Experimental study on adaptogenic and antiulcer activity of *Rasayana Ghana* tablet (a tri-herbal formulation) in albino rats

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Distress is one of the most dangerous epidemics having potential to cause severe somatic and psychiatric disorders. Rasayana (rejuvenation therapy) drugs in Ayurveda can be an answer to prevent stress induced disorders. Rasayana powder, an Ayurvedic formulation, is used for health promoting and anti-ageing benefits. The present study was conducted to evaluate the adaptogenic and anti-ulcer potency of *Rasayana Ghana* tablet (RGT) in experimental animals in reverse pharmacological approach. Charles Foster strain albino rats of either sex were divided into five groups and treated as Water control, Stress control, Ghee and Honey treated (Vehicle control), RGT with vehicle control and Standard control. All the animals were subjected to pharmacological protocol of forced swimming stress. RGT was administered along with vehicle in the dose of 180 mg/kg orally for 7 consecutive days before subjecting to stress. The adaptogenic and anti-ulcer activities were assessed on the basis of changes in rectal temperature, body weight, ulcer index, haematological parameters and antioxidant activities. In forced swimming hypothermia, pre-treatment with RGT caused significant attenuation of rectal temperature when compared with both stress control group. The RGT showed significant attenuation on stress induced gastric ulcers by quenching free radicals. Further it significantly prevented stress induced leucopenia. Thus, it can be concluded that RGT along with ghee and honey as vehicle possess anti-stress and adaptogenic activity.

**Keywords:** Adaptogen, Antioxidant, Anti-stress, Antiulcer, *Tinospora cordifolia, Tribulus terrestris, Emblica officinalis, Rasayana Ghana.*

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**Introduction**

Stress has been postulated to be an important etio-pathological factor in variety of diseases, ranging from psychiatric disorders such as depression and anxiety, cognitive dysfunctions, endocrine disorders including *diabetes mellitus*, male sexual dysfunction, immunosuppression, peptic ulcer, hypertension and ulcerative colitis\(^1\). It is a globally recognized problem getting worsened by advancement of industrialization and a demanding civilization\(^2\). Every individual today faces stressful situations in day to day life\(^2\).

Ayurvedic classical texts are enriched with many rejuvenator formulations described in context of Rasayana\(^3,4\). It is needed to explore this traditional knowledge, in order to improve quality of life in modern distressed society, by discovering anti-stress formulations, available in the classical pharmacopoeias of Ayurveda, with less side effects. *Rasayana Churna* (powdered drug) is one among the most commonly used formulation\(^5\) comprising three potent rejuvenator herbs, viz. dried stem of *Guduchi* (*Tinospora cordifolia* (Willd.) Miers, ex Hook. f. & Thoms.,)\(^6,7\) dried fruit rind of *Aamalaki* (*Emblica officinalis* Garten.,)\(^8\) and whole part of *Gokshura* (*Tribulus terrestris* L.,)\(^9,10\). This formulation is indicated after mixing with unequal quantity of ghee and honey as *anupana* (vehicle) in maintenance of sexual vigor (*Vrishya*), physical and mental stability and steadiness (*Sthiratva*), peacefulness and eradication of diseases (*Shanta vikara-dukkham*), balance in mind and body (*Samah*) and longevity (*Shatam Jeevati*) with black hairs (it restricts graying of hairs)\(^11\). However, in *Churna* (Powder) form this

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formulation has certain disadvantages in terms of palatability, storage, administration, etc. for clinical use. Therefore, this powder formulation was converted to Ghana (a solid form, prepared mostly from decoction) tablets which is concentrated, more efficacious, easy to administer and palatable form of drug than that of Churna, which will provide an accurately measured dosage of the active ingredient in a convenient portable package. Further, clinically it is observed that patients, especially those with psychological disorders (here stress related disorders), prefer to take tablet form rather than any other forms like powder, decoction etc. Thus in the present study prepared Ghana tablet form of test formulation was evaluated for anti-stress and adaptogenic activity in albino rats. The proportion of Anupana (vehicle) i.e. ghee and honey were taken in the ratio of 2:1 considering the Vata-Paittika doshik dominance of the disease as per Ayurvedic pathology. The classics quote that mixing honey and ghee in equal quantity is Matra viruddha (dose incompatible) and may produce hazardous effects.

Materials and Methods

Test formulation

The genuine and authenticated raw materials (Table 1) of the test formulation were procured from pharmacy attached to Gujarat Ayurved University, Jamnagar. Coarse powder was prepared and mixed in equal proportions. Decoction was prepared by following the classical method. It was then filtered and further heated up to concentrated form i.e. Ghana was formed and then tablets (500 mg each) were made and coded as RGT. Honey was purchased from forest department office of Gujarat, Jamnagar. Cow’s ghee manufactured by Schreiber Dynamix Dairies Ltd. Baramati was procured from Khadi Gramodyoga Bhandar, Jamnagar.

