

Pharmacological Research

Evaluation of adaptogenic and anti-stress effects of *Ranahamsa Rasayanaya*-A Sri Lankan classical *Rasayana* drug on experimental animals

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Abstract

Various types of stress not only harm the mental function, but also cause diseases by weakening body defenses. *Rasayana* therapy has an advantage over the conventional *Kayachikitsa* treatment in such conditions, as it is capable of counteracting the stress, promote the adaptogenic abilities of the body, enhance mental endurance, etc. These are the some of parameters for evaluation the *rasayana* effect of a drug, therefore the same have been studied to assess the *rasayana* effect of *Ranahamsa Rasayanaya* (RR). Experimental models such as forced swimming induced hypothermia and stress induced gastric ulcer formation have been carried out befitting on Charles Foster strain albino rats to determine the *rasayana* effect of RR. Statistically highly significant decrease in forced swimming induced hypothermia and non-significant decrease in gastric ulcer formation were observed in the treatment groups, when compared to the stress control group. These results show the probable adaptogenic and anti-stress activities of the test drug. The study results support the claims made by the Sri Lankan traditional practitioners that, the test drug is a potent *rasayana* formulation.

Key words: *Ranahamsa Rasayanaya*, *Rasayana* therapy, hypothermia, gastro-cytoprotective, adaptogenic, anti-stress activities, traditional practitioners.

Introduction

Influence of the mind over the body has been studied extensively in the fields of psychology, psychoanalysis, psycholinguistics, psychoneuro-immunology, etc. These studies demonstrated repeatedly, a sound mind is a prelude to a healthy body. The science of *Ayurveda* also substantiates such claims by its unique and intricate understanding of mind over matter in the following ways. The harmonious interaction of the mind and body is the key to well-being as they are the seats of the health and ill-health¹, and to counteract the psychic disorders, it professes introspection (*Atmajnana*²) as well as restraining the mind (*manonigraha*) in *Satvavajaya*³ therapy. *Rasayana* approach for the treatment of same

diseases extends further by prescription of *medhya rasayana*⁴ as well as *Achara rasayana*⁵ (good conduct). The usage of drugs for mental illnesses is justified with *medhya rasayana*. Such claims made by the great teachers of *Ayurveda* should be put to use for the betterment of humanity, lack of evidence-based data hampers achieving the said goal. Pharmacological and clinical studies will address this critical issue. *Ranahamsa Rasayanaya* (RR) is a well known classical Sri Lankan *rasayana* drug among the traditional practitioners (*desiya vaidya*⁶), which contains several *medhya* (brain tonics) ingredients as well as many *rasayana* (rejuvenation) drugs plus *brimhana* (anabolic) and *balya* (roborant) drugs. Similar modalities have been postulated to explain the effect of *rasayana* therapy, some of them are nootropic, anti-stress, adaptogenic and anabolic effects. Bioassays for pharmacological screening is recommended along with other parameters by World Health Organization (WHO) in 1992, for the authentication and standardization of plant drugs. The present experimental study explores the test drugs efficacy in the said experimental modalities.

Aims and Objectives

1. Evaluation of the effect of *Ranahamsa rasayana*

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(RR) for its adaptogenic activity.

2. Evaluation of the effect of *Ranahamsa rasayana* for cytoprotective activity.

Plan of study: The pharmacological study has been carefully planned according to the current scientific and ethical guidelines to evaluate the key objectives on adaptogenic properties and anti-gastric-ulcer activity, and accordingly the experiments were carried out. The following pharmacological studies were approved by the Institutional animal ethics committee (IAEC).

Experimental models:

1. Adaptogenic and anti-ulcer activity.
2. Forced swimming induced hypothermia and stress induced gastric ulcer formation.

