



Pharmacological Research

Evaluation of anti-depressant and anxiolytic activity of *Rasayana Ghana* Tablet (A compound Ayurvedic formulation) in albino mice

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Abstract

In recent years, many Ayurvedic formulations are being researched to provide an effective antidepressant and anxiolytic drug in the field of psycho-pharmacology. The present study was planned to evaluate the anti-depressant and anxiolytic activity of *Rasayana Ghana* Tablet comprising three herbs *Guduchi* (*Tinospora cordifolia* Miers), *Aamalaki* (*Emblica officinalis* Garten) (RGT) and *Gokshura* (*Tribulus terrestris* Linn). Swiss albino mice were divided into four groups of six animals each, comprising of both male and female in each group. Group I received water served as normal control (WC), group II received vehicle and served as vehicle control (VC), group III received *Rasayana Ghana* tablet and group IV received standard drug diazepam (2 mg/kg) for anxiolytic study in elevated plus maze and standard antidepressant imipramine (5 mg/kg) for anti-depressant activity in behavior despair test. *Rasayana Ghana* tablet along with ghee and honey as vehicle is found to be having antidepressant and anxiolytic activity in experimental animals. Thus, this formulation can be used in prevention and treatment of depression and anxiety.

Key words: *Aamalaki* (*Emblica officinalis* Garten), Anti-depressant, anxiolytic, *Gokshura* (*Tribulus terrestris* Linn), *Guduchi* (*Tinospora cordifolia* Miers), *Rasayana Ghana*

Introduction

Depression and anxiety are the two most prevalent psychiatric disorders challenging medical science. Stress has significant impact on causation of these two diseases. Modern medical science is always in search of better treatment options with minimum side effects. *Ayurveda* also bears the responsibility to provide an effective psycho-active drug to combat with disease. There are ample references for psycho-neuro-pharmacological effects of herbal medicines, and factors affecting the psyche in the classics^[1] which are to be revealed scientifically.

In Ayurvedic classical texts, potent psycho active and

rejuvenator formulations are quoted which can be utilized for improving the quality of life in modern distressed society. *Rasayana churna* is one among the most commonly used classical formulations comprising three potent well established rejuvenator herbs viz., *Guduchi* (*Tinospora cordifolia* Miers), *Aamalaki* (*Emblica officinalis* Garten), and *Gokshura* (*Tribulus terrestris* Linn) advocated along with unequal quantity of ghee and honey as *Anupana* (vehicle). As per classics, ghee and honey mixed in equal quantity are *Matra viruddha* (incompatible in same quantity).^[2] Therefore, the quantity of vehicle, i.e. ghee and honey was calculated unequal as 2:1. Classics quote that this *Rasayana* formulation with *anupana* of ghee and honey is beneficial in maintenance of sexual vigor (*Vrishah*), 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 restriction graying of hair.^[3] In this study, to overcome the disadvantages of *Churna* (Powder) form in terms of palatability, storage etc. for clinical use, *Rasayana Churna* was converted to *Ghana* (solid form prepared from decoction) tablets, which is a more concentrated and

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palatable form of drug than that of *Churna*. The tablet form (*Rasayana Ghana* Tablet) also provides an accurately measured dosage of the active ingredient in a convenient portable package.^[4] Furthermore, as per clinical experience of author, it is observed that patients, especially those with psychological disorders (here stress related disorders), prefer to take tablet form due to convenience and palatability rather than any other forms like powder, decoction etc. Thus prepared *Rasayana Ghana* Tablet was subjected to experimental study to evaluate antidepressant and anxiolytic activities.

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. They were made to coarse powder form and mixed in equal proportions. The decoction was prepared by following the classical method.^[5] The decoction was then filtered and further heated up to a concentrated form, i.e. *Ghana* was formed and then tablets (each of 500 mg) were made and coded as RGT. The honey and *ghee* of standard brands were purchased from local market.

Animals and husbandry conditions

Swiss albino mice (24±04 g) of either sex were procured from animal house (Registration No. 548/2002/Committee for the purpose of control and supervision on experiments on animals) attached to the pharmacology laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar. They were housed in the groups of six under the standard laboratory conditions (Temperature 23±2°C, relative humidity 50-60%) with food (Amrut brand) and tap water. Tests were performed only after the animals had acclimated to the laboratory conditions for at least 7 days and the experiments were performed during morning hours (08.00-10.00 hours). The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC 05/09-10/Ph.D.03) and the care of animals was taken as per the Committee for the purpose of control and supervision on experiments on animals (CPCSEA) guidelines.

