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Review Article

Protective Role of Herbal Drugs against Stress Induced Immunosuppression and the Possible Mechanism

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Abstract

Herbal drug research and development has emerged as one of the leading priority areas in biology and medicine, and the scientific use of traditional medicinal remedies are being strongly advocated. Interactions between herbalists and biomedical scientists/clinicians have led to the integration of traditional with modern medical concepts with special reference to new drug discovery and development. Considerable interest has been generated globally in the subject and herbal drugs are being considered effective alternative therapy in several diseases. A favorable safety profile has supported their use and modern methodology is being utilized to delineate cellular and molecular mechanisms of action. Modern day living is associated with heightened levels of psychological stress caused by the fast pace of modern life and ever increasing demands. Stress is a complex phenomenon that triggers multiple physiological responses in the nervous, endocrine, and immune systems referred to as 'stress response'. There is no specific treatment for the management of stress and we generally rely on symptomatic treatment. Herbal products contain phytochemicals which may have the potential to act as preventive or therapeutic agents against various human diseases. Life style diseases which are commonly precipitated by stress are an area where they could be of great benefit. In addition; the available drugs have unacceptable adverse effect profiles which are generally not associated with herbal drugs. Interactions between traditional and modern systems of medicine are now prevalent and experiments are being designed for the validation of their demonstrated effects and explore possible mechanism of action. As a result, several medicinal plants may emerge as effective supplements/alternatives in pharmacotherapy of stress and related disorders. This could revolutionize drug treatment in several pathophysiological states and have crucial pharmacoeconomic implications. This review describes the effects of *Ocimum sanctum* (OS) and *Azadirachta indica* (AI) leaf extracts on stress induced immunomodulation and the possible mechanism in experimental animal models.

Keywords: *Stress; Immunomodulation; Ocimum sanctum; Azadirachta indica; Herbal Drugs*

Introduction

Traditional medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They primarily use medicinal plant (both mono and poly herbals) preparations for therapy. They show better compatibility with human body as the chemical constituents present in the drugs are the part of physiologic function of living flora. Herbal drug therapy thus has the potential to provide rational treatment of many respiratory, cardiovascular, gastrointestinal and metabolic disorders. The earliest recorded evidence of the use of herbal agents in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. The classical Indian texts include Rigveda, Atharvaveda, Charak Samhita and Sushruta Samhita. Traditional knowledge can provide us with valuable guidelines to the selection, preparation and application of herbal formulation [1].

The World Health Organization currently encourages, recommends and promotes traditional/herbal drugs in National Health Care Programmes because of their easy availability, low cost, safety and the faith of people in such remedies [2]. In India, medicinal plants constitute the principal health care resources for the majority of population and per capita annual consumption of modern drugs is very low. Under such changed world health picture, it is quite reasonable to explore the use of plants as potential sources of medicines, and also to determine and identify scientifically the responsible cellular and molecular mechanisms involved for validation of their effects. This will help in the discovery of new pharmaceutical compounds from traditional medicine/natural products. The Golden Triangle Project initiated by the Govt. of India (involving ICMR, CSIR and AYUSH) has lent further impetus to such efforts. Medicinal plants have been used as a rich source of therapeutic agents for prevention and treatment of diseases all over the world since long.

Stressors are known to influence the physiological milieu and disturb the homeostasis resulting in either disease states or development of adaptive mechanisms [3]. These coordinated responses are composed of altered behaviour, immunity, autonomic function and secretion of multiple hormones. Effects of stress on an organism depend upon various factors viz. type, intensity, and the duration of a particular stressor or physiological factors like strain, gender and age of the subjects [3-7]. In addition, differential responses to acute and chronic stress have been documented on neuroendocrine, visceral and immune systems. The immune system is particularly susceptible to stressors and could result in inflammatory, immunological and infectious disorders. The optimal functioning of the immune system is crucial and immunomodulation by drugs plays an important role in determining state of health and disease. Herbal/traditional drugs could influence physiological and pathophysiological states by modulating immune responses. Although, modern medicine has several drugs to cope with stress, but still the results are unsatisfactory as most of the drugs produce troublesome adverse effects and the problem is further complicated by the fact that development of insensitivity or refractoriness occurs to many of these drugs. Thus, the prevention and management of stress and stress related disorders is still far from satisfactory. There is a dire need to explore adjuncts from alternative forms of therapy to complement/supplement the conventional treatment of the disease. Herbal drugs play an important role in the traditional systems and have exhibited efficacy in treatment of a number of diseases which are not otherwise cured by synthetic drugs. This review paper describes the effects of *Ocimum sanctum* (OS) and *Azadirachta indica* (AI) leaf extracts on stress induced immunomodulation and the possible mechanism in experimental animal models.

