Research Article

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A PRELIMINARY PHYTOCHEMICAL EXPERIMENTAL STUDY ON GILODYA (*CEROPEGIA BULBOSA* ROXB. VAR. *BULBOSA*) WITH SPECIAL REFERENCE TO ITS BALYA KARMA IN MALNOURISHED RATS

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ABSTRACT

In the present day scenario of nutritional crisis, it is the need of the hour to search for new alternate sources to serve as food. Ayurveda have many potential sources described in name of Balya Dravya, one of which is Gilodya Tuber, Tuber of this plant has been used for Balavardhan and nutritional purpose by villagers of Rajasthan (mainly tribal area). The present study was aimed to evaluate the Balya Karma (Strength and Malnutrition) of Powder of Gilodya (*Ceropegia bulbosa* Roxb. var. *bulbosa*) (Test drug) in Malnutrition Effect, Weight Loaded Forced Swim Test and Rota Rod Test in experimental animals. Test drug was found safe up to 5000 mg/kg in oral acute dose in rats. In malnutrition model, test drug at dose 540 mg/kg was statistically significant on total protein (0.262 g/dl), haemoglobin (1.01 g/dl), and body weight (14.67 gm) and Standard drug (powder of Bala Root-*Sida cordifolia* Linn., 270 mg/kg) was found total protein (0.402 g/dl), haemoglobin (1.83 g/dl), and body weight (18.33 gm) but test sample at dose 540 mg was found 3.94 % having lesser response than standard drug. Weight loaded force swim test and Rota Road Test, test drug 540 mg/kg, after comparison with negative control group (group 1), the test drug was found statistically significant and It was 32.35% lesser action in comparison to standard drug (Bala – 270 mg/kg).

Keywords: Gilodya, Balya karma, Experimental Study, Albino Wistar rats

INTRODUCTION

Ayurveda is the ancient science of life stress upon the maintenance of health through its preventive and curative aspects. In Ayurveda text Sharira is defined as the combination of Doshas, Dhatu and Mala[1].

Charaka has stated that the maintenance of health entirely depends upon the Bala[2]. Acharya Sushruta says that Patient whose Bala has been extremely reduced becomes incapable of being treated[3]. Thus, in a broad sense, Bala can be described as the inherent or acquired strength that aids in the maintenance of health and the sustenance of life[4].

Under nutrition and Malnutrition seems to be a major health issue in the under developed countries. As per FAO, about 805 million people are estimated to be chronically undernourished in 2012-14 down by more than 100 million over the last decade and by 209 million since 1990-92.However, about one in every nine people in the world still has insufficient food for an active and healthy life style[5].

Dietary and medicinal substances have been used for various purposes including nutritional value from time immemorial. Various activities such as immunomodulator, bulk promoting, nutritional value, etc. that enhance strength, immunity, bulk of the body resulted by the use of dietary or medicinal substance are termed in total as Balya in Ayurveda. The term Balya originally stands for all those action that enhance the "Bala". The word "Bala" refers to the strength and ability of the body or part of the body to cope up with various physical stressors[6]. Charaka Samhita explained the assessment of Bala as per Vyayamshakti. He further says Vyayamshakti is assessed by Karmashakti i.e. capacity to do work[7].

Sushruta Samhita described Gilodya as under "Madhura Varga"[8] and Prameha Chikitsa[9]. Acharya Dalhan has been commented on Sushruta Samhita in Prameha Chikitsa[10] about the Gilodya and on the basis of morphological character and habitat of this plant given by Dalhan, Acharya Priyavrat Sharma has told in fifth part of Dravayaguna Vigyana that Gilodya is *Ceropegia bulbosa* Roxb. According to Charaka and Sushruta Samhita, Madhura rasa is responsible for Balya karma[11].

Gilodya (*Ceropegia bulbosa* Roxb. var. *bulbosa*) belonging to family Asclepiadaceae is founded distributed throughout India, and in Rajasthan its local name is "Khadula". Parts of the plant like leaves, tuber, roots and seeds are used in various diseases. The tuber is reported to contains an active alkaloid namely Cerpegin. Roots also contain starch, sugars, gums, albuminoids, Fats and crude fiber.[12]

Overall when we conclude the definition and dimensions of Bala from different classic text book. Prakrita Shleshma, Vyaayamshakti, Oja, nourishment and stability of Mamsa Dhatu are responsible factors for Balya. So, the Balya is multidimensional factor and assessed by multi- factors but due to time constraint and cost effective point of view present study focus on only malnutrition (deficiency of protein) and muscular strength for Vyaayamshakti will be evaluated on animal model.

