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ORIGINAL ARTICLE

Protective Effect of *Withania somnifera* on SO_2 Induced Osmotic Fragility Alterations in Albino Rats

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ABSTRACT

The present study is designed to evaluate the modulatory effect of Withania somnifera on osmotic fragility after exposure to SO_2 gas (80ppm, 1h/day) for 30 and 60 days. The findings of the present study showed that there is significant elevation in the osmotic fragility ratio after 30 and 60 days exposure to SO_2 gas. However, SO_2 induced alterations were modulated by oral supplementation of aqueous extract of Withania somnifera (5mg/100gb.wt./day) due to its anti-stress antioxidant defense mechanism against toxic action of SO_2 gas inhalation.
***Key words**: Albino rat, SO_2 gas, Withania somnifera, osmotic fragility*

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INTRODUCTION

Air pollution is a major environmental issue which continues to threat human health for a long time all over the world. Air is polluted by the release of gaseous pollutants like carbon monoxide, sulphur dioxide, chlorofluorocarbons (CFC) and nitrogen oxides produced by industries and motor vehicles into the atmosphere. Exhaust gases from automobiles are supposed to generate about 75% of total air pollution. WHO estimates that 2.4 million people die each year causes directly attributable to air pollution (Gulati, *et al*., 2010). The gases which beyond the certain limit cause undesirable and disastrous effect on human and environment. The chief gaseous pollutants are oxides of sulphur and nitrogen. From several major pollutants in the atmosphere, increasing sulphur dioxide is considered to be an evil of the development. Sulphur dioxide gas is a common industrial pollutant of the air that arises mainly from industrial process and domestic combustion of fossil fuels, burning of fuels and oil. It is also present in appreciable quantities in air where coal is used as fuel, example electric power plants, smelters where sulphur bearing ore is roasted, oil refineries, sulphuric acid manufacturing industries, fertilizers and paper and pulp industries etc.

It is the fact that blood is an index of the state of health of an organism. It is the closest tissue to air pollutants after they enter through respiratory apparatus. It is a fluid constituent of the body that flows through vascular channels and transport the vital requirement and waste product of the body.The immune response, the second challenge occurs more quickly than the first, is stronger and often is more effective in neutralizing and clearing the pathogens. The major cellular agents of adaptive immunity are lymphocytes and the antibodies and other molecules they produce. Adaptive immune

responses require some time to marshal, innate immunity provides the first line of defense during the critical period just after the host's exposure to a pathogen.

*Tinosporacordifolia*commonly known as guduchi or gelloy, a glabrous climbing shrub, widely used in folk and ayurvedic system of medicine in India since ancient times. It is distributed throughout tropical Indian subcontinent and China, ascending to an altitude of 300m. The stem of *Tinosporacordifolia*are rather succulent with long filiform fleshy aerial roots from the branches. *Withania somnifera*, commonly known as ashwagandha and winter cherry grows in the temperate climate. It grows up to a height of 5000 feet in western India and Himalayan regions. The stem is around 3 to 4 feet in height. One plant survives for upto 4 to 5 years. The leaves are bowl shaped, small and without thorns. Its stem contains fibre like texture.

Thus considering the fact, present study is designed to assess the ameliorative effect of adaptogens on SO_2 induced hematotoxicity and immunotoxicity in albino rats. The albino rats will be selected for the present study because of its easy adaptability and having physiological similarity to the human beings and can be easily reared in laboratory conditions.

MATERIAL AND METHODS

EXPERIMENTAL ANIMAL: Wistar albino rat, *Rattus norvegicus* (Berkenhout) of both the sexes have been selected for the present study.

MAINTENANCE AND FEEDING OF EXPERIMENTAL ANIMALS: Healthy and adult albino rats of both the sexes of almost equal size and weight ranging from 150-200g were taken for the present study. They were kept in clean, well ventilated rooms in cages, measuring 45x27x15cm at temperature $25^0\pm2^0$C and, relative humidity 50±5% and desired light dark cycle (12-12hrs). The top and sides of the cages were made of galvanized steel mesh. A sliding removable tray was placed below the cage to hold excreta which was cleaned regularly to avoid any infection and undesirable odour in the laboratory. Each cage was equipped with a metallic food plate and water cup.The rats were fed on Golden feed, New Delhi and water was provided *ad libitum*. The experimental albino rats were acclimated for one month prior to experiment.

