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Attenuation of acute and chronic restraint stress-induced perturbations in experimental animals by *Zingiber officinale* Roscoe

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ABSTRACT

Ethanolic extract of rhizomes of *Zingiber officinale* was investigated on anoxia stress tolerance test in Swiss mice. The animals were also subjected to acute physical stress (swimming endurance test) to gauge the anti-stress potential of the extract. Further to evaluate the anti-stress activity of *Z. officinale* in chronic stress condition, fresh Wistar rats were subjected to cold restraint stress (4° for 2 h) for 10 days. Stimulation of hypothalamus pituitary adrenal axis in stressful condition alters plasma glucose, triglyceride, cholesterol, BUN and corticosterone levels. There is also alteration in the blood cell counts. Pretreatment with the extract significantly ameliorated the stress-induced variations in these biochemical levels and blood cell counts in both acute and chronic stress models. The extract treated animals showed increase in swimming endurance time and increase in anoxia tolerance time in physical and anoxia stress models, respectively. Treatment groups also reverted back increase in liver, adrenal gland weights and atrophy of spleen caused by cold chronic stress and swimming endurance stress models. The results indicate that ethanolic extract of *Z. officinale* has significant adaptogenic activity against a variety of biochemical and physiological perturbations in different stress models.

1. Introduction

Stress basically is a reaction of mind and body against change in the homeostasis. The productive stress is called Eustress while harmful stress is called Distress. If the stress is extreme, the homeostatic mechanisms of the organism become deficit and the survival of the organism is threatened. Under these conditions, stress triggers a wide range of body changes called General Adaptation Syndrome (GAS). The stimuli, which produce GAS, are called the stressors and range from physical to psychological factors including cold, heat, infection, toxins, major personal disappointment, etc. (Selye, 1973). In the stress-filled environment we live in, successful adaptation to stress is a prerequisite for survival. In the indigenous system of medicine, there are many herbal drugs and formulations recommended to enable one to withstand stress without altering the physiological functions of the body. This, drug induced state of resistance against aversive stimuli is termed as adaptogenic activity and the drugs, named adaptogens. Stress alters the equilibrium of various hormones which have a significant impact on the immune response in general. The status of immune system-immunosuppression versus immunopotentiation-will depend upon the net effect of these changes. Stress and depression have been shown to affect immune system functioning, with both immunosuppression and immune activation (Raison and Miller, 2001). Correlations between depression and elevated susceptibility for infections or mortality rates have been observed and are associated with immune suppression (Irwin, 2002). The physiological reaction to stress involves alteration in the autonomic nervous system, the endocrine system and the immune system. The secretion of glucocorticoids is a classic endocrine response to stress (Sapolsky et al., 2000). Stressful stimulation influences antigen-specific as well as non-specific reactions (Ader and Cohen, 1993).

Many herbs reported in ancient literature have potent anti-stress activity and their utilities in current scenario need to be unveiled. *Zingiber officinalis*, Roscoe is a rhizome that is widely used as culinary herb and herbal remedy for some common ailments. It is used as carminative, antipyretic, antiemetic in pregnancy and as anticancer adjunct (Evans and Trease, 1979). It also ameliorates motion sickness and it is a known thromboxane synthesis and platelet aggregation inhibitors, and diaphoretic agent (Evans and Trease, 1979). It contains about 1–2% of volatile oil and 5–8% of resinous matter, starch and mucilage (Evans and Trease, 1979). The volatile oil contains monoterpenes, sesquiterpenes and sesquiterpene alcohol zingiberol (Evans and Trease, 1979), gingerol and

Abbreviations: Fig., figure; i.p., intra peritoneal; kg, kilogram; mg, milligram; ml, millilitre; p.o., per oral; w/w, weight/weight; w/v, weight/volume; ZO, *Zingiber officinale*.

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shagoals. Most of the pharmacologically active constituents reside in the volatile oils. Gingerols have cardio tonic (Kobayashi et al., 1988), analgesic, anti-inflammatory (Young et al., 2005), antipyretic (Yoshikawa et al., 1993) and antibacterial effects both *in vitro* and *in vivo* (Mascolo et al., 1989). Shagoal has antiemetic, antispasmodic, anxiolytic and anticonvulsant activity (Vishwakarma et al., 2002). Scientific reports show that it is also used for conditions such as anti-ulcerogenic (Al-Yahya et al., 1989), antidiabetic (Akhani et al., 1981), anti-oxidant (Jeyakumar et al., 1999) and anti-hepatotoxic (Omoniyi et al., 2006) activities. Ginger has been reported to possess a potent anti-oxidant activity *in vitro* (Jeyakumar et al., 1999) which reduces the oxidative stress in the body. Since *Zingiber officinale* has a number of medicinal properties and is a potent anti-oxidant, the present study was undertaken to evaluate the potential usefulness of fresh rhizomes of *Z. officinale* for anti-stress and adaptogenic activity in experimental animals. *Withania somnifera*, an established ayurvedic herb used as an adaptogen is used as reference standard (Singh et al., 2005).

2. Materials and methods

2.1. Plant material and extraction

Z. officinale rhizomes were collected from the local market of Rangareddy District, Hyderabad, in the month of February–March, the botanical authentication was done by the Department of Botany, Osmania University, Hyderabad and voucher specimen (MRCP-104) is lodged in our research laboratory for the future reference. The fresh rhizomes were sliced using a home slicer and the slices obtained were shade-dried, pulverized and passed through a 20-mesh sieve. The dried, coarsely powdered plant material was extracted with 99% ethanol using Soxhlet apparatus at a temperature below 60 °C for 24 h. The solvent was evaporated under vacuum, which gave semisolid mass (yield: 26% w/w) with respect to the dried powder. Oral suspensions containing 50, 100 and 200 mg/ml of the ethanolic extract of *Z. officinale* were prepared in 1% w/v gum acacia.

