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Rapid Communication

Study of Antistress Activity of NR-A2 in Active Anaphylaxis-Induced Allergic Rats

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Abstract : NR-A2, is a polyherbal formulation of Natural Remedies Pvt. Ltd., Bangalore, developed for the treatment of allergic rhinitis. A new experimental model was developed to study the antistress activity of NR-A2 in allergic condition. The rats were divided into 4 groups of 6 animals each. Group I (Control I) and Group II (Control II or Stressed control) animals received distilled water p.o. for 30 days. Group III animals received NR-A2 250 mg/kg p.o. for 30 days and Group IV animals received *Ocimum sanctum*, 100 mg/kg i.p. on last day. The rats of group II, III and IV were sensitized on 17th day of 30 days treatment with subcutaneous injection of 0.5 ml horse serum and 0.5 ml of triple antigen containing 20,000 million B. purtussis organism s.c. All the animals were subjected to stress by Swim endurance on the last day after 1 hour of last dose of drugs and rechallenged with 0.5 ml horse serum and 0.5 ml triple antigen i.p. At the end of swim endurance test, plasma corticosterone estimation was done. Active anaphylaxis induced animals showed significantly increased levels of stress. NR-A2 treated rats showed a significant increase in swimming time and reduction of the increased levels of Plasma corticosterone as compared to sensitized control rats.

Key words : NR-A2, Active anaphylaxis, Antistress activity, Plasma corticosterone, Swimming time

Introduction

Allergic rhinitis is an inflammation of the nasal passages, usually associated with watery nasal discharge and itching of the nose and eyes. Dust mites, cockroaches, molds and animal dander, are some of the allergens, which cause allergic rhinitis. Characteristic symptoms of allergic rhinitis include repetitive sneezing; rhinorrhea (runny nose); post-nasal drip; nasal congestion; pruritic (itchy) eyes, ears, nose or throat; and generalized fatigue. Symptoms can also include wheezing, eye tearing, sore throat, and impaired smell. A chronic cough may be secondary to postnasal drip, but should not be mistaken for asthma. Sinus headaches and ear plugging are also common. Classic signs of allergic rhinitis may include swelling of the eyelids, injected sclerae (the whites of the eyes may be red), allergic shiners (darkened areas under the lower eyelids thought to result from venous pooling of blood), and extra skin folds in the lower eyelids. Skin testing may confirm the diagnosis of allergic rhinitis. Since allergic rhinitis is a stress related allergic disease, the goal of treatment is to reduce the allergy symptoms. Antihistamines and nasal Decongestants as well as Immunotherapy may control the allergic rhinitis. (http://www.healthscout.com/ency/68/208/main.html)

NR-A2, is a polyherbal formulation of Natural Remedies Pvt. Ltd., Bangalore, developed for the treatment of allergic rhinitis. The two major ingredients of NR-A2 are *Albizzia lebbeck* and *Embelica officinalis*. *Albizzia lebbeck* possess immunomodulatory (Barua et.al., 2000) activity and *Embelica*

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officinalis is hypolipidemic, antioxidant, antiulcer, antimutagenic, and antiallergic. (Muruganandan et. al., 2002). The formulation needs to be validated for its proposed antiallergic and antistress activity. We have reported anti-stress and anti-allergic activity of formulation NR-A2. It is important to prove the effectiveness of NR-A2 by studying the antistress effect in allergic condition. The extensive literature search for an experimental model to study the relationship between stress and allergy yielded no results. The present study was taken up to evaluate antistress activity of the formulation in an allergic model.

