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Research Article

COMPARATIVE ANALYSIS OF TEN MADHURSKANDHA DRUGS DELINEATED IN CHARAKASAMHITA W.S.R TO ADAPTOGENIC AND ANTIOXIDANT ACTIVITIES

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ABSTRACT

Objective: Acharya Charaka had classified Asthapanabastidravya (corrective enema) based on Rasa (taste), called as Rasaskandha (a group of drugs having similar taste). He ascertained some criteria to include drugs in the group such as drug having either similar Rasa (taste) or Vipaka (biotransformation) or Prabhava (principle responsible for a specific action). The Rasayana Karma of Madhuraskandha dravyas which possess either Madhurarasa or Madhuravipaka and Madhuraprabhava requires scientific validation to determine the skandha classification. Keeping this in view, the study was planned to evaluate Rasayana karma of Madhuraskandha drugs for their adaptogenic and *in vivo* antioxidant activities.

Materials and Methods: Ten selected drugs from Madhuraskandha were conveniently formulated into three Groups A, B, and C and used in powder form to evaluate the pharmacological activities. Adaptogenic activity of powders of Madhuraskandha drugs against swimming-induced hypothermia was evaluated. *In vivo* antioxidant activity was assessed by enzymatic assay. The data were analyzed by parametric test, i.e., Student paired and unpaired t-test and ANOVA followed by Dunnett's t-test.

Results: Adaptogenic activity of drugs was assessed on the basis of reduction in hypothermia, body weight, physical activity in terms of cage rotations, sr. corticosterone, and total protein level. *In vivo* antioxidant activity was carried out by measuring catalase, glutathione peroxidase, lipid peroxidation, and superoxide dismutase enzymes assay and tabulated in mean±standard error of mean format.

Conclusions: The ten selected drugs of Madhuraskandha possess weak-to-moderate Rasayana (adaptogenic and *in vivo* antioxidant) activity either through Madhurarasa or Vipaka or Madhuraprabhava. The result obtained in the present study may help to boost the Caraka's Rasaskandha classification. Madhuraskandha drug can be used as a natural source of antioxidants as they are attributed with Rasayana Karma.

Keywords: Madhuraskandha, Adaptogenic activity, Antioxidant activity.

INTRODUCTION

Drugs have been classified in Samhitas based on Karya-karaṇa (cause–effect), Yoni (origin), Rasa (taste), etc. Acharya Charaka had classified Asthapana basti dravyas (corrective enema) based on the Rasa. He stated that the drugs included in each category do possess either same Rasa or Vipaka (transformation) or Prabhava (principle responsible for specific action), for example, the drugs enlisted in the Madhuraskandha have Madhurarasa or Madhuravipaka or Madhuraprabhava (drugs not having either Madhurarasa or Madhuravipaka but show the actions such as Madhurarasa and Vipaka) [1]. Madhurarasa attributed for Balya (strength promoting), Bṛhmaṇiya (increasing muscle bulk of the body), Jivaniya (invigorating), and Rasayana (rejuvenating) karmas while Madhuravipaka has Śukrala (spermatopoeitic) property [2]. Madhuraprabhava attributes the same karmas as that of Madhurarasa and Vipaka. In the present study, exclusively ten Madhuraskandha drugs were selected and assessed for Rasayana Karma to boost Madhuraprabhava phenomenon. Ten drugs were divided into three groups to reduce the complexity of the study. The scientific validation of Rasayana karma on the basis of modern pharmacology can be done by antioxidant and adaptogenic activities. Hence, a combination of Madhuraskandha drugs was evaluated for adaptogenic activity and *in vivo* antioxidant activities in rats to endorse the grouping of Rasaskandha (Madhuraskandha) drugs and Madhuraprabhava phenomenon.

MATERIALS AND METHODS

Collection of plant drugs

All the ten drugs were collected as per the part used according to seasons from May 2014 to November 2014 from the periphery of Jamnagar and Junagadh as per availability. The list of drugs, botanical source, family, and part used are summarized in Table 1.

