

Final Report



Health Reactive

192, HPSIDC, Ind.Area,
Baddi, Solan, HP-173205, India
March-June, 2016

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Project No.	03/16/BR-006
Project Status	Completed
Study Period	March-June, 2016
Nature of Study	Analytical Toxicological & Pharmacological
Number of Product	10
Product Name(s)	Body Revival and it's 9 ingredients

Product Nature	Natural Herbal extract Natural formulation
Intended of Use	Rejuvenation Therapeutic
Study Sponsor	Health Reactive 192, HPSID Ind. Area, Baddi, Dist. Solan (H.P.), India
Testing Facility	Department of Pharmacology Institute of Post Graduate Medical Education & Research 244B, Acharya J.C Bose Road Kolkata 700 020, West Bengal, India
Investigator	Dr. Tapas Kumar Sur Senior Researcher Department of Pharmacology Institute of Post Graduate Medical Education & Research 244B, Acharya J.C Bose Road, Kolkata 700020, India e-mail: drdbdrts@gmail.com Cell: +91 8017575428
Sponsor's Representative	Mr. P.K. Rane Senior Manager Health Reactive 192, HPSID Ind. Area, Baddi, Dist. Solan (H.P.), India



Health Reactive R & D

Health Reactive has well defined Research and Development policy. The promoters and tech. staff of the company have not only launched a product but their zeal and mission to eradicate the ailments of the human suffering and to achieve the same devoid of the most dreaded evil monster of modern diseases including side effects. The pursuit of this mission gave birth to a passion to develop such a product which should be a pinnacle in the *Shodhana-RasayanaKayakalp* segment as laid out by Maharishi Charaka in his Ayurvedic text compilation "*Charaka Samhita*". Inventor and Promoter of the company, Natural Born Mr. Munir Khan has Invented '**Body Revival**' which is an Micro-Herbal, 100% plant based formula which dissolves the harmful deposits and flushes out the toxins (*Mala*) through urine and stool. It also maintains and revives the tissue cells in their state of excellent health by clearing all the channels of circulation in the body, thus, bringing the body in the state of equilibrium and forcing the disease to abandon the body in the form of various excretions (*Mala*). Health Reactive blends Ayurvedic expertise with modern technology to authentically the efficacy and safety of such herbs and the ultimate formulations. Not only Body Revival is itself free from side-effects but relieves the ailing body from the side effects of other medicines, therapies, pollutants and toxins. This is the special feature of Body Revival. The R & D department is focused on developing specialized products, quality control and standardization. Body Revival is derived after intensive in house research and produced in the state of the art production facilities. Health Reactive and Body Revival in pursuit of excellence of International Standards is benchmarked at par with worldwide accepted protocols such as, A GMP Certified Ayurvedic Quality Medicine.

Sur TK, Auddy B, Bhattacharyya D. Effects of Body Revival (herbal formulation) on human platelet aggregation and myocardial ischemia in rats. *Journal of Chinese Integrative Medicine* 2011; 9(7): 746-751.

Label Claim of Body Revival

Indian System of medicine, Aurveda is unique in recognizing at first rejuvenation, not only as possible, but assigning it priority over treatment of other ailments, To achieve rejuvenation, various body purification processes (*Shodhana*) were adopted by the ancient hermits / ascetics which we term as 'detoxification' in modern science. After detoxification, to further revive the ageing body, *Rasayana* (rejuvenation) therapy was initiated. The precise selection of *divya* herbs and where then action of each plants was determined by the doctrine of *Rasa*, *Virya*, *Vipaca*, *Guna* and *Karma* of the plant. *Rasayanas* were thus, specific remedies for

rejuvenation (body revival) especially for the aged and were so powerful that they could also treat incurable diseases. With this therapy, all tissues, glands and organs, however inactive, started to function in harmony. *Rasayana* is the Ayurvedic science of rejuvenation and longevity. Body-Revival is one such “*Rasayana*” developed by Health Reactive, is a unique micro herbal formulation which dissolves completely the harmful deposits and flushes out the toxins (*Mala*) through urine and stool.

With the consumption of antibiotics, drugs or alcohol or due to a poor diet or fast food lifestyle, the body becomes unhealthy. Further, the health is affected by environmental pollution and intake of contaminated water. Body revival is needed to detoxify the toxins and pollutants entered in our body and to regenerate new cells.

Composition

Each 5ml contains:

Aegle marmelos, Acorus calamus, Withania somnifera, Blumea lacera, Rumex vesicarius, Rubia cardifolia, Cucumis melo, Symplocos racemosa, Honey.

Indication

As an adjuvant, recuperative and rejuvenator in various modern, faulty, stress induced wear and tear syndrome of middle age, hypertension, drug induced toxicity, wasting diseases and premature ageing.

Mode of Administration

Take the required oral dosage at night before retiring to bed.

Diet Restriction

Very sour edibles like lime, lime juice, vinegar, raw mango, pickle etc., to be avoided during medication. Milk, curd and milk products to be completely avoided since this may hamper with proper assimilation of the drug in the body and lower its efficacy.

Side Effects

Safe time tested, Ayurvedic product with no side effect. Dosage recommended on alternate days to accommodate the patient's slow healing response to the product's high potency and fast acting effect.

Self-Life

Shelf life remains the same as per expiry period and stability remains unaffected. Just replace the cap tight after use.

Contra Indication

None. Just follow the diet restrictions.

Precaution

Do not touch the medicine with stale/ bare hands/ object to avoid any contamination of the product.

Storage

Store in a cool place, away from moist area

Warning

To be taken under medical supervision only.

Packing

100 ml plastic bottle with seal cap.