Material & Methods

Procurement of test drug: *Ranahamsa Rasayanaya*, a Sri Lankan classical compound rasayana formulation, is referred in *Ayurvedic Pharmacopoeia of Sri Lanka*, published by Department of Ayurveda. This formulation is in *avaleha* form and consists of ingredients like *Abies spectabilis* (*Talisapatra*), *Abrus precatorius* (*Gunja*), *Allium sativum* (*Lashuna*), *Aloe vera* (*Kumari*), *Amomum subulatum* (*Sthula ela*), *Anacyclus pyrethrum* (*Akarakarabha*), *Bacopa monnieri* (*Brahmi*), *Benincasa hispida* (*Kushmanda*) *Bombax ceiba* (*Shalmali*) *Borassus flabellifer* (*Tala*), *Cannabis sativa* (*Vijaya*), *Cassia auriculata* (*Hemadhruma*), *Cinnamomum zeylanicum* (*Twak*), *Cocos nucifera* (*Narikola*), *Coptis teeta* (*Pitamula*), *Coriandrum sativum* (*Dhanyaka*), *Crocus sativus* (*Kunkuma*), *Cuminum cyminum* (*Jiraka*), *Cyclea burmanni* (*Pathabheda*), *Elettaria cardomomum* (*Suksmela*), *Embelia ribes* (*Vidanga*), *Glycyrrhiza glabra* (*Yashtimadhu*), *Gynura pseudochina* (*Chopachini*), *Hemidesmus indicus* (*Sariva*), *Ipomoea mauritiana* (*Kshiravidari*), *Myristica fragrance* (*Jatiphala*), *Myristica fragrance* (*Jatipatri*), *Nigella sativa* (*Karavi*), *Nymphaea stellata* (*Nilotpala*), *Papaver somniferum* (*Ahiphena*), *Phyllanthus emblica* (*Amalaki dried*), *Phyllanthus emblica* (*Amalaki fresh*), *Piper cubeba* (*Kankola*), *Piper longum* (*Pippali*), *Plumbago rosea* (*Chitraka*), *Pogostemon heyneanus* (*Parpati*), *Saccharum officinarum* (*Ikshu*), *Santalum album* (*Shweta Candana*), *Saussurea lappa* (*Kushtha*), *Semecarpus anacardium* (*Bhallataka*), *Syzygium aromaticum* (*Lavanga*), *Terminalia bellerica* (*Vibhitaki*), *Terminalia chebula* (*Haritaki*), *Tinospora cordifolia* (*Guduchi*), *Trachyspermum ammi* (*Yavani*), *Tribulus terrestris* (*Gokhura*), *Vitis vinifera* (*Draksha*), *Sugar* (*Sharkara*), *Cows milk* (*Go-dugdha*), *Cows ghee* (*Go-ghrita*), *Honey* (*Madhu*). This formulation of two different batches was bought from Sri Lanka *Ayurvedic Drugs Corporation* and coded as RR I, and RR II).

Animals

1. Charles Foster strain albino rats of either sex weighing between 210 g to 270 g were used for experimental study.
2. The animals were obtained from the animal house attached to the pharmacology laboratory of I. P. G. T. & R.A.
3. Animals were exposed to natural day and night cycles with ideal laboratory condition in terms of ambient temperature ($22 \pm 2^\circ \text{C}$) and humidity (50 - 60%). They were fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water given *ad libitum*.
4. All the experiments were carried out after obtaining permission from "Institutional animal ethics committee" (IAEC).

Dose fixation and schedule: The human dose of the test drug is 10 g per day. The dose calculation was done on the basis of body surface area ratio using the table of Paget and Barnes⁷ and a dose of 900 mg/kg/day from both the batches of the test drug and the vehicle were administered according to the body weight of the individual animals orally to the respective groups, with the help of a gastric catheter of suitable size sleeved to a syringe nozzle. The test drug was administered from a fresh stock solution prepared by mixing the test formulation with tap water to suitable concentration.

Statistical analysis: Evaluation of the data through statistical estimations within the group and comparison between the groups after treatment (AT) were assessed by using paired and unpaired Student's t test respectively. Being number of observations (n) in each group below 30, Student's t test was employed for the purpose. The statistical estimations particularly sample mean, standard deviation (SD), standard error of mean (SEM), calculated t value and probability (P) value were obtained by applying the standard formulas. Probability (P) values of t are tabulated for various degrees of freedom (df) according to the number of observations. If the obtained 'P' value is less than 0.05, then the result is considered as statistically significant.