Animals and husbandry conditions

Charles Foster strain albino rats of either sex weighing between 230 ± 30 g were selected and procured from the animal house (Registration No. 548/2002/CPCSEA) attached to pharmacology laboratory, IPGT & RA, Gujarat Ayurved University, Jamnagar. They were housed in polypropylene cages and fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries, Baroda and tap water given ad libitum. The animals were acclimatized for one week in lab condition before commencement of the experiment in standard laboratory conditions 12 ± 01 h day and night rhythm, maintained at 25 ± 3°C and 40 to 60% humidity. Before the test, the animals were fasted for 12 h. Institutional Animal Ethics Committee had approved the experimental protocol (Approval number; IAEC 05/09-10/Ph. D.03) and care of animals was taken as per the CPCSEA guidelines.

Dose selection and schedule

The dose of Rasayana Ghana tablet in human beings in the clinical trial was designed as 2 g/day (two tablets of 500 mg each) in two divided doses. This dose was calculated considering the Roga bala (disease) and applicability of the modified drug dosage form apart from classics. The dose of Rasayana powder is 6-10 g per day. Therefore, the dose of Ghana tablet is designed as equal to 1/4th of the mean powder dose. The dose for experimental animals was calculated by extrapolating the human dose to animals (180 mg/kg) based on the body surface area ratio by referring to the standard table of Paget and Barnes (1964). The vehicle for this drug, as advocated in classical text is ghee and honey. Classics quote not to give ghee and honey in equal proportion, hence considering the doshik pathophysiology of disease stress and premature ageing, the dose was designed in unequal proportion as two parts ghee mixed with one part honey. Hence, fine powder of drug was made and suspended and mixed properly in unequal quantity of ghee (1080 mg/kg) and honey (540 mg/kg) as per the

Table 1—Ingredients of Rasayana Ghana tablet and Ayurvedic pharmacological properties

<table>
<thead>
<tr>
<th>Common name of the plant &amp; part used</th>
<th>Latin name</th>
<th>Quantity</th>
<th>Rasa</th>
<th>Guna</th>
<th>Virya</th>
<th>Vipaka</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gokshura (fruit &amp; root)</td>
<td>Tribulus terrestris L.</td>
<td>1 part</td>
<td>Madhura</td>
<td>Guru</td>
<td>Sheeta</td>
<td>Madhura</td>
</tr>
<tr>
<td>Aamalki (Fruit)</td>
<td>Embelica officinalis Gaertn.</td>
<td>1 part</td>
<td>Madhura, Amla, Katu, Tikta, Kashaya</td>
<td>Guru, Raksha</td>
<td>Sheeta</td>
<td>Madhura</td>
</tr>
<tr>
<td>Guduchi (stem)</td>
<td>Tinospora cordifolia (Wild.) Miers. ex Hook. f. &amp; Thoms.</td>
<td>1 part</td>
<td>Tikta, Kashaya</td>
<td>Guru, Snigdha</td>
<td>Ushna</td>
<td>Madhura</td>
</tr>
</tbody>
</table>
classical indication and administered to animals orally with the help of gastric catheter sleeved to syringe. The drugs were administered to overnight fasted animals.

**Study design**

Animals were divided into five groups of six animals each, comprising of both male and female in each group. Normal control (WC) animals were kept under standard laboratory conditions, left undisturbed in their home cages without any stress exposures. Second group received distilled water and served as stress control (SC) group. Third group received combination of ghee (1080 mg/kg) and honey (540 mg/kg) and served as vehicle control (VC). Fourth group was administered with the Rasayana Ghana Tablet (RGT-180 mg/kg) which was made to fine powder and mixed with vehicles. For third and fourth groups, vehicle and RGT were given for seven consecutive days. To fifth group diazepam as standard reference drug control (RS-20 mg/kg) was administered orally at 48 h, 24 h and 1 h prior to swimming. On sixth day the rats were kept in individual metabolic cages to prevent coprophagy and fasted for 36 h with access to water ad libitum. On seventh day one hour after drug administration, the initial rectal temperatures of individual rats was noted and were kept inside specially arranged containers which were made up of plexiglass with holed lids. The water level was maintained up to 25 cm height and temperature of water was maintained at 22 ± 2°C. Rats were placed in the container and exactly after 20 minutes of exposure to stressed condition, the rats were taken out individually and final rectal temperature of each rat was noted. The drop in rectal temperature was noted down.

Effect of drugs on stress-induced ulcer was evaluated by following the method of Parmar and Jagruti. After noting the final rectal temperature rats were again exposed to swimming stress inside the same container for 16 h. At the end of 16 h blood was obtained from the retro-orbital puncture under mild ether anaesthesia using capillary tubes. The body weight was noted and they were sacrificed by excess dose of ether. Blood samples were collected for assessing different types of haematological parameters, using automatic haematological analyzer (ACRUS automated haematology auto-analyzer). The adrenal glands were dissected out, cleaned for extraneous tissues, blotted with tissue paper and utilized for estimation of adrenal ascorbic acid contents.

