

Evaluation of adaptogenic activity profile of a compound Ayurvedic formulation - *Amalakayas Rasayana*

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The present study was undertaken to evaluate adaptogenic activity profile of a classical compound Ayurvedic formulation *Amalakayas Rasayana* in experimental animals. *Amalakayas Rasayana* (AR) was tested for its adaptogenic activity by determining anti-stress and anti-ulcer activity in forced swimming induced hypothermia and stress induced gastric ulcers. AR was administered in the dose of 270 mg/kg orally for 7 consecutive days prior to forced swimming induced hypothermia and stress ulcers. The adaptogenic and anti-ulcer activities were assessed by determining and comparing the changes in rectal temperature, ponderal changes, ulcer index, haematological parameters and anti-oxidant activity in the test drug group with that of stress control group as well as vehicle control group. In forced swimming hypothermia pre-treatment with AR caused significant attenuation of rectal temperature when compared with both stress control and vehicle control groups. It has shown a significant reduction in ulcer index and lipid peroxidation. Moreover, AR did show a significant increase in total glutathione content. The results suggest that AR possesses significant adaptogenic and anti-stress activity which could be either due to attenuation of stress induced stimulation of HPA axis and cytoprotective action of the test formulation.

Keywords: *Amalakayas Rasayana*, Adaptogenic activity, *Ghrita*, *Madhu*, Ulcer index

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Ageing is the accumulation of changes in an organism over time which in humans refers to a multi-dimensional process of physical, psychological, and social changes¹. The ageing process is of course a biological reality which has its own dynamic, largely beyond human control. Ageing has been 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 disease².

In Ayurveda, ageing has been mentioned in two ways, viz. *Kalaja jara* (physiological ageing) which is a natural process of ageing and *Akalaja jara* (premature ageing)³. Ayurveda describes various rejuvenative therapies with help of special class of medicinal preparations called *Rasayana* that are believed to rebuild the body, mind, prevent degeneration and postpone aging or rather reverse the aging process⁴. *Amalakayas Rasayana* (AR) is one

among the many *Rasayana* formulations mentioned in Ayurvedic classical text '*Charaka Samhita*' for the treatment of ageing related disorder⁵ and used by Ayurvedic physicians clinically to treat ageing related disorders. However, no reports on the pharmacological screening on this formulation is available; hence the present study was designed to verify adaptogenic and anti-stress activities of *Amalakayas Rasayana* to provide pharmacological basis to clinical claims.

Materials and methods

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 evaluate the authenticity. From the raw materials, the test drug *Amalakayas Rasayana* was prepared following the classical guidelines⁶ in the Pharmacy. The vehicles, viz. honey and ghee of standard brands were purchased from local market.

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Table 1—Formulation composition of *Amalakash Rasayana*

Ingredients	Botanical name	Parts used	Quantity
<i>Amalakai</i>	<i>Emblica officinalis</i> Gaertn.	Fruit	11 Parts
<i>Shweta</i>	<i>Alpenia galanga</i> Willd.	Rhizome	1 Part
<i>Shatavari</i>	<i>Asparagus racemosus</i> Willd.	Root	1 Part
<i>Punarnava</i>	<i>Boerhavia diffusa</i> Linn.	Root	1 Part
<i>Manduka parni</i>	<i>Centella asiatica</i> Linn.	Whole plant	1 Part
<i>Shalaparni</i>	<i>Desmodium gangeticum</i> Linn.	Root	1 Part
<i>Jivanti</i>	<i>Leptadenia reticulata</i> Retz. Wt. & Arn.	Root	1 Part
<i>Rasna</i>	<i>Pluchea lanceolata</i> Oliver & Hiern.	Root	1 Part
<i>Haritaki</i>	<i>Terminalia chebula</i> Retz.	Fruit	1 Part
<i>Guduchi</i>	<i>Tinospora cordifolia</i> Willd.	Stem	1 Part
<i>Lauha Bhasma</i>	Incinerated Iron	--	1.5 Part

All the chemicals or reagents used in the experimental study were procured from standard and reputed firms and are of analytical grade (EXLR) regularly used in the laboratory.

Charles Foster strain albino rats of either sex weighing between 200 ± 30 gm 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 laboratory 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 - 60 % humidity. Before the test, the animals were fasted for at least 12 hrs. 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.