Dose selection and schedule

The dose of *Rasayana Ghana* tablet in human beings is 2 g/day in two divided doses. This dose was calculated considering the *Roga bala* (disease) and applicability of the modified drug dosage form. The dose for experimental animals was calculated by extrapolating the human dose to animals (260 mg/kg) based on the body surface area ratio by referring to the standard table of Paget and Barnes (1964).^[6] The vehicle for this drug advocated in classical

text is *ghee* and honey in unequal quantity (2:1). Hence, the drug solution was made by suspending the fine powder of *Rasayana Ghana* in a triturated mixture of unequal quantity of *ghee* (1040 mg/kg) and honey (520 mg/kg). This was 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 in to four groups of six animals each, comprising of both male and female in each group. Group I received water served as normal water control (WC), group II received vehicle and served as vehicle control (VC), group III received *Rasayana Ghana* and group IV received standard drug diazepam (2 mg/kg) for anxiolytic study and standard antidepressant imipramine (5 mg/kg) for anti-depressant activity.

Elevated plus maze

The plus-maze apparatus, consisting of two open arms (16 × 5 cm) and two closed arms (16 × 5 × 12 cm) having an open roof, with the plus-maze elevated (25 cm) from the floor, was used to observe anxiolytic behaviour in mice. Mice were given a single oral dose of the vehicle, test drug and standard drug one hour before their placement on the Elevated plus maze (EPM). Dose administration schedule was adjusted so that each mouse took its turn on the elevated plus-maze apparatus one hour after administration of the dose. To begin a test session, mice were placed on the open arm facing the center of the maze. An entry into an arm was defined as the animal placing all four paws over the line marking that area. The number of entries and the time spent in the open and closed arms were recorded during a 5-minute test period. During the entire experiment, mice were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of the plus-maze could invoke maze anxiety.^[7]

Behavioral despair test

Mice were forced to swim individually in glass jar (25 × 12 × 25 cm³) containing fresh water to a height of 15 cm and maintained at 25°±2°C. After an initial two minutes period of vigorous activity each animal assumed a typical immobile posture. A mouse was considered to be immobile when it remained floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4 minutes of a total 6 minute test. The changes in immobility periods were studied after administering drugs in separate groups of animals.^[8] Each animal was used only once.

Statistical analysis

Results from the pharmacological screening were expressed as Mean ± standard error of the mean (SEM). Differences

Table 1: Ingredients of *Rasayana Ghana* tablet and their Ayurvedic pharmacological properties

Plant and part used	Latin name	Quantity	Rasa	Guna	Virya	Vipaka
Gokshura (fruit and root)	<i>Tribulus terrestris</i> Linn.	1 part	Madhura	Guru	Sheeta	Madhura
Aamalki (Fruit)	<i>Embelica officinalis</i> Gaertn.	1 part	Madhura, Amla, Katu, Tikta, Kashaya	Guru, Ruksha	Sheeta	Madhura
Guduchi (stem)	<i>Tinospora cordifolia</i> Miers.	1 part	Tikta, Kashaya	Guru, Snigdha	Ushna	Madhura

between the control and treatment groups in the experiments' were tested for significance using unpaired student's 't' test. values of $P < 0.05$ were considered as statistically significant.

Results

Diazepam which is a well established anti-anxiety drug significantly increased the latency of first entry to closed arm and highly significantly increased the time spent in open arm in comparison to control group. Pre-treatment with *Rasayana Ghana* tablet (RGT) also significantly increased the latency of first entry to closed arm and significantly increased the time spent in open arm. Further it also increased the number of entries from closed to open arm which was found to be not affected by administration of diazepam. However, in comparison to vehicle control only, latency of first entry to closed arm is found to be statistically significant in RGT treated group [Table 2].

Pre-treatment with RGT and imipramine significantly reduced the immobility duration of mice in comparison to control group. However, in comparison to vehicle control, RGT non-significantly reduced the immobility duration [Table 3].

