

AN EXPERIMENTAL EVALUATION OF *BALYA MAHAKASHAYA* ON MENTAL STRENGTH AND PHYSICAL PERFORMANCE IN HEALTHY ALBINO RATS

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ABSTRACT

Balya Mahakashaya, described in *Charak Samhita*, traditionally indicated for enhancing physical (*Sharirika Bala*). However its potential role in improving mental strength has not been extensively explored. Thus, a study was designed to evaluate the effect of *Balya Mahakashaya* (Test formulation) on mental strength in healthy albino rats using *In vitro* and *In vivo* experimental models. 78 Healthy albino Wistar rats were selected for the study. Test formulation was administered orally for 15 days consecutively and observations were recorded on Day 0, 5th and 15th. Statistical analysis was performed using two-way ANNOVA followed by Dunnett's multiple comparison tests. Motor coordination was assessed using rota rod apparatus, endurance by weight-loaded swim test, and anxiety behavior by light-dark model. The test formulation showed antioxidant activity in DPPH and SOD assays. The

acute toxicity studies revealed no mortality or significant toxic effects indicates the safety of the test formulation. The test formulation showed significant improvement in motor coordination and endurance, with minimal effect on anxiety with statistically significant results ($p < 0.05$). Thus, support its traditional use as a strength-promoting formulation.

KEYWORDS: *Balya Mahakashaya*, Test formulation.

INTRODUCTION

In *Ayurveda*, health is not solely the absence of disease, but a harmonious balance that unites body, mind, and soul. In today's era, poor lifestyle, mental & emotional stress, and multiple factors weaken *Ojas* (Immunity) and *Samdosha* hence have less *prana*. The primary crux of all fatal diseases is mental stress or power. As per WHO, more than 264 million people suffer from depression alone worldwide. Mental illness is a leading cause of disability around the world and contributes greatly to the global burden of disease.^[1] Mental health is prioritised in *Ayurveda*. In terms of mental health, *Vishada* and *Avasada* are two states that are quite comparable to depression. विषादौरोर्ग्वर्धनानां (Agya) says by *Acharya Charaka*^[2] To eradicate the infirmity, this study was done through an approach by *Balya Mahakashaya*. *Acharya Charak* has classified the drugs according to the *Karmas* into 50 *Mahakashaya*^[4] *Balya Mahakashaya* is one of them that is precisely indicated for *bala*. These drugs are *Aindri*, *Rishabhi*, *Atirasa*, *Rishyaprokta*, *Payasya*, *Ashwagandha*, *Sthira*, *Rohini*, *Bala*, *Atibala*^[3] Each drug is carrying a unique quality and action on vitality and support healthy state of mind. *Bala* is not just physical strength, but includes mental resilience, immunity, endurance, and energy (*Ojas*), cognitive clarity etc. It has the ability to strengthen and nourish the body with its characteristics. The study was designed to assess the role of *Balya Mahakashaya* (Test formulation) on motor coordination and endurance, anxiety.

MATERIALS AND METHODS

Authentication and Preparation of Test Formulation Drugs

Ten drugs of *Balya Mahakashaya* have been analysed thoroughly. *Brahmi*, *Atirasa*, *Shalaparni*, *Bala*, *Atibala* were collected and acquired from commercial source. The drugs were authenticated by the Botany Department of Rajasthan University, Jaipur and by the Raw Material, Herbarium and Museum, Delhi (RHMD) of the National Institute of Science Communication and Policy Research (NIScPR) having Reference No. respectively **RUBL211818 - RUBL211820** and **NIScPR/RHMD/Consult/2021/348-49-1-10**. The test formulation was made in *kwatha* form and prepared by following the standards for *kwath kalpana*.

Experimental Study

1. *In-Vitro* model anti-oxidation

- i. Super Oxide Anti-oxidation (SOD) assay
- ii. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay.

2. *In-Vivo* model

1. Acute Toxicity study

2. Efficacy study

a) Light and Dark Model

b) Weight Loaded Forced Swim Test (WLFST)

c) Rota Rod Test.

Ethical Clearance

The experimental study was conducted by getting approval from Institutional Animal Ethical Committee vide Approval No. **NIA/IAEC/2021/25 on 26-08-2021.**

Animals

78 healthy albino wistar rats of both sexes weighing 110-160 gm were selected. Rats were accustomed to the laboratory environment for seven days before the experiment. The temperature was maintained at $\pm 22^{\circ}\text{C}$. Adequate lighting was maintained under 12:12 hr light and dark cycle. The relative humidity was kept at approximately 50% with free access to food and water ad libitum.

