Indian Journal of Ancient Medicine and Yoga
Volume 5 Number 1, January - March 2012

Evaluation of Antioxidant Activity of *Amalakayas Rasayana* in Tissue Homogenate of Albino Rats

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Abstract

This study was designed to evaluate the antioxidant activity of classical poly-herbal compound Ayurvedic formulation "Amalakayas Rasayana" (AR) on experimental animals. AR was administered in standard dose orally for seven consecutive days prior to forced swimming- induced hypothermia and stress ulcers. The antioxidant potential was assessed by determining and comparing the changes in adrenal ascorbic acid, catalase, glutathione, total protein and lipid peroxidation in test drug group and vehicle control group with that of stress control group. Pretreatment with AR and vehicle caused highly significant attenuation of lipid per oxidation ($p<0.001$) in comparison to stress control group. Both AR and vehicle increased total glutathione content in stomach tissue homogenate in highly significant manner ($p<0.001$). Moreover, AR attenuated adrenal ascorbic acid (10.47%), catalase (08.11%), and total protein (06.11%), but the values are statistically insignificant. The results suggest that AR possesses significant anti-oxidant activity which could be due to its cytoprotective activity.

Keywords: Amalakayas Rasayana; Antioxidant; Ghee; Honey; Lipid peroxidation.

Introduction

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

Ageing is mentioned in Ayurveda in two ways viz., *Kalaja jara* (timely ageing) and *Akalaja jara* (premature ageing)[3]. Ayurveda describes various rejuvenative therapies with the help of special class of medicinal preparations called Rasayana that are believed to revitalize the body and mind, prevent degeneration and postpone ageing rather than reverse it[4]. Amalakayas Rasayana (AR) is one among many Rasayana formulations mentioned in Charaka Samhita in the treatment of age related disorders[5]. However, reports on screening of antioxidant activity of this formulation are not available; hence, this study was designed to verify its antioxidant activity for providing pharmacological basis and justify its use as a Rasayana drug.

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(Received on 01.08.2011, accepted on 24.10.2011)

Materials and methods

Test drugs

The raw materials (Table 1) of the test formulation were collected from the pharmacy of Gujarat Ayurveda University and subjected to pharmacognostic study to evaluate their authenticity, and found to be authentic[6]. The test drug Amalakayas Rasayana was formulated based on the classical guidelines[7]. The vehicles viz. honey and ghee of standard brands were purchased from local market.

Chemicals

All the chemicals and reagents used in the experimental study were procured from Krishna Pharmaceuticals, Rajkot, and were of analytical grade (EXLR) regularly used in the laboratory.

Animals

Charles Foster strain albino rats of either sex weighing between 200 ± 30g were procured from the animal house attached to the institute. (Reg. 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, temperature at 25 ± 3°C and humidity 40- 60 %). Before the experiment, the animals were fasted for 12 hours. Institutional Animal Ethics Committee had approved the experimental protocol (Approval number; IAEC 05/09-10/Ph.D.08) and the animals were cared as per the CPCSEA guidelines.

Dose schedule

The classical dose of Amalakayas Rasayana in human beings is 3g/day[8]. The dose for experimental animals was calculated by extrapolating the human dose to animals (270mg/kg) based on the standard table of Paget and Barnes (1964)[9]. The drug solution was made by adding unequal quantity of ghee (700mg/kg) and honey (1350mg/kg) as per the classical indication and administered to the animals orally with the help of gastric catheter.

Anti-oxidant activity[10]

The selected animals were divided into four groups of six animals each. The first group of normal control (WC) animals was kept under standard laboratory conditions and left undisturbed in their home cages without stress exposures. The second group of animals received only distilled water and labeled as stress control (SC) group. The third group received combination of ghee (700mg/kg) and honey, (1350mg/kg) and labeled as vehicle control (VC). The fourth group (AR) was administered Amalakayas Rasayana (270mg/kg) along with vehicle and named as drug control group (DC). Drugs were given for seven consecutive days. On the sixth day, the rats were kept in individual metabolic cages to prevent coprophagy and fasted for 16 hours with access to water *ad libitum*. On seventh day one hour after drug administration, rats 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 to expose them to swimming stress inside the container for the 16-hours. At the end of the 16- hour period, blood was obtained from the retro-orbital puncture under light ether anesthesia using capillary tubes. The body weight was noticed 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 stomach was excised, cleaned and opened along the greater curvature. The inner surface was cleaned gently by washing with cold saline solution. The weighed mucosal part of stomach tissue

was taken and homogenized in ice cold normal saline for estimation of total protein[11], catalase[12], and lipid peroxidation[13]. The second fragment was homogenized in 3% metaphosphoric acid solution for estimation of glutathione content[14]. Further, weighed adrenal glands were homogenized with 4 ml of 6% trichloroacetic acid for estimation of adrenal ascorbic acid[15].

