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AMELIORATIVE EFFECT OF ASHWAGANDHA AGAINST SO_2 TOXICITY ON SERUM IMMUNOGLOBULINS IN ALBINO RAT

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ABSTRACT

The present study is designed to investigate the serum immunoglobulins viz. IgA, IgG and IgM after sub-acute (80ppm, 1h/d for 30 days) and sub-chronic (80ppm, 1h/d for 60 days) exposure to SO_2 gas in albino rat. The rats six sets- control set (I) and (IV) exposed to ambient air for 30 and 60 days, experimental set (II) and (V) exposed to SO_2 gas for 30 and 60 days and experimental set (III) and (VI)exposed to SO_2 gas with pre-exposure supplementation of aqueous extract of ashwagandha root (5mg/rat/day) for 30 and 60 days. The result of the present study shows a significant decrease in IgA, IgG and IgM after 30 days exposure to SO_2 gas, while a very highly significant decrease after 60 days exposure to SO_2 gas. However, an increase in values of serum immunoglobulins were observed after supplementation of aqueous extract of Ashwagandha. Modulation in serum immunoglobulins after supplementation of Ashwagandha extract is due to antioxidant defense mechanism against SO_2 toxicity.

KEYWORDS:Ashwagandha, immunoglobulins, SO_2, albino rat.

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INTRODUCTION

Ashwagandha, *Withania somnifera* Dunal belongs to family solanaceae, is widely used in Ayurvedic medicine, the traditional medical system of India. Traditionally all parts of the plant are used as herbal remedies but the root, which has a damp horse smell, is much more effective. Many pharmacological studies have been conducted to investigate the properties of Ashwagandha in an attempt to authenticate its use as a multi-purpose medicinal agent[1]. Main active constituents withaferin, withanolides, withanine, sitoindosides, somniferine, alkaloids and ethanolides. Withanolides are believed to account for the multiple medicinal application of ashwagandha. Ashwagandha is a main constituent of various adaptogenic and antistress tonics[2]. SO_2 gas is an ubiquitous air pollutant, that arises mainly from industrial processes and domestic combustion of fossil fuels, burning of fuels and oils, occur in the atmosphere in the large quantity[3]. Exposure of air pollutants and the development of certain pathological conditions lead to oxidative stress consequently increase production of free radicals. When SO_2 emitted into the atmosphere reacts with water and other compounds to form various acidic compounds, fine particles and ozone. Blood is closest to air pollutants after they enter through the respiratory tract. Most air pollutants reach the blood quickly without previous bio-transformation and have been shown to produce harmful effect on the blood, bone marrow and lymph nodes[4]. Immunity is an integral part of the blood which provides protection from infectious diseases. any means by which these defense system can be catalyzed or enhanced, will prove to boost the overall immune response and well being of the host. The present study was aimed to evaluating the ameliorative effect of ashwagandha on SO_2 induced serum immunoglobulins in albino rats.

MATERIALS AND METHODS

- ***Experimental animals*** –

Experimental rats (100-150 g) reared in polypropylene cages in standard conditions of temperature 25±0.5^{0}C, relative humidity 60±57 and Photoperiod 12 h/d. The rats were feed on commercial food pellets (Golden Feed, New Delhi) and water *ad libitum*. Experimental rats were acclimated for one month prior to experiment. The institutional ethics committee approved the experimental protocol.

- ***Exposure to SO_2 gas –***

The sulphur dioxide gas was prepared by controlled action of 5% sulphuric acid on sodium sulphite[5] in SO_2 gas generator. Experimental rats were exposed to SO_2 gas in the fumigation chamber (Model AP-07, SFC-120) manufactured by Standard Appliances, Varanasi.

Preparation of aqueous extract of Ashwagandha –

Ashwagandha plant was collected from botanical garden of School of life sciences, Khandari Campus, Agra, and identified by a plant taxonomist of the institute. For aqueous extraction of root, heat distillation process was used. The root powder was boiled in water (1:5 ratio) at 100^{0}C for 30 minutes[6] after 30 minutes the mixture was filtered and the filtrate was stored in a refrigerator until use.

Experimental protocol –

Experimental rats were divided in six sets containing 5 rats each Control set (I) and (IV) – exposed to ambient air for 30 and 60 days respectively. Experimental sets (II) and (V) – Exposed to 80 ppm SO_2 gas 1 h/d for 30 and 60 days respectively. Experimental sets (III) and (VI) – Exposed to 80 ppm SO_2 gas 1 h/d with simultaneous supplementation of aqueous extract of Ashwagandha root (5 mg/100 g.b.wt./day) for 30 and 60 days respectively.

Collection of blood samples –

Rats of each set were sacrificed after 30 and 60 days exposure to SO_2 gas and blood samples were collected directly from the ventricle of heart of the dissected rats with the help of sterilized disposable syringes fitted with hypodermic needles and were taken in the sterilized centrifuge tubes for the separation of serum.

