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Research Article

EVALUATION OF SERUM IgG, IgA & IgM IN IMMOBILIZED STRESSED RATS UNDER INFLUENCE OF PLANT EXTRACTS

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ABSTRACT
Asparagus racemosus (Shatavari) and *Tinospora cardifolia* (Guduchi) and *Withania somnifera* (Ashwagandha) are conventional herbal medicine. Indian system of medicine used as aphrodisiac, sedative, rejuvenative, in the treatment of jaundice, diabetes, and rheumatoid arthritis, and is also used as an immunostimulant. Experiments have examined its antineoplastic, antioxidant, hepatoprotective, hypolipidemic, and immunologic properties; however, clinical trials done using antistress model to evaluate the pattern of IgA, IgG and IgM. The dried roots of *A. racemosus, W. somnifera* and stem of *Tinospora cardifolia* were powdered and the ethanolic extract was obtained. The dried extract was administered orally as 0.1% suspension of carboxymethylcellulose at a dose mentioned in table1. After 15 days of oral administration serum Immunoglobulins were evaluated in Charle Foster strain and all selected herbs possess antistress effects.

Keywords: *Immobilization stress, Extract, Serum IgA, IgG, IgM*

INTRODUCTION
In Ayurveda, *Asparagus racemosus* (Shatavari) of family Asparagaceae,has been described as a rasayana herb and has been used extensively as an adaptogen to increase the non-specific resistance of organisms against a variety of stresses (Bopana and Saxena, 2007). *Asparagus racemosus* in Ayurveda used as a tonic remedy to promote fertility and reducing menopausal symptoms (Gaur and Kaushik, 2011). It is also used successfully for nervous disorders, inflammation, liver diseases, certain infectious diseases, immune modulator, increases corticosteroid production, ischemia and promotes cell regeneration (Potduang *et al.*, 2008; Nandagopal *et al.*, 2011; Velavan and Begum, 2007). The juice of fresh root of A. racemosus has curative effect in patients with duodenal ulcers. Oral administration of decoction of powered root enhances the immuno-modulatory effect (Uma *et al.*, 2009).
T. cardifolia acts as immunomodulator which act by modifying the immune system and affect a therapeutic benefit (Aher *et al.*, 2010). *Tinospora cardifolia* used to treat general weakness, fever, dyspepsia, dysentery, gonorrhea, secondary syphilis, urinary diseases, impotency, gout, viral hepatitis, skin diseases, and anemia. In compound formulations, guduchi is used clinically to treat jaundice, rheumatoid arthritis, and diabetes. The root is considered to be a strong emetic and is used for bowel obstruction (Chintalwar *et al.*, 1999; Gupta *et al.*, 1967). T. cordifolia is widely used in the Indian Ayurvedic system of medicine as an immunostimulant (Nair *et al.*, 2004). Sohni *et al.*, (1996) and Thatte *et al.*, (1994) reported the immunomodulatory activity of *Tinospora cardifolia*.
Withania somnifera an Indian ginsen used as herbal remedy in a variety of ailments to promote general debility. It possess a large number of alkaloids and steroidal lactones (Grover *et al*., 2010; Singh *et al*., 2011). *W. somnifera* possess anti-inflammatory, antioxidant, anti-tumour and immunomodulatory properties (Pretorius *et al*., 2009; Singh *et al*., 2011). EuMil, a herbal preparation of Withania somnifera, Ocimum sanctum, Asparagus racemosus and Embelica officinalis shown adaptogenic and antistress activity (Muruganandam *et al.*, 2002). Ziauddin (1996) reported the immunomodulatory activity of *W. somnifera*. Various plants like *H. perforatum, Ocimum sonactum, Calophyllum, Catanospermum, Echinaceae, Pinus, Andorgraphis and Trichopus* possess immunomodulatory property (Chauhan, 2002). Several workers have reported that stress is responsible for alteration of body immunity which was

reflected by reducing the level of IgG, IgA and IgM (Jons *et al.,* 1996; Rozen *et al.,* 1996). Several studies have conducted to correlate relationship between stress and humoral immunity. There are 3 ways of characterizing the response of the humoral immunity to stress, total Ig; Ab to latent viruses and Ab to vaccines.