Statistical analysis

The results were presented as Mean ± SEM for six rats in each group. The data were calculated statistically by using 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) for both the treated groups and the level of significance was set at $p<0.05$.

Results

Table 1. Composition of Amalakayas Rasayana

	Ingredients	Botanical Name	Part used	Quantity
1	*Amalakai*	*Emblica officinalis* Gaertn.	Fruit	11 Parts
2	*Shweta*	*Alpenia galanga* Willd.	Rhizome	1 Part
3	*Shatavari*	*Asparagus racemosus* Willd.	Root	1 Part
4	*Punarnava*	*Boerhavia diffusa* Linn.	Root	1 Part
5	*Manduka parni*	*Centella asciatica* Linn. Urban.	Whole plant	1 Part
6	*Shalaparni*	*Desmodium gangiticum* Linn. DC.	Root	1 Part
7	*Jivanti*	*Leptadenia reticulate* Retz. Wt. & Arn.	Root	1 Part
8	*Rasna*	*Pluchea lanceolata* Oliver & Hiern.	Root	1 Part
9	*Haritaki*	*Terminalia chebula* Retz.	Fruit	1 Part
10	*Guduchi*	*Tinospora cordifolia* Willd.	Stem	1 Part
11	*Lauha Bhasma*	*Incinerated Iron*	--	1.5 Part

Effect on lipid peroxidation

In forced swimming-induced stress, statistically highly significant increase in lipid peroxidation in stomach homogenate was observed in stress control group in comparison to normal control group ($p<0.001$). Administration of both test drug (AR) and vehicle (ghee + honey) attenuated lipid peroxidation to a highly significant extent ($p<0.001$) (Table 2). Further, the observed effect is almost similar in both vehicle and AR treated groups.

Effect on adrenal ascorbic acid

An apparent decrease in ascorbic acid content (34.84%) in adrenal gland homogenate was observed in stress control group in comparison to normal control. The vehicle (11.02%) and AR (10.47%) treated groups showed moderate but statistically non-significant increase in ascorbic acid content in comparison to stress control group (Table 3).

Effect on catalase

A moderate but statistically non-significant decrease in catalase activity was observed in stress control group (16.34 %) in comparison to normal control. Administration of vehicle (10.64%) and AR (08.11%) lead to moderate and statistically non-significant increase in catalase activity in comparison to stress control group (Table 4).

Effect on total protein

Statistically non-significant moderate increase in total protein content in stomach homogenate was observed in stress control group (10.00%) in comparison to normal control. The vehicle (06.86%) and AR (06.11 %) did not affect the total protein content to a

significant extent in comparison to stress control group (Table 5).

Effect on glutathione

An apparent and statistically non-significant decrease in glutathione content was observed in stress control group (34.37 %) in comparison to normal control. Administration of AR (88.12 %) and vehicle (98.64 %) increased glutathione content to a highly significant extent ($p < 0.001$) in comparison to stress control group (Table 6).

Table 2. Effect of Amalakayas Rasayana on lipid peroxidation in stomach homogenate in rats subjected to forced swimming stress

Group	Dose (g/kg)	Lipid peroxidation (µ moles MDA released /g wet tissue)	% change
WC	QS	10.107 ± 2.848	--
SC	QS	23.792 ± 1.164***	135.40
VC	0.7+1.4	7.750 ± 3.697ΨΨΨ	67.42
AR	0.27+0.7+1.4	9.938 ± 3.223ΨΨΨ	58.23

Mean ± SEM; ↑-Increase; ↓-Decrease; (***p<0.001 in comparison to normal control; ØØØp<0.001 in comparison to stress control)

Table 3. Effect of Amalakayas Rasayana on adrenal ascorbic acid content in rats subjected to forced swimming stress

Group	Dose (g/kg)	Adrenal ascorbic acid (µg/mg wet tissue)	% change
WC	QS	28.27 ± 04.78	--
SC	QS	18.42 ± 04.21	34.84 ↓
VC	0.7+1.4	20.45 ± 03.98	11.02 ↑
AR	0.27+0.7+1.4	18.12 ± 03.63	10.47 ↑

Mean ± SEM; ↑-Increase; ↓-Decrease

Table 4. Effect of Amalakayas Rasayana on catalase activity in stomach homogenate in rats subjected to forced swimming stress

