60 days	90 days	180 days
Set A	Control	96.4±1.43	103±2.21	112.4±4.03	114.8±3.73	116.8±4.11
Set B	80ppm SO_2	104.6±2.20$^{***\uparrow}$	112±2.21$^{***\uparrow}$	123.4±3.54$^{***\uparrow}$	127±3.83$^{***\uparrow}$	132.8±4.11$^{*\uparrow}$
Set C	80ppm SO_2+ *Emblica officinalis*	103.2±2.35$^{*\downarrow}$	104.6±2.20$^{***\downarrow}$	113±2.34$^{***\downarrow}$	115.4±2.35$^{**\downarrow}$	111.4±3.55$^{***\downarrow}$

Table 2: Effect of *Emblica officinalis* on serum AST conc. (IU/L) in Nitrogen dioxideexposed albino rat

Experimental sets	Groups	Mean ±S.Em.				
		15 days	30 days	60 days	90 days	180 days
Set D	Control	96.6±1.91	102.8±2.78	113.4±4.24	116±3.84	115.6±4.0
Set E	80ppm NO_2	108.4±2.13$^{***\uparrow}$	113.6±2.54$^{***\uparrow}$	124.8±3.62$^{***\uparrow}$	128.4±3.64$^{***\uparrow}$	133±4.6$^{***\uparrow}$
Set F	80ppm NO_2+ *Emblica officinalis*	101.6±2.29$^{***\downarrow}$	105.4±2.48$^{***\downarrow}$	114±2.21$^{***\downarrow}$	117.6±2.42$^{***\downarrow}$	113.8±3.87$^{***\downarrow}$

Table 3: Effect of *Emblica officinalis* on serum ALT conc. (IU/L) in Sulphur dioxideexposed albino rat

Experimental sets	Groups	Mean ±S.Em.				
		15 days	30 days	60 days	90 days	180 days
Set A	Control	36.6±2.33	39.8±2.47	47±3.33	44.8±3.18	49.4±4.73
Set B	80ppm SO_2	46.2±2.78$^{***\uparrow}$	49.8±3.91$^{**\uparrow}$	57.6±3.23$^{***\uparrow}$	61.6±3.20$^{***\uparrow}$	66.8±3.02$^{**\uparrow}$
Set C	80ppm SO_2+ *Emblica officinalis*	44±2.62$^{**\downarrow}$	46.2±3.91$^{***\downarrow}$	46.8±2.99$^{***\downarrow}$	43.4±3.20$^{***\downarrow}$	44.2±2.62$^{***\downarrow}$

Table 4: Effect of *Emblica officinalis* on serum ALT conc. (IU/L) in Nitrogen dioxideexposed albino rat

Experimental sets	Groups	Mean ±S.Em.				
		15 days	30 days	60 days	90 days	180 days
Set D	Control	38.2±2.55	37.6±1.86	44.8±3.18	47.4±3.64	46.6±2.99
Set E	80ppm NO_2	45±2.82$^{***\uparrow}$	46.8±3.46$^{**\uparrow}$	58.6±3.14$^{***\uparrow}$	64.4±3.77$^{***\uparrow}$	66.2±4.11$^{***\uparrow}$
Set F	80ppm NO_2+ *Emblica officinalis*	41±2.12$^{**\downarrow}$	41.2±2.26$^{**\downarrow}$	48.2±3.67$^{***\downarrow}$	45.2±2.57$^{***\downarrow}$	46.4±2.9$^{***\downarrow}$

Table 5: Effect of *Emblica officinalis* on serum ALP conc. (IU/L) in Sulphur dioxideexposed albino rat

Experimental sets	Groups	Mean ±S.Em.				
		15 days	30 days	60 days	90 days	180 days
Set A	Control	145.8±1.65	151.2±1.93	156.4±2.63	159.4±2.80	168.4±3.64
Set B	80ppm SO_2	154±2.12$^{***\uparrow}$	156.4±2.63$^{**\uparrow}$	181±6.50$^{**\uparrow}$	193.2±10.28$^{**\uparrow}$	196±11.61$^{*\uparrow}$
Set C	80ppm SO_2+ *Emblica officinalis*	152±2.07$^{ns\downarrow}$	151.4±1.86$^{**\downarrow}$	158.2±2.78$^{**\downarrow}$	156.8±3.07$^{**\downarrow}$	159.8±3.12$^{**\downarrow}$

Table 6: Effect of *Emblica officinalis* on serum ALP conc. (IU/L) in Nitrogen dioxideexposed albino rat

Experimental sets	Groups	Mean ±S.Em.				
		15 days	30 days	60 days	90 days	180 days
Set D	Control	146.8±1.42	152.2±2.08	156.2±1.93	159.6±3.52	169±3.34
Set E	80ppm NO_2	155.4±2.48$^{**\uparrow}$	158.4±3.15$^{**\uparrow}$	182.4±6.53$^{**\uparrow}$	194.6±11.13$^{**\uparrow}$	200.8±13.3$^{*\uparrow}$
Set F	80ppm NO_2+ *Emblica officinalis*	152.8±2.35$^{**\downarrow}$	151.4±2.54$^{***\downarrow}$	158.6±2.83$^{**\downarrow}$	157±2.94$^{**\downarrow}$	163.2±2.95$^{*\downarrow}$