MATERIALS AND METHODS

Table 1: Details of studied plants and doses-

SN	Botanical Name	Plant	Family	Parts Used	Dose
1	*Asparagus racemosus*	Satavari	Liliaceae	Root	540mg/kg bw
2	*Tinospora cardifolia*	Guruchi	Menispermaceae	Stem	180mg/kg bw
3	*Withania somnifera*	Ashwagandha	Solanaceae	Root	130mg/kg bw

Plant Material Procuring and Crude Organic Extraction

Plants samples were obtain from Department of pharmacy, Faculty of Ayurveda, IMS.

The test drug's air dried roots and stem were coarsely powdered and exhaustively extracted in a Soxhlet apparatus with ethanol (90%) for 72 h. The yield of the extract was found to be 30%. The extract was concentrated and dried on rotary flash evaporator to get a dark brown mass.

Animal Selection

Adult Charles Foster albino rats strains (175-250g) of either sex were procured from Central Animal House of the Institute animal house. The rats were housed in gp of six in polypropylene cages at ambient temp. of 25˚C and 45-55% RH, with a 12:12 h light/dark cycle. Animal were provided with commercial food pellets (Brooke Brand-Lipton, India) and water ad libitum unless stated otherwise. ("Principles of laboratory animal care" (NIH publication no. 85-23, revised 1985) guideline were followed.

Experimentation

This experiment has been divided into three groups and in each group 06 rats of Charles foster strain weighing 175 gm to 250 gm were selected. Experiment was divided into three groups.

1. Control group and received only vehicles

2. Stress group; Immobilization stress was given to all rats for 15 minutes daily for 15 days and received only DW.

3. Stress + drug treated group; stress was given to all the rats alongs with various mentioned doses of alcoholic extracts of Ashwagandha, Guruchi and Satavari daily for 15 days orally.

At 15th day blood samples were collected and immunoglobulins were quantitatively measured.

Collection of Blood Sample

Three ml. blood was collected from the rats in a sterile tube. Serum was separated and preserved at 4ºC after adding sodium azide powder as preservative.

Immunoogical Investigation

Quantitative estimation of serum Immunoglobulins was done by the method of single radial immuno diffusion technique of Maccini (1965) modified by Fahey (1968) and animal model for immobilization stress followed method of Roger *et al.*, (1978).

Evaluation of Immunoglobulin Concentration of the Sample

After appropriate time the diameter of the antigen antibody diffusion rings were measured with the measuring templet. The ring diameters (mm) of the standards are plotted as the abscaissa on a linear scale against their respective immunoglobulin concentration which yield a curve line (a straight line can be obtained by plotting the squares of the diameters).

The corresponding protein concentration can be read from the standard curve, this was multiplied by the dilution factor to give the concentration of each immunoglobulins in the undiluted samples.

RESULTS AND DISCUSSION

Observation and Results

The data were statistically analyzed obtained on IgA, IgG and IgM in blood serum of immobilized stressed rat groups and in group of stress plus ethanolic extract of *Asparagus racemosus, Tinospora cardifolia and Withania somnifera* along with a group of normal control.

Table 2: Effect of plants extracts on serum Immunoglobulins following Immobilization stress in rats (values are in mean+-SD)

SN	GROUP	IgG mg/100ml		IgA mg/100ml		IgM mg/100ml	
		0Days	15Days	0Days	15Days	0Days	15Days
1	Control	1260.85	1270.25	260.46	270.30	128.20	140.30
	n=6	±45.35	±54.84	±14.85	±16.36	±8.36	±7.97
2	Immobilization	1025.38	983.28	245.48	240.36	110.25	80.70
	n=6	±46.36	± 57.33	±13.85	±10.86	±7.46	±6.98
3	I.S.+*A. racemosus*	1120.25	1140.78	245.75	260.25	100.50	55.84
	n=6	±45.36	±48.38	±14.35	±12.74	±8.36	±6.75
4	I. S.+*T. cardifolia*	1005.25	1050.50	235.45	260.50	115.25	125.25
	n=6	±48.32	±47.36	±14.07	±13.76	±7.46	±6.95
5	I. S.+*W.somnifera*	1020.35	1108.25	235.78	250.47	115.35	125.35
	n=6	±47.94	±46.72	±11.87	±18.92	±8.04	±7.34

Asparagus Racemosus

Serum IgG: Serum IgG level was reduced significantly in stressed rats as compared to normal rats($p<0.01$) whereas, the level of sIgG was significantly increased in *Asparagus racemosus* treated stressed rats as compared to stressed rats($p<0.02$).

