

Experimental evaluation of gastroprotective and adaptogenic activity of *Amalakyas Rasayana* and its vehicle (ghee and honey)

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Abstract

Amalakyas Rasayana (AR) was tested for its anti-ulcer activity in forced swimming induced hypothermia and stress induced gastric ulceration. AR was administered in the dose of 270 mg/kg orally for 7 consecutive days prior to the experiment. The adaptogenic activity was assessed by determining and comparing the changes in rectal temperature and ulcer index and compared in AR and vehicle treated group against stress control group. In forced swimming induced gastric ulceration, pretreatment with AR caused significant attenuation of ulcer index when compared with both stress control ($p < 0.001$) and vehicle control ($p < 0.05$) groups. AR exhibits significant reduction in ulcer index in comparison to stress control group ($p < 0.05$) and vehicle control ($p < 0.001$) groups. The results suggest that AR possesses significant adaptogenic and gastro protective activities.

Introduction

Ageing is the accumulation of changes in humans refers to a multi-dimensional process of physical, psychological, and social changes [1]. The ageing process is a biological reality which has its own dynamic course that is beyond human control. Ageing is defined as a progressive generalized impairment of function resulting in a loss of adaptive response to stress and in a growing risk of age associated disease [2].

Ayurveda has classified ageing into two viz., *Kalaja jara* (Physiological ageing) which is natural process of ageing and *Akalaja jara* (premature ageing) [3]. Ayurveda has described various rejuvenative therapies with the help of special class of medicinal preparations called *Rasayana* that are believed to rebuild the body, mind, prevent degeneration and postpone ageing [4]. *Amalakyas Rasayana* (AR) is one among many *Rasayana* formulations mentioned in Ayurvedic classical text Charaka Samhita for the treatment of ageing related disorders [5] and used by

Ayurvedic physicians. However, no report on the pharmacological screening on this formulation is available; hence, this study was designed to assess adaptogenic and anti-stress activities of AR to provide pharmacological basis to clinical claims and to justify its use as anti-ageing medicine.

Materials and Methods

Test drug: The raw materials (Table 1) of the test formulation were collected from Gujarat Ayurveda University pharmacy and were subjected to pharmacognostical studies in order to establish their authenticity. From the raw materials, the test drug AR was prepared following the classical guidelines [6]. The vehicle viz., honey and ghee of standard brands were purchased from the local market.

Chemicals: All the chemicals and reagents used in the experimental study were procured from standard and reputed firms and are of analytical grade regularly being used in the laboratory.

Animals: Charles Foster strain albino rats of either sex weighing between 200 ± 30 g were selected and procured from the animal house attached to the institute (Registration No.548/2002/CPCSEA). They were housed in large spacious polypropylene cages and fed with *Amrut brand* rat pellet feed supplied by Pranav Agro Industries and tap water given *ad libitum*. The animals were acclimatized for at least one week in lab condition before commencement of the experiment in standard laboratory conditions 12 ± 01 hour day and night rhythm, maintained at $25 \pm 3^\circ\text{C}$ and 40 to 60% humidity. Before the test, the animals were kept fasting for 12 hours. Institutional Animal Ethics Committee had approved the experimental protocol (Approval number: IAEC 05/09-10/Ph.D.08) and the care of animals was taken as per the CPCSEA Guidelines (Committee for the Purpose of Control and Supervision on Experiments on Animals) [7].

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Table 1: Formulation composition of *Amalakash Rasayana*

	<i>Ingredients</i>	<i>Botanical Name</i>	<i>Part used</i>	<i>Quantity</i>
1	Amalakai	<i>Emblica officinalis</i>	Fruit	11 Part
2	Shweta	<i>Alpenia galanga</i>	Rhizome	1 Part
3	Shatavari	<i>Asparagus racemosus</i>	Root	1 Part
4	Punarnava	<i>Boerhavia diffusa</i>	Root	1 Part
5	Manduka parni	<i>Centella asiatica</i>	Whole plant	1 Part
6	Shalaparni	<i>Desmodium gangeticum</i>	Root	1 Part
7	Jivanti	<i>Leptadenia reticulata</i>	Root	1 Part
8	Rasna	<i>Pluchea lanceolata</i>	Root	1 Part
9	Haritaki	<i>Terminalia chebula</i>	Fruit	1 Part
10	Guduchi	<i>Tinospora cordifolia</i>	Stem	1 Part
11	Lauha Bhasma	Incinerated Iron	–	1.5 Part

Dose selection and schedule: The classical dose of AR in human beings is 3 g/day [8]. The dose for experimental animals was calculated by extrapolating the human dose to animals (270 mg/kg) based on the body surface area ratio by referring to the standard table of Paget and Barnes (1964) [9]. The drug solution was made by adding unequal quantity of ghee (700 mg/kg) and honey (1350 mg/kg) as per the classical indication [10] and administered to animals orally with the help of gastric catheter sleeved to syringe. The drugs were administered to over night fasted animals.

