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Evaluation of antioxidant potential of *Rasayana* drugs in healthy human volunteers

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Abstract

Background: It is increasingly being realized that many of today's diseases are due to "oxidative stress" that results from an imbalance between formation and neutralization of free radicals. *Rasayana Chikitsa* is a unique branch of Ayurveda. The word *Rasayana* means the way for attaining excellent *Rasadi Dhatus*. Several medicinal plants have been described as *Rasayanas* in Ayurveda. *Ashwagandha* and *Guduchi* are the best among the *Rasayanas* described by Charaka. **Aim:** To study the efficacy of *Ashwagandha* and *Guduchi* in oxidative stress in healthy volunteers. **Materials and Methods:** The study was carried out on 30 healthy volunteers after obtaining written informed consent. They were randomly distributed in three groups. Each group was treated with three different colored capsules containing *Ashwagandha*, *Guduchi* and placebo in the dose of 1 capsule (500 mg) twice a day for 6 months. The parameters such as hemoglobin%, Erythorcyte Sedimentation Rate (ESR), Malondialdehyde (MDA), Super-Oxide Dismutase (SOD) level, etc., were assessed before and after treatment. The Student's *t*-test was applied to assess significant variations in all of the studied parameters. **Results:** In this study, there was a significant increase in SOD level and decrease in MDA level in *Ashwagandha* and *Guduchi* groups. **Conclusion:** *Ashwagandha* and *Guduchi* may be helpful in preventing the oxidative stress and premature aging.

Key words: *Ashwagandha*, *Guduchi,* malondialdehyde, oxidative stress, *Rasayana*, super-oxide dismutase

Introduction

It is increasingly being realized that many of today's diseases are due to "oxidative stress" that results from an imbalance between formation and neutralization of free radicals. Free radicals are produced in the body as byproducts of normal metabolism, as a result of exposure to radiation and some environmental pollutants. Because they are highly reactive, they can damage cellular components and are implicated in a variety of diseases. Free radicals are normally neutralized by efficient systems in the body that include the antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) and the nutrient-derived antioxidant small molecules (vitamin E, vitamin C, carotenes, flavonoids, glutathione, uric acid, and taurine). In healthy individuals, a delicate balance exists between free radicals and antioxidants.

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In some pathologic conditions such as diabetes and in critically ill patients, oxidative stress causes the level of antioxidants to fall below normal. Antioxidant supplements for such conditions are expected to be of benefit. As a preventive measure against certain diseases, the best approach for healthy individuals is to regularly consume an adequate amount of antioxidant-rich foods or herbs.[1]

Rasayana Chikitsa (rejuvenation) is a unique branch of Ayurveda. The word "*Rasayana*" means the way for attaining excellent *Rasadi Dhatus*. The improved nutritional status and the better qualities of *Dhatus* lead to a series of secondary attributes of *Rasayana*, which bestow longevity, impart strength, *Ojabala*, etc.[2]

Several medicinal plants have been described as *Rasayanas* in Ayurveda. *Ashwagandha* and *Guduchi* are the best of the *Rasayanas* described by Charaka. *Ashwagandha* (*Withania somnifera* (L.) Dunal.), is also known as Indian ginseng, or winter cherry. It has been an important herb in the Ayurvedic and indigenous medical systems for over 3000 years. The roots of the plant are categorized as *Rasayanas* and described to promote health and longevity by augmenting defenses

against disease, arresting the ageing process, revitalizing the body in debilitated conditions and thus creating a sense of well-being.[3] The extract of *W. somnifera* has analgesic, mildly sedative, anti-inflammatory, and anabolic activities.[4] It is useful in stress, strain, fatigue, skin diseases, diabetes, gastrointestinal diseases, rheumatoid arthritis, epilepsy,[5] and even during pregnancy without any side-effects.[6]

Guduchi (*Tinospora cordifolia* (Thunb.) Miers.) has been used in Ayurvedic preparations for the treatment of various ailments throughout the centuries. It is used as a *Rasayana* to improve the immune system and body resistance against infections. The whole plant is used medicinally; however, the stem is approved for use in medicine as listed by the Ayurvedic Pharmacopoeia of India.[7] This is due to higher alkaloid content in the stems than in the leaves.

Objectives

1. To study the concept of *Rasayana* with reference to antioxidation
2. To study and compare the effect of *Ashwagandha* and *Guduchi* in oxidative stress in healthy volunteers
3. To study the changes in the hematocrit levels of both drugs.

Materials and Methods

This was a randomized, double-blind study. The study was approved by the Institutional Ethics Committee (IEC). The content of the study was explained to the volunteers. Written informed consent was obtained from subjects prior to inclusion in the study.