Discussion

Psychopharmacology is the study of drug-induced changes in

mood, sensation, thinking, and behavior.^[9] It encompasses a wide range of substances with various types of psychoactive properties. Psychoactive drugs interact at particular target sites or receptors found in the nervous system to induce widespread changes in physiological or psychological functions. Researchers are interested in any substance that crosses the blood-brain barrier, and thus has an effect on behavior, mood or cognition. Drugs are researched for their physicochemical properties, physical side effects, and psychological side effects.^[10] Therefore, the effects are evaluated in experimental animals. Recent researches suggest that animal models are valid for studying the antidepressant effects of drug because of strong resemblances between stressed animals and depressed humans in terms of changes in various body functions, such as, the regulation of endocrine systems, learning and memory, histology of certain parts of the brain, behavior and others. Therefore, stress-induced brain changes in animal models of depression may validly represent the brain changes in depressed humans, and antidepressant-induced changes in animal models may validly represent antidepressant mechanisms in depressed humans.^[11]

Elevated plus maze is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the gamma-aminobutyric acid (GABA)-benzodiazepine complex.^[12] Rodents have a natural aversion for high and open spaces and prefer closed arms, which have a burrow like ambience and therefore, spend greater amount of time in the closed arm. When exposed to the novel maze alley, the animals experienced an approach-avoidance conflict, which was stronger in the open arms as compared to closed arms. The decrease aversion to the open arms was the result of an anxiolytic effect expressed by an increased number of open arm entries, and time spent in the elevated plus maze, and the decreased time spent on the central platform was another indication of a reduced decision making behavior. Pre-treatment with RGT along with *ghee* and honey significantly increased the latency of first entry to closed arm and significantly increased the time spent in open arm. Further, it also increased the number of entries from closed to open arm which was found to be not affected by administration of diazepam. This indicates anxiolytic effect of *ghee* and honey potentiated by formulation. Benzodiazepines act by facilitating inhibitory GABAergic transmission.^[13] RGT with the vehicle of *ghee* and honey may modulate the GABAergic system.

Antidepressant effect on forced swimming model of depression provides a rapid and reliable behavior screening test for anti-depressants. The model is valid for a broad spectrum of antidepressants mainly including tricyclics and Mono-amine oxidase (MAO) inhibitors, which significantly decrease immobility time in forced swimming test (FST).^[14] Immobility is thought to reflect either a failure to persist in escape directed behavior after persistent stress, or the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli.^[15] Several antidepressants reduce the immobility after forced swimming.^[16] In the present study, RGT significantly decreased the immobility time of mice in FST and was comparable with standard anti-depressant drug imipramine. The observed effect may be attributed to blockage of 5-HT reuptake or MAO inhibition.

Table 2: Results of elevated plus maze test in different groups of Swiss albino mice during the experimental study

Groups	Latency of first entry to closed arm (Seconds)	Time spent in open arm (Seconds)	Number of entries from closed to open arm
WC	2.40±1.03	11.50±05.27	06.40±1.78
VC	0.83±0.83	35.33±10.72	11.17±1.70
<i>Rasayana Ghana</i> tablet	5.50±1.78 [#]	45.50±11.02 ^{**}	12.00±1.00 [*]
Drug group (Diazepam (2 mg/kg))	24.30±6.75 ^{**}	136.67±27.77 ^{**}	2.50±1.06

Data: Mean ± SEM, WC- Water control, VC -Vehicle control, * $P < 0.05$ as compared to water control, ** $P < 0.01$ as compared to water control, [#] $P < 0.05$ as compared to vehicle control

Table 3: Results of behavior despair test in different groups of Swiss albino mice during the experimental study

Groups	Immobility time (Sec)
WC	152.17±06.00
VC	122.67±22.24
<i>Rasayana Ghana</i> tablet	106.33±15.83 [*]
Drug group (Imipramine (5 mg/kg))	069.13±13.94 ^{**}

Data: Mean ± SEM, WC- Water control, VC -Vehicle control, * $P < 0.05$ as compared to water control, ** $P < 0.001$ as compared to water control