The classical dose of *Amalakash Rasayana* in human beings is 3 gm/day⁷. 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)⁸. The drug solution was made with unequal quantity of ghee (700 mg/kg) and honey (1350 mg/kg) as per the classical indication 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 activity⁹: The selected animals were divided in to 4 groups of 6 animals each. Normal control animals were kept under standard laboratory conditions, left undisturbed

in their home cages without any stress exposures. Second group received only distilled water and served as stress control group. Third group received combination of ghee (700 mg/kg) and honey (1350 gm/kg) and served as vehicle control. Fourth group was administered with the *Amalakash Rasayana* (270 mg/kg). 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 hrs with access to water *ad libitum*. On 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 $22 \pm 2^\circ\text{C}$. 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 Jagruti¹⁰ and modified according to experimental need. The rats after noting their final rectal temperature were again exposed to the swimming stress inside the same container for 16 hrs. At the end of 16 hrs period, blood was obtained from the retro-orbital puncture under light ether anesthesia using capillary tubes. The body weight was noted and they were sacrificed. Blood samples were collected for assessing different types of hematological parameters by using automatic hematological 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 and weighed and were computed per 100 gm 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 by following the method of Kulkarni & Goel (1996)¹¹. 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 protein¹², catalase¹³, and lipid peroxidation¹⁴. Second fragment was homogenized in 3% metaphosphoric acid solution for estimation of glutathione content¹⁵. Further weighed adrenal glands were homogenized with 4 ml of 6% trichloroacetic acid for estimation of adrenal ascorbic acid¹⁶.

The results were presented as Mean \pm SEM for 6 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) for both the treated groups with the level of significance set at $p < 0.05$.

Results

Pre-treatment with both vehicle and AR significantly attenuated the stress induced hypothermia and gastric ulcers in rats subjected to forced swimming. [Figs 1a & 1b - normal cyto-architecture; Fig:1c & 1d- stress control group, note: Severe destruction of epithelial layer; Fig:1e-photomicrograph of representative sections of stomach of albino rats from vehicle treated group (1 \times 100 magnification), note: comparatively less epithelial destruction; Fig:1f-photomicrograph of representative sections of stomach of albino rats from vehicle treated group, note: comparatively less epithelial destruction; Fig:1g- photomicrograph of representative sections of stomach of albino rats from AR treated group, note: almost normal cyto-architecture; Fig: 1h- photomicrograph of representative sections of stomach of albino rats from AR treated group, note: almost normal cyto-architecture.]. Further, the observed effect in AR treated group is

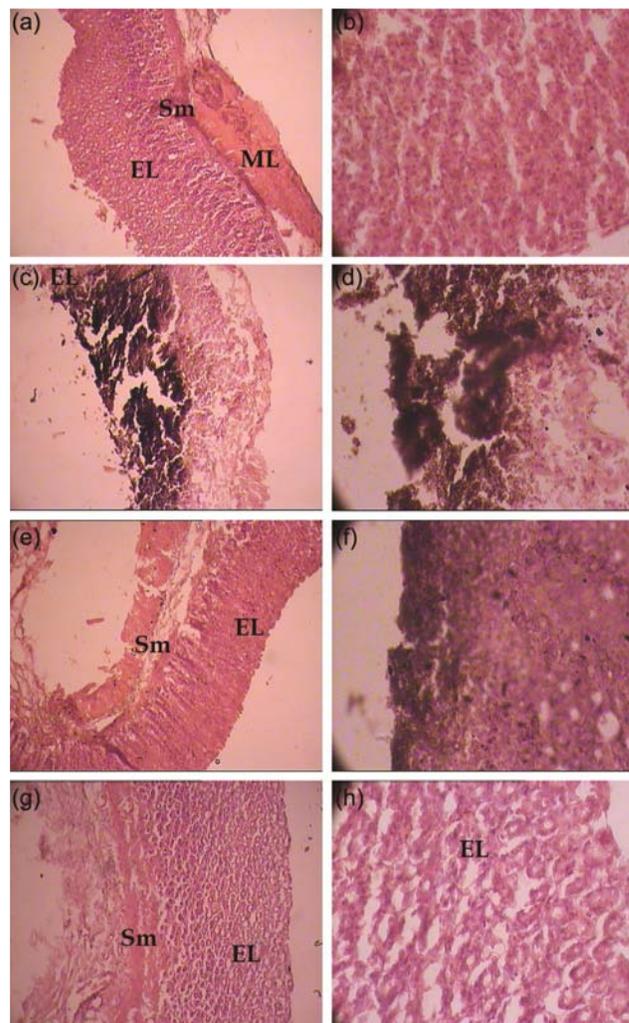


Fig.1a-h—Photomicrographs of sections of stomach from stress induced ulcers in rats