All the drugs were authenticated and identified by microscopical, physicochemical, and phytochemical studies at the Pharmacognosy and Pharmaceutical Laboratory, Institute for Postgraduate Teaching and Research in Ayurveda (IPGT and RA). Drugs were washed, cleaned, and dried in shade for several days and then powdered coarsely with the help of grinder and stored in airtight containers.

Drug

The shade-dried drugs were pulverized individually with the help of grinder and passed through mesh no. 120 to obtain fine powder [3].

Group A: It contains the powders of Atibala, Vidari, Eranda, Kantakari, and Gokshura in equal quantity.

Group B: It includes Guduchi, Shalaparni, Jivanti, Shatavari, and Punarnava in equal proportion.

Group C: It is a combination of Groups A and B in equal quantity.

The drug was made into fine powder (mesh no. 120) and stored in airtight container. Then, a stock solution of suitable concentration was prepared freshly with distilled water just before administration. The test drugs were administered by oral route with the help of oral feeding cannula.

Instrument and chemicals

Chemicals used for analytical experiment were purchased from reputed firm. Double-beam ultraviolet–visible spectrophotometer was used to measure the absorbance. Digital thermometer and swimming test apparatus were used for adaptogenic activity.

Animals
Charles Foster rats of both the sexes weighing between 200±20 g were used for the experimentation. The animals were obtained from the animal house attached to IPGT and RA., Gujarat Ayurved University, Jamnagar. The experimental protocols were approved by the Institutional Animal Ethics Committee (Ph.D/IAEC/15/2013/32) in accordance with the guidelines formulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals, India. The animals were housed in cage made up of polypropylene with stainless steel top grill. The dry wheat (post hulled) husk was used as bedding material and was changed every morning. The selected animals were kept under acclimatization for 7 days before dosing.

The animals were exposed to 12 h light and 12 h dark cycle with the relative humidity of 50–70% and the ambient temperature during the period of experimentation was 22±03°C. Animals were fed with Amrut brand rat pellet feed supplied by Pranav Agro Mills Pvt. Limited. The drinking water was given *ad libitum*.

Dose fixation
Dose of the drug was calculated by extrapolating the human therapeutic dose to rat on the basis of body surface area ratio (conversion factor 0.018 for rat) by referring to the table of Paget's and Barnes [4].

Human dose of powder [5]: 12 g/day.

Dose for rat = Human adult dose × Body surface area ratio convertible factor

= 12g × 0.018 = 0.216 g/200 g = 1.08 g/Kg body weight of rat

Adaptogenic activity
The effect of test drugs on swimming-induced hypothermia was studied in the Charles Foster albino rats as per the method described by Sheth [6] and Parmar and Jagruti [7] with slight modification.

The albino rats of either sex weighing about 200±20 g were selected randomly and divided into four groups each comprised of six rats. The first group received distilled water (10 ml/kg, p.o) and served as control group. Second, third, and fourth groups received a stock solution of powder of Groups A, B, and C, respectively (1.08 g/kg, p.o.). The drugs or distilled water was administered to respective group for 7 consecutive days and fed with normal diet.

After acclimatization for 7 days, they were subjected to overnight fasting. Next day, the initial rectal temperature has been recorded and single dose of drugs/distilled water was administered to respective groups. 1 h after drug administration, rats were subjected for forced swimming for 15 min in digital swimming test apparatus. After exposure to swimming stress again, rectal temperature was recorded immediately. Rotation counts were also noted for each rat during experimental period. The same procedure was repeated on the 7th day. The swimming time was increased up to 30 min on the 7th day. Adaptogenic activity of drugs was evaluated on the basis of reduction in hypothermia, increase in body weight and physical activity in terms of cage rotations, and changes observed in serum corticosterone and total protein levels.