EXPERIMENTAL GAS: Sulphur dioxide gas was selected for the present study. 80ppmsulphur dioxide was selected as sub-lethal concentration for the present study. Sulphur dioxide gas was generated in gas generator. In sulphur dioxide generator solutions of sodium sulphite and sulphuric acid are fed to the reactor unit where the reaction takes place and reagents remain for a set period of time. The sulphur dioxide gas is continuously released in the reactor loop. Flusher- evacuater unit equipped with a system to maintain dynamic vacuum or partial pressure in the reactor instantly takes up the gas being produced and dispenses it out by selection of proper concentration of sodium sulphite and sulphuric acid. The desired gas concentration can be obtained.

FUMIGATION CHAMBER: Fumigation chamber (Model AP-07, SFC-120) manufactured by Standard Appliances, Varanasi was used for the exposure of rats. The fumigation chamber measuring 45x45x60cms is made of metallic frame with glass walls. A glass door is fitted in the front, while a water chamber on the top of the fumigation chamber. Various control knobs for air circulation, water circulation, air pressure are located above and below the door of the fumigation chamber. Sulphur dioxide gas with ambient air circulate in the chamber was maintained with the help of the speed blow fan located at the base of fumigation chamber.

PROCUREMENT OF PLANT MATERIALS: In the present study, two adaptogens *Tinosporacordifolia* and *Withania somnifera* were used. They were collected from the

botanical garden of School of Life Sciences, Khandari Campus, Dr. B.R.A. University, Agra and identified by plant taxonomist.

The stem of *Tinosporacordifolia* is rather succulent with long filiform fleshy aerial roots from the branches. The bark is creamy white to grey, deeply left spirally, the space in between being spotted with large rosette like lenticels. The leaves are membranous and chordate. The flowers are small and greenish yellow.Main effective constituents are alkaloids, diterpenoids, lactones, glycosides, steroids, sesqueterpenoids, phenolics, aliphatic compounds and polysaccharides (Singh, *et al*., 2003).

The stem of *Withania somnifera*is around 3 to 4 feet in height. One plant survives for upto 4 to 5 years. The leaves are bowl shaped, small and without thorns. Its stem contains fibre like texture. The flowers are blooming at the base of the stem are small and yellowish green in colour. The seeds are small, heart shaped, smooth and flat. The roots are rough, white from within strong, transparent, thick and one to one and half feet long.Main effective constituents are steroidal lactones, withaferin, withanolides, saponins, iron, somniferin, alkaloids (Bharti, *et al*., 2013).

PREPARATION OF EXTRACTS: For aqueous extraction of *Tinosporacordifolia* stem and *Withaniasomnifera* root, heat distillation process was used. The stem of *Tinosporacordifolia* and root of *Withaniasomnifera* cut into small pieces and powdered in electric mixer. 200g powder of plant material was boiled in 1 litre water at 1:5 ratio at 100^0C for 30 minutes (Sonia *et al*., 2001). After 30 minute the mixture was filtered and the filtrate was stored in a refrigerator until used.

EXPERIMENTAL PROTOCOL: The albino rats of both the sexes were grouped into eight sets- four sets (Ia, IIb, IIIc, IVd) for 30 days and four sets (IA, IIB, IIIC, IVD) for 60 days, each set contains 5 rats.

Control sets (Ia and IA)– were kept in separate room without exposure for 30 and 60 days

Experimental sets (IIb and IIB)– exposed to 80ppmSO_2 gas one hour per day for 30 and 60 days

Experimental sets (IIIc and IIIC)– exposed to 80ppmSO_2 gas one hour per day with supplementation of aqueous extract of *Tinosporacordifolia* stem (5mg/rat/day) for 30 and 60 days.

Experimental sets (IVd and IVD)– exposed to 80ppmSO_2 gas one hour per day with supplementation of aqueous extract of *Withaniasomnifera* root (5mg/rat/day) for 30 and 60 days.