*Values are mean ± S.E.M. * p <0.05, ** p<0.01, *** p<0.001 significantly different from the group treated with NO_2, N.S.= Non- Significant*

SO_2 gas is an oxidant and produces free radicals that damage our body systems by means of disrupting living cells. The damage or death of the tissue usually leads to the leakage of enzymes in the effective tissue and subsequent release in the blood stream (Pant, 2004). In the present study, an increase in serum enzyme activity alterations in the rat liver is the indication of hepatocellular injury accompanied with inflammatory responses of SO_2 gas (Gopal and Rosan, 2000). The SO_2 causes hepatic cell injury which leads to the release of serum enzymes in blood stream, is an indication of impaired liver function (Al-Malki *et al.,* 2008; Agarwal *et al.,* 2009 and Yadav *et al.,* 2014). Inhalation of SO_2 gas causes pathological changes in liver including necrosis in hepatocytes and degeneration of hepatocytes due to inflammation in liver cells of mice (Meng and Liu, 2007; Rajaii, *et al.,* 2008 and Zhao, *et al.,* 2008).

The NO_2 causes cellular damage, protein reduction in serum by translocation which leads in the elevation of ALT, which is an indication of liver damage (Agarwal *et al.,* 2010). An increased level of AST and ALT has been reported after exposure of NO_2 and SO_2 air pollutants in albino rat (Olajire and Azeez, 2012). The NO_2 directly affects the several tissues after gets metabolized into nitrites and nitrates which damages liver during detoxification, which is probably a cause of alterations in AST and ALP concentrations in liver. The findings of the present study is also affirmed by the observations of Dey, *et al.* (2015) who also observed that gaseous air pollutants, particularly NO_2, significantly enhance the level of serum enzymes AST and ALT along with significant reduction in ALP, an indication of hepatic injury.

The fruits of *Emblica officinalis* Gaertn. commonly known as amla or Indian gooseberry is known for its medicinal and therapeutic properties from ancient time in India and considered as a wonder fruit for health conscious population. It is extensively found throughout India and some other Asian countries. The fruits are widely consumed raw, cooked, or pickled. The fruits of plant form a major constituent of many potent Ayurveda preparations and these preparations are widely used for their preventive, curative, and health restorative properties. Amla contains highest amount of Vitamin C (ascorbic acid), low and high molecular weight tannins 30%, phyllembin (2.4%), phyllemblic acid (6.3%), gallic acid (1.32%), ellagic acid in natural form and cytokine like substances identified as Zeatin, Z riboside, Z nucleotide (Abbas *et al*, 2017) Amla fruit ash contains chromium, 2.5; zinc, 4; and copper, 3 ppm. Presence of chromium is of therapeutic value in diabetes. The fruit contains 482.14 units of superoxide dismutase/g fresh weight, and exhibited anti senescent activity (Ghosal, *et al.,* 1996). In traditional medicine, the medicinal plants play a major role and constitute backbone of traditional medicine Synthetic hypoglycemic agents can produce serious side effects including hematological effects, coma and disturbances of liver and kidney. In addition, they are not suitable for use during pregnancy. Compared to synthetic drugs, herbal preparations are frequently considered

to be less toxic with fewer side effects. Therefore search for more effective and safer anti hyperglycemic has become an area of current research. *Emblica officinalis* act as antioxidant by scavenging pro-oxidant free radicals and stimulate the regeneration of liver cells. It also exhibits a regulatory action of cellular membrane permeability and increase in its stability against hepatic injury. Aqueous extract of *Emblica officinalis* significantly capable of restoring integrity of hepatocytes indicated by improvement in liver function. The extract of *Emblica officinalis* improved liver function by decreasing the level of AST, ALT, ALP in serum of rats (Bhuvaneshwari, *et al*., 2014). Similarly, the extract of *Emblica officinalis* decreased the level of ALT and ALP for their hepatoprotective activity in rats (Jose and Kuttan, 2000).The amla has hepatoprotective effects by decreasing the level of transaminases (AST and ALT) and ALP in serum of ethanol induced rat hepatic injury (Abbas, *et al*, 2017). Supporting findings indicated that the normalcy of AST, ALT and ALP towards the respective normal values by herbal formula is an indication of the stabilization of plasma membranes as well as repair hepatic tissue damage caused by carbon tetrachloride (Prakash, *et al.,* 2008). This effect is in agreement with the commonly accepted view that serum levels of transaminases and ALP return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes in wistar albino rats (Bhuvaneshwari, *et al*., 2014). The treatment with aqueous extract of *Emblica officinalis* showed a significant reduction in ALT activity in diabetic rats (Deori, *et al*., 2017).

Present study will help us to know the role of *Emblica officinalis* to modulate the toxic effects of nitrogen dioxide and sulphur dioxide gas in mammals.

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