Antioxidant activity
The same sets of rats were continued with drug administration for 7 more days to assess the *in vivo* antioxidant activity. On the 16th day, the blood was collected under light ether anesthesia from retro-orbital plexus. The serum was separated for the analysis of parameters, namely catalase, glutathione (GSH) peroxidase (GPx), lipid peroxidation (LPO), and superoxide dismutase (SOD).

Catalase in serum was measured according to the procedures of Sinha [8]. 50 µl of serum was taken for estimation. Catalase content in the serum is expressed as µmoles H_2O_2 consumed/mg protein/min. The activity of GPx in serum was assayed by the method of Rotruck *et al.* [9]. Reduced GSH was used as standard and run in similar fashion. GPx activity in serum is expressed as µg of GSH utilized/dl serum/min at 37°C. The activity of tissue LPO is determined by measuring the content of the thiobarbituric acid reactive substances following the procedure of Ohkawa *et al.* [10]. Standard malondialdehyde (MDA) was also processed in a similar fashion. The level of lipid peroxide is expressed as µmoles of MDA formed/dl serum. SOD in the serum was estimated by the method of McCord and Fridovich [11]. SOD in the serum sample is expressed as unit/mg protein.

Statistical analysis
The data were analyzed by parametric test, i.e., Student paired and unpaired t-test and ANOVA followed by Dunnett's t-test as applicable. $p<0.05$ was considered as statistically significant using SigmaStat software 3.5 version.

RESULTS

Adaptogenic activity
The effect of test drugs on rectal temperature of albino rats subjected to forced swimming stress on 1st day showed that Group A (20.93%) and Group C (27.54%) had shown statistically non-significant reduction in the fall of rectal temperature, while Group B treated rats did not show any effect in the rectal temperature as compared to control group on the 1st day of experiment. The effect of test drugs on rectal temperature of albino rats subjected to forced swimming stress on the 7th day revealed that Group A (7.97%) had shown statistically non-significant reduction in fall of rectal temperature when compared to control group on the 7th day. Group B and Group C did not produce any hypothermia effects in rats as compared to control group (Table 2).

Table 1: Details of ten drugs of Madhuraskandha

Name of drug	Botanical source	Family	Part used
Atibala	*Abutilon indicum* Linn. Sweet	*Malvaceae*	Root
Vidari	*Pueraria tuberosa* DC.	*Fabaceae*	Tuber
Kantakari	*Solanum virginianum* Linn.	*Solanaceae*	Whole plant
Eranda	*Ricinus communis* Linn.	*Euphorbiaceae*	Root
Gokshura	*Tribulus terrestris* Linn.	*Zygophyllaceae*	Fruit
Guduchi	*Tinospora cordifolia* (Willd.) Miers ex Hook. f. and Thoms	*Menispermaceae*	Stem
Shalparni	*Desmodium gangeticum* DC.	*Fabaceae*	Root
Jivanti	*Leptadenia reticulata* W. and A.	*Asclepiadaceae*	Leaves
Shatavari	*Asparagus racemosus* Willd.	*Asparagaceae*	Tuberous root
Punarnava	*Boerhavia diffusa* Linn.	*Nyctaginaceae*	Root

Table 2: Effects of test drugs on rectal temperature of albino rats subjected to forced swimming stress on the 1st and 7th day

Group	Dose (g/kg)	Decrease in rectal temperature (°C)	
		1st day	7th day
Control	–	3.27±0.40	2.08±0.29
Group A	1.08	2.58±0.23	1.92±0.38
Group B	1.08	3.33±0.39	2.25±0.37
Group C	1.08	2.37±0.28	2.35±0.48

Data: Mean±standard error of mean; n=6 in each group.

The statistically significant gain in body weight had been observed in Group B and Group C treated group as compared to initial body weight as well as control group on 7th day. In case of Group A, there is a non-significant increase in the body weight as compared to initial body weight and control group on 7th day (Table 3).