Fig- 1a: Photomicrograph of representative sections of stomach of albino rats from normal control group (1 \times 100 magnification). **EL** - Epithelial layer; **Sm** - Sub-mucosal layer; **ML** - Muscular layer. **Note:** Normal cyto-architecture; **Fig- 1b:** Photomicrograph of representative sections of stomach of albino rats from normal control group (1 \times 400 magnification). **Note:** Normal cyto-architecture; **Fig - 1c:** Photomicrograph of representative sections of stomach of albino rats from stress control group (1 \times 100 magnification). **Note:** Severe destruction of epithelial layer; **Fig - 1d:** Photomicrograph of representative sections of stomach of albino rats from stress control group (1 \times 400 magnification). **Note:** Severe destruction of epithelial layer; **Fig- 1e:** Photomicrograph of representative sections of stomach of albino rats from vehicle treated group (1 \times 100 magnification). **Note:** Comparatively less epithelial destruction; **Fig- 1f:** Photomicrograph of representative sections of stomach of albino rats from vehicle treated group (1 \times 400 magnification). **Note:** Comparatively less epithelial destruction; **Fig - 1g:** Photomicrograph of representative sections of stomach of albino rats from AR treated group (1 \times 100 magnification). **Note:** Almost normal cyto-architecture; **Fig - 1h:** Photomicrograph of representative sections of stomach of albino rats from AR treated group (1 \times 400 magnification). **Note:** Almost normal cyto-architecture.

found to be statistically significant in comparison to vehicle control group (Table 2).

Table 3 shows data related to effect of test drug on body weight and weight of different organs of albino rats subjected to forced swimming induced stress. A normal range increase in body weight was observed in control group when the values were compared with initial body weight. Contrary to this an apparent decrease in bodyweight was observed in stress control rats in comparison to their initial body weight. Both the vehicle and AR significantly reversed this effect.

A marginal decrease in liver weight and increase in heart weight was observed in stress control rats in comparison to normal control rats. Pre-treatment with vehicle and AR did not affect the weight of heart and liver to significant extent. The weight of kidney and

adrenal glands in forced swimming control group was found to be significantly higher in comparison to normal control group. Pre-treatment with both vehicle and AR failed to attenuate weight of kidney and adrenal glands to significant extent.

Effect of AR on different oxidant and oxidant levels has been given in Table 4. Forced swimming induced stress leads to remarkable and statistically significant increase in lipid peroxidation and decrease in total glutathione content in comparison to stomach homogenate obtained from normal control rats. The vehicle and AR significantly reversed increased lipid peroxidation and depleted total glutathione in comparison to stress control group. Further the observed effect is almost similar in both vehicle and AR treated groups.

Table 2—Effect on ulcer index and rectal temperature

Groups	Ulcer Index	Percentage decrease in rectal Temperature (°C)
SC	47.58 ± 2.44	28.05 ± 0.83
VC	20.70 ± 2.55 [#]	22.61 ± 1.90*
AR	05.90 ± 0.48 ^{#¶¶}	16.47 ± 0.53 ^{***¶¶}

[#] One way ANOVA - F value 102.50; p<0.001: DMTT - p<0.05for VC and AR Vs stress control.

*p<0.05, ***p<0.001 Vs stress control (Unpaired t test)

¶p<0.05, ¶¶p<0.001 Vs vehicle control (Unpaired t test)

Table 3 — Effect on ponderal changes

Groups	Percentage change in body wt	Heart (mg/100gm body wt)	Liver (mg/100gm body wt)	Kidney (mg/100gm body wt)	Adrenals (mg/100gm body wt)
WC	6.57 ± 1.86	307 ± 10	2734 ± 99	599 ± 32	19 ± 01
SC	-11.46 ± 0.85 ^{¶¶¶¶}	323 ± 10	2606 ± 189	690 ± 15 [¶]	25 ± 01 ^{¶¶}
VC	3.20 ± 2.14 ^{***}	313 ± 09	2598 ± 81	623 ± 39	22 ± 02
AR	5.89 ± 3.16 ^{***}	318 ± 15	2461 ± 133	699 ± 39	21 ± 02

[¶]p<0.05 ^{¶¶}p<0.01, compared with normal control (Unpaired t test)

Table 4 — Effect on tissue biochemical parameters

Groups	Total protein (mg/gm wet tissue)	Catalase (μ moles H ₂ O ₂ consumed/mg wet tissue/ min)	Lipid Px (μ moles MDA released / gm wet tissue)	Glutathione (n moles / gm wet tissue)	Adrenal ascorbic acid (μg/mg wet tissue)
WC	11.10 ± 1.24	13.14 ± 1.04	10.10 ± 2.85	10.72 ± 1.02	28.27 ± 04.78
SC	12.21 ± 1.54	10.99 ± 0.89	23.79 ± 1.16 ^{¶¶¶¶}	07.04 ± 1.16 [¶]	18.42 ± 04.21
VC	13.05 ± 1.58	12.17 ± 1.13	7.75 ± 3.70 ^{***}	13.98 ± 2.00 ^{**}	20.45 ± 03.98
AR	12.23 ± 0.45	11.89 ± 0.94	9.94 ± 3.22 ^{***}	13.24 ± 2.22 ^{**}	18.12 ± 03.63