EXPOSURE TO SULPHUR DIOXIDE GAS: For generation of 80ppmsulphur dioxide gas the solution of sodium sulphite (*vide supra*) and 5% sulphuric acid solution were taken separately in the plastic bottle of the SO_2 generator. Each bottle is equipped with two tube and stop cock to control the flow of the solution. The flow of reagent to reactor was set to 1.25ml/min to obtain the required concentration of the sulphur dioxide gas and the flow of the gas and the air is controlled by adjusting the speed of blow fan at 0.5 m^3/min. Finally adjusting all the controlling knobs and connections of the fumigation chamber it was switched on each day. The rats of sets IIb and IIB, IIIc and IIIC, IVd and IVD were exposed in the fumigation chamber according to the experimental protocol (*vide supra*) for one hour per day for 30 and 60 days.

DOSES AND ADMINISTRATION OF EXTRACTS OF *Tinosporacordifolia* and *Withaniasomnifera:* The doses range was employed as guidelines as per traditional medical system (TMS) (WHO, 2001). In the present study, aqueous extract of *Tinosporacordifolia* stem (5mg/rat/day) and *Withaniasomnifera* root (5mg/rat/day) were administered in rats by gavaging tube.

PREPARATION OF DOUBLE OXALATE VIALS AND COLLECTION OF BLOOD AND SEPARATION OF SERUM: 800mg potassium oxalate and 1200mg ammonium oxalate were dissolved in 100ml of distilled water. After shaking well 0.1ml/ml of blood were taken in each sterilized empty glass vials and dried at 80^{0}C in an oven. The double oxalate vials were used for various haematological parameters.Blood samples of each rat of each set were taken in double oxalate vials (*vide supra*) for determination of various hematological parameters and for the estimation of immune response blood will be transferred to the empty centrifuge tubes for the separation of serum.

OSMOTIC FRAGILITY: The osmotic fragility of erythrocytes was done by the method described by Beutler (1983).Anticoagulated blood is placed in a series of tubes of decreasing concentrations of sodium chloride solutions and incubated at room temperature for one hour. Hemolysis is used as an index of the degree of susceptibility of erythrocytes to hypotonic damage.

STATISTICAL CALCULATIONS: In the present investigation, the following formulae were used for different statistical calculations (Fisher and Yates, 1963).

RESULTS AND DISCUSSION

Table 1: Values of Osmotic fragility (per cent hemolysis) after 80ppmSO_2 exposure and supplementation of *Tinosporacordifolia* and *Withaniasomnifera* in albino rats for 30 days

NaCl conc. (%)	Control set I_a (Non-exposed)	Experimental set II_b (80ppmSO_2)	Significance difference from corresponding	Experimental set III_c (80ppmSO_2+ *Tinosporacordifolia*)	Significance difference from corresponding		Experimental set IV_d (80ppmSO_2+ *Withaniasomnifera*)	Significance difference from corresponding	
	Range (Mean±S.Em.)	Range (Mean±S.Em.)	Set I_a	Range (Mean±S.Em.)	Set I_a	Set II_b	Range (Mean±S.Em.)	Set I_a	Set II_b
0.1	90.90-95.68 93.67±0.93	94.44-95.98 95.38±0.28	↑•	81.81-96.00 92.32±2.73	↓•	↓•	88.46-96.66 93.34±1.42	↓•	↓•
0.2	74.98-78.68 76.76±0.77	80.69-84.86 83.28±0.74	↑***	67.63-72.64 70.35±0.96	↓***	↓***	76.56-79.98 77.91±0.64	↑•	↓***
0.3	50.00-69.23 61.63±3.25	63.68-67.89 65.69±0.77	↑•	50.00-59.18 55.20±1.83	↓•	↓***	60.00-62.96 61.50±0.63	↓•	↓***
0.4	25.00-32.14 28.01±1.34	33.30-35.96 34.57±0.56	↑***	22.72-42.30 33.00±3.21	↑•	↓•	31.81-35.28 33.19±0.77	↑***	↓•
0.5	4.34-9.80 6.57±1.03	8.00-12.00 9.87±0.66	↑•	4.54-10.04 6.97±0.92	↑•	↓*	5.36-11.53 7.52±1.05	↑•	↓*

Table 2: Values of Osmotic fragility (per cent hemolysis) after 80ppmSO_2 exposure and supplementation of *Tinosporacordifolia* and *Withaniasomnifera* in albino rats for 60 days