Short Description of Product & Ingredients

Ingredient	Action on Human Body	Proved by
<i>Aegle marmelos</i>	Antioxidant, hepatoprotective, controlling early tumor events.	J. Enzyme. Inhib, Med. Chem. 2008, Oct1:1.
	Anti-hyperglycemic, anti-dyslipidemic.	Bioorg. Med. Chem. Lett. 2007.
	Anti-thyroid, antioxidative, anti-hyperglycemic	Phytother Res. 2006, Dec 20.
	Hypoglycemic	J. Ethnopharmacol. 2003, Aug 7.
<i>Acorus calamus</i>	Anti-Stress	Biol. Pharma. Bull. 2005, Dec 28.
	Hypolipidemic activity	Elsevier Science. B:V. 2002.
	Middle cerebral artery occulusionindused ischemia.	Hum. Exp. Toxicol. 2006, April 25.

	Anti-carcinogenic, activation of xasarone on human cancer cells.	Xi Yi Jie He Za Zhi. 1986, Aug 6.
	Anticonvulsant, anti-arrhythmic	Int. Pharmacodyn. Ther, 1960, eb1.
<i>Blumea lacera</i>	Physiological disorders such as rheumatism and hypertension.	Dept. Food & Human Health Sciences, Graduate School of Human Life Science, Oaska City University.
	Leukemia, provides cell resistance	Blood, 2006, January 15.
<i>Rumex vesicarius</i>	Analgesic, antipyretic, astringent, diuretic, laxative, stomachic.	Handbook of Medicinal herbs, by James A. Duke.
<i>Rubia cardifolia</i>	Hypoglycemic effects, memory impairment.	Maharashtra Institute of Pharmacy, Kothrud, Pune
<i>Cucumis melo</i>	Hypothyroidism.	Chem Biol Interact. 2009, Feb 12.
<i>Symplocos racemosa</i>	All Female Disorders.	Phenolic Glycosides from Symplocos, Racemosa, Natural Inhibitors of phosphodiesterase I.
Honey	Antibacterial, antioxidant, anti-inflammatory, antiviral, antitumor, antimicrobial, immune modulation.	Universidad Miguel Hernandez, Ctra, Orihuela Alihuela, Spain.

Body Revival

Product Name	Body Revival
Physical State	Liquid Suspension
Manufacturer	HEALTH REACTIVE 192, HPSID, Industrial Area Baddi, Distt. Solan (H.P.) Pin-173 205 (India) GMP Certified
Mfg. Lic. No.	HP-177-AY
Batch No.	HRB0026/Export
Mfg. No.	12/2015
Exp. Date	11/2018

Color	Dark Brown
Physical State	Liquid Suspension
Dosage	Oral
Net Volume	100 ml
Product Claim	Helps to increase energy, endurance and mental focus
Indications	Body Revival has herbal active ingredients which works as <i>Balya, Rasayana and Medhya</i> and useful in general debilities and life style related disorders etc.

®

Scientific Evidence: Body Revival

Sur TK, Auddy B, Bhattacharyya D. Effects of Body Revival (herbal formulation) on human platelet aggregation and myocardial ischemia in rats. *Journal of Chinese Integrative Medicine* 2011; 9(7): 746-751.

Disclaimer:

The above physicochemical data of test substance was supplied by the Sponsor. All responsibilities with regards to the accuracy and authenticity of these remains with the Sponsor. The test lab is not responsible for any variations with the batch number supplied.

Composition: Each 5 ml contains extract of -			
<i>Aegle marmelos</i>	150mg	<i>Rubia cordifolia</i>	200mg
<i>Acorus calamus</i>	175mg	<i>Cucumis melo</i>	200mg
<i>Withania somnifera</i>	325mg	<i>Symplocos racemosa</i>	95mg
<i>Blumea lacera</i>	115mg	Honey	Q.S.
<i>Rumex vesicarius</i>	240mg	Permitted Preservative (Sodium benzoate)	Q.S.



Testing Materials

Code Name	Form	De-code Name
001	Dry powder extract	<i>Aegle mermelos</i>
002	Dry powder extract	<i>Acorus calamus</i>
003	Dry powder extract	<i>Rumex vesicarius</i>
004	Dry powder extract	<i>Blumea lacera</i>
005	Dry powder extract	<i>Cucumis melo</i>
006	Dry powder extract	<i>Symplocos racemosa</i>
007	Dry powder extract	<i>Withania somnifera</i>
008	Dry powder extract	<i>Rubia cordifolia</i>
009	Liquid	Honey

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Purpose of Study

Objectives

- ✓ **Analytical Studies.** The purpose of the analytical studies was to find out the nutritive values of herbal and natural ingredients presence in Body Revival.
- ✓ **Pharmacological Studies.** The purpose of the pharmacological studies was to find out the safety parameters and efficacies (claims like, Balya, Rasayana and Medhya) of Body Revival in animals.

Analytical Studies

Assay of Nutritive Values

The following parameters were assessed chemically by standard Indian Pharmacopeia's guided method:

1. Ash Value (%).
2. Carbohydrate (%).
3. Protein (%).
4. Fat (%).
5. Fibre (%).
6. Energy (Kcal/100 g).
7. Sodium (mg/100 g).
8. Potassium (mg/100 g).
9. Calcium (mg/100 g).
10. Specific Gravity (Final Product only).
11. HPLC (Final Product only).
12. HPTLC (Final Product only).

Methods

Estimation of Ash. Five gm of each sample was weighed in a silica crucible and heated in muffle furnace for about 5-6 h at 500°C. It was cooled in a desiccator and weighed. It was heated again in the furnace for half an hour, cooled and weighed. This was repeated consequently till the weight became constant.

Estimation of Moisture. Two gm of each sample was taken in a flat-bottom dish and kept overnight in an air oven at 100-110°C and weighed. The loss in weight was regarded as a measure of moisture content.