MATERIAL AND METHODS

Test Sample

Gilodya (Ceropegia bulbosa Roxb. var. *bulbosa)* were collected from Tehsil- Piplda, Dist.-Kota, Rajasthan in the August-September 2017 The plant was taxonomically identified and authenticated by Botany Department, University of Rajasthan,

vide reference number RUBL211710. pharmacognostical and preliminary phytochemical evaluation of test drug.

Experimental animal

Source of animal from CPCSEA approved animal house. Animal house attached to Bilwal Medchem and Research Laboratory Pvt. Ltd. H-9 SKS Reengus Industrial Area, Reengus, Rajasthan. The experimental protocol was submitted to the animal ethics committee of the institute, and approval was obtained for conducting the experiment (Approval No: BMRL/AD/CPCSEA/IAEC/2018/3/III). Test Animals Strain taken was Wistar Albino Rat, 54 in Number, each weighing 140-200 gm. they were kept in polypropylene cages. Based on paget and Barnes (1964) formula involving body surface area ratio, human dosage has been converted in to animal dosage[13] (200 gm of Rats = 0.018X Adult dose)

Study Design

Oral Acute Toxicity Study

Acute oral toxicity study has been conducted according to OECD guideline 423 ANNEX 2c. 6 albino Wistar rats were divided in two groups each group have 3 wistar rats. Group A, 3 Wistar rats has been received test sample at dose 2000 mg/kg/ orally. Group B, 3 Wistar rats has been received test sample at dose 5000 mg/kg/ orally.

Malnutrition Effect in Albino Wistar Rats

Trial period- Pre trial period (Inducing Malnutrition effect -1 month) Malnutrition was induced in 24 wistar rats by oral administration of low protein diet which contains protein 50, gelatin 150, lipid 70, carbohydrate 532.5, sucrose 100, fiber 50, mix mineral 35, mix Vitamin 10 choline 2.5 gm/100 gm of chow for 30 days. Animal has been feed non protein diet at duration of experiment.

Group Design

(Control Group), 6 Rats of group 1 was treated with 1% CMC solution for 1 month per oral twice a day. 6 Rats of group 2 was treated with X (270 mg/kg) dose of Gilodya for 1 month per oral twice a day. 6 Rats of group 3 was treated with 2X (540 mg/kg) dose of Gilodya for 1 month per oral twice a day. 6 Rats of group 4 was treated with Standard Drug (Bala 270 mg/kg) for 1 month per oral twice a day.

Evaluation

Blood was collected form retro orbital plexus for analysis of Total Protein, Total Albumin, Cholesterol, Haemoglobin, Weight before initiation of experiment, and after 30 days of administration of test and standard drugs.

Evaluation Period – Before inducing of malnutrition effect, Before Administration of drug (0 day) and After Administration of drug (30 day)

Weight loaded forced swim test and Rota Rod test

Twenty four healthy adult Albino rats weighing between 140-200 gm has been selected randomly and divided in 4 groups. Each group has 6 animals, Same Group Design Malnutrition Model.

Weight Loaded Forced Swimming Test (WLFST)

The rats of Test drug administered groups and control group is taken for swimming exercise with support of constant loads (attached to the tails) corresponding to 5% of their body weight. The swimming exercise is carried out in small tank with 30 cm deep with water maintained at 25 ± 2°C. Exhaustion is determined by observing loss of coordinated movements and failure to return to the surface within 10s. This experiment is repeated every alternate day for a period over two weeks.

Rota Rod Test

The apparatus consists of a horizontal wooden rod or metal rod coated with rubber with 3 cm diameter attached to a motor with the speed adjusted to 2 rotations per minute. The rod is 75 cm in length and is divided into 6 sections by plastic discs, thereby allowing the simultaneous testing of 6 mice. The rod is in a height of about 50 cm above the table top in order to discourage the animals from jumping off the roller. Cages below the sections serve to restrict the movements of the animals when they fall from the roller. Only those animals which will demonstrate their ability to remain on the revolving rod for at least 1 minute has been used for the test. Time spent in the rota rod by each animal will consider the key for evaluation.

Evaluation

Time of swimming in weight loaded forced swim test.
Time spent on Rod in Rota Rod test.

Route of Administration

All the drugs have been administered orally with the help of feeding needle.

Housing and feeding conditions

The temperature in the experimental animal room had been 22ºC (+ 3ºC). Although the relative humidity had been at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water.

