



ANTISTRESS ACTIVITY OF THREE MEDICINAL PLANTS AND THEIR COMBINATION USING TAIL SUSPENSION TEST

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ABSTRACT

Stress is the generator of several diseases. Stress can harm the body in several ways. Thus nowadays stress can lead to various complications. Various relaxation techniques and meditation techniques have been tried to counteract stress. Also various allopathic medicines have been tried in stress. However medicinal plants find special use in the treatment of stress. Various adaptogenic plants have been used in Ayurvedic system of medicine to counter stress and have been successful in this regard. The current review focuses on the antistress activity of three medicinal plants and their combination in fighting stress.

DOI Number: 10.14704/nq.2022.20.8.NQ44871

NeuroQuantology 2022; 20(8): 8469-8474

INTRODUCTION

Stress can lead to a variety of diseases (Chrousos et al., 2009). Stress can be an external stimuli or internal stimuli (Yaribeygi et al., 2017). It can change the physical or mental homeostasis of an animal or human (Chrousos and Gold, 1992). It can be good or harmful to an organism (Sapolsky RM, 2004). It plays a useful role by maintenance of homeostasis in the body but it can also produce a deleterious effect since it can lead to the generation of several diseases (Chrousos and Gold, 1992). Stress can be psychological in nature and it can also be physiological in nature (Marketon and Glaser, 2008). Various factors can lead to the production of stress (Papathanasiou et al., 2015). Stress can lead to various changes inside the body. The respiratory and cardiovascular systems function effectively and the blood flow is enhanced to regions like brain, heart and

eISSN1303-5150

muscles under the influence of stress (Habib et al., 2001; Sapolsky, 2000; Sapolsky et al., 2000). Various factors can lead to stress like infection, chemical exposure, death of a near one, dangerous situation etc. (Papathanasiou et al., 2015). Stress occurs when a person is not able to cope with day to day complications of life. Stress can also lead to generation of several oxidative species and free radicals. This can result in aging and several types of diseases. Although stress is not itself a disease, however it can lead to various other diseases. Complications related to stress are on a rise in the modern world (Radovanovic and Rankovic, 2004; Esterbauer, 1996; Packer and Ong, 1998; Chrousos et al., 2009). The hypothalamic pituitary adrenal axis and the sympathetic nervous system are the two pathways that are activated in stress. Due to activation of hypothalamic pituitary adrenal axis

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glucocorticoids are released and due to activation of the sympathetic nervous system adrenaline and noradrenaline are released (Marketon and Glaser, 2008). Glucocorticoids lead to various effects in the body like they increase the blood glucose levels, increase the destruction of proteins and fats and decrease the inflammatory and immune response. Epinephrine and norepinephrine also produce various effects in the body like they enhance blood pressure, blood glucose and heart rate in the body. They produce dilation of bronchioles and decrease digestive activities (Waugh and Grant, 2003). With the rise in cases related to stress, newer treatment strategies are being explored to counteract stress. Stress can be treated with various behavioral therapies like cognitive behavioral therapy. Yoga can also be tried to reduce the mental complications associated with stress (Varvogli and Darviri, 2011). Also various allopathic medicines have been tried in the treatment of stress. Medicinal plants have now become an important source for the treatment of stress and its complications. Allopathic medicines only treat the symptoms of stress and produce several complications and side effects. Use of medicinal plants for stress has been tried since time immemorial. Many medicinal plants have been mentioned in Ayurveda which have legendary adaptogenic activities. Ashwagandha, Tulsi have potent antistress effects. Thus medicinal plants can be very useful in countering stress and complications associated with stress. Medicinal plants also have an advantage over allopathic drugs that they are relatively safer (devries and Wilkerson, 2003; Kathleen and Kelly, 2009). In the present study ethanolic extracts of three medicinal plants *Ocimum kilimandscharicum* (OCM), *Thymus serpyllum* (THY), *Spilanthes acmella* (SPL) and their combination in equal ratio (1:1:1) (COMB) were evaluated for antistress activity using tail suspension test.