Estimation of Crude Fat. Two gm moisture free of each sample was extracted with petroleum ether (60-80°C) in a Soxhlet apparatus for about 6-8h. After boiling with petrol, the residual petrol was filtered using Whatman No. 40 filter paper and the filtrate was evaporated in a pre-weighed beaker. Increase in weight of beaker gave crude fat.

Estimation of Crude Fibre. Two gm of moisture and fat-free material of each sample was treated with 200 ml of 1.25% H₂SO₄. After filtration and washing, the residue was treated with 1.25% NaOH. It was filtered, washed with hot water and then 1% HNO₃ and again with hot water. The washed residue was dried in an oven at 130°C to constant weight and cooled in a dessicator. The residue was scraped into a preweighed porcelain crucible, weighed, ashed at 550°C for two hours, cooled in a dessicator and reweighed. Crude fibre content was expressed as percentage loss in weight on ignition.

Estimation of Crude Protein. The crude protein was determined using micro Kjeldahl method. Two gm of each sample compound was decomposed by digestion with concentrated sulphuric acid in the presence of a catalyst, ammonium sulphate is produced. An excess of sodium hydroxide solution was added to the diluted reaction mixture, the liberated ammonia was distilled in steam and absorbed in a measured excess of standard sulphuric acid. Titration of the residual mineral acid with standard sodium hydroxide gives the equivalent of ammonia obtained from the weight of the sample taken. From this the percentage of nitrogen in the compound can be calculated. On the basis of early determinations, the average nitrogen (N) content of proteins was found to be about 16 percent, which led to use of the calculation $N \times 6.25$ ($1/0.16 = 6.25$) to convert nitrogen content into protein content.

Estimation of Carbohydrate. Percentage of available carbohydrate was given by:

$100 - (\text{ash}\% + \text{fat}\% + \text{protein}\% + \text{crude fibre}\%).$

Estimation of Nutritive Value (Energy). The three components of foods which provide energy are protein, carbohydrate and fat. One gram carbohydrate and protein yield 4 kcal energy whereas one gram fat yield 9 kcal energy. The energy content of each plant samples were determined by multiplying the values obtained for protein, fat and available carbohydrate by 4.00, 9.00 and 4.00 respectively and adding up the values.

Estimation of Minerals. Sample was taken in a silica crucible and heated in a muffle furnace at 400°C till there was no evolution of smoke. The crucible was cooled at room temperature in a desiccator and carbon-free ash was moistened with concentrated sulphuric acid and heated on a heating mantle till fumes of sulphuric acid ceased to evolve. The crucible with sulphated ash was then heated in a muffle furnace at 600°C till the weight of the content was constant (~2-3 h). One gram of sulphated ash obtained above was dissolved in 100 ml of 5% HCl to obtain the solution ready for determination of mineral elements (sodium, potassium and calcium) through atomic absorption spectroscopy (AA 800, Perkin- Elmer Germany).

HPLC Chromatographic Analysis of Phenolic Compounds. HPLC analyses were performed with Dionex Ultimate 3000 liquid chromatograph (Germany) with four solvent delivery system quaternary pump (LPG 3400 SD) including a diode array detector (DAD 3000) with 5 cm flow cell, a manual sample injection valve equipped with a 20 µl loop and Chromeleon 6.8 system manager as data processor. The separation was achieved by a reversed-phase AcclaimTM120 C₁₈ column (5 µm particle size, i.d. 4.6 x 250 mm). The mobile phase contains 1% aqueous acetic acid solution (Solvent A) and acetonitrile (Solvent B), the flow rate was adjusted to 0.7 ml/min, the column was thermostatically controlled at 28°C and the injection volume was kept at 20 µl. A gradient elution was performed by varying the proportion of solvent B to solvent A. The gradient elution was changed from 10% to 40% B in a linear fashion for duration of 28 min, from 40 to 60% B in 39 min, from 60 to 90 % B in 50 min. The mobile phase composition back to initial condition (solvent B : solvent A : 10 : 90) in 55 min and allowed to run for another 10

min, before the injection of another sample. Total analysis time per sample was 115 min. HPLC Chromatograms were detected using a photo diode array UV detector at 280 nm according to absorption maxima of analysed compounds. Each compound was identified by its retention time and by spiking with standards under the same conditions. The quantification of the sample was done by the measurement of the integrated peak area and the content was calculated using the calibration curve by plotting peak area against concentration of the respective standard sample. The data were reported with convergence limit in six times. According to the USP and ICH guidelines, there are various parameters to validate the reproducibility of the method viz. the effectiveness, the limit of detection (LOD), the limit of quantitation (LOQ), the linearity, the precision and the accuracy.

HPTLC Chromatographic analysis

Densitometric HPTLC fingerprint of Body Revival was carried out. Body Revival was diluted into 1 mg/ml in methanol and spotted (10 µl) in the form of bands with on a pre-coated silica gel plates (Merck, 60 F 254, 20x20 cm) by automated Camag Linomat 5. The plates were developed in a mobile solvent system (toluene : ethyl acetate : formic acid = 4.5:3:0.2) for 30 min and scanned at 254 nm using Camag TLC Scanner 3.