Dose Calculation

The dose of Test formulation was derived from Acute Toxicity Study. The dose for the albino wistar rats was calculated by referring the Paget and Barne's table i.e

Animal dose (dose of rat in mg/kg) = Human dose x 0.018 x 5

= 20ml (min. dose of test formulation) x 0.018 x 5 = 1.8 ml/kg body weight

= 40ml (max. dose of test formulation) x 0.018 x 5 = 3.6 ml/kg body weight

Standard drug Diazepam dose: 2mg/kg/dayPO^[4]

Group Design

The experiment was conducted by designing two groups for acute toxicity study and four groups for efficacy study [Table No.1 and 2 Table No.2]

For Acute-Toxicity^[5]

Table No. 1:

Groups	No. of Rats	Intervention
Group 1	3	300 mg/kg test formulation.
Group 2	3	2000 mg/Kg test formulation

Table No. 2:

Groups	No. of Rats	Intervention	Duration
Group 1	6	Distilled water 5ml /kg/day PO.	15 days
Group 2	6	Diazepam(2mg/kg/dayPO)	15 days
Group 3	6	Test formulation 1.8 ml/kg/dayPO.	15 days
Group 4	6	Test formulation 3.6 ml/kg/dayPO	15 days

RESULTS

❖ *In- Vitro* Study

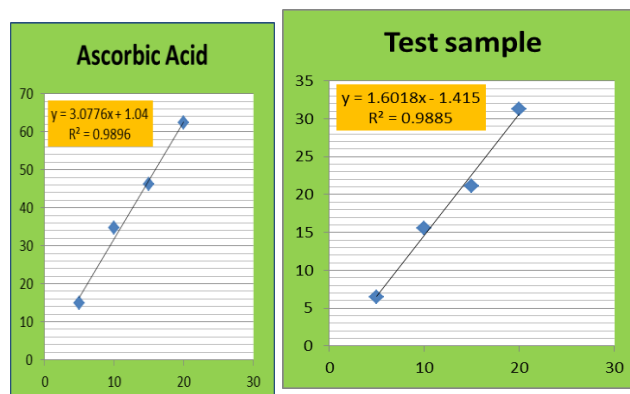
1. DPPH Free Radical Scavenging Assay

DPPH free radical scavenging activity (%) from the absorbance was calculated as inhibition percentage.

DPPH scavenging activity (%) = $\frac{\text{Absorbance of control} - \text{absorbance of sample}}{\text{Absorbance of control}} \times 100$.

Table No. 3: DPPH scavenging activity (%) against different concentrations.

Concentration (µg/ml)	Ascorbic acid% inhibition	Test Sample% inhibition
5	14.82	6.47
10	34.76	15.55
15	46.14	21.09
20	62.32	31.32
EC₅₀ value	253.12	967.00



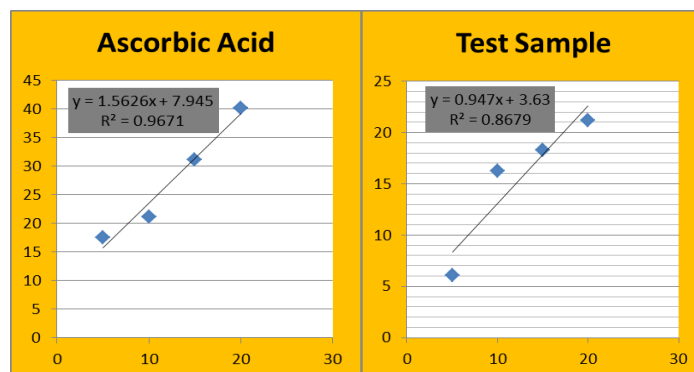
Graph 1: DPPH scavenging activity between Ascorbic acid and test sample.

Test sample showed positive result in DPPH scavenging activity which increases with the increase of concentration.

Determination of Superoxide-Scavenging properties

Table No. 4: Superoxide scavenging activity of test sample.

Concentration (µg/ml)	Ascorbic acid % inhibition	Test Sample % inhibition
5	17.51	6.11
10	21.08	16.24
15	31.11	18.32
20	40.21	21.20
EC₅₀ value	729.68	2397.08



Graph 2: Comparison of Superoxide scavenging activity between Ascorbic acid and test sample.