Ocimum sanctum

Ocimum sanctum (OS) is an aromatic shrub in the basil family Lamiaceae (tribe ocimeae) that originated in north central India and now grows native throughout the eastern world tropics. Within Ayurveda, *Ocimum sanctum* is known as "The Incomparable One," "Mother Medicine of Nature" and is revered as an "elixir of life" that provide a vast array of health benefits that are being confirmed by modern science [8]. *Ocimum sanctum* is commonly known as Tulsi or Holy Basil and the leaf is the most commonly used part of the plant. In Ayurveda, its role in enhancing immune and metabolic function and in respiratory disease has been documented. The whole plant has got medicinal values like digestive, anabolic, hypoglycemic, hypolipidemic, smooth muscle relaxant, cardiac depressant, antifertility, adaptogenic, antiallergic, antimicrobial etc. Its constituents are eugenol, euginal, urosolic acid, vicenin, carvacrol, linalool, limatrol, caryophyllene, methyl carvicol while the seeds are rich in fatty acids, sitosterol and leaves have mainly sugars and anthocyanins. OS has been well documented to have several anti-stress effects [9]. Anti-fatigue effects of OS (300 mg/kg) in rats have been reported by observing increase in swimming time, change in body weight, lipid peroxidation, lactic acid, glycogen and biochemical parameters like haemoglobin (Hb%), Blood Urea Nitrogen (BUN) and Creatine Kinase (CK) [10].

Azadirachta indica

Another plant, *Azadirachta indica* (AI) is commonly known as Neem and almost every part of the tree has been used to treat different human ailments. It is regarded as a household pesticide. The extract of bark, leaves, fruits and roots has been used to treat leprosy, intestinal helminthiasis and respiratory disorders. The bark extract is used as tonic, astringent and is useful in relieving fever, thirst, nausea, vomiting and skin diseases. Other reports on the biological and pharmacological studies showed antiviral, anti-inflammatory, im-

munomodulatory, antipyretic, antioxidant and adaptogenic properties. AI leaf extract has been reported to cause upregulation of marker enzymes, such as alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase and the oxidative damage during skin tumor induction is inhibited [11]. It has been widely used in Ayurvedic, Unani and Chinese medicines globally mainly in Indian subcontinent for the treatment and prevention of several diseases. It is reported to be a rich source of antioxidant which may be responsible for its role as a health-promoter. Several studies showed that AI and its constituents play an important role in the scavenging of free radical generation and prevention of disease pathogenesis. The animal model based experimental studies showed that AI and its chief constituents play key role in the management of cancer through the modulation of various molecular pathways such as NF-κB, PI3K/Akt, Bcl-2, p53, pTEN and VEGF. It is considered as safe medicinal plants and modulates the numerous biological processes without any side effect [12].

Our studies showed that OS and AI leaf extracts could influence physiological and pathophysiological states by modulating immune responses. In our experiments we evaluated the effects of OS and AI on stress induced immunomodulation, with a view to explore possible interactions between traditional and modern medical concepts for validating their effects in various reported disorders.

Effects of *Ocimum sanctum* on stress induced immunomodulation in rats

Stress is known to influence immune function and a plethora of mechanisms have been proposed. Studies on stress and immunity in both experimental animals and humans suggest that psychological challenges are capable of modifying various features of immune response and both innate and adaptive immunity are affected by a variety of stressors [13].

