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Original Article

Stress effect on humoral and cell mediated immune response: Indispensable part of corticosterone and cytokine in neutrophil function

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ABSTRACT

Background: The objective of this study is to evaluate the immunization and stress dormant role in humoral and cell mediated response after sub-acute exposure of noise stress and immunomodulatory activity of *Indigofera tinctoria* (*I. tinctoria*).

Method: Noise stress was done by broadband white noise generator (0–26 kHz), 100 dB, 4 h daily for 15 days and *I. tinctoria* (300 mg/kg b.w.) administered orally. The animals were divided into eight groups with six animals in each group. All the rats were housed under condition of controlled temperature (26 ± 2 °C) with 12 h light and 12 h dark exposure.

Results: In the present study, noise stress significantly increased the corticosterone level in both immunized (76.55 ± 5.17) and un-immunized (66.25 ± 4.87). In sub-acute stress TLC level decreased in un-immunized and increased in immunized. A significant decrease in neutrophil (14.5 ± 3.01) and increase in lymphocyte (86.166 ± 4.83) level was noticed on un-immunized after noise exposure. NAT level was decreased in the un-immunized (40.745 ± 1.95) and increased in immunized (72.625 ± 2.88). The noise stress increased the NBT levels in un-immunized (19.5 ± 1.87) and decreased in immunized (24 ± 2.10). Noise stress shows decreases phagocytic index, avidity index, organ weight and cell count of the spleen, thymus, lymph node in irrespective of whether un-immunized and immunized. Subacute exposure of noise significantly affects humoral (SIC, antibody titter) and cell mediated (LMI, FPT) immunity. Stress further decrease the IL-2, TNF-α, IFN-γ and increase IL-4 cytokine level in serum.

Conclusion: This result further concludes that prior immunization of SRBC in animal's act as a vaccination, which helps to prevent noise stress induced impairment in immune system. Orally administered *I. tinctoria* prevented noise altered immune system. These results also concluded that *I. tinctoria* supplementation could act as an immunomodulators and suggesting its therapeutic efficacy as an antistressor.

1. Introduction

The immune system can be divided into two main subsystems, the innate immune response has no immunologic memory and adaptive immunity has immunologic memory, therefore, it involves a lag time between exposure to the antigen and maximal response [1]. Neutrophils are primary line of defence against invading pathogens. The primed neutrophils are then mobilized to the site of infection or inflammation, where they encounter activating signals to trigger bacterial killing [2]. Immunizations are among the top 10 great public health achievements of the 20th century for their success in realizing substantial declines in cases, hospitalizations, deaths, and health care costs associated with vaccine-preventable diseases [3]. Immunization information systems (IISs) provide a potentially powerful tool to allow collaboration between vaccination providers and public health agencies and for coordination of population based interventions [4]. Stress immunisation makes us handle better with stress in later years.

Environmental noise is a threat to public health according to the World Health Organization (WHO). There are seven categories associated with adverse health effects of noise: hearing impairment, interference with spoken communication, sleep disturbance, cardiovascular disturbances, impaired task performance, disturbances in mental health, negative social behavior and annoyance reactions [5]. Studies on stress-associated immune dysregulation have interested scientists and clinicians in the field of psychoneuroimmunology (PNI). However, the hypothalamic–pituitary–adre

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nal (HPA) and the sympathetic-adrenal medullary (SAM) axes are the two major pathways through which immune function can be altered [6]. Lymphocytes, monocytes or macrophages and granulocytes, exhibit receptors for many neuroendocrine products of the HPA and SAM axes [7] such as cortisol and catecholamines, which can cause changes in cellular trafficking, proliferation, cytokine secretion, antibody production and cytolytic activity [8]. The previous reports states that overproduction of corticosterone during severe sepsis results in increased apoptosis of immune cells [9], which may result in immunosuppression and impairment. The effects of noise stress on the immune system status have already been reported [10]. Previous findings suggest that pro inflammatory cytokine production increases in response to acute psychological stress in humans [11]. The most underappreciated effects of stress on the immune system is its ability to induce significant changes in leukocyte distribution in the body [12]. Acute stress can affect dendritic cell, neutrophil, macrophage, and lymphocyte trafficking, maturation, or function in ways that can enhance innate and adaptive immunity [13]. Chronic stress suppresses or dysregulates immune function, while acute stress has an immunoenhancing effects.

The immunomodulators may be defined as a substance, biological of synthetic, which can stimulate, suppress or modulate any of the components of the immune system including both innate and adaptive arms of the immune response [14]. Drugs from natural source either herbal or mineral have been used as to alter the human immune system [15]. There are several medicinal plants are employed in different system of medicine throughout the world to improve the immunological disorders [16]. According to World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for primary healthcare needs and the reason is the broader degree of chemical diversity and novelty found than any other source [17].