Subjects

Subjects of age group 18-45 years were recruited from the staff and students of Mahatma Gandhi Ayurved College, Hospital and Research Center, Salod. Screening included a medical history, physical examination and blood tests such as hemoglobin % (Hb%), Total Leukocyte Count (TLC), Differential Leukocyte Count (DLC) and Erythrocyte Sedimentation Rate (ESR). Subjects with any medication or any acute or chronic disease were excluded. Thirty-four subjects were screened for this study. Four subjects were excluded on the ground of medical history and medication. A total of 30 subjects were included in the study. Subjects were randomly allocated (by using table of random numbers) into following three groups.

1. Group W,
2. Group R
3. Group B

As it was double blind study, the names to the group were given as per color of the capsules, i.e. White, Red and Blue respectively. Out of 30 volunteers, Group W had 9 volunteers, Group R had 11 volunteers and Group B had 10 volunteers.

Test drugs

Ashwagandha and *Guduchi* were used in the *Ghana* form (dried aqueous extract) of its root. The extracts were prepared in Sushrut Natural Health Care Pvt., Ltd. Ahamadnagar. The raw material was procured from Bhavamishra Vatika of MGACH and RC. After authentication of crude drugs by Department of Dravyaguna, MGACH and RC, it was sent to above pharmaceutical for preparation of *Ghana*.

Preparation of *Ghana*: Crude drug was soaked in eight times of water and made decoction of it. The decoction was reheated until it became semisolid and dried in oven at 55°C. After complete drying, it was collected and made into a fine powder through mixer grinder machine and filled in 500 mg gelatin capsules. The capsules were stored at room temperatures (24-32°C) and protected from light.

Standardization of *Ghana* was done in the analytical laboratory of MGACH and RC, Salod.

Macroscopic, microscopic, physicochemical, and microbiological tests were done.

The subjects were supplemented the capsules in the dose of 1 capsule (500 mg). Two times a day (at 8-9 am and 5-6 pm) orally with water for 6 months continuously. All subjects were free living and consumed their routine diet throughout the study as before. During 6 months of treatment, the volunteers were instructed to report the physician every month.

Follow up and assessment

Following variables were assessed at baseline and at 180th day.

1. Hb% and ESR (mm in 1st h)
2. Mean Corpuscular Volume (MCV) in μm^3
3. Mean Corpuscular Hemoglobin Concentration (MCHC) in %
4. Mean Corpuscular Hemoglobin (MCH) in pg
5. Malondialdehyde (MDA) in nmol/ml
6. Super-Oxide Dismutase (SOD) in U/g Hb.

Fully automatic hematology cell counter was used for Hb%, MCV, MCHC, MCH.

The MDA and SOD were carried out with thiobarbituric acid and Marklund and Marklund method respectively by spectrophotometer.

Statistical analysis

All values were expressed as mean ± SD. The Student's *t*-test was applied to assess significant variations in all of the studied parameters. All statistical computations were performed using Statistical Package for Social Science (SPSS) for windows statistical software version 13 (SPSS Inc., Illinois, USA).

Observations and Results

Three were staff and 27 were the students of second BAMS. Ten were male and 20 were female. All were belonging to middle socio-economic group. The capsules were decoded after completion of 6 months study. Red capsules, Blue capsules, and White capsules contained *Ashwagandha*, *Guduchi* and placebo (i.e., calcium carbonate), respectively.

The results obtained in 9 volunteers of W group and 10 volunteers of B group showed no improvement in Hb level. Group R of 11 volunteers showed increase in Hb level. No significant changes were found in the blood indices, i.e. MCV, MCHC and MCH in Group W and Group B. Group R showed increase in MCV, MCHC and MCH [Table 1].

In Group R and Group B, there were increase in SOD and decrease in MDA. Group W showed no changes in SOD and MDA [Table 2].

Table 1: Total blood indices at 0 day and 180th day in different groups

Parameters	Group	BT	AT	% change	*t*	*P*
Hb (in %)	W	12.43±1.35	12.50±1.37	0.56	0.109	>0.05
	R	13.52±1.43	14.37±0.93	6.29	1.653	>0.05
	B	12.12±1.25	12.57±1.14	3.71	0.841	>0.05
MCV (in μm³)	W	82.5±2.96	82.6±26.07	0.12	0.011	>0.05
	R	82.5±3.88	89.2±3.78	8.12	4.102	<0.05
	B	77.9±10.10	77.7±1.83	0.26	0.062	>0.05
MCHC (in %)	W	31.88±0.63	32.21±3.0	1.03	0.323	>0.05
	R	31.88±0.54	33.3±0.74	4.45	5.141	<0.05
	B	32.11±1.11	32.85±0.71	2.30	1.776	>0.05
MCH (in %)	W	27.01±1.68	27.19±10.3	0.67	0.052	>0.05
	R	27.01±1.77	29.21±2.34	8.15	2.487	<0.05
	B	25.3±3.65	26.05±0.71	2.96	0.638	>0.05

Data: Mean±SD, BT: Before treatment, AT: After treatment, Hb%: Hemoglobin Percentage, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration, MCH: Mean corpuscular hemoglobin

Table 2: MDA and SOD levels at 0 day and 180th day in different groups

Parameters	Group	BT	AT	% change	*t*	*P*
MDA (in %)	W	15.37±1.47	15.6±7.99	1.50	0.085	>0.05
	R	17.72±3.40	14.48±2.46	18.28	2.561	<0.05
	B	18.07±3.56	14.55±2.41	19.48	2.589	<0.05
SOD (in μm³)	W	0.14±0.02	0.14±0.02	-	-	-
	R	0.11±0.09	0.13±0.01	18.18	2.733	<0.05
	B	0.10±0.02	0.13±0.02	30.00	3.354	<0.05

Data: Mean±SD, BT: Before treatment, AT: After treatment, MDA: Malondialdehyde, SOD: Super-oxide dismutase

No any adverse effects were found in the trial period of 6 months.