Test sample showed positive results in Superoxide scavenging activity which increases with the increase of the concentration of the solution.

In-Vivo Study

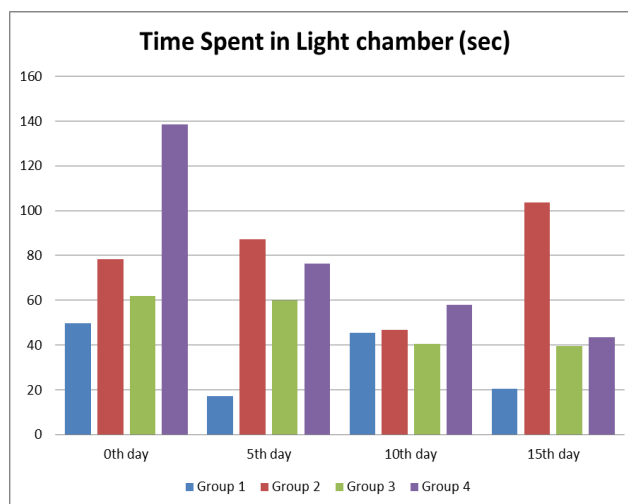
1. Toxicity Study

Behavioural analysis of Group 1 at dose of 300 mg/kg test formulation and Group 2 at dose of 2000mg/kg did not show any toxicological observations. Morbidity and mortality both were not reported in this study.

2. Light and Dark Model for assessment of anti-anxiety effect of Test Formulation^[6]

Table No. 5: Mean values of Time spent in Light Chamber.

Observation Day	Time Spent on Light chamber (sec)			
	Group 1 Mean ± SEM	Group 2 Mean± SEM	Group 3 Mean ±SEM	Group 4 Mean ±SEM
0 th day	49.83±27.70	78.17±25.31	62.00±17.70	138.50±31.66
5 th day	17.17±8.80	87.17±25.62	60.00±20.68	76.33±23.80
10 th day	45.50±27.16	46.83±21.73	40.50±14.21	58.00±14.54
15 th day	20.33±4.00	103.50±34.16	39.67±12.90	43.50±15.15

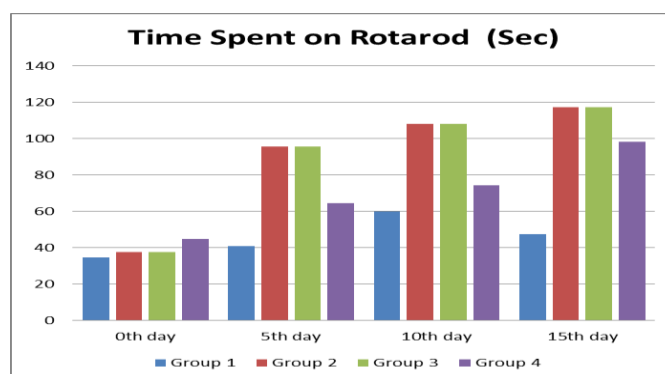


Graph 3

3. Rota rod test for the assessment of motor coordination and motor functions^[7]

Table No. 6: Mean values of the time spent on Rota Rod.

Time Spent on Rotarod (Sec)				
Observation day	Group 1	Group 2	Group 3	Group 4
	Mean ±SEM	Mean± SEM	Mean ±SEM	Mean ±SEM
0 th day	34.54±10.55	37.50±13.46	37.50±13.46	44.70±11.58
5 th day	40.94±7.87	95.76±7.27	95.76±7.27	64.55±10.85
10 th day	59.95±3.02	108.03±11.54	108.03±11.54	74.48±4.65
15 th day	47.58±8.58	117.20±10.95	117.20±10.95	98.15±5.08



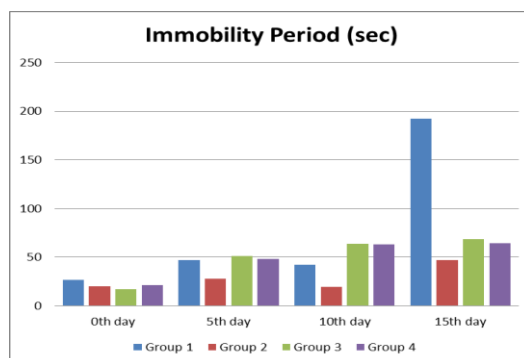
Graph 4

4. Weight Loaded Force Swim Test (WLFST) for the assessment of endurance^[8]

Table No. 7: Mean values of immobility period of four groups.