MATERIAL AND METHODS

PLANT COLLECTION AND AUTHENTICATION

The aerial parts of OCM, THY and SPL were collected from the herbal garden of Defence Institute of Bioenergy Research, Pithoragarh, India. The final authentication of the plant samples was carried out by Botanical Survey of India, Northern Regional Center, Dehradun, India.

Plant extraction

The collected plants were dried and extracted by absolute ethanol using Soxhlet's Assembly. The plant extracts were then dried using rotator vacuum flash evaporator (Kokate et al., 2010).

Chemicals and Instruments Used

The following chemicals were used in the study: Absolute ethanol (99.9 %) (Changshu yangyuan Chemical, China); Tween 80 (5% v/v) (Loba Chemie PVT. LTD, Mumbai). Geriforte (Himalaya). All other reagents and chemicals used were of good quality. The instruments and materials used in the study were: Adhesive tapes, Rotatory vacuum flash evaporator and cages to house the animals. All other types of equipments used were of good quality.

Animals

Albino mice weighing 20-30 grams were used in the experiment. They were housed in well ventilated conditions in the departmental animal house of Department of Pharmaceutical Sciences, Bhimtal on a natural 12 hour light and 12 hour dark cycle with food and water ad libitum. The experimental protocol was approved in the meeting of the Institutional animal ethics committee of the Department of Pharmaceutical Sciences, Bhimtal, Kumaun University, Nainital, Uttarakhand.

Statistical Analysis

The results of the study were analyzed using graph pad prism. One way anova was applied followed by tukey's test.

Tail Suspension test

The animals were divided into 10 groups each containing six animals. They were administered the vehicle, standard and the test extracts according to their respective body weight. Group one received vehicle (5% Tween 80, 10 ml/kg), the second group received Geriforte



(Himalaya) (100 mg/kg) (standard drug). The third and the fourth group received OCM 200 mg/kg and OCM 400 mg/kg. The fifth and the sixth group received THY200 mg/kg and THY 400 mg/kg, seventh and the eighth group received extracts of SPL 200 mg/kg and SPL 400 mg/kg. Group nine and tenth received COMB 200 and 400 mg/kg. All the treatments were given for fifteen days. On the fifteenth day one

hour after the administration of the specific treatments the mice were suspended by attaching adhesive tapes one centimeter from the tip of the tail. They were suspended at a height of 50 centimeters above the ground. The total immobility time for each animal was noted for duration of six minutes (Steru et al., 1985; Lotankar et al., 2016; Roy et al., 2011).



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Figure 1. Albino mice in tail suspension test.

Results

The results of the study show that the standard drug (100 mg/kg), OCM 200 mg/kg, OCM 400 mg/kg, THY 200 mg/kg, THY 400 mg/kg, SPL 400 mg/kg, COMB 200 mg/kg and COMB 400 mg/kg

showed a significant decrease in immobility time in comparison to the vehicle control group. SPL 200 mg/kg did not show a significant effect in comparison to the vehicle control group. (Figure 2.)