The stomach was excised, cleaned and opened along the greater curvature. The inner surface was cleaned gently by washing with cold saline solution and spreaded on wax board with the mucous surface upwards avoiding corrugation and examined for ulceration with a magnifying lens. Severity of ulcer and total number of ulcers in each rat was recorded for calculating ulcer index which was calculated by method of Kulkarni and Goel. Mean ulcer scores for each experimental group were calculated and expressed as the ulcer index.

The weighed mucosal part of stomach tissue was taken and homogenized in ice cold normal saline for estimation of total protein, catalase and lipid peroxidation. Second fragment was homogenized in 3% metaphosphoric acid solution for estimation of glutathione content and glutathione peroxidase activity. Further weighed adrenal glands were homogenized with 4 ml of 6% trichloroacetic acid for estimation of adrenal ascorbic acid.

**Statistical analysis**

The results were presented as Mean ± SEM. Statistical comparisons were performed by unpaired student’s t test and one way ANOVA with Dunnets’ multiple ‘t’ test as post-hoc test by using Sigma stat software (version 3.1). The level of significance was set at P<0.05.

**Results**

Effect of test drug on ulcer index, hypothermia and body weight of rats subjected to forced swimming stress is shown in Table 2. Exposing rats to forced swimming stress for 16 h lead to formation of extensive gastric ulceration. The stomach from the rats pretreated with vehicle, RGT and diazepam showed very few gastric ulcers and observed effect was statistically significant. Further, in comparison to vehicle control RGT treated group showed significant attenuation of gastric ulceration.

Reduction in rectal temperature (hypothermia) was observed in rats subjected to forced swimming for 20 minutes. Pre-treatment with vehicle, RGT and diazepam significantly attenuated forced swimming hypothermia. The effect observed in vehicle and test drug treated group was better than that of diazepam treated group.
A normal range increase in body weight was observed in rats of control group, contrary to this, an apparent decrease in body weight was observed in stress control rats. In treated groups, decrease in body weight was also observed; however, the magnitude of decrease was comparatively less.

When the rats were subjected to swimming stress significant decrease in total WBC count, lymphocyte percentage and neutrophil percentage was observed (Table 3). Pre-treatment with vehicle and RGT significantly attenuated total WBC count, whereas they have not shown any significant impact on lymphocyte percentage and neutrophil percentages.

Rats subjected to stress significantly elevated the total protein, lipid peroxidation and significantly decreased the antioxidant levels, viz. catalase activity, glutathione and glutathione peroxidase activity (Table 4). Treatment with RGT significantly attenuated all most all these parameters as that of Diazepam.

The adrenal ascorbic acid content was found to be significantly decreased in stress control group in comparison to normal control group. Pre-treatment with vehicle RC and Diazepam significantly attenuated adrenal ascorbic acid content significantly.

Discussion

Forced swimming test in laboratory animals has been widely used for studying the physiological changes and the capacity of the organism to adjust in response to stress². Even short single bout of stress like one day forced swimming stress is as effective as prolonged stressor in bringing the stress induced alterations in the body²⁸.
Swimming induced hypothermia is an inevitable outcome of swimming at water temperature lower than the animal’s core temperature. Reduction in rectal temperature (hypothermia) was observed in rats subjected to forced swimming stress for 20 minutes. In present study forced swimming lead to remarkable hypothermia which was significantly attenuated by pre-treatment with both vehicle and RGT. The magnitude of attenuation observed in both vehicle and RGT treated group is almost similar and better than that of reference standard treated group.

Gastrointestinal erosion is one of the consistent findings in man and in experimental animals subjected to different types of stress. In present study, exposing rats to forced swimming stress for 16 h lead to formation of extensive gastric ulceration. It was proposed that stress ulcers are due to both physiological and psychological factors, which is crucial for gastrointestinal defense and increased accumulation of acid and pepsin leading to autodigestion of the gastric mucosa. Stress in animals is known to increase gastric motility and acidity which could lead to ulceration manifested by severe mucosal damage and haemorrhage. The other factors that may be involved are platelet-activating factor increase in gastric motility, vagal over activity, mast cell degranulation and decreased prosta-glandine (PG) synthesis. The reactive oxygen species generated by the metabolism of arachidonic acid, platelets, macrophages and smooth muscle cells may also contribute to gastric mucosal damage. Contrary to observation of stress control group, the stomach from the rats pretreated with vehicle, RGT and Diazepam showed very few gastric ulcers and observed effect was statistically significant. Further, in comparison to vehicle control, RGT treated group shows significant attenuation of gastric ulceration.