Group	Dose (g/kg)	Catalase (µ moles H_2O_2 consumed/mg wet tissue/min)	% change
WC	QS	13.142 ± 1.044	--
SC	QS	10.995 ± 0.896	16.34 ↓
VC	0.7+1.4	12.165 ± 1.129	10.64 ↑
AR	0.27+0.7+1.4	11.887 ± 0.936	08.11 ↑

Mean ± SEM; ↑-Increase; ↓-Decrease

Table 5. Effect of Amalakayas Rasayana on total protein content stomach homogenate in rats subjected to forced swimming stress

Group	Dose (g/kg)	Total protein (mg/g wet tissue)	% change
WC	QS	11.100 ± 1.242	--
SC	QS	12.212 ± 1.541	10.00 ↓
VC	0.7+1.4	13.050 ± 1.577	06.86 ↑
AR	0.27+0.7+1.4	12.233 ± 0.448	06.11 ↑

Mean ± SEM; ↑-Increase; ↓-Decrease

Table 6. Effect of Amalakayas Rasayana on glutathione content in stomach homogenate in rats subjected to forced swimming stress

Group	Dose (g/kg)	Glutathione (n moles /g wet tissue)	% change
WC	QS	10.722 ± 2.085	--
SC	QS	07.037 ± 1.158	34.37 ↓
VC	0.7+1.4	13.978 ± 2.002ΨΨΨ	98.64 ↑
AR	0.27+0.7+1.4	13.238 ± 2.217ΨΨΨ	88.12 ↑

Mean ± SEM; ↑-Increase; ↓-Decrease; ØØØp<0.001 in comparison to stress control

Discussion

Ageing is a complex biological process with common and definite manifestations characterized by impairment of various functions and decreased ability to respond to stress. Rasayana is a specialized branch of Ayurveda, which mainly deals with the preservation and promotion of health by revitalizing metabolism and enhancing immunity. Rasayana therapy encompasses procedures of revitalization and rejuvenation to increase the body's resistance to disease and is supposed to retard ageing.

Swimming of small laboratory animals has been widely used for studying the physiological changes and the capacity of the organism to adjust the response to stress[16]. 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]. It is a well known fact that forced swimming -induced stress brings about various physiological changes in the body; hence, the effect of AR was tested on different types of parameters in rats subjected to forced swimming stress. Analysis of the biochemical parameters in the stomach homogenate obtained from stress control rats (SC) indicated marked elevation in lipid peroxidation, marked depletion in glutathione, and moderate decrease in catalase, total protein and adrenal ascorbic acid.

These stress-induced changes were found to be reversed by treatment with both the vehicle and AR, especially the marked elevation observed in lipid peroxidation which was found to be markedly reversed. The result suggests that AR may have antioxidant activity and enhance endogenous antioxidant production which may minimize stress - induced free radical production. Moreover, oral administration of vehicle (ghee and honey) also showed nearly similar results. Ghee contains vitamin A, B, E and K and catalase. Vitamin A, E and catalase are antioxidant and are helpful in preventing oxidative injury to the body[18]. Honey contains catalase, flavonoids, selenium, and vitamin E and C which are well known antioxidants to fight against free radicals[19].

Results showed that oral administration of AR increased total glutathione content in gastric mucosa in statistically highly significant manner. Oral administration of ghee and honey also showed nearly same

result. Post swimming -induced stress decreased adrenal ascorbic acid, total protein and catalase content in tissue homogenate. Both AR and vehicle attenuated adrenal ascorbic acid (10.47%), catalase (08.11%), and total protein (06.11%), but the values are statistically insignificant. Catalase, ascorbic acid, superoxide dismutase and glutathione are endogenous antioxidants which prevent free radical damage in human body[20].

Many of the drugs used in AR formulations are reported to have adaptogenic activity, and are rich source of natural antiantioxidant. viz., *Emblica officinalis[21]*, *Asparagus racemosus[22]*, *Boerhavia diffusa[23]*, *Centella asciatica[24]*, *Terminalia chebula[25]* and *Tinospora cordifolia[26,27]*. All ingredients of AR have polyphenols or phenolic compounds which are well known natural antioxidants[28]. A previous phytochemical study on Amalakayas Rasayana demonstrated that AR possesses phenolic compounds and has superoxide radical scavenging activity[29]. Further, the vehicle which is a combination of ghee[30,31] and honey[32,33] is also reported to have antioxidant activity. In another *in-vitro* study on AmalakayasRasayana, it was proved that AR possesses remarkable antioxidant activity[34].

Conclusion

Thus the observed antioxidant 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 has significant antioxidant activity and can be used for treatment of age related disorders and thus justify its clinical claims as anti-ageing rasayana drug.

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