The plant *Indigofera tinctoria* (*I. tinctoria*) belongs to the family Fabaceae. *I. tinctoria* is distributed in south and south East Africa. *I. tinctoria* useful for constipation, liver disease, heart palpitation, gout [18] and dry leaf powder is used in the treatment of asthma [19]. It also possesses antibacterial, antioxidant, cytotoxic, hepatoprotective activity [20], anti-toxic [21], antidyslipidemic activity [22], anti-proliferative activity [23], anti-inflammatory activity [24], phytochemical compounds and *in vitro* free radical scavenging capacity [25], antimicrobial, larvicidal activity [26] and neuroprotective activity [27]. The present study is aim to investigate on both immunization and stress dormant role in humoral and cell mediated response conducive to corticosterone and cytokine in neutrophil function. With this evidence of numerous activities, additional examine the immunomodulatory activity of *I. tinctoria.*

2. Materials and methods

2.1. Extraction of plant extract

The plant *I. tinctoria* collected from the KSG Enterprises (Tindivanam, Tamil Nadu, India) and authenticated by Dr. D. Aravind (Department of Medical Botany, National Institute of Siddha, Chennai, India). Voucher specimens have been deposited at the Herbarium of National institute of Siddha, Chennai, India Reg No: NIS/MB/83/2013. The collected plant were separated from unwanted materials and dried in shade. The leaves were grounded to coarse powder then stored in an airtight container, kept in a cool, dark and dry place until the analysis commence. *I. tinctoria* dried plant leaves of 30 g were extracted with 250 mL of sterile distilled water using the Soxhlet apparatus. The extracts were then filtered with Whatman No 1 filter paper and then freeze dried stored at 4 °C for further investigation.

2.2. Experimental design

Wister strain male albino rats weighing 180–220 g were randomly selected. The animals were maintained under standard laboratory condition and fed ad libitum with food (M/S Hindustan Lever Limited, Bombay, India) and water. All the rats were housed under condition of controlled temperature (26 ± 2 °C) with 12 h light and 12 h dark exposure. The animals were divided into eight groups with six animals in each group. Group I served as the control, Group II animals were subjected to noise-stress 4 h daily for 15 days (Sub-acute exposure), Group III were treated with *I. tinctoria* (IT) for 48 days and experiments were carried out on 49th day and Group IV consisted of noise stress with IT-treated animals, these animals were pre-treated with IT for 33 days and then exposed to noise stress for 15 days. During the noise stress period, they were also given IT extract by the oral route and all the experiments were done on the 49th day. Group V served as the control immunized, Group VI animals were subjected to noise-stress 4 h daily for 15 days (Sub-acute exposure-immunized), Group VII were treated with *I. tinctoria* (IT) (immunized) for 48 days and experiments were carried out on 49th day and Group VIII consisted of noise stress with IT-treated animals (immunized). These animals were pre-treated with IT for 33 days and then exposed to noise stress for 15 days. During the noise stress period, they were also given IT extract by the oral route and all the experiments were done on the 49th day. Ethical clearance was obtained before the commencement of experiments from the ethical committee (IAEC No: 22/02/2013) and the Committee for the purpose of control and supervision of experiments on animals (CPCSEA).

2.3. Noise stress induction

Noise was produced by two loudspeakers (15 W), driven by a white-noise generator (0–26 kHz), and installed 30-cm above the cage. The noise level was set at 100 dB uniformly throughout the cage and monitored by a sound level meter D2023(S.NO-F02199; Cygnet Systems, Gurgaon, Haryana, India). A sound level meter was used to measure the intensity of the noise [28].

2.4. Immunization

The sheep red blood cells (SRBC) were used to immunize the animals, which were collected in a sterile alsever's solution and washed thrice with pyrogen free normal saline and adjusted to 5×10^9 cells per mL. The animals were immunized by injecting 20% (1 mL) SRBC intraperitoneally (i.p). The day of immunization was considered as day zero. On the 5th day, the blood samples were collected to carry out the immunological parameters.

2.5. Sample collection

Blood samples and isolation of spleen, thymus, lymph node and bone marrow was done between 8 and 10 a.m. to avoid circadian rhythm induced changes. At the end of experimental period all the animals were exposed to mild anesthesia and blood was collected from internal jugular vein, plasma and serum was separated respectively by centrifugation at 3000 rpm at 4 °C for 15 min. Later all the animals were sacrificed under deep anesthesia using Pentothiol sodium (40 mg/kg b.w.). The spleen, thymus and lymph node was excised, washed in ice cold saline and blotted to dryness. Quickly weighed and the spleen, thymus lymph node and bone marrow sample were taken and stored.