Results

Nutritive Values

Product No.	001
Sample Name	<i>Aegle mermelos</i>
Form	Dry Powder Extract
Study	April-May, 2016

	Mean \pm SD	Range
Ash (%)	7.10 \pm 0.01	7-7.3
Carbohydrate (%)	74.66 \pm 0.21	72.14-76.25
Protein (%)	2.40 \pm 0.15	2.1-2.8
Fat (%)	0.49 \pm 0.01	0.45-0.52
Fibre (%)	15.25 \pm 0.04	15-16.1
Energy (Kcal/100 g)	314.30 \pm 1.01	310-318.5
Sodium (mg/100 g)	3.64 \pm 0.01	3.51-3.83
Potassium (mg/100 g)	24.96 \pm 0.05	24-25.52
Calcium (mg/100 g)	43.54 \pm 0.48	42.1-44.5

N=6 in each test

Product No.	002
Sample Name	<i>Acorus calamus</i>
Form	Dry Powder Extract
Study	April-May, 2016

	Mean \pm SD	Range
Ash (%)	12.43 \pm 0.20	12.1-12.84

Results

Nutritive Values

Carbohydrate (%)	73.71±0.06	72.6-75.62
Protein (%)	4.21±0.04	4-4.54
Fat (%)	4.07±0.05	3.8-4.28
Fibre (%)	5.61±0.02	5.4-5.78
Energy (Kcal/100 g)	348.03±0.27	344.6-350.9
Sodium (mg/100 g)	6.17±0.04	6-6.38
Potassium (mg/100 g)	31.56±0.30	31.1-32.6
Calcium (mg/100 g)	35.34±0.14	35-36.8

N=6 in each test

Product No.	003
Sample Name	<i>Rumex vesicarius</i>
Form	Dry Powder Extract
Study	April-May, 2016

	Mean ± SD	Range
Ash (%)	11.80±1.01	10.9-12.2
Carbohydrate (%)	76.64±0.13	75.3-77.6
Protein (%)	4.55±0.03	4.4-4.6
Fat (%)	4.32±0.04	4.2-4.4
Fibre (%)	2.91±0.02	2.8-3.1
Energy (Kcal/100 g)	363.01±0.45	360.4-36.7

Results

Nutritive Values

Sodium (mg/100 g)	5.01±0.07	4.9-5.2
Potassium (mg/100 g)	30.52±0.08	29.6-31.2
Calcium (mg/100 g)	33.74±0.06	32.8-34.5

N=6 in each test

Product No.	004
Sample Name	<i>Blumea lacera</i>
Form	Dry Powder Extract
Study	April-May, 2016

	Mean ± SD	Range
Ash (%)	25.66±0.25	25.1-26.4
Carbohydrate (%)	60.21±0.40	59.3-61.8
Protein (%)	7.06±0.05	6.9-7.2
Fat (%)	0.74±0.03	0.7-0.78
Fibre (%)	7.20±0.26	7-7.5
Energy (Kcal/100 g)	274.45±0.43	270-280
Sodium (mg/100 g)	3.45±0.02	3.3-3.5
Potassium (mg/100 g)	6.16±0.04	6-6.2
Calcium (mg/100 g)	41.67±0.18	40-42

N=6 in each test

Product No.	005
Sample Name	<i>Cucumis melo</i>
Form	Dry Powder Extract

Results

Nutritive Values

Study	April-May, 2016	
	Mean ± SD	Range
Ash (%)	14.50±0.21	14-15
Carbohydrate (%)	8.40±0.02	8.2-8.5
Protein (%)	23.86±0.10	23-24
Fat (%)	47.51±0.12	47-48
Fibre (%)	6.14±0.03	6-6.4
Energy (Kcal/100 g)	555.40±1.26	550-560
Sodium (mg/100 g)	2.93±0.04	2.9-2.98
Potassium (mg/100 g)	11.56±0.05	11-11.8
Calcium (mg/100 g)	14.37±0.06	14-14.6

N=6 in each test

Product No.	006
Sample Name	<i>Symplocos racemosa</i>
Form	Dry Powder Extract
Study	April-May, 2016

	Mean ± SD	Range
Ash (%)	16.56±0.20	16.4-16.6
Carbohydrate (%)	73.16±0.09	72-73.5
Protein (%)	1.88±0.03	1.8-1.9
Fat (%)	0.40±0.10	0.38-0.42

Results

Nutritive Values

Fibre (%)	8.35±0.05	8-8.8
Energy (Kcal/100 g)	302.85±0.32	300-318.3
Sodium (mg/100 g)	3.37±0.03	3.31-3.48
Potassium (mg/100 g)	13.91±0.04	13.6-14.1
Calcium (mg/100 g)	17.76±0.15	17.3-18.4

N=6 in each test

Product No.	007
Sample Name	<i>Withania somnifera</i>
Form	Dry Powder Extract
Study	April-May, 2016

	Mean ± SD	Range
Ash (%)	10.46±0.25	10.2-10.8
Carbohydrate (%)	80.93±0.04	80.4-81.2
Protein (%)	1.90±0.10	1.8-1.9
Fat (%)	0.86±0.02	0.83-0.9
Fibre (%)	6.15±0.03	6-6.4
Energy (Kcal/100 g)	338.84±0.65	335.4-342.1
Sodium (mg/100 g)	4.72±0.06	4.68-4.75
Potassium (mg/100 g)	27.58±0.03	27.3-28.2
Calcium (mg/100 g)	43.73±0.11	43.4-44.2

N=6 in each test

Results

Nutritive Values

Product No.	008
Sample Name	<i>Rubia cordifolia</i>
Form	Dry Powder Extract
Study	April-May, 2016

	Mean \pm SD	Range
Ash (%)	13.63 \pm 0.15	13.2-13.8
Carbohydrate (%)	81.43 \pm 0.06	80.8-81.9
Protein (%)	1.10 \pm 0.01	1-1.3
Fat (%)	0.44 \pm 0.02	0.4-0.48
Fibre (%)	3.66 \pm 0.03	3.2-3.8
Energy (Kcal/100 g)	335.21 \pm 0.70	331-329
Sodium (mg/100 g)	3.34 \pm 0.04	3.3-3.5
Potassium (mg/100 g)	36.88 \pm 0.03	36.6-36.9
Calcium (mg/100 g)	36.43 \pm 0.35	36.1-36.9