The delayed type of hypersensitivity (DTH) reaction is a well validated test for assessment of cell mediated immune (CMI) function and sheep red blood cells (SRBC) is a highly immunogenic and stable T cell dependent antigen. Rats were immunized with SRBC [0.5 x 10^9 cells/100 ml] on the day 0. They were then divided in five different groups and administered different treatments from day 0 to day 5; group 1 being control group received saline, group 2 received OS (100 mg/kg). Rats of group 3 - 5 were subjected to restraint stress (RS) for 1hour/day from day 0 to day 5. For RS, rats were immobilized in specific Plexiglas restrainers. This method involves minimum pain with minimum movement of tail. This restraint or immobilization technique is widely validated animal model of stress because it induces activation of sympatho-adrenal medullary system, the HPA axis, elevation of blood pressure and heart rate [14]. Group 3 was treated with saline, group 4 with OS (100 mg/kg) and group 5 with haloperidol (0.5 mg/kg) and OS (100 mg/kg). After 5 days of immunization, all rats were challenged by injection of 0.1 ml of SRBC into the left hind foot pad and isotonic saline in right hind paw. Foot pad thickness was measured after 24h of challenge using a Plethysmometer (Ugo-Basile) by the volume displacement and oedema was expressed as percentage increase in paw thickness (ΔT) and was calculated from the formula: ΔT= (Tr-Tl)/Tl *100; where Tr = right hind paw thickness (mm), Tl = left hind paw thickness (mm). The results showed that control group rats showed a DTH response with 20% increase in left hind foot volume. After the administration of OS (100mg/kg), it was increased to 28%. Exposure to RS(x5) led to significant decrease in DTH response as evidenced by the reduced increase in left foot volume as compared to controls. Interestingly, pre-treatment with OS (100 mg/kg) induced attenuations in the RS-induced suppression of DTH response. The results are summarized in figure 1.

Haemagglutination Assay

Rats were immunized with fresh SRBC [0.5 x 10^9 cells/100 ml] on day 0. The animals were divided in five groups as mentioned above and given various treatments from day 1 to 5. After various drug treatments rats were bled on day 6 under light ketamine anaesthesia from retro-orbital plexus using microcapillary technique. The serum was assayed for haemagglutination titre [15]. The serum samples 0.025 ml were diluted two folds in phosphate buffer saline. To each well, 0.025 ml of 1% v/v SRBC was added. The plates were incubated at 37°C for 1 hour and then observed for haemagglutination. The highest dilution giving haemagglutination was taken as the antibody titre. The antibody titre was expressed in a graded manner, the minimum dilution (1/2) being ranked as one and the mean rank of different group was analyzed.

The results showed that control group rats had an antibody titre of 6.6 ± 0.6 (-log2), which was increased to 7.6 ± 1.5 by OS (100

mg/kg) treatment. Although there was an increase in response by 15% but it was not significant. Exposure to RS(x5) led to significant decrease in anti-SRBC antibody response as evidenced by the low hemagglutination titre as compared to controls. Interestingly, pre-treatment with OS (100 mg/kg) induced attenuations in the RS-induced suppression of antibody response (Figure 1).

Dopamine (DA) and stress

Dopamine (DA) is an important neurotransmitter in CNS. The role of DA as stress modulator is reported. DA agonists protect whereas, DA antagonists aggravate stress responses. DA is also involved in the effects of other agents/transmitters. The possible role of DA in anti-stress effects of other agents not clearly defined. To evaluate the role of DA in mediating the immunomodulation in response to stress by OS, dopamine antagonist, haloperidol (0.5 mg/kg) was administered prior to OS for 5 days in separate groups to study humoral immune response (anti-SRBC antibody) and CMI response (DTH). Surprisingly, the protective effect of OS was blocked/suppressed as compared to that seen with OS alone. It suggested that haloperidol shuts out the DA which is probably involved in mediating the immunopotentiating effects of OS (Figure 1).