NaCl conc. (%)	Control set I_A (Non-exposed)	Experimental set II_B (80ppmSO_2)	Significance difference from corresponding	Experimental set III_C (80ppmSO_2+ *Tinosporacordifolia*)	Significance difference from corresponding		Experimental set IV_D (80ppmSO_2+ *Withaniasomnifera*)	Significance difference from corresponding	
	Range (Mean±S.Em.)	Range (Mean±S.Em.)	Set I_A	Range (Mean±S.Em.)	Set I_A	Set II_B	Range (Mean±S.Em.)	Set I_A	Set II_B
0.1	90.89-95.36 93.41±0.95	96.50-98.80 97.67±0.42	↑***	93.75-94.73 94.18±0.15	↑•	↓***	92.68-96.99 94.79±0.78	↑•	↓***
0.2	74.98-78.68 76.76±0.77	84.16-93.33 88.83±1.78	↑***	64.69-70.58 68.37±1.00	↓***	↓***	75.63-78.80 77.41±0.59	↑•	↓***
0.3	60.00-62.97 61.50±0.63	63.68-67.89 65.69±0.77	↑***	52.63-58.88 55.94±0.99	↓***	↓***	60.16-64.16 62.24±0.74	↑•	↓***
0.4	31.81-35.28 33.19±0.77	47.82-53.20 50.62±0.93	↑***	29.41-37.50 33.69±1.44	↑•	↓***	30.69-40.42 34.70±1.85	↑•	↓***
0.5	5.86-8.88 7.13±0.52	14.28-20.00 17.04±1.02	↑***	5.88-10.52 7.88±0.85	↑•	↓***	5.26-9.29 7.35±0.69	↑•	↓***

(5) = Number of albino rats in each set •Non–significant (P>0.05) ↓Decrease
I_a, II_b, III_c, IV_d= Sets for 30 days ***Very highly significant (P<0.001) ↑Increase
I_A, II_B, III_C, IV_D= Sets for 60 days

In the present study, very highly significant increase in osmotic fragility is the indication of oxidative damage in rats after inhalation of SO_2 gas. Further, the erythrocyte membrane

is rich in unsaturated fatty acids which may susceptible to lipid peroxidation induced by products may cause some change including membrane protein cross linking and oxidation of heme protein resulting in their cross linking to skeletal proteins that is spectrin, actin and the cytoplasmic components of band-3 (Azeez*et al*., 2011). Oxidative denaturation of hemoglobin into methemoglobin and sulphemoglobin is the process that accompanies the formation of hydrogen peroxide. Effects of hydrogen peroxide on erythrocyte membrane include change in passive cation permeability and cross linking between proteins (Wintrobe, *et al*., 1981).

Etlik, *et al*. (1995) and Etlik, *et al*. (1997) reported increase in osmotic fragility in sulphur dioxide exposed rats. Similar to the present findings, Etlik and Tomar (2006) observed an increased level of osmotic fragility due to oxidative damage after exposure to SO_2 gas in albino rats. Guleria (2008) has also reported enhancement in osmotic fragility after combined exposure to SO_2 and NO_2 gas in albino rat.

In accordance of present findings, Baskurt, *et al*. (1990), have observed increase in osmotic fragility in SO_2 exposed male military students. Similarly, Etlik, *et al*. (1995) and (1997) and Agar, *et al*. (2000) reported elevation in osmotic fragility in experimentals after inhalation of SO_2 gas.Supporting findings are given by Senturk*et al*. (2001) who studied oxidative stress contribute to deterioration of erythrocytes with increase in osmotic fragility in rats. Similar to the present findings, Meng, *et al*. (2003a&b), and Zhang, *et al*. (2005) have observed an increase in osmotic fragility with higher MDA levels in sulphur dioxide exposed mice. Adenkola and Ayo (2009) have also reported an increase in osmotic fragility due to oxidative stress in pigs. Supporting findings are also given by Zhao, *et al*. (2012) who have reported oxidative damage of erythrocytes *in vitro* after SO_2 gas exposure.

In the present study, the supplementation of adaptogens *Tinosporacordifolia* and *Withaniasomnifera* individually have potential in attenuating the toxic effects of sulphur dioxide gas. Aqueous extract of *Tinosporacordifolia* stem and *Withaniasomnifera* root decreases the inflammatory responses induced by free radicals, they inhibited the chain of free radicals and have ameliorative effects against oxidative stress in albino rats.

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