Generally when animals are subjected to stress by different means like swimming for long duration, etc. body weight loss is seen. In present study, the body weight decreased significantly after 16 h of swimming stress in stress control group in comparison to non-stressed rats. Pre-treatment with RGT remarkably attenuated stress induced weight loss in rats which may be due to presence of adaptogenic activity in test formulation.

When the rats were subjected to swimming stress significant decrease in total WBC count and lymphocyte percentage and marked decrease in neutrophil percentage was observed. The leucopenic response observed in the study might be attributed to the action of adrenal steroids. Jensen and Rasmussen have shown that involvement of adrenal glands on the white cell number. Under the influence of the stress neutrophils might exit from the circulation as there could have been increased chance of local tissue damage leading to increased capillary permeability after swimming stress. Pre-treatment with vehicle and RGT significantly attenuated total WBC count, and attenuated neutrophil percentage non-significantly. Lymphocyte percentage was not affected by both vehicle and RGT.

Increase in the activity of endogenous antioxidant system can be an important mechanism for expressing adaptogenic activity. Increase in the activity of this system may lead to enhancement in scavenging of free radicals, the reactive oxygen metabolites that might have been produced in higher quantity due to increased lipid peroxidation during stress stimuli. Pre-treatment with RGT significantly decreased lipid peroxidation and increased antioxidant system in terms of catalase, total glutathione and glutathione peroxidase activities. Further, stress lead to significant increase in total protein content of stomach tissue and this factor was also significantly attenuated by RGT. The exact mechanism involved needs to be elucidated.

Stress leads to significant decrease of adrenal ascorbic acid content in rats. Pre-treatment with vehicle, RGT and reference standard drugs significantly increased ascorbic acid content in comparison to stress control rats. Among them RGT showed more effect. Many previous researchers showed that pretreatment with adaptogenic drugs increases the adrenal ascorbic acid values of the adrenal cortex and prevent the loss of adrenal weight may be through corticosteroid sparing effect. Thus the effect observed in present study also may be attributed to adaptogenic effect of RGT.

As stated in introductory part, RGT is a polyherbal compound comprising of T. cordifolia, E. officinalis and T. terrestris (Table 1). Among these T. cordifolia is with proven stress-attenuating activity. In addition, T. cordifolia has several properties generally associated with adaptogens, including immunomodulatory, anti-inflammatory and antioxidant activity in experimental animals. E. officinalis possess immunomodulatory, adaptogenic and antioxidant activity. The studies on T. terrestris (Gokshura) suggest that the Harmine content of
Tribulus acts as Monoamine Oxidase (MAO) inhibitor, leading to higher levels of dopamine in the brain. Due to higher levels of dopamine, the mood is elevated slowly and the stronger and better is the feeling. These actions may have been supportive in substantiating adaptogenic effect of the formulation through a different mechanism in the study. Further honey and ghee are also proven for their adaptogenic activities. Thus the anti-stress and adaptogenic profile of RGT may be attributed to one or more bioactive principles present in these drugs. There may be synergetic herb-herb interactions enhancing the total efficacy of the formulation.

Thus the cumulative Ayurvedic pharmacological properties of Rasayana Ghana tablet are as follows: RGT has predominantly Madhura rasa (sweet taste), Madhura Vipaka (sweet after digestion) Sheeta veerya (cold potency), Snigdha (unctuous), Guru guna (heavy) and Vata-pitta dosha shamaka properties. Rasayana Ghana tablets is finally converted in Madhura rasa (sweetness) after metabolism by Agni (digestive bio-fire). As per the classics, Madhura vipaka promotes normal Kapha helps in proper elimination of stool and urine. Madhura (sweetness) also can cause Aalhada, i.e. soothing and delightfulness. Sushruta states that Madhura can produce Soumansya (sense of well being), Bala (power), Utsaha (enthusiasm), Harshana (pleasure), Sukham (happiness). These are important and symbiotic qualities to enhance the adaptogenic activity and anti-ulcer properties of the formulation. With reference to the Sheeta Virya (cold potency), it is stated as at psychic level, Sheeta is known to produce Lhadana (delightening of mind) while at physical level it can aggravate Vata and Kapha due to similarity in qualities. Sheeta Virya has soothing and calming effect which is necessary in adapting the given situation in stress prone patients for relaxation. Its sedative property may be helpful in agitated and anxious patients. Furthermore, Pitta is pacified by Sheeta, which is of great significance in preventing ulcer formation. Rest of the work is done by the synergetic activity of ghee and honey which are found equally efficient in preventing ulcer and having adaptogenic activity.

Conclusion

From the present study it can be concluded that Rasayana Ghana tablet along with ghee and honey as vehicle possesses potent anti-stress, ulcer preventing and adaptogenic activity in experimental animals.

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