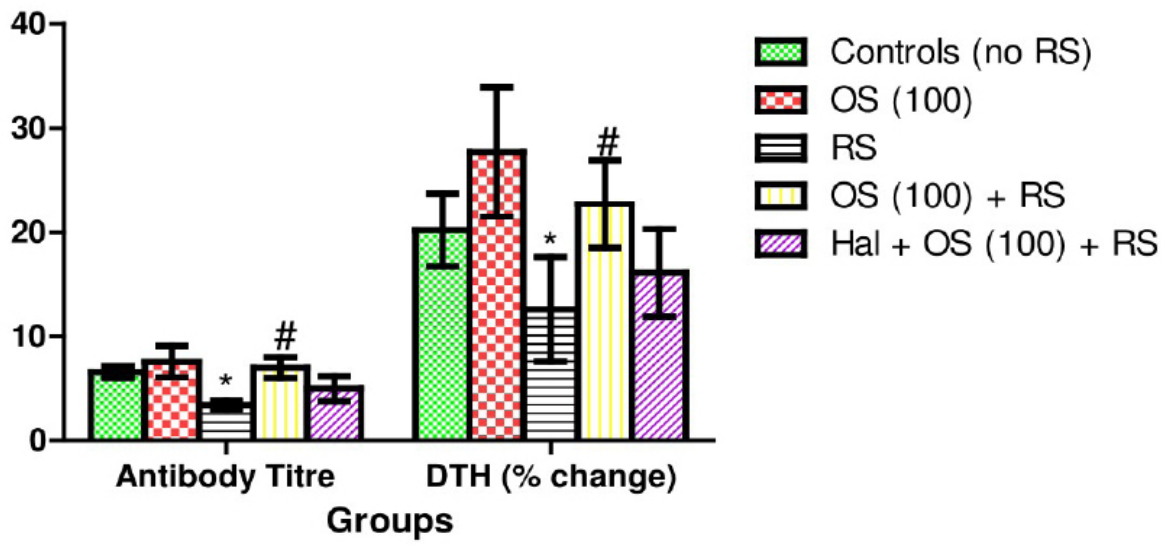

Figure 1: *Effects of Ocimum sanctum (OS) on Immunological parameters. All data are expressed as Mean ± SEM. RS: Restraint Stress; Hal: Haloperidol (0.5 mg/kg), *p < 0.05 vs controls (no RS); #p < 0.05 vs RS.*

Effects of *Azadirachta indica* leaf extracts on stress induced immunomodulation in rats

As mentioned above, DTH reaction is a well-established test for assessment of cell mediated immune response and SRBC is a highly immunogenic and stable T cell dependent antigen. Rats were sensitized with SRBC [0.5 x 10^9 cells/100 ml] on the day 0. They were then divided in five different groups and administered different treatments from day 0 to day 5; group 1 being control group received saline, group 2 received AI (100 mg/kg). Rats of group 3 - 5 were subjected to restraint stress (RS) for 1 hour daily. For RS, rats were immobilized in specific Plexiglas restrainers which inflicted minimum pain with minimum movement of tail. Group 3 animals were treated with saline; group 4 animals were administered AI (100 mg/kg) and group 5 with haloperidol (0.5 mg/kg) and AI (100 mg/kg). After 5 days of immunization, all animals were challenged by injection of 0.1 ml of SRBC into the left hind foot pad and isotonic saline in right hind paw. Foot pad thickness was measured after 24h of challenge using a Plethysmometer (Ugo-Basile)by the volume displacement and oedema

was expressed as percentage increase in paw thickness (ΔT) and was calculated from the formula: ΔT = (Tr-Tl)/Tl *100; where Tr = right hind paw thickness (mm), Tl = left hind paw thickness (mm). The results showed that control group rats showed a DTH response with 20% increase in left hind foot volume. Administration of AI (100mg/kg), further increased the response by 8%. Exposure to RS(x5) led to significant decrease in DTH response as evidenced by the reduced increase in left foot volume as compared to controls. Interestingly, pre-treatment with AI (100mg/kg) induced attenuations in the RS-induced suppression of DTH response. The results are summarized in figure 2.