Serum IgA: Serum IgA level was just reduced in stressed rats as compared to normal rats. The value of p was ($p<0.05$) whereas the level of Serum IgA in *Asparagus racemosus* treated stressed rats was increased significantly as compared to stressed rats($p<0.01$).

Serum IgM: The level of serum IgM significantly reduced in stressed rats as compared to normal rats($p<0.001$). Serum IgM was just increased in *Asparagus racemosus* treated stressed rats as compared to stressed rats ($p<0.05$).

Tinospora Cardifolia

Serum IgG: The serum IgG level reduced significantly in stressed rats as compared to normal rats ($p<0.01$). On the contrary, serum IgG level was significantly increased in *Tinospora cardifolia* treated stressed rats as compared to stressed rats($p<0.02$).

Serum IgA: Serum IgA level of stressed rats was just reduced in stressed rats as compared to normal rats($p<0.05$). Where as serum IgA level was significantly increased in *Tinospora cardifolia* treated stressed rats as compared to stressed rats($p<0.05$).

Serum IgM: The level of Serum IgM was reduced significantly in stressed group as compared to normal group($p<0.001$) where as serum IgM was increased significantly in *Tinospora cardifolia* treated stressed group as compared to stressed group($p<0.01$).

Withania Somnifera

Serum IgG: Serum IgG level in stressed rats treated with *Withania somnifera was found increased significantly* as compared to stressed group *($p<0.02$)*.

Serum IgA: Serum IgA level was just reduced in stressed rats as compared to normal ($p<0.05$). However Serum IgA level was just increased in *Withania somnifera* treated stressed rats group compared to stressed group(*$p<0.05$*).

Serum IgM: The level of serum IgM was significantly reduced in stressed group as compared to normal group ($p<0.001$). On the other hand Serum IgM level was significantly increased ($p<0.01$) in *Withania somnifera* treated stressed rat group.

Discussion

Among various stressor mechanisms, immobilization stress has been selected for experimental study. Physical restraint is one of the most widely used methods of applying stressor in experimental studies. The very first investigation of alarm reaction was preferred on imobilization rats (Selye, 1976). Imobilization stress (restraint) proved to be a very effective in eliciting typical nonspecific stress manifestation.

In this studied the levels of immunoglobulins were reduced significantly in stress gp in comparison to control gp the level of sIgG ($p<0.01$), sIgA($p<0.05$) and IgM ($p<0.001$).

In *Asparagus racemosus* treated stressed gp a significant increased in sIgG ($p<0.02$), sIgA (0.01) and sIgM ($p<0.05$) was found in comparison to stressed gp.

The level were significantly increased in *Tinospora cardifolia* treated stress gp in comparison to stress gp and the values of sIgG ($p<0.02$), sIgA($p<0.05$) and IgM ($p<0.01$).

Similarly in *Withania somnifera* treated stressed gp a significantly increased in sIgG ($p<0.02$), sIgA($p<0.05$) and sIgM ($p<0.01$) level as compared to stressed rats.

Conclusion

A diversity of experimental model shows that laboratory stressor such as force exercise, avoidance learning, restraint isolation and cold exposure make animal more susceptible to disease infection. It can lead to reduced Immunoglobulin levels, immune deficiency and increased susceptibility to infection. Immune system has a fundamental role in the maintenance of body homostasis by eliminating the substance which is not recognized as self (Austen, 1977; 78; Bellant and Green, 1971). Considerable emphasis has also been laid in the research programme of pharmaceutist world wide during the last 20 years to tap the plant kingdom for treatment of such diseases for which the modern medicine does not have any effective treatment.

The data emerging out of this study as presented in result and observations is of a greatly beneficiaries may be utilized to understand the role of Immunoglobulins in diagnosis and pathophysiology of stress.

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