N=6 in each test

Product No.	009
Sample Name	Honey
Form	Semi Liquid
Study	April-May, 2016

	Mean \pm SD	Range
Ash (%)	0.32 \pm 0.01	0.3-0.35

Results

Nutritive Values

Carbohydrate (%)	88.08±0.71	87.6-89.1
Protein (%)	0.13±0.01	0.1-0.15
Fat (%)	0.0006±0.00001	0.0005-0.0007
Fibre (%)	10.66±0.08	10.2-10.9
Energy (Kcal/100 g)	356.61±0.54	353.8-359.6
Sodium (mg/100 g)	2.96±0.02	2.9-2.99
Potassium (mg/100 g)	0.95±0.03	0.92-0.96
Calcium (mg/100 g)	3.54±0.02	3.5-3.7

N=6 in each test

Product No.	010
Sample Name	Body Revival
Form	Liquid / Suspension
Study	April-May, 2016

	Mean ± SD	Range
Ash (%)	8.35±0.02	8.2-8.5
Carbohydrate (%)	82.26±0.08	81.6-82.8
Protein (%)	0.18±0.01	0.16-0.19
Fat (%)	0.0004±0.00006	0.0003-0.0005
Fibre (%)	9.16±0.01	8.8-9.6
Energy (Kcal/100 g)	330.79±0.65	328-333

Results

Nutritive Values

Sodium (mg/100 g)	4.18±0.005	4-4.3
Potassium (mg/100 g)	1.15±0.01	1-1.18
Calcium (mg/100 g)	3.76±0.03	3.7-3.8

N=6 in each test

Results

Special Tests

Product No.	010
Sample Name	Body Revival
Form	Liquid / Suspension
Study	April-May, 2016
Specific Gravity	1.31±0.002

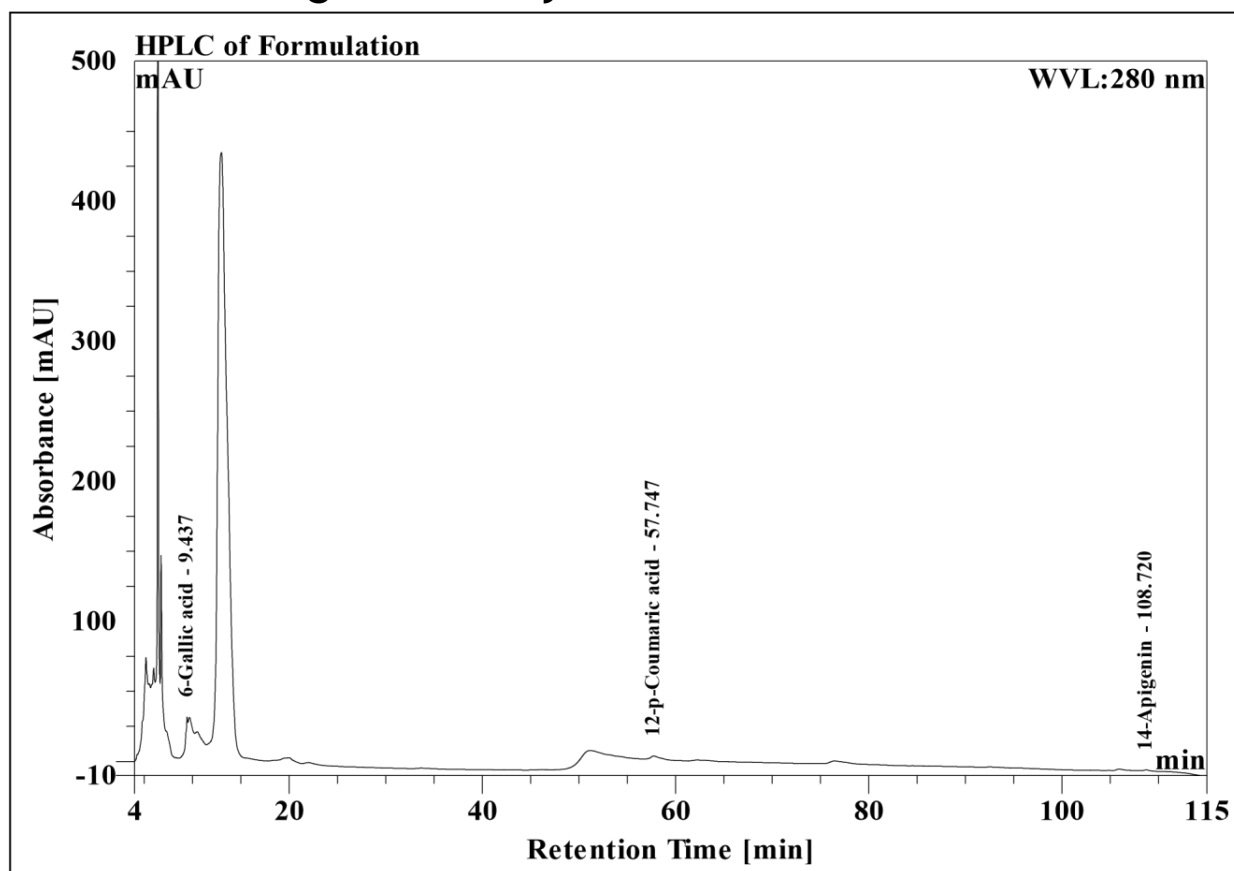
	Mean ± SD	Range
HPLC Analysis		
Gallic acid (µg/g)	18.40±0.015	18.3-18.4
p-Coumaric acid (µg/g)	2.41±0.02	2.4-2.44
Apigenin (µg/g)	0.29±0.0004	0.28-0.30

N=6 in each test

	Peaks (Rf)	Area %
HPTLC Analysis	Mean ± SD	Mean ± SD
Peak 1	0.06±0.001	1.18±0.002
Peak 2	0.11±0.004	5.72±0.006
Peak 3	0.2±0.003	1.04±0.001
Peak 4	0.26±0.006	2.59±0.003
Peak 5	0.54±0.012	84.19±0.015
Peak 6	0.71±0.002	5.27±0.004