Haemagglutination Assay

Rats were immunized with fresh SRBC [0.5 x 10^9 cells/100 ml] on day 0. The animals were divided in five groups as mentioned above and given various treatments from day 1 to 5. After various drug treatments rats were bled on day 6 under light ketamine anesthesia from retro-orbital plexus using microcapillary technique. The serum was assayed for haemagglutination titre [15]. The serum samples 0.025 ml were diluted two folds in phosphate buffer saline. To each well, 0.025ml of 1% v/v SRBC was added. The plates were incubated at 37°C for 1 hour and then observed for haemagglutination. The highest dilution giving haemagglutination was taken as the antibody titre. The antibody titre was expressed in a graded manner, the minimum dilution (1/2) being ranked as one and the mean rank of different group was analyzed.

The results showed that control group rats had an antibody titre of 6.6 ± 0.6 (-log2), which was increased to 7.2 ± 1.5 by AI (100 mg/kg) treatment. Although there was an increase in response by 9% but it was not significant. Exposure to RS(x5) led to significant decrease in anti-SRBC antibody response as evidenced by the low hemagglutination titre as compared to controls. Interestingly, pre-treatment with AI (100 mg/kg) induced attenuations in the RS-induced suppression of antibody response (Figure 2). As suggested by experiment with OS that DA is involved in mediating the immunomodulatory response to stress, dopamine antagonist, haloperidol (0.5 mg/kg) was administered along with AI for 5 days to study its role in humoral immune response (anti-SRBC antibody) and CMI response (DTH) to AI. Similar to OS results, the protective effect of AI was also suppressed as compared to that seen with AI alone in a consistent manner. The results strengthened the hypothesis that the DA is probably involved in mediating the immunopotentiating effects of OS (Figure 2).

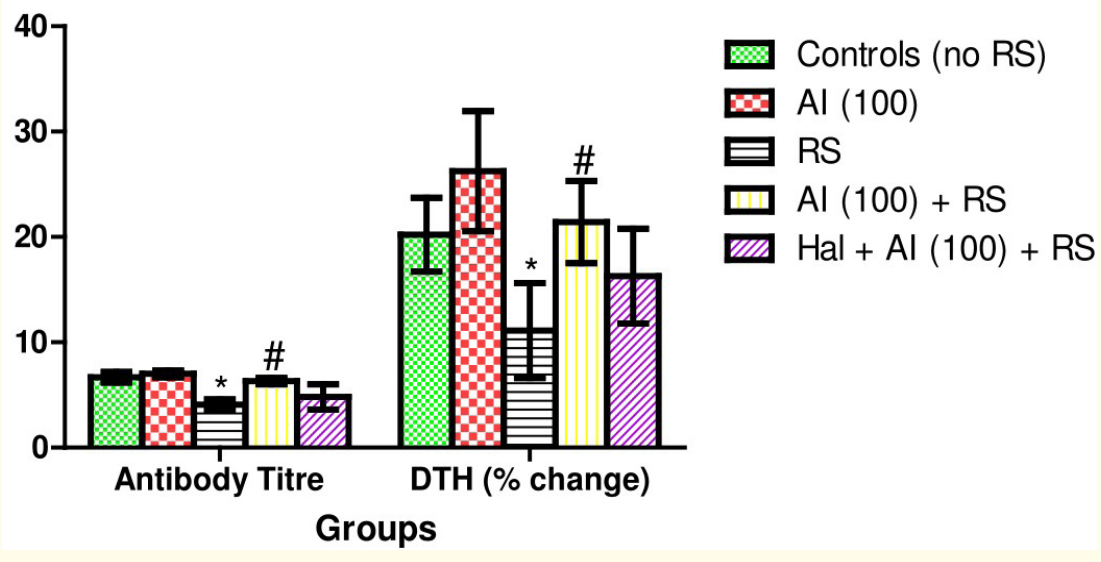

Figure 2: *Effects of Azadirachta indica (AI) on Immunological parameters. All data are expressed as Mean ± SEM. RS: Restraint Stress; Hal: Haloperidol (0.5 mg/kg), *p < 0.05 vs controls (no RS); #p < 0.05 vs RS.*