The physical activity of the rats has been observed in terms of cage rotations in swimming apparatus. It showed the Group A (24.53%) and Group B (6.01%) treated rats have shown statistically non-significant increase, while Group C treated rats noticed with a non-significant decrease in the physical activity on 1st day as compared to control group. On the 7th day, Group A (75.12%), Group B (88.03%), and Group C (128.20%) all the drugs treated groups showed statistically non-significant increase in physical activity as compared to control group (Table 4).

Drug-treated group showed that serum corticosterone level has shown statistically non-significant decrease in Group A (8.5%) treated rats as compared to control group, while Group B and Group C treated rats were observed with statistically non-significant increase in the serum corticosterone level as compared to control group.

The effect of test drugs on total protein level of albino rats subjected to swimming induced stress revealed that Group A (4.71%) and Group C (10.49%) have shown an apparent increase in total protein level, while Group B has shown an apparent decrease in total protein level when compared to control group (Table 5).

***In vivo* antioxidant activity**

The effect of test drugs on serum catalase level of albino rats when they were subjected to swimming-induced stress showed that Group A and Group C have shown a decrease in catalase level, while Group B (2.31%) has shown statistically non-significant increase in the catalase level when compared to control group. The effects of test drugs on GPx level in swimming-induced stressed albino rats revealed the statistically non-significant increase in GPx in Group A (2.22%), Group B (5.14%), and Group C (12.87%) all the treated groups in comparison to control group.

Group A has revealed a non-significant decrease, while Group B (21.29%) has shown a statistically non-significant increase in SOD level when compared to control group. Group C did not produce any effects in comparison to control group. The effect of test drugs on serum LPO level in stressed albino rats showed the statistically non-significant decrease in LPO has been observed in Group A, while Group B (2.98%) shown non-significant increase and Group C did not produce any effects in comparison to control group (Table 6).

Table 3: Effect of test drugs on body weight of albino rats subjected to forced swimming stress

Groups	Dose (g/kg)	Body weight (g)		
		1st day	7th day	Actual change
Control	–	173.33±7.15	176.67±17.79	3.33±2.47
Group A	1.08	172.50±10.63	179.17±14.17	6.67±3.58
Group B	1.08	173.33±4.22	200.00±7.42**	26.67±4.77@
Group C	1.08	170.00±7.30	196.67±7.92***	26.67±3.58@

Data: Mean±standard error of mean; n=6 in each group; @p<0.001 when compared to control group (ANOVA followed by Dunnett's test); **p<0.01, ***p<0.001 when compared to control group (paired t-test)

Table 4: Effect of test drugs on physical activity of albino rats subjected to forced swimming stress

Group	Physical activity (No of rotations)	
	1st day	7th day
Control	36.00±06.74	19.50±09.21
Group A	44.83±20.35	34.17±14.09
Group B	38.17±8.90	36.67±13.94
Group C	31.50±11.83	44.50±11.62

Data: Mean±standard error of mean; n=6 in each group

Table 5: Effects of test drugs on serum corticosterone level and total protein of rats subjected to forced swimming stress

Group	Serum corticosterone (µg/dl)	Total protein (g/dl)
Control	0.83±0.16	6.58±0.53
Group A	0.76±0.14	6.89±0.38
Group B	1.09±0.09	6.23±0.51
Group C	1.03±0.07	7.27±0.06

Data: Mean±standard error of mean; n=6 in each group

DISCUSSION

The drugs of Madhurarasa or Vipaka are attributed mainly with Balya, Jivaniya, Prinana, Brihmaniya, Saptadhatuvardhana, and Shukrala activities indicating cell protective and promotive activities which fall under antioxidant pharmacological profile. Madhuraskandha drugs possess either Madhurarasa or Madhuravipaka or Madhuraprabhava. Hence, their Rasayana Karma was assessed by adaptogenic and *in vivo* antioxidant activities to endorse this classification.