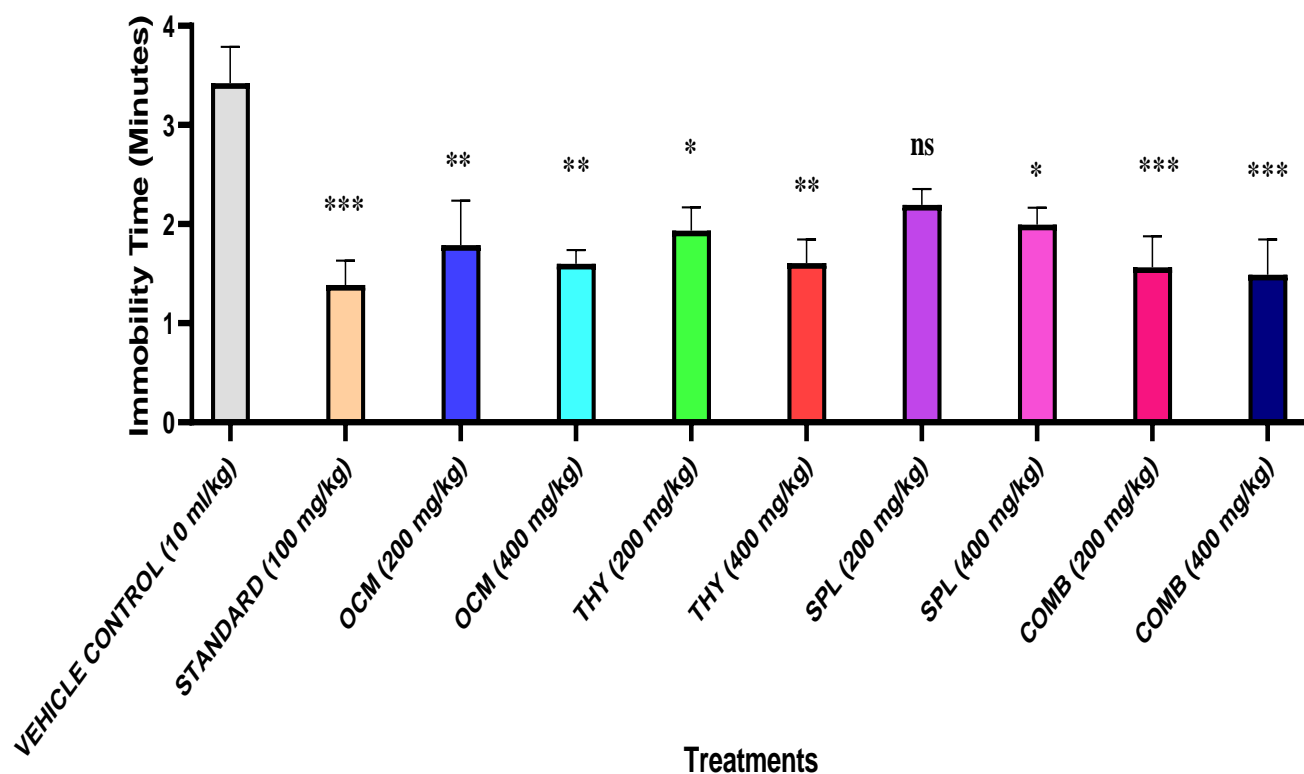


Figure 2. Effect of individual extracts and the combination extract on immobility time in tail suspension test. Values are represented as Mean \pm SEM of six animals.

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Discussion

Tail suspension test is based on the lines that when rodents are put in stressful situations from which they cannot escape, they assume an immobile posture after initial movements attempted at escape. The time of the test is usually around 6 minutes (Cryan et al., 2005). Comparison was done between the vehicle control group and groups receiving the extracts of OCM, THY, SPL and COMB. In the groups pretreated with OCM at 200 mg/kg and 400 mg/kg a very significant ($p < 0.01$) effect was observed in comparison to the vehicle control group. In comparison to the vehicle control group THY at 200 mg/kg showed a significant ($p < 0.05$) effect and THY at 400 mg/kg showed a

very significant ($p < 0.01$) effect. At the 200 mg/kg SPL did not produce a significant effect but at 400 mg/kg it showed a significant effect ($p < 0.05$) in comparison to the vehicle control group. COMB 200 mg/kg and 400 mg produced an extremely significant ($p < 0.001$) effect in comparison to the vehicle control group. The standard group also produced an extremely significant effect ($p < 0.001$) in comparison to the vehicle control group. As revealed by phytochemical screening ethanolic extracts of OCM, THY, SPL and COMB contain various phytochemicals like carbohydrates, saponins, flavanoids, phenols. One or more of these phytochemicals might be responsible for the



antistress activity of the extracts in the tail suspension test.

Conclusion

As discussed above stress is itself not a disease but it can lead to the progression of numerous diseases. Thus effective treatment of stress can help in the elimination of various diseases. Allopathic medicine can be used for the symptomatic treatment of stress but they are not very helpful in eliminating stress. Medicinal plants have the advantage that they can treat stress efficiently without many side effects. The individual plant extracts and their combination used in the study have proven their potential in countering stress by reducing immobility time in the tail suspension test. Thus medicinal plants can prove to be highly effective medicines in treatment of diseases and disorders related to stress.

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