In an earlier study, in mice immunized with ovalbumin and Freund's adjuvant, oral administration of 100 mg/kg of AI leaf extract has been shown to elevate IgM and IgG levels. The most marked elevations were seen in the IgM levels which were elevated from 12.2 mg/ml in controls to 15.1 mg/ml in the AI treated group (approximately 24% rise). There was also a 32% increase in the antibody responses to the antigen in the AI treated group as compared to controls. In test of CMI, AI treatment resulted in (a) increases in macrophage migration - wherein AI enhanced this parameter by 50% and (b) enhancements in the footpad thickness response (increase by 30% over controls) – indicating clear immunopotentiating effects of AI [16]. In another study, the effects of RS and its modulation by AI leaf extract were assessed on gamma glutamyltranspeptidase (GGT) activity in different tissues of the lymphoid system. Restraint stress suppressed GGT activity differentially (by 30 – 50%) in lymphocytes, the spleen, the thymus and the macrophage, with maximal effect being seen in the spleen (a approximately 30% reduction in GGT activity). AI leaf extract consistently reversed this suppression in GGT activity in the lymphoid tissue and nearly normalized the same, in a manner similar to that seen with the classical adaptogen, chlordiazepoxide. Further, AI also markedly stimulated GGT activity, per se, in these lymphoid tissue components which were greater than that of normal animals. GGT levels are a sensitive indicator of immune responsiveness in health and disease and the present findings were also strongly suggestive of an immunopotentiating effect of AI leaf extract.

Discussion and Conclusion

A variety of stressors have been shown to affect both innate and adaptive immunity and CNS plays a crucial role in such stress-induced immunomodulation. Several neuromodulators are known to influence stress-immune interactions and centrally acting psychoactive drugs differentially influence such changes. Adaptogens have been reported in traditional Ayurvedic literature as rejuvenators and immune enhancers, and these were kept in mind while evaluating the immunomodulatory effects of OS and AI. In rats immunized with SRBC, RS consistently suppressed the humoral immune and cell mediated immune responses. The anti-SRBC antibody titres were lowered after RS as compared to controls which were normalized after treatment with doses of OS and AI leaf extracts. Further, in the test for CMI in these rats, the footpad thickness response was attenuated after RS, and these attenuations in changes in paw volume after antigenic challenge were reversed after pretreatment with OS and AI leaf extracts. Further, the immune enhancing property of these herbs was also evident in the absence of RS. Thus, both the herbal extracts had immune-potentiating effects in stressed animals as well as normal animals – albeit to lesser extent as compared to those seen in stressed rats. Administration of the OS and AI leaf extracts for the duration of these experiments did not have any deleterious effects in experimental animals. Neither general behavioral patterns nor biochemical markers of organ function were much interfered in both stressed and non-stressed situations – indicating a reasonable safety profile of OS and AI leaf extracts (data not shown). The experimental data available to date shows that both OS and AI neutralize the neurobehavioral deficits, gastric responses, and immunomodulatory effects of restraint stress (a widely accepted laboratory stress model for rodents). Interactions between traditional and modern medicines are a key feature in confirming the beneficial effects of some traditionally used herbal agents, and these studies clearly go a long way in projecting AI and OS as potential adaptogens for medicinal use in stress and stress related immunomodulation. The experimental data available to date shows that both plants extracts neutralize the neurobehavioral deficits, gastric responses, and immunomodulatory effects of restraint stress. Further, both the herbal drugs also had immunopotentiating effects in normal animals. Taken together, it is clear that OS and AI leaf extracts have considerable adaptogenic potential.

Investigations into the possible mechanisms of these observed beneficial effects of OS and AI revealed that a CNS-immune axis may regulate these immunomodulatory effects during stress. Further, interaction studies with the anti-stress neurotransmitter DA showed that its receptor blockade could shut out the adaptogenic effects of both herbs, though by differing degrees. Similar interactions of OS/AI were also seen in behavioral and endocrinal markers. It is suggestive of a neural-immune interaction during stress reactions and such concept can play a key role in defining strategies for the treatment of various diseases where stress is a major precipitating/contributing factor. Interactions between traditional and modern medicines are a key feature in confirming the beneficial effects of some traditionally used herbal agents, and these studies clearly go a long way in projecting OS and AI as potential adaptogens for medicinal use in stress and stress related disorders. This in turn could change drug treatment in several pathophysiological states and have crucial pharmacoeconomic implications [17].

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