Materials And Methods

Drug and Chemicals: NR-A2, a polyherbal formulation, and *Ocimum sanctum* used as reference antistress agent,) were gift from Natural Remedies Pvt. Ltd., Bangalore. Horse serum (Hyclone) and Triple antigen (Serum institute of India) were used as active anaphylactic agents. Some of the other drugs and chemicals used in the study were Dichloromethane, Heparin, HPLC grade methylene chloride, HPLC grade methanol, corticosterone, (Sigma Chemicals, USA)

Animal: Albino rats, Wistar strain, 150-200 g of either sex, maintained on natural day-night cycle, at a temperature of 25^0 C ± 2^0 C, commercial pellet diet (Amrut feeds, Bangalore) and water *ad libitum* were used. Institutional Animal Ethics Committee's permission was obtained before starting the experimentation.

Experimental

Antistress activity in allergic rat model: The rats were divided into 4 groups of 6 animals each. Group I (Control I or normal control) and Group II (Control II or stressed control) animals received distilled water p.o. for 30 days. Group III animals received NR-A2 250 mg/kg p.o. for 30 days and Group IV animals received *Ocimum sanctum*, 100 mg/kg i.p. on last day. The dose of NR-A2 was calculated, based on reported LD_{50} of it's various ingradients and also the proposed human dose of formulation. The rats of group II, III and IV were sensitized on 17^{th} day of 30 days treatment with subcutaneous injection of 0.5 ml horse serum and 0.5 ml of triple antigen containing 20,000 million B. purtussis organism s.c. Animals of all the groups were subjected to stress by Swim endurance on the last day after 1 hour of last dose of drugs and rechallenged with 0.5 ml horse serum and 0.5 ml triple antigen i.p. The immobility time was measured. At the end of swim endurance test, blood was withdrawn from carotid artery and corticosterone (Singh *et al*., 2000) levels were estimated.

Estimation of plasma corticosterone: Blood was collected into heparinized tubes and centrifuged, 2 ml of plasma was pipetted into 15 ml of methylene chloride to extract the steroids. The top aqueous layer was aspirated and discarded and the solvent phase evaporated to dryness in a water bath at 50^0C. (Bhargava and Singh, 1981). The dried residues were dissolved in methanol and 20 µl was injected into the HPLC analyser. (LC 8-A Shimadizer, Photodiode array detector- SPD-M 10 A UP Shimadzu, Rheodyne injector, 0.45 nm filter system, Richrocort 250-4 column, Water **:** Acetonitrite (55: 45) as mobile phase with flow rate 1.5 ml/m.) Similarly 20 µl of 10 µg/ml, 5 µg/ml, 1 µg/ml and 0.5 µg/ml of corticosterone in HPLC grade methanol were injected and eluted through the column at a particular RT and wavelength of 240-250 nm. The accuracy and reproducibility of the method was investigated by replicate analysis. The corticosterone peaks in chromatograms of biological samples were identified by comparison of their retention times (RT) with that of pure corticosterone. A standard graph of the peak areas of standard corticosterone in various concentrations was plotted and sample was quantified using it's peak value. (Pacak *et al.*, 1998)

Statistical analysis: All the data was statistical analyzed using one way ANOVA. P< 0.01 was considered statistically significant. The post-hock analysis was done by Dunnet's test for the significance of difference between the groups.

Results

Evaluation of antistress activity in allergic model: As seen from Table 1, active anaphylaxis induced rats showed significantly increased levels of stress as compared to animal which were not sensitized (Control I). Sensitized rats (Control II), when subjected to swim endurance swam for lesser time. The NR-A2 treated rats showed a significant (P< 0.01) increase in swimming time as compared to sensitized rats.

Estimation of plasma corticosterone: As shown in Fig 1, there is a significant increase in plasma corticosterone in rats sensitized and subjected to swim stress (Control II) compared to the unsensitized rats (Control I). Pretreatment with NR-A2 resulted in significant (P < 0.01; F= 0.38) reduction of corticosterone in the sensitized and stressed rats.