Serum immunoglobulins –
IgG, IgM and IgA levels were estimated in serum using a sandwich Enzyme-Linked Immunosorbent Assay (ELISA)[7]. The results were expressed as Mean±S.Em. by using student 't' test and statistical analysis were done by one-way ANOVA with the help of Computer Statistical programme K_p K_y plot (Version 3.0).

RESULTS

The results of the present study shows the ameliorative effect of aqueous extract of ashwagandha in reducing the toxic effects of SO_2 gas inhalation. A significant decrease in serum immunoglobulins – IgG, IgM and IgA were observed after 30days, while very highly significant decrease after 60days exposure to SO_2 gas, whereas increase in serum immunoglobulins after 30 and 60days exposure to SO_2 with supplementation of aqueous extract of ashwagandha root were observed (Table-1 and 2).

Table-1
Values of IgA IgG and IgM after SO_2 exposure and Treatment with ashwagandha for 30 days in albino rats

Sets (5)	Treatment (1h/d for 30 days)	IgA (SU) Mean±S.Em.	IgG (SU) Mean±S.Em.	IgM (SU) Mean±S.Em.
Control set (I)	Ambient air	201.4±3.318	125.2±1.984	0.872±0.032
Experimental sets				
Set (II)	80ppm SO_2	189.0±2.932↓*	108.8±2.009↓*	0.684±0.0361↓*
Set (III)	80ppm SO_2+ashwagandha	206.6±1.720↑• ↑HS	129.2±1.496↑• ↑VHS	0.88±0.013↑* ↑VHS

(5) Number of albino rats ***Correspondence to set (I)*** ***• Non-significant (P>0.05)*** ****Significant (P<0.05)*** ***Correspondence to set (II)*** ***HS- Highly significant (P<0.01)*** ***VHS-Very highly significant (P<0.001)***

Table 2
Values of IgA IgG and IgM after SO_2 exposure and treatment with ashwagandha for 60 days in albino rats

Sets (5)	Treatment (1h/d for 60 days)	IgA (SU) Mean±S.Em.	IgG (SU) Mean±S.Em.	IgM (SU) Mean±S.Em.
Control set (IV)	Ambient air	204.2±2.973	124.2±1.854	0.79±0.007
Experimental sets				
Set (V)	80ppm SO_2	129.4±4.190↓***	109.2±2.576↓***	0.598±0.018↓***
Set (VI)	80ppm SO_2+ashwagandha	211.8±1.428↑* ↑VHS	129.4±1.077↑• ↑VHS	0.818±0.006↑* ↑VHS

(5) Number of albino rats ***Correspondence to set (IV)*** ***• Non-significant (P>0.05*** ****Significant (P<0.05)*** ***Correspondence to set (V)*** ***VHS- Very highly significant (P<0.001)*** ******Very highly significant (P<0.001)***

DISCUSSION

Exposure of pollutants leads to oxidative stress consequently increase production of oxyradicals[8]. SO_2 gas imposes oxidative stress to the body[9]. In the present study, a significant decrease in serum immunoglobulins is due to toxic effect of SO_2 gas on haemopoietic tissue. Sulphur dioxide gas make contact with the internal environment of the body through the respiratory tract and interfere in normal lymphocyte cell production and its immunoglobulin contents which results in immunosuppression in albino rats. Oxidative stress contribute to depress the lymphocytic response[10]. Lymphocyte cells interact with SO_2 contents which causes oxidative damage to the immunocompetent cells, lacking immune function. An imbalance of the immune system resulting from cellular injury might be expressed as either immune enhancement or immune suppression[11]. The results are

consistent with the similar findings of Sapakal *et al*.[12] who observed that the cell damage caused by free radicals appear to be a major contributor in a compromised immune system. Secondary or acquired immunodeficiency is due to loss of immune function and results from exposure to various physical, chemical and biological agents[13]. Environmental agents may act as immunotoxicants[14]. Rutowski *et al*.[15] have observed a negative correlation between NO concentration in air in work place and serum level of IgA, IgM and IgG. Alterations in serum immunoglobulins are modulated after supplementation of root extract of ashwagandha, due to its antistress and antioxidative properties of active components, which accelerates hematopoesis to stimulate antibody production. Ashwagandha root extract has a significant antioxidative property[16]. It is an herb counteract the effects of stress and promotes general wellness and is found to offer protection against biological stressor[17]. Ashwagandha root powder possesses free radical scavenging activity[18] and intake of ashwagandha is improved hematopoiesis[19]. Similar to the present findings Ziaauddin *et al*.[20] observed that the treatment with ashwagandha was accompanied with a significant increase in hemolytic antibody responses. Methanolic extract of the ashwagandha root powder exhibited significant antistress property[21]. Experimental studies have reported that *Withania somnifera* have immunomodulatory activities[22]. The present study suggests that the supplementation of ashwagandha root extract have potential to modulate the toxic effects of SO_2 gas in mammals.

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