As stated in introductory part, RGT is a poly herbal compound comprising of *Tinospora cordifolia*, *Emblica officinalis* and *Tribulus terrestris*. Among these, *Tinospora cordifolia* is with proven stress-attenuating activity.^[17] In addition, *Tinospora cordifolia* has several properties generally associated with adaptogenic^[18,19] antioxidant^[20,21] properties. It is also known to possess beneficial effects on learning, stress and memory.^[22] *Emblica officinalis* is having immunomodulatory activity, adoptogenic and antioxidant activity.^[23-30] Earlier studies on formulation containing *Guduchi* (*Guduchyadi ghrita*) and *Aamalaki* (*Bhringarajadi rasayana*) showed potent antidepressant, anti stress, anxiolytic activity.^[31] The studies on *Tribulus 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.^[32] Further honey^[33-35] and *ghee*^[36] are also proven for their anti-oxidant and adaptogenic activities. Thus, the observed and anxiolytic and anti-depressant profile of RGT may be attributed to one or more bioactive principles present in these drugs. There may be synergistic herb-herb interactions enhancing the total efficacy of the formulation. The exact mechanism of action of the drug needs to be evaluated by further extensive studies.

In Ayurvedic parlance, both anxiety and depression can be stemmed at the vitiation of *Vata dosha*, imbalance of *Kapha dosha* and *Pitta dosha* at the somatic level. Therefore, to prevent these pathological events, the *dosha* states must be kept in balance. RGT may act as to maintain the homeostasis of *dosha* in either of following ways. 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 possess *Madhura rasa* and *Madhura vipaka*, which means that it is finally converted in *Madhura rasa* after metabolism by *Agni*.^[37] As per the classics, *Madhura vipaka* promotes the formation of normal *Kapha*, and helps in proper elimination of stool and urine.^[38] *Madhura* also can cause *Aalhada*, i.e. soothing and delightfulness.^[39] Sushruta states that *Madhura* can produce *Soumansya* (sense of well being), *Bala* (power), *Utsaha* (enthusiasm), *Harshana* (pleasure) and *Sukham* (happiness).^[40] These are important and symbiotic qualities to enhance the anti-depressant, anxiolytic and adaptogenic activities of the formulation. With reference to the *Sheeta Virya* (cold potency), it produces *Alhadana* (delighting of mind) at the psychological level,^[41] while at physical level it can aggravate *Vata* and *Kapha* due to similarity in qualities.^[42] *Sheeta Virya* has soothing and calming effect, which is necessary in adapting to the given situations 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 correcting balance of *dosha*. Rest of the work is done by the synergistic activity of *ghee* and honey which are having adaptogenic activity.

Conclusion

From the present study, it can be concluded that *Rasayana Ghana* tablet along with *ghee* and honey as vehicle is found to

be having antidepressant and anxiolytic activity in experimental animals. Thus, this formulation can be used in prevention and treatment of depression and anxiety. The present study opens new windows for further researches on this classical combination at different dose levels, as adjuvant with and in comparison with various anti-depressant and anxiolytic drugs. This may lead to a better integrative management of these psychiatric disorders in future.

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हिन्दी सारांश

अल्बिनो चूहों में रसायन घन वटी के अवसादहर (एन्ड्रिओलायटिक) प्रभाव का मूल्यांकन

योगेश देवले, सुलक्षण एस. चव्हाण, बी. के. अशोक, बी. रविशंकर, ए. बी. ठाकर, एच. एम. चन्दोला

रसायन घन वटी गुडूची, आमलकी एवं गोक्षुर से बना हुआ एक रसायन योग है। प्रस्तुत प्रयोगात्मक अध्ययन इस योग के अवसादहर (एन्ड्रिओलायटिक) एवं चित्तोद्वेगहर (एन्ड्रिओलायटिक) प्रभाव के मूल्यांकन के लिए किया गया। स्विस अल्बिनो चूहों को चार समूहों में बाँटा गया। प्रथम समूह को केवल जल (नियंत्रण समूह), द्वितीय समूह में घी और शहद का अनुपान, तृतीय समूह में रसायन घन वटी एवं चतुर्थ समूह में मानक औषधी डायजेपाम (चित्तोद्वेगहर प्रभाव के मूल्यांकन के लिए) दी गयी। इस प्रयोग में एलिवेटेड प्लस मेज, ओपन फिल्ड बिहेवीअर, ग्रास बिहेवीअर, बिहेवीअर डिस्पेअर इन प्रतिक्रिया मानकों का उपयोग किया गया। प्राप्त परिणामों के अनुसार रसायन घन वटी में मध्यम अवसादहर एवं अल्प चित्तोद्वेगहर प्रभाव पाया गया।