Discussion and Conclusion

The aim of present study was to study antistress activity of the formulation NR-A2 in an allergic model. We subjected the active anaphylaxis induced allergic rats to swim stress. Swim endurance as a stress model is used by many researchers to study antistress activity of drugs. There is a marked elevation of plasma ACTH and corticosterone concentrations in the rats subjected to swim stress, which is used as a marker to study stress. (Paul *et al*. 2002). Our study showed an increase in corticosterone concentrations in rats sensitized and swim stressed. NR-A2 in a dose of 250 mg/kg p.o. produced a significant reduction in corticosterone levels indicating an antistress property.

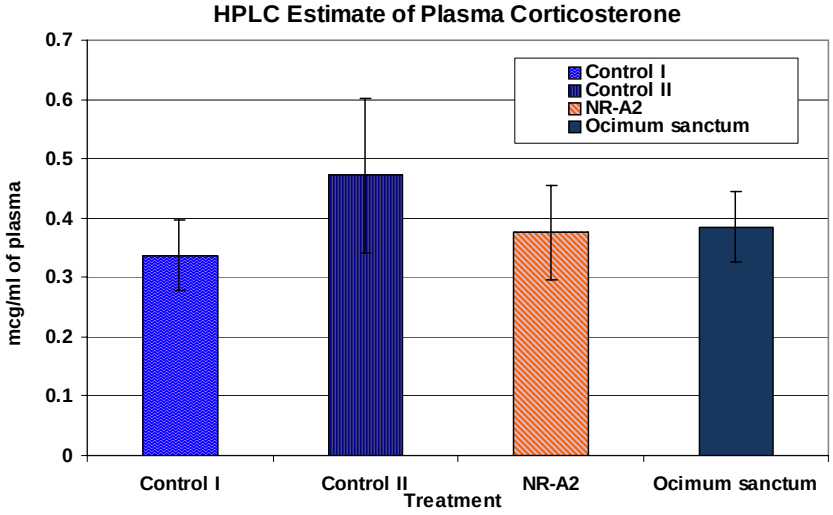
Immunological challenge by active anaphylaxis causes the release of mediators such as histamine, cytokine, leukotrienes which causes neuroendocrine changes similar to those provoked by psychogenic stressor. (Hung, 2001). It is reported that during stressful event, neuroendocrine hormones like CRH, ACTH, Cortisol, NE and epinephrine are released and they can alter immune functions. (Cannon, 1935). In our studies, we found that, there is a significant increase in plasma corticosterone in rats sensitized and subjected to swim stress (Control II) compared to the unsensitized rats (Control I). Earlier studies indicate that allergy through immune system plays a vital role in psychological and neurological diseases particularly stress and depression. (Popovic *et al*., 1997). In this study, there was a significant reduction in swimming time of the rats sensitized with horse serum as compared to unsensitized control rats. The NR-A2 pretreated, sensitized rats showed a considerable increase in swimming time as that of unsensitized rats.

Table 1 : Antistress activity of NR-A2 in active anaphylaxis induced rats

Treatment	Swimming time (minutes)
Control I (unsensitized)	142.00 ± 8.38
Control II (sensitized,stressed)	48.67 ± 3.60**
NR-A2 (250 mg/kg)	108.33 ± 7.60***
Ocimum sanctum (100 mg/kg)	74.17 ± 3.51

Values are Mean ± SEM. n=6, one way ANOVA, F = 43.062

**P < 0.001 as compared to control I, *** P < 0.001 as compared to control II



Values are Mean ± SEM, n=6, *** $p < 0.001$ as compared to control
Control I-Unsensitized, stressed rats,
Control II- Rats sensitized with 0.5 ml horse serum and 0.5 ml triple antigen s.c., stressed

Fig. 1 : Effect of NR-A2 on plasma corticosterone levels in active anaphylaxis induced rats

To summarize and to conclude, antistress activity of formulation NR-A2 was evaluated in active anaphylaxix-induced allergic rats. The formulation possesses antistress property. Hence, it has therapeutic application in treatment of allergic rhinitis.

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