Among ten drugs of the Madhuraskandha, majority of the drugs are attributed with Rasayana Karma. The three combinations of the ten drugs of Madhuraskandha have been evaluated for adaptogenic activity using swimming-induced hypothermia model. Forced swimming stress involves physical exercise and psychological stress which result in increased serum corticosterone and protein levels [12]. Rats treated with Group A have shown a non-significant decrease in hypothermia and an increase in physical activity on the 1st and 7th day as compared to control group. Serum corticosterone level has an apparent decrease as compared to control group. There was a non-significant increase in body weight as compared to control group. Rats treated with Group B were not able to reverse the swimming stress-induced hypothermia on the 1st and 7th day in comparison to control. Physical activity was increased on 7th day all the three groups and statistically highly significant increase in body weight on the 7th day in Groups B and C as compared to control group. Group C treated rats have shown a statistically non-significant decrease in hypothermia and increase in physical activity as compared to control. Total protein and sr. corticosterone level have shown an apparent increase as compared to control. A statistically highly significant increase in body weight was observed on the 7th day as compared to control group.

Under stressful conditions, corticosterone is secreted by adrenal cortex in rats. Hypersecretion of cortisol helps in the maintenance of internal homeostasis through the process of gluconeogenesis and lipogenesis. Treatment with Group A reduced the hyperactivity of the adrenal cortex by decreasing the secretion of sr. corticosterone and maintained the homeostasis [13]. Stress induces adrenomedullary response in man to release adrenaline which, in turn, stimulates β2 receptors on the pituitary gland. It leads to greater release of adrenocorticotropic hormone that can stimulate the adrenal medulla as well as cortex resulting in further release of adrenaline and increase in weight of adrenal gland to a greater extent. Cortisol increases messenger ribonucleic acid (m-RNA) levels in liver cells since the protein required for repair of wear and tear in swimming stress is more along with higher metabolic changes too that lead to show an increase in weight. Hence, rats treated with Group C had shown an increase in total protein level and body weight which indicates the increase in the protein synthesis [14].

The rats treated with Group A and Group C had shown the decrease in the hypothermia on 1st day, while only Group A was reported to decrease in the hypothermia on the 7th day. Drugs which are able to reduce the stress-induced hypothermia can be considered to have adaptogenic activity.

Table 6: Effect of test drugs on serum catalase, GSH, SOD, and LPO level of albino rats subjected to forced swimming stress

Group	Catalase (µmol/min/mg protein)	GPx (µg GSH/dL)	SOD (unit/mg protein)	LPO (µmol MDA formed/dL)
Control	1.17±0.09	836.42±61.57	2.02±0.25	13.77±3.29
Group A	1.09±0.19	855.03±145.63	1.80±0.45	11.05±2.97
Group B	1.19±0.07	879.39±85.61	2.45±0.20	14.18±3.78
Group C	0.99±0.02	944.05±53.64	2.03±0.27	13.57±1.83

Data: Mean±standard error of mean; n=6 in each group, MDA: Malondialdehyde, SOD: Superoxide dismutase, GPx: GSH peroxidase, LPO: Lipid peroxidation, GSH: Glutathione

Selye (1936) stated about the long- and short-term response to stress as "General adaptation syndrome" which consists of three-phased reactions to nervous tension such as alarm reaction stage, resistance stage, and adaptation stage with respect to handling stress [15].

Alarm reaction stage provides a burst of energy; it releases stress hormones such as adrenaline and cortisol. These hormones become disruptive to effective cellular function for a long period of time. Cortisol acts as immunosuppressor. It also has a negative effect on energy regulation. Prolonged stress leads to the formation of β-lipoprotein molecule which inhibits the passage of energy through cell wall. As a result of this physiological response to stress, cells do not receive enough energy and their ability to perform many of their critical functions is greatly hindered. In the second stage, known as the resistance stage, the body attempts to resist or adapt to the stressor. When adaptation occurs, the individual returns to homeostasis. However, when the capacity of individual is overwhelmed, it goes to the last stage, known as the exhaustion stage, because energy is depleted and entered into the risk illness or injury [16].