N=6 in each test

HPLC Chromatogram of Body Revival



Sample Name:	HPLC of Body Revival	Injection Volume:	20.0
Vial Number:	94	Channel:	UV_VIS_1
Sample Type:	unknown	Wavelength:	280.0
Control Program:	FLAVONOID	Bandwidth:	4
Quantif. Method:	FLAVONOID	Dilution Factor:	1.0000
Recording Time:	5/23/2016 11:01	Sample Weight:	1.0000
Run Time (min):	116.00	Sample Amount:	1.0000

HPLC Peaks Body Revival: Quantitative Analysis

No.	Ret.Time	Peakname	Height	Width	Type	Resol.	Asym.	Plates
	min	min	mAU	min		(EP)	(EP)	(EP)

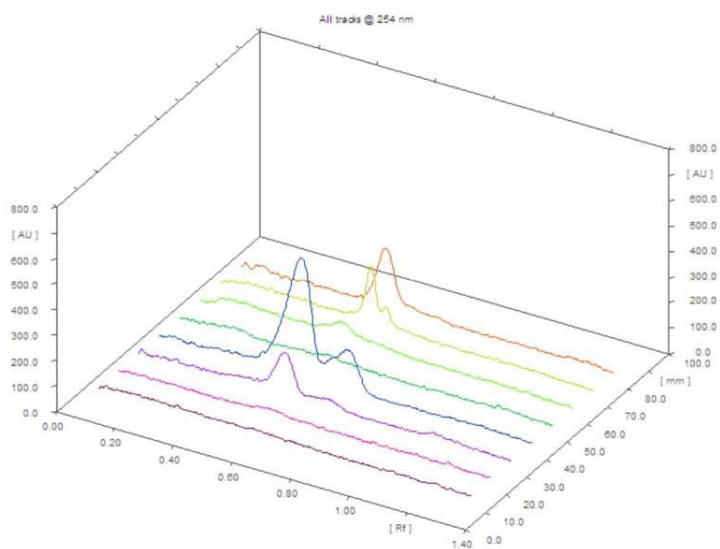
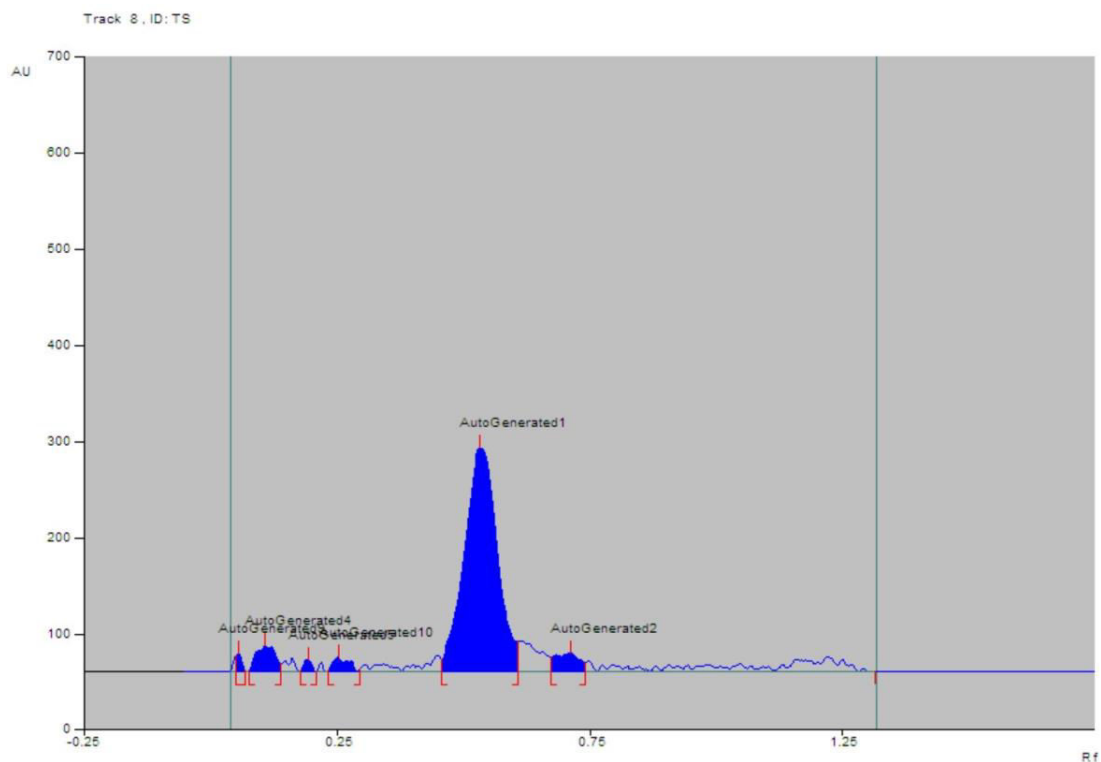
n.a.	n.a.	Naringenin	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	Vanilic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	Salicylic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	Gentisic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1	5.183	n.a.	35.178	0.352	BMB	n.a.	1.31	1697
2	5.500	n.a.	1.373	n.a.	Rd	n.a.	n.a.	n.a.
3	5.970	n.a.	20.352	0.368	bM	n.a.	n.a.	n.a.
4	6.403	n.a.	936.661	0.130	MB	2.40	1.29	39327
5	6.740	n.a.	97.793	n.a.	Rd	n.a.	n.a.	n.a.
6	9.437	Gallic acid	29.636	0.608	BM	1.68	n.a.	246
7	9.693	n.a.	5.613	n.a.	Rd	n.a.	n.a.	n.a.
8	10.490	n.a.	3.135	n.a.	Rd	n.a.	n.a.	n.a.
9	12.990	n.a.	433.360	1.622	MB	3.46	1.35	794
n.a.	n.a.	Protocatechuic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	Aesculin	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
10	19.953	n.a.	3.576	3.123	BMB	6.53	0.77	1320
n.a.	n.a.	Chlorogenic	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	Catechin	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	pOH Benz acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	Caffeic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	Syringic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
11	51.157	n.a.	12.139	6.720	BM	n.a.	n.a.	768
12	57.747	p-Coumaric acid	5.088	2.882	MB	n.a.	n.a.	n.a.
n.a.	n.a.	Rutin	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	Ellagic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	Ferulic acid	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	Sinapic	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	Naringin	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
13	76.473	n.a.	1.930	4.011	BMB	16.06	1.88	9763
n.a.	n.a.	Myrecetin	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	Coumarin	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	Luteolin	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	n.a.	Quercetin	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
14	108.720	Apigenin	0.645	0.885	BMB	n.a.	1.37	217923
n.a.	n.a.	Kaempferol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Average:			113.320	2.070		6.02	1.33	33980