Adaptogenic and anti-ulcer activity (Sheth 1972⁸, Parmar 1993⁹)

Charles Foster strain of albino rats weighing 210g - 270 g of were randomly divided in to 4 groups. Group A received tap water and served as the control. Group B also received tap water and served as the stress control. Individually calculated doses of both the samples of RR I and RR II were administered to Group C and D respectively. The test drugs were administered for ten consecutive days. Animals were fasted for 36 hours from 8th day onwards and the coprophagia was prevented by using metabolic cages. On 10th day, animals of Groups C and D were fed with the test drug and one hour after

the feeding, their rectal temperature were measured and documented individually. After recording the initial rectal temperature, they were exposed to forced swimming for 20 minutes. Exactly after 20 minute exposure to forced swimming, the rats were taken out and again their rectal temperature was measured and recorded individually. The drop in rectal temperature is calculated and evaluated to determine the adaptogenic activity of the test drug.

Stress produced by various methods leads to the formation of stress ulcers such as body restraint, cold, ligation of the pylorus, etc¹⁰. To assess the gastro-cytoprotective activity, the rats were exposed to the swimming stress for 16hr. After 16 hr, they were removed and individual body weight was measured, followed by sacrifice. The stomach was removed by making midline incision and was cut opened along the greater curvature. The inner surface of the stomach was carefully washed

with normal saline and examined with a magnifying lens to calculate the ulcer index. The severity of an ulcer and total number of ulcer index were calculated according to the rating given by Kulkarni (1999)¹¹.

Statistically highly significant reduction in fall of rectal temperature was observed with both the treatment groups in comparison to control group (Table 1).

In both the test drug administered groups an apparent body weight decrease was observed which was of higher magnitude in comparison to stress control groups however, the difference was found to be statistically non-significant. (Table 2).

An apparent and statistically non-significant reduction in gastric ulcer formation was observed in both the treatment groups in comparison to stress control group (Table 3).

Observations & Results

Adaptogenic and anti-ulcer effect

Table 1: *Ranahamsa Rasayanaya* on rectal temperature in albino rats subjected to forced swimming

| Groups | Rectal temperature (°C) | | | |
|--------------|-------------------------|---------------|----------------|--------------------------|
| | Initial | After 20 min. | Actual change | % change of rectal temp. |
| Stress Ctrl. | 38.78 ± 0.46 | 31.92 ± 0.16 | 6.86 ± 0.52 | 17.68 ± 01.59 |
| R.H.R. I | 37.97 ± 0.10 | 34.33 ± 0.54 | 3.63 ± 0.46*** | 09.57 ± 01.37** |
| R.H.R. II | 38.27 ± 0.16 | 33.78 ± 0.45 | 4.48 ± 0.29** | 11.71 ± 01.02** |

Data: Mean ± SEM, ** = P<0.01 *** = P<0.001

Table 2: *Ranahamsa Rasayanaya* on the body weight of albino rats subjected to forced swimming stress

| Groups | Body Weight (g) | | |
|--------------|-----------------|----------------|-----------------|
| | Initial | Final | Actual Change |
| Control | 210.00 ± 00.46 | 213.33 ± 06.67 | 03.33 ± 02.11 ↑ |
| Stress Ctrl. | 225.00 ± 00.10 | 211.67 ± 19.90 | 13.33 ± 02.11 ↓ |
| R.H.R. I | 258.33 ± 29.03 | 236.67 ± 26.67 | 21.67 ± 04.01 ↓ |
| R.H.R. II | 263.33 ± 23.05 | 241.67 ± 18.87 | 21.67 ± 04.77 ↓ |

Data: Mean ± SEM, ↓ - Decrease

Table 3: *Ranahamsa Rasayanaya* on stress induced gastric ulcer formation in albino rats subjected to forced swimming stress

| Groups | Dose (mg/kg) | Ulcer index (mm ²) | % change |
|--------------|--------------|--------------------------------|----------|
| Stress Ctrl. | Q.S. | 03.78 ± 00.09 | - |
| R.H.R. I | 900 | 01.73 ± 01.55 | 54.53 ↓ |
| R.H.R. II | 900 | 01.35 ± 01.21 | 64.29 ↓ |

Data: Mean ± SEM, ↓ - Decrease

Discussion

The obtained results show, the well responsiveness of the employed experimental models in the study to the trial rasayana drug. The analysis of the results and the

outcomes are discussed below in the context of the aims and objectives of the present study.

1. *Effect on body weight:* All the three groups including stress control group show an apparent decrease in

body weight in comparison to initial body weight. However the magnitude in loss of body weight in both the treated group is more in comparison to stress control group.