The biologically active substances such as flavonoids, carbohydrates, phenolic compounds, and triterpenoids can modify the alarm stage and increase resistance stage. This is done by preventing the formation and accumulation of the harmful β-lipoprotein caused by stress and also allows the hexokinase enzymes to more readily convert glucose into usable energy for the cells. It also increases the capacity of the cells to build up mRNA and transfer ribonucleic acid for protein synthesis.

The drugs of Madhuraskandha do possess flavonoids, carbohydrates, triterpenoids, amino acid, and protein and also shown *in vitro* antioxidant activity [17]. The synergistic effect of these phytoconstituents may contribute to the adaptogenic activity [18]. Group A may act as adaptogenic by reducing the serum corticosterone level in the first alarm phase.

Anxiety can cause hypothermia in humans due to the restricted blood flow to the skin due to the constriction of blood vessels caused by the stress response. Hence, rats treated with Group A may adopt resistance to stress by reversing the fall in the body temperature and help in maintenance of homeostasis [19].

GPx plays a pivotal role in H_2O_2 catabolism and in the detoxification of endogenous metabolic peroxides and hydroperoxides which catalyzes GSH. Decreased activity of GPx may result from radical-induced inactivation [20]. LPO is oxidative degeneration of lipids. Its antioxidant activity prevents to steal the electron from the lipids of the cell membrane and thereby prevents the cell damage [21]. Catalase removes the H_2O_2 (by-product of many metabolic reactions) and prevents the damage to cell and tissue [22]. SOD eradicates superoxide radical (by-product of O_2 metabolism) and prevents cell damage [23].

The assessment of *in vivo* antioxidant assay showed that Groups B and C produced a marked increase in GSH peroxidation. LPO was found to be decreased in rats treated with Group A indicating the effective antioxidant property of the herbal drug in the moderation of tissue damage. An increased level of SOD was observed with Group B, while Group A was found to decrease SOD level. Group A and Group C had reduced the level of serum catalase. SOD is an important defense enzyme which catalyzes the dismutation of superoxide radicals [24]. Catalase is a heme protein which catalyzes the reduction of hydrogen peroxides and protects the tissues from hydroxyl radicals [8]. Therefore, reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide anion and hydrogen peroxide.

All the three formulations were subjected to *in vitro* and *in vivo* antioxidant activity evaluation. Group C has shown potent antioxidant activity by both the methods, i.e., *in vitro* (α, α-diphenyl-β-picrylhydrazyl [DPPH] radical scavenging activity) and *in vivo* (GPx, LPO, and SOD), and hence, Group C can exhibit antioxidant activity by both the mechanisms, i.e., preventive and radical scavenging aspect. Group B can act as a preventive antioxidant by suppressing the free radicals through enzyme formation such as GPx and SOD. Group A has shown significant DPPH radical scavenging activity, and hence, it exhibits antioxidant activity by radical scavenging mechanism.

CONCLUSIONS

The assessment of pharmacological activities suggests that Group A has moderate, while Group B and Group C have weak adaptogenic activity in swimming-induced hypothermia model in rats. Group B has shown moderate activity, while Groups A and C have exhibited weak antioxidant activity (*in vivo*). The ten selected drugs of Madhurskandha, i.e., Atibala, Vidari, Kantkari, Eranda, Gokshura, Guduchi, Shalaparni, Jivanti, Shatavari, and Punarnava possess weak-to-moderate Rasayana (~antioxidant and adaptogenic activities) activity either through Madhurarasa or Vipaka or Madhuraprabhava. The results obtained in the present study may help to boost the Caraka's Rasaskandha classification. Madhuraskandha's drug can be used as a natural source of antioxidants as they are attributed with Rasayana Karma. Further clinical studies should be carried out to confirm Rasayana Karma of Madhuraskandha.

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