[¶]p< 0.05, ^{¶¶¶¶}p< 0.001 compared with normal control (Unpaired t test)

^{**}p<0.01, ^{***}p<0.001 compared with stress control (Unpaired t test)

Table 5 — Effect on hematological parameters

Groups	WBC (cells/cu mm)	Neutrophils (%)	Lymphocytes (%)	RBC (10e ⁶ / μl)	PCV (%)	Hb (gm/dl)
WC	7150.00 ± 353.78	39.00 ± 01.57	77.50 ± 2.156	8.08 ± 0.319	42.20 ± 1.218	13.68 ± 0.421
SC	9283.33 ± 937.87	47.17 ± 6.585	45.33 ± 6.888 ^{ψψψ}	8.81 ± 0.0611 ^ψ	46.40 ± 0.786 ^{ψψ}	14.57 ± 0.496
VC	9566.67 ± 1220.56	52.17 ± 2.868	42.33 ± 3.018	8.32 ± 0.247	46.60 ± 0.83	14.57 ± 0.257
AR	10150.00 ± 1241.16	55.83 ± 3.311	39.00 ± 3.266	8.57 ± 0.255	46.08 ± 0.969	15.48 ± 0.239 [¶]

^ψp<0.05, ^{ψψ}P<0.01, ^{ψψψ} p<0.001 compared with normal control (Unpaired t test)

[¶]p<0.05 compared with vehicle control (Unpaired t test)

The adrenal ascorbic acid and catalase activity were found to be non-significantly decreased in stress control group in comparison to normal control group. Pre-treatment with vehicle and AR attenuated them in non-significant manner.

Forced swimming induced stress in rats resulted in significant decrease in lymphocyte percentage, total RBC count and packed cell volume (PCV) in comparison to normal rats (Table 5). Pre-treatment with vehicle and AR did not attenuate these parameters to significant extent. Other parameters like total WBC count, neutrophil percentage and haemoglobin contents were not affected by swimming stress. Further the haemoglobin content of AR treated group was found to be statistically significant in comparison to vehicle control group.

Discussion

Ageing is universal but complex biological process with proverbial and unambiguous manifestations characterized by impairment of various functions and decreased ability to respond stress. *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 aging also¹⁷.

Swimming in small laboratory animals has been widely used for studying the physiological changes and the capacity of the organism to adjust in response to stress¹⁸. 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 body¹⁹. 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 also forced swimming lead to remarkable hypothermia and pre-treatment with both vehicle and AR attenuated it in significant manner. The magnitude of attenuation observed in AR treated group is remarkably high in comparison to vehicle. Thus the observed adaptogenic effect is mainly due to the active principles contained in the plant products used in the preparation of the AR.

It is a well known fact that forced swimming induced stress brings about various physiological changes in the body hence the effect of test formulation was tested on different types of parameters in rats subjected to forced swimming stress. 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 mucosa²⁰. Stress in animals is known to increase gastric motility and acidity which could lead to ulceration manifested by severe mucosal damage and haemorrhage²¹. Importance of impaired mucosal blood flow also appears among the important factors in the pathogenesis of stress-induced ulcers²².

The other factors that may be involved are platelet-activating factor²³, increase in gastric motility, vagal over activity²⁴, mast cell degranulation²⁵ and decreased PG synthesis²⁶. The reactive oxygen species generated by the metabolism of arachidonic acid, platelets, macrophages, and smooth muscle cells may also contribute to gastric mucosal damage²⁷. Results presented in this work show that oral administration of AR before stress induction decreased the incidence and severity of stress induced gastric ulcers in rats. It also reduced lipid peroxidation and increased total glutathione content in gastric

mucosa. Also, it attenuated the weight loss and weight of kidney and adrenal glands. The observed adaptogenic and anti-stress effect may be through attenuation of stress induced stimulation of HPA axis, quenching of free radicals, enhancement of cell proliferation and cellular detoxification mechanisms.

Many of the drugs used in AR formulations are reported to have anti-stress and adaptogenic activity, viz. *Embllica officinalis*²⁸, *Asparagus racemosus*²⁹, *Boerhavia diffusa*³⁰, *Centella asiatica*³¹, *Terminalia chebula*³² and *Tinospora cordifolia*^{33,34}. Further, the vehicle a combination of ghee^{35,36} and honey^{37,38} were 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 activity and can be used for treatment of ageing related disorders.

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