2.2. Animals

Swiss albino mice weighing 20–25 g and Albino Wistar rats weighing 150–250 g of either sex, 4 months of age were used for this study. The experimental animals were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25 ± 3 °C and 35–60% humidity). Standard pelletized feed and tap water were provided *ad libitum*. The Institutional Animal Ethical Committee (IAEC) of Malla Reddy College of Pharmacy, Hyderabad, approved the study.

2.3. Anoxia stress tolerance test in mice

Swiss mice of either sex were divided randomly into five groups, each group containing six mice. Group I mice received 0.1% gum acacia in saline; (vehicle control). Group II mice were treated with *W. somnifera* (100 mg/kg, p.o.) and stress; Group III, IV and V mice were treated with ethanolic extract at doses of 50, 100 and 200 mg/kg, p.o. and stress. The drug treatment was carried out daily for a period of 21 days. At the end of each week, i.e., 1st, 2nd and 3rd weeks of drug treatment, the animals were exposed to the anoxia stress and anoxia tolerance time was noted. Hermetic vessel of one litre air capacity was used to induce anoxia stress (Krupavaram et al., 2007). Each animal was kept in the hermetic vessel and the time to show the first sign of convulsion was noted, and were immediately removed from the vessel and resuscitated if needed.

2.4. Forced swimming endurance test (physical stress)

Rats of either sex (200–250 g) were used for forced swim endurance stress. Group I rats received 0.1% gum acacia in saline; (vehicle control). Group II mice were treated with 0.1% gum acacia in saline and stress; (negative control). Group III rats were treated with *W. somnifera* (100 mg/kg, p.o.) and stress; (positive control). Group IV, V and VI mice were treated with ethanolic extract at 50, 100 and 200 mg/kg, p.o. and stress. The rats were subjected to swimming stress by keeping them in propylene tank of dimension (37 × 37 × 30 cm), filled with water to a height of 25 cm. Extracts were given to rats, once daily for period of 7 days. On 8th day the rats were allowed to swim till complete exhaustion and the endpoint was taken when the animal started drowning. The mean swimming time for each group was calculated (Kannur et al., 2006). Then animals were killed and blood was collected by cardiac puncture to estimate biochemical parameters like serum glucose, triglycerides, cholesterol, BUN, corticosterone and blood cell count (RBC, WBC and DLC). The weights of organs such as liver, adrenals, spleen were recorded after washing with alcohol.

2.5. Chronic cold restraint stress

Treatment groups were similar to forced swimming endurance stress. Rats were subjected to cold stress by exposing them to 4 ± 1 °C, daily for 2 h for a period of 10 days (Bhattacharya and Ghosal, 1994). Animals were sacrificed at the end of the study period and blood was collected for estimation of various biochemical parameters such as Serum cortisol, glucose levels, RBC count, total leukocyte count, differential count as well as lipid profile. Similarly the weights of organs, i.e., liver, spleen and adrenal glands were also recorded.

2.6. Statistical analysis

All the values are expressed as mean ± SEM and data was analyzed by one-way ANOVA, using Graph pad INSTAT. The post hoc analysis was carried out by Dunnett's multiple comparison test to estimate the significance of difference between individual groups.

3. Results

3.1. Effect of ethanolic extract in anoxia stress tolerance test

In the anoxia tolerance test (Table 1), the extract at 50,100 and 200 mg kg^{-1} doses statistically produced a dose dependant significant ($P < 0.05$) increase in mean time to convulsion in mice subjected to anoxia stress.

3.2. Effect of ethanolic extract in forced swimming endurance stress

The results of the study revealed that the extract possess anti-stress property as it significantly ($P < 0.05$) increased the swimming time (Fig. 1). Swimming endurance stress resulted in significant increase in adrenal gland weight and liver weight with concomitant decrease in spleen weight in stress control group, which was significantly reverted by *Z. officinale* pretreatment at 50, 100 and 200 mg/kg. Similarly stress-induced elevated blood cell counts of RBC and DLC, i.e., lymphocytes, neutrophils, eosinophils and monocytes have been significantly ($P < 0.01$) reduced by the ethanolic extract in a dose dependant manner (Table 2). Pretreatment of animals with *Z. officinale* at three doses also significantly ($P < 0.05$) restored back forced swimming stress-induced alterations in plasma corticosterone, glucose, triglyceride, BUN and cholesterol (Table 3).

3.3. Effect of ethanolic extract in cold restraint stress

In cold restraint stress, ethanolic extract at 50, 100 and 200 mg/kg offered significant ($P < 0.05$) protection against the change in the weights of liver, spleen and adrenal gland when compared to stress control (Figs. 2 and 3). The extract dose dependently reduced the elevated levels of biochemical parameters ($P < 0.05$) (Table 4). The extract at 200 mg/kg significantly ($P < 0.01$) reduced all the blood cell counts (Table 5).

4. Discussion

Adaptogens are the substances meant to put the organisms into a state of non-specific heightened resistance in order to better resist stressor and adapt to extraordinary challengers. They normalize body functions, strengthen systems and functions that are compromised by stress and have a protective effect against a wide variety of environmental and emotional stress.

The forced swimming is the most widely used method for assessing the anti-stress property of a novel compound (Anisman and Zacharko, 1991; Subarnas et al., 1993). This paradigm is based