2. *Effect on rectal temperature:* Reduction in rectal temperature (hypothermia) was observed in rats subjected to forced swimming stress for 20 minutes. Drugs which are having adaptogenic properties will reverse the hypothermia in stress conditions. In the present study the data on the effect on forced swimming induced hypothermia reveals that both the test formulations prevented the fall of rectal temperature in highly significant manner in comparison to stress control group. The observed result is more significant in RR I treated group may indicates better adaptogenic activity.
3. *Effect on stress ulcer index:* Stress ulcers most commonly seen in the stomach caused by psychological, physiological or physical stress¹². It is not clear how the mucosal erosions occur in stress ulcers, because actual hyper-secretion of gastric acid is demonstrable in only Cushing's ulcers caused by brain trauma, intracranial surgery, etc. In all other etiologic factors, gastric acid secretion is normal or below normal. In these conditions possible genesis of stress ulcers is due to ischemic hypoxic injury to the mucosal cells and depletion of the gastric mucus barrier rendering the mucosa susceptible to attack by acid-peptic secretions¹³. But at the same time, Stressful life experiences have long been considered to negatively affect ulcer formation, principally via gastric secretions¹⁴. However, stresses may lead to various pathophysiological and biochemical changes¹⁵. One such pathological change in the biochemistry may be prostaglandin secretion, which play an important role in the regulating mucosal blood flow. It is possible that, the test formulations may enhance the formation and turnover of prostaglandins. Next, the hypocalcemia caused by stress is also responsible for gastric ulcer formation¹⁶. That may also be possible, the test drug replenish the depleted calcium level in the blood. In present study both the batches of the test drug decreased the ulcer formation in comparison to standard stress control group; however the observed effect failed to reach statistically significant level. Higher dose may bring the gastro-cytoprotection into a statistically significant level. The magnitude of observed effect is more in RR II treated group.

Conclusion

This paper discusses the first part of the pharmacological study¹⁷ of *Ranahamsa Rasayanaya*. The experimental studies were carried out as a part of standardization of the test drug. According to the results of these studies, it

was found that the test drug is efficacious in decreasing the hypothermia induced by forced swimming in statistically highly significant manner and reducing the gastric ulcer index caused by forced swimming stress in statistically non-significant manner. At the same time, the test drug failed to counteract the catabolic effect produced by the severe stress. It has been proven that, occurrence of complex interactions¹⁸ among behaviour, the brain, the immune system, and the type of stress in animal studies on psycho-neuroimmunology. Similarly, the immune system mediates the effects of psychosocial factors on the susceptibility to and/or the progression of infectious and other immunologically mediated disease processes¹⁹. These findings compared with the established facts yield that, both the batches of the test drug were able to counteract the psychological, physiological and physical stresses. It is safer to infer that, this effect may be resulted from the manipulation of psycho-neuro-endocrine-immune (PNI) axis by the test drug. The analysis of the data obtained from the above studies points out that, both the batches of the test formulations have strong adaptogenic activity and anti-stress activities. Further they also possess powerful *rasayana* effect in the form of adaptogenic and anti-stress activities. Outcomes of these studies validate the potent *rasayana* effect of the test drug - *Ranahamsa Rasayanaya* claimed by the traditional practitioners of Sri Lanka.

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हिन्दी सारांश

श्रीलंकन पारम्परिक औषधि – रणहंस रसायन का प्रायोगिक प्राणीओं में अनुकूलन क्षमता तथा तनाव निवारकता पर प्रभाव

के. इंद्रजित डब्लु. के. सोमरथन, एच. एम. चन्दोला, बी. रविशंकर, के. एन. पण्ड्या, ए. एम. पी. अट्टानायके, एवं बी. के. अशोक

अनेक प्रकार के तनाव मानसिक क्षमता तथा व्याधि प्रतिकारक्षमता कम करते हैं। ऐसी परिस्थितियों में आयुर्वेदीय रसायन चिकित्सा वरदान है। प्रस्तुत शोधकार्य में श्रीलंका में प्रयुक्त होनेवाली रसायन औषधि - रणहंस रसायन का प्रायोगिक प्राणीओं के अनुकूलन क्षमता तथा तनाव निवारकता प्रभाव का अध्ययन किया गया है। प्राप्त परिणामों के अनुसार उपरोक्त औषधि तनाव निवारण क्षमता एवं अनुकूलन क्षमता बढ़ाने में उपयुक्त है।