Immobility Period (sec)				
Observation day	Group 1	Group 2	Group 3	Group 4
	Mean ± SEM	Mean± SEM	Mean ±SEM	Mean ±SEM
0 th day	26.67±4.49	19.83±2.95	17.17±3.12	21.17±4.70
5 th day	46.67±8.14	27.50±6.80	51.33±7.72	48.17±7.29

10th day	42.33±7.28	19.50±2.49	63.83±15.07	63.33±8.09
15th day	192.17±66.92	47.17±23.01	68.50±21.21	64.50±9.32



Graph 5

DISCUSSION

Reactive Oxygen Species delivers a role in signaling the events that control cell proliferation, wound healing, hypertrophy, neuronal signaling, thyroid hormone metabolism, angiogenesis, and vasomotor tone. Superoxide anion radical ($-O_2$) is one of the strongest reactive Oxygen species among free radicals produced during metabolic reactions.^[15] All these radicals exert oxidative stress on the human body cells, leading to several physiological disorders. Both DPPH scavenging activity and Superoxide scavenging activity assays showed significant antioxidant capacity. Though test formulation has the antioxidant capacity, but it is not greater than ascorbic acid. The scavenging ability of test formulation showed an increasing tendency with the increase of concentration. Light dark model is considered as one of the best apparatus to measure anxiety in rodents. It can measure the locomotor action of rodents and positive drive to explore the novel environment. After oral administration of test formulation, at two different doses i.e 1.8 ml/kg & 3.6 ml/kg, standard drug diazepam at 2 mg/kg and Negative control at 5 ml/kg and observed for 15 days which showed that in Group 1 (Negative Control Group) on 0th day and 15th day time spent by rats in light chamber was decreased that indicate anxiety/depression state of rat was increased. In Group 2 (Standard Drug Group) time spent by rats in light chamber was increased in comparison of 0th day and 15th day that showed anxiety/depression state of rat was resolve. In Group 3 (1.8 ml/kg) and Group 4 (3.6 ml/kg), time spent in light chamber was decrease in comparison to 0th day and 15th day, showed anxiety/depression state of rats was increase. Therefore, the Test formulation at both dose have no any significant action on anxiety/depression/mental strength. The rota rod apparatus is used to assess the motor functions of rodents.^[16] It includes the muscle coordination, balance and strength. In this study after comparing

Negative control Group 1 with the Group 2 showed statistically in-significant on 0th day, and significant on 5th day, 10th day ($p=0.0005$, 0.0024) and highly significant on 15th day ($p<0.0001$). The Group 3 showed statistically in-significant on 0th day, significant on 5th day and 10th ($p=0.0005$, 0.0024) day and highly significant on 15th day ($p<0.0001$). The Group 4 showed statistically in-significant on 0th day, 5th day & 10th day and significant on 15th day ($p=0.0013$). Therefore, the Group 3 (1.8ml/kg) showed more significant result as compared to Group 4 (3.6 ml/kg). Weight loaded forced swim test assess the time of exhaustion in rodents. From the present study, it has been observed that after comparing Negative control Group 1 with, the Group 2 showed statistically non-significant on 0th, 5th, 10th day highly significant on 15th day ($p<0.0001$) and Group 3 and Group 4 showed statistically in-significant on 0th, 5th, 10th days and highly significant on 15th day ($p<0.0001$) results (Table No.4.2.13). The test formulation showed significant results from 5th day onwards in Rota rod test and on 15th day in Weight loaded force swim test which signified that *Balya Mahakashaya* drugs were insignificant for *sadhya bala* and significant in *dhatushaya (Rajyakshma)* where *bala* can be gradually increases by these drugs.

CONCLUSION

The test formulation showed a potent anti-oxidant activity, and it has proportional trending with concentration. The test formulation was found to be safe when given at 300 mg/kg body wt. and 2000 mg/kg body wt. in healthy albino wistar rats. The test formulation at both doses was found statistically insignificant in light and dark model. The test formulation at low dose i.e 1.8 ml/kg showed significant results in rota rod test and weight loaded force swim test (WLFST). Hence, these two models, rota rod and weight loaded forced swim test proved to be significant for physical strength & mental strength whereas light and dark model proved as insignificant for mental strength.

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