* 24 known standard polyphenolics were used as biomarkers.

** Out of 24, only 3 biomarkers named Gallic acid, p-Coumaric acid and Apigenin were identified.

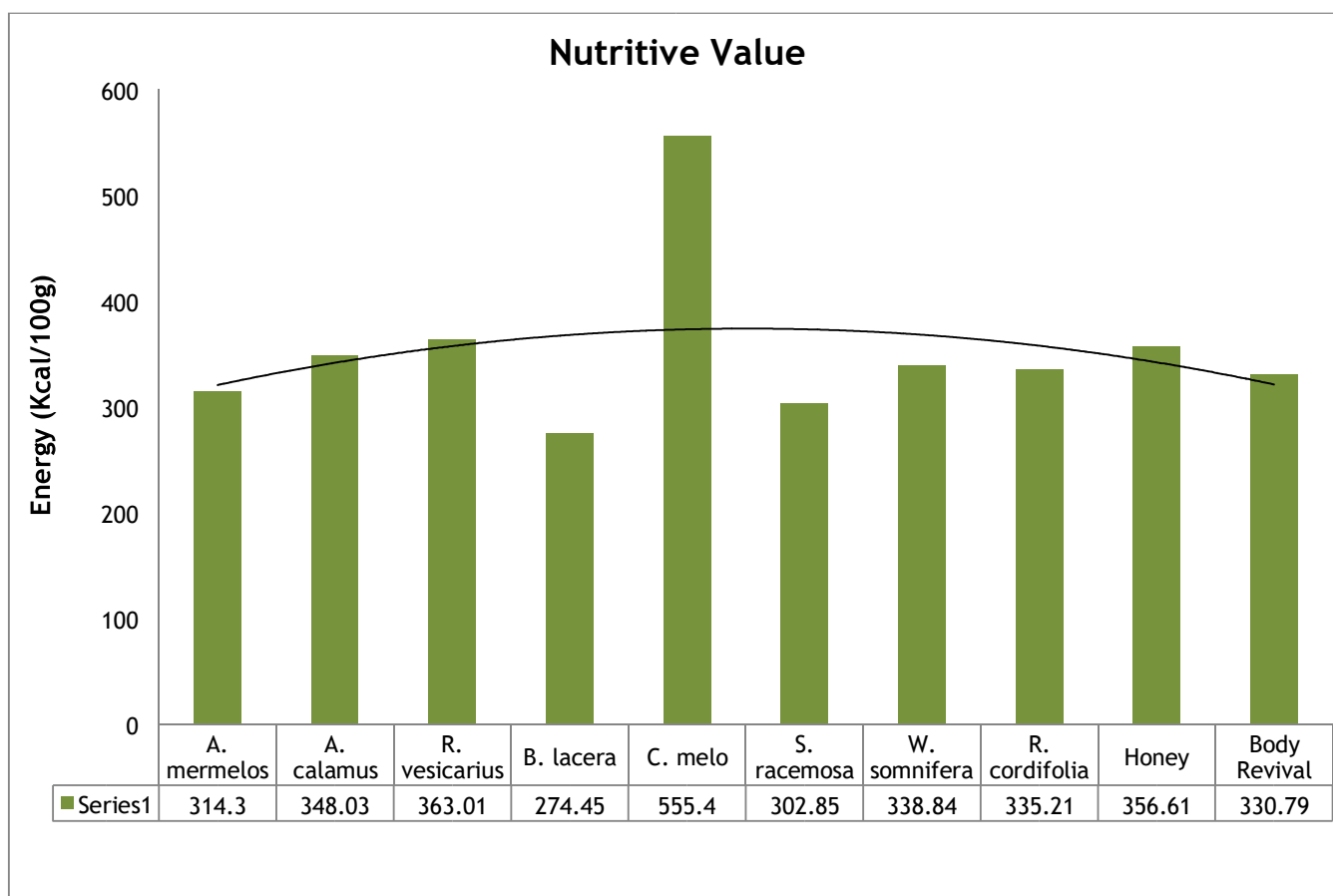
***At 12.99 min (RT) a sharp unidentified peak (433.360 mAU) was noted. Probably it is the peak of sodium benzoate (preservative in Body Revival).

HPTLC Chromatogram of Body Revival



* HPTLC chromatogram showed 6 prominent peaks.

* Peak 6 showed maximum area (84.19%) at Rf 0.54. Probably it is peak of Gallic acid.