Discussion

The present study was aimed to assess the efficacy of *Ashwagandha* and *Guduchi* in oxidative stress and hematocrit levels. Group R and B showed significant increase in antioxidant, i.e., SOD. Group R showed increase in Hb and blood indices with antioxidant activity. Group B did not show any effect on Hb and blood indices.

These drugs are known as *Rasayana* drugs. *Rasayana* promotes nutrition by direct enrichment of the nutritional quality of *Rasa* by improving *Agni*, i.e., digestion, metabolism and by promoting the patency of *Srotas* (microcirculatory channels in body). Their antistress actions have made them therapeutically more important.[8] Hence any medicine that improves the quality of *Rasa* should strengthen or promote the health of all tissues of the body. A significant part of Ayurvedic therapeutics is preventive in nature.

A recent report has also confirmed the rejuvenating effect of *Ashwagandha*. In a double-blind study, the growth-promoting effect of *Ashwagandha* was studied for 60 days in 60 healthy children, age 8-12 years. The results indicate that *Ashwagandha* may be used as growth promoter and hematinic in growing children.[9] Clinical investigations with the *Ashwagandha* root extracts indicate that it exerts significant anti-aging effect in normal healthy but aged subjects.[10]

Guduchi is known to be a rich source of trace elements (zinc and copper) which act as antioxidants and protects cells from the damaging effects of oxygen radicals generated during immune activation.[11]

The rejuvenating drugs act on mind, body through three modes-*Rasa*, *Agni* and *Srotas*. The actual pharmacotherapeutics in Ayurveda appears to have been based on the preservation of equilibrium of *Tridoshas* through the principle of *Samanya-Vishesha* principle.

Modern free radical therapy is also based on homeostasis called as Redox state of the cell, i.e. the dynamic balance between the number of antioxidants and the number of free radicals in our body. *Ashwagandha* has been referred as Indian Ginseng and *Guduchi* is mentioned as *Vishaghni*, and *Tridoshashamaka* in various texts of Ayurveda.[3,12,13]

Guduchi and *Ashwagandha* pacify *Tridoshas* and maintain the balance; it might be maintaining oxidation process by maintaining equilibrium state of *Tridoshas*.

Further studies are required in larger sample to confirm the result and to isolate and identify the antioxidant compounds present in these plants.

Conclusion

On the basis of the result obtained in this study, it is concluded that *Ashwagandha* and *Guduchi* may be helpful in preventing the oxidative stress and premature aging. Oxidative stress is

thought to contribute to the development of a wide range of diseases including Alzheimer's disease, Parkinson's disease, diabetes, rheumatoid arthritis, and neurodegeneration in motor neuron diseases. So *Ashwagandha* and *Guduchi* may prove beneficial in combating these problems. *Ashwagandha* and *Guduchi* may be very helpful to maintain healthy body and mind in today's environmental condition and life style. Both drugs appear to be safe for young adults when given for mentioned dosage and duration.

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हिन्दी सारांश

स्वस्थ व्यक्तियों में रसायन औषधियों का एण्टीओक्सिडेन्ट प्रभाव

वैशाली व्ही. कुचेवार, मंगल ए. बोरकर, मिलींद ए. निसर्गंध

रसायन चिकित्सा आयुर्वेद का विशेष अंग है, जो मुख्यतः स्वस्थ व्यक्ति के स्वास्थ्य के रक्षण हेतु है । अनेक रसायन औषधियों का वर्णन आयुर्वेद में है, जिसमे अश्वगंधा और गुडूची प्रमुख है । इन औषधियों का आक्सिडेटिव स्ट्रेस और हिमेटोक्रिट पर प्रभाव ३० स्वस्थ विद्यार्थी एवं कर्मचारीयों पर देखा गया जिनकी आयु १८ से ४५ वर्ष की थी । उनको तीन समूहों में विभाजित किया गया और क्रमशः प्लेसीबो, अश्वगंधा और गुडूची कैप्सूल (५०० मि.ग्रा.) स्वरुप मे ६ माह तक दी गयी । अश्वगंधा का प्रभाव हिमेटोक्रिट और आक्सिडेशन के मानकों पर एवं गुडूची का प्रभाव आक्सिडेशन के मानकों पर प्रभावकारी पाया गया ।