Marking of albino Wistar rat for identification

The albino rat was marked with Picric acid in each group as H, B, T, HB, BT and HT where: H stand for head of albino rat, B stand for Back of albino rat, T stand for Tail of albino rat, HB stand for head back of albino rat, BT stand for Back Tail of albino rat, HT stand for head tail of albino rat.

Statistical analysis

All the values were expressed as mean ± standard error of the mean (S.E.M) of six animals each across the groups. Statistical analysis of data was carried out using one-way analysis of variance (ANOVA) with help of Graph pad Prism software. Dunnett's multiple comparisons test. P value < 0.05 was considered to be statistically significant.

RESULT

Oral Acute Toxicity Study

After oral administration of test sample at dose 5000 mg/kg in single dose and observation up to 14 days was found that test sample found safe no any mortality behaviour and haematological abnormality was found test sample was found safe up to 5000 mg/kg in oral acute dose in rats.

Malnutrition Effect

Table 1: Total Protein

Total Protein(g/dL)	Before inducing Mean ± SEM	0 day Mean ± SEM	30 day Mean ± SEM	Difference Mean ± SEM
Group 1	7.938 ± 0.1026	7.345 ± 0.0698	7.3183 ± 0.0285	0.0266 ± 0.0480
Group 2	7.878 ± 0.0546	7.350 ± 0.0402	7.482 ± 0.0400	0.132 ± 0.0374
Group 3	7.998 ± 0.0648	7.387 ± 0.0533	7.648 ± 0.0594	0.262 ± 0.0215
Group 4	7.805 ± 0.0922	7.342 ± 0.0465	7.743 ± 0.0454	0.402 ± 0.0366

Table 2: Total Protein

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	-0.1583	-0.2916 to -0.02511	Yes	*	0.0180
Group 1 vs. Group 3	-0.2883	-0.4216 to -0.1551	Yes	****	< 0.0001
Group 1 vs. Group 4	-0.4283	-0.5616 to -0.2951	Yes	****	< 0.0001

Table 3: Total Albumin

Total Albumin	Before inducing Mean ± SEM	0 day Mean ± SEM	30 day Mean ± SEM	Difference Mean ± SEM
Group 1	3.253 ± 0.0238	3.162 ± 0.0306	3.185 ± 0.0150	0.023 ± 0.0313
Group 2	3.233 ± 0.0229	3.160 ± 0.0183	3.192 ± 0.0252	0.032 ± 0.0166
Group 3	3.200 ± 0.0363	3.125 ± 0.0384	3.167 ± 0.0373	0.042 ± 0.0095
Group 4	3.222 ± 0.0419	3.123 ± 0.0332	3.192 ± 0.0394	0.068 ± 0.0065

Table 4: Total Albumin

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	-0.008333	-0.07522 to 0.05855	No	Ns	0.9783
Group 1 vs. Group 3	-0.01833	-0.08522 to 0.04855	No	Ns	0.8274
Group 1 vs. Group 4	-0.04500	-0.1119 to 0.02188	No	Ns	0.2372

Table 5: Cholesterol

Cholesterol	Before inducing Mean ± SEM	0 day Mean ± SEM	30 day Mean ± SEM	Difference Mean ± SEM
Group 1	1.32 ± 0.061	1.26 ± 0.027	1.29 ± 0.018	0.03 ± 0.038
Group 2	1.30 ± 0.038	1.26 ± 0.045	1.29 ± 0.023	0.027 ± 0.046
Group 3	1.24 ± 0.021	1.19 ± 0.013	1.28 ± 0.041	0.093 ± 0.043
Group 4	1.24 ± 0.030	1.20 ± 0.010	1.26 ± 0.022	0.055 ± 0.020

Table 6: Cholesterol

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	0.003333	-0.1329 to 0.1396	No	Ns	0.9999
Group 1 vs. Group 3	-0.06333	-0.1996 to 0.07294	No	Ns	0.5134
Group 1 vs. Group 4	-0.025	-0.1613 to 0.1113	No	Ns	0.9369

Table 7: Haemoglobin

Hemoglobin	Before inducing Mean ± SEM	0 day Mean ± SEM	30 day Mean ± SEM	Difference Mean ± SEM
Group 1	12.97 ± 0.3084	11.24 ± 0.1789	11.05 ± 0.170	0.19 ± 0.2411
Group 2	13.02 ± 0.191	11.28 ± 0.2665	11.67 ± 0.079	0.39 ± 0.2775
Group 3	13.63 ± 0.2919	11.14 ± 0.2681	12.15 ± 0.1931	1.01 ± 0.1102
Group 4	13.52 ± 0.187	11.00 ± 0.239	12.83 ± 0.145	1.83 ± 0.221