Adaptogenic and anti-ulcer activity [11]: The selected animals were divided in to four groups of six animals in each. Normal control (water control – WC) animals were kept under standard laboratory conditions, left undisturbed in their home cages without stress exposures. Second group received only distilled water and served as stress control (SC) group where as the third group received combination of ghee (700 mg/kg) and honey (1350 g/kg) and served as vehicle control (VC). Fourth group (AR) was administered with the AR (270 mg/kg) plus vehicle. For the experimental group, drugs were given for seven consecutive days. On sixth day the rats were kept in individual metabolic cages to prevent coprophagy and fasted for 36 hours with access to water *ad libitum*. On the seventh day one hour after drug administration, the initial rectal temperature of individual rats was noted. After noting initial rectal temperature rats are 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 [12]. which was modified according to the experimental need. The rats after noting their final rectal temperature were again exposed to the swimming stress inside the same container for 16 hours. At the end of 16 hour period blood was obtained from the retro-orbital puncture under light ether anaesthesia using capillary tubes. The body weight was noted and then they were sacrificed. Blood samples were collected for assessing different types of haematological parameters by using automatic haematological analyzer (ACRUS automated haematology auto-analyzer). The vital organs like liver, heart, kidney and adrenals were dissected out, cleaned for extraneous tissues, blotted with tissue paper, weighed and computed per 100 g body weight.

The stomach was excised, cleaned and opened along the greater curvature. The inner surface was cleaned gently by washing with cold saline solution and spread 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. Ulcer index was calculated according to the method described by Kulkarni and Goel [13]. Mean ulcer scores for each experimental group were calculated and expressed as the ulcer index.

Statistical analysis: The results were presented as mean \pm SEM for six rats in each group. Statistical comparisons were performed by unpaired student's t test and one way Anova with Dunnett's multiple t test as post-hoc test by using Sigma Stat Software (version 3.1) and the level of significance was set at $p < 0.05$.

Results

Effect of AR on rectal temperature

Table 2: Effect of AR and vehicle on rectal temperature in rats subjected to forced swimming stress

Group	Dose (g/kg)	Percentage decrease in rectal temperature (°C)	% change
SC	QS	28.046 ± 0.835	–
VC	0.7+1.4	22.608 ± 1.909 *	19.39 ↓
AR	0.27+0.7+1.4	16.470 ± 0.530 **	41.32 ↓

*p<0.05 **p<0.001 Vs stress control (unpaired t test); p<0.05 Vs vehicle control (unpaired t test)

Administration of vehicle and test drug significantly decreased rectal temperature in comparison to stress control group. Further, test drug shows statistically significant decrease in rectal temperature in comparison to vehicle control group (Table 2).

Effect of AR and vehicle on ulcer index

Table 3: Effect of AR and vehicle on ulcer index in rats subjected to forced swimming stress

Group	Dose (g/kg)	Ulcer index	% change
SC	QS	47.58 ± 2.44	–
VC	0.7+1.4	20.70 ± 2.55#	56.49 ↓
AR	0.27+0.7+1.4	05.90 ± 0.48**	87.60 ↓

#One way Anova - F value 102.50; p<0.001; p<0.05 for VC and AR Vs stress control.

*p<0.001 Vs vehicle control (Unpaired t test)

Administration of vehicle and test drug significantly attenuated stress induced ulceration in comparison to stress control group. Further, test drug shows statistically highly significant decrease in ulcer index in comparison to vehicle control group (Table 3).

Discussion

Ageing is universal but complex biological process with definite manifestations characterized by impairment of various functions and decreased ability to respond to stress [14]. *Rasayana chikitsa* is a specialized section of Ayurveda, which mainly deals with the preservation and

promotion of health by revitalizing the metabolism and enhancing immunity. *Rasayana* therapy encompasses procedures of revitalization and rejuvenation to increase the body's power of resistance to disease and supposed to slowdown the advancement of ageing [15].

Swimming stress in small laboratory animals has been widely used for studying the physiological changes and the capacity of the organism to adjust in response to stress [16]. Swimming is not always a simple exercise stress, because emotional factors are difficult to be eliminated. Even short single stress like one day forced swimming stress is as effective as prolonged stressor in bringing about the stress induced alterations in the body [17]. Swimming induced hypothermia is an inevitable outcome of swimming at water temperature lower than the animal's core temperature. In this study, forced swimming lead to remarkable hypothermia and pre-treatment with both vehicle (p<0.05) and AR (p<0.001) attenuated it in significant manner. The magnitude of attenuation observed in AR treated group is comparatively high in comparison to vehicle (p<0.05). Thus, the observed adaptogenic effect can mainly be due to adaptogenic properties of AR.

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 auto-digestion of the gastric mucosa [18]. Stress in animals is known to increase gastric motility and acidity which could lead to ulceration manifested by severe mucosal damage and haemorrhage [19]. Importance of impaired mucosal blood flow also appears among the important factors in the pathogenesis of stress-induced ulcers [20].

The other factors that may be involved are platelet-activating factor [21], increase in gastric motility, vagal over activity [22], mast cell degranulation [23] and decreased prostaglandin (PG) synthesis [24]. The reactive oxygen species generated by the metabolism of arachidonic acid, platelets, macrophages, and smooth muscle cells may also contribute to gastric mucosal damage [25]. Results presented in this work showed that oral administration of AR and vehicle before stress induction decreased the incidence and severity of stress induced gastric ulcers in rats (p<0.05). Attenuation of ulcer index in pretreated with AR against vehicle control group is highly significant (p<0.001).

Conclusion

Many of the drugs in AR are reported to have anti-stress and adaptogenic activity. Further the vehicle; combination of ghee and honey is also reported to have adaptogenic activity. Thus, the observed adaptogenic profile of AR may be attributed to one or more bioactive principles present in these drugs. From this study, it can

be concluded that AR is having significant adaptogenic and gastroprotective activity. The observed adaptogenic and anti-stress effect may probably be either through attenuation of stress induced stimulation of hypothalamus-pituitary-axis (HPA), quenching of free radicals, and enhancement of cell proliferation or cellular detoxification mechanisms.

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