Table 8: Haemoglobin

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	-0.5833	-1.379 to 0.2121	No	Ns	0.1827
Group 1 vs. Group 3	-1.203	-1.999 to -0.4079	Yes	**	0.0028
Group 1 vs. Group 4	-2.018	-2.814 to -1.223	Yes	****	0.0001

Table 9: Weight

Weight	Before inducing Mean ± SEM	0 day Mean ± SEM	30 day Mean ± SEM	Difference Mean ± SEM
Group 1	170.67 ± 3.676	153.17 ± 2.651	152.50 ± 1.839	0.67 ± 3.084
Group 2	174.83 ± 4.362	157.00 ± 1.983	162.17 ± 1.778	5.17 ± 2.242
Group 3	175.17 ± 3.825	156.33 ± 2.060	171.00 ± 4.107	14.67 ± 3.159
Group 4	164.67 ± 5.432	143.00 ± 4.973	161.33 ± 3.930	18.33 ± 3.648

Table 10: Weight

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	-5.833	-16.88 to 5.216	No	Ns	0.4158
Group 1 vs. Group 3	-15.33	-26.38 to -4.284	Yes	**	0.0058
Group 1 vs. Group 4	-19.00	-30.05 to -7.951	Yes	***	0.0008

Table 11: Weight loaded forced swim test and Rota Rod test

Evaluation	Group 1 Mean ± SEM	Group 2 Mean ± SEM	Group 3 Mean ± SEM	Group 4 Mean ± SEM
Time spent on Rota Rod (sec)	116.50 ± 1.9451	124.50 ± 1.839	131.33 ± 1.801	152.17 ± 3.627
Time of swimming (sec)	55.33 ± 3.7476	66.17 ± 3.5158	76.00 ± 2.3381	84.67 ± 1.7448

Table 12: Time spent on Rota Rod

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	-8	-16.72 to 0.722	No	Ns	0.0764
Group 1 vs. Group 3	-14.83	-23.56 to -6.111	Yes	***	0.0009
Group 1 vs. Group 4	-35.67	-44.39 to -26.94	Yes	****	0.0001

Table 13: Time of swimming

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	-10.83	-21.45 to -0.218	Yes	*	0.0449
Group 1 vs. Group 3	-20.67	-31.28 to -10.05	Yes	***	0.0002
Group 1 vs. Group 4	-29.33	-39.95 to -18.72	Yes	****	0.0001

DISCUSSION

Oral Acute Toxicity Study

After oral administration of test sample at dose 5000 mg/kg in single dose and observation up to 14 days was found that test sample found safe no any mortality behaviour and haematological abnormality was found test sample was found safe up to 5000 mg/kg in oral acute dose in rats.

Malnutrition effect in albino Wistar rats

Malnutrition was induced in 24 wistar rats by oral administration of low protein diet. Rats were divided into four different treatment groups and observed that total protein, albumin, haemoglobin and body weight was found significant after administration of low protein diet for 30 days in total protein albumin, weight, haemoglobin.

After treatment of test sample at two different doses (270 mg/kg and 540 mg/kg), standard drug Bala (270 mg/kg) and vehicle for 30 days, it is found that test sample at dose 540 mg/kg was statistically significant on total protein (0.262 g/dl), haemoglobin (1.01 g/dl), and body weight (14.67 gm) and Standard drug was found total protein (0.402g/dl), haemoglobin (1.83g/dl), and body weight (18.33 gm) but test sample at dose 540 mg was found 3.94 % having lesser response than standard drug (Table 1 to 10).

Weight loaded force swim test and Rota Road Test

After 30 days, orally administration of test sample i.e. standard and vehicle group Physical strength was estimated with the help of Weight loaded force swim test and Rota rod test.

Time spent on Rota rod 116.50, 124.50, 131.33, 152.17 sec. respectively in Group 1, 2, 3, 4 and time of swimming 55.33, 66.17, 76.00, 84.67 sec. In test sample 540 mg/kg, after comparison with negative control group (group 1), the test sample 540 mg/kg was found statistically significant and It was 32.35% lesser action in comparison to standard drug (Table 11 to 13).

CONCLUSION

Based on the findings of the study, the conclusion can be put forth – In present experimental study Gilodya (*Ceropegia bulbosa* Roxb. var. *bulbosa*) was found to be effective against malnutrition. It was found to increase physical strength. Hence, Balya karma of gilodya was established.

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