Interpretation of Results

- ✓ The proximate composition and nutraceutical role of ingredients present in Body Revival and its formulation by chemical analysis provide unequivocal testimony to the fact.
- ✓ All the ingredients of Body Revival shows rich sources of carbohydrate, protein, fat, fibres and microelements like sodium, potassium and calcium.
- ✓ The nutritive values (energy) of all components of Body Revival establish a good co-relation and match with the final formulation. But, only one ingredient

Cucumis melo (seed) extract has higher nutritive value. It is assumed that seed contains high fat.

- ✓ Chemical analysis reveals Body Revival has maximum carbohydrate and fibres with lesser amount of protein and practically zero fat. Further, Body Revival has low sodium concentration. Hence, it may be helpful for many therapeutic aspects like, cardiac disorders, digestive dysfunctions, neurological problems, renal failures etc.
- ✓ Besides that, a remarkable amount of three major biologically active polyphenolics like, Gallic acid, p-Coumaric acid and Apigenin are found in Body Revival. All these three components have known therapeutic measures in human.

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Pharmacological Studies

Safety Studies

Acute Toxicity Studies. Assurance of safety, quality and efficacy of medicinal plants and herbal products is a key issue, which needs to be addressed. Both the general consumer and health care professional need up-to-date authoritative information on the safety and efficacy of herbal medicines. In clinical practice, Ayurvedic physicians rarely use single drugs, but employ compound formulations, which, while enhancing the overall therapeutic efficacy and also aim to reduce the side effects/toxicity of the main ingredients of the formulations. In this context, Body Revival was examined for its safety measure following the OECD guidelines 423 (adopted 27th Dec., 2001).^[1]

It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, *i.e.*;

- no further testing is needed,
- dosing of three additional animals, with the same dose
- dosing of three additional animals at the next higher or the next lower dose level.

Body Revival was given to the 18 h fasted Swiss albino female mice (30g body weight) as arithmetically progressive manner by oral route at 0.5 ml/100 g, 1 ml/100 g, 1.5 ml/100 g and 2.0 ml/100 g, in a single dose and observed for three days. The rate of mortality up to 5th day was recorded for the selection of 50% lethal dose of test formulation.

Table 1. Acute Oral Toxicity of Body Revival in Mice

Group	Dose	Log dose	Dead/Total	Dead%	LD ₅₀
	(µl/100g)				

			Day 1	Day 2	Day 3	Day 5		
1	500	2.698	0/6	0/6	0/6	0/6	0	ND
2	1000	3.000	0/6	0/6	0/6	0/6	0	ND
3	1500	3.1760	0/6	0/6	0/6	0/6	0	ND
4	2000	3.3010	0/6	0/6	0/6	0/6	0	ND

N=6 in each group; ND= not determined

The test drug formulation, Body Revival did not showed any signs of toxicity or mortality up to 2.0 ml/100 g per oral dose in mice. Therefore, the LD₅₀ of Body Revival could not determine. It could not possible to enhance the dosage further as it exceeds the optimal level of volume for mice. The dose up to 2.0 ml/100 g orally in mice is safe and practically non-toxic. Hence, the test drug formulation 2.0 ml/100 g could be assumed to have no lethal effects.

Interpretation of Results

- ✓ No mortality observed in animals after oral ingestion of herbal formulation Body Revival even in large dose.
- ✓ In this limit test, 2.0 ml/100 g in mice by oral route is considered safe.
- ✓ Dose conversion rule confirms this dose is similar to 240 ml (20 X 12) for human oral dose (60 kg body weight).
- ✓ Therefore, it may assume, Body Revival do not possess any harmful chemical or ingredients and it is safe for oral use by human.

Pharmacological Efficacy Studies

Studies on exercise physiology use animal models to simulate conditions of physical stress commonly observed in humans, with the aim of better understanding changes that occur in humans at the systemic, cellular, and molecular levels.^[2-3] Swimming protocols are frequently used with rats, mainly because rats have an innate swimming

ability and acclimate well to training, which can be done for little cost compared to treadmill running.^[3] Studies of swimming in rats have shown acclimations similar to those observed in humans. ^[4-5] Studies using animal models should use physical exercise protocols that simulate situations to which humans are exposed daily, such as continuous aerobic exercise of low and moderate intensity, in addition to intermittent exercise of high intensity as observed in team sports. The intensity of the exercise can differentiate acclimation to training by determining the metabolic pathway, the energetic substrate and the energy source used, as well as the tension overload on the cardiovascular system.

The forced swim test is one commonly used stressor, where rats (or mice) are forced to swim in an environment from which escape is not possible. In this paradigm, rats go through a period of behavioral activation characterized by vigorous swimming and diving to search for alternate routes of escape. This period of behavioral activation may persist for as much as 3-5 min, after which the rats cease attempts to escape and adopt a characteristic posture of immobility. This second, more passive phase of behavior is thought to reflect a state of depressed mood, and has been referred to as behavioral despair.^[6-8] This conclusion has been reified by the findings that the immobility response observed during the forced swim test is potentially diminished by administration of common anti-depressant agents.^[6] As a result, the forced swim test has been used extensively as a diagnostic for the screening of compounds with potential anti-depressant activity.^[9] In this context, the effect of Body Revival (a proprietary formulation of Health Reactive, Baddi, Solan, H.P., India) was studied on physical and mental endurance during forced swim test regimen in rats.

Randomization & Grouping of Animals:

Wistar strain adult male healthy rats, body weight 150-160 g were selected for the study. The rats were divided into four groups (N=6) randomly. Animals were allowed to acclimatization for 15 days to laboratory conditions prior to the initiation of treatments/dosing.

Species	Rats
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Strain	Wistar
Source	CPCSEA registered animal vendor
Sex	Male
Age	Adult, 2-3 month
Body weight	150-160 g rat
Acclimatization	15 days prior to treatment / dosing
Diet	Pelleted feed supplied Provimi, Bangalore
Water	Aqua guard pure water in glass bottle <i>ad libitum</i>
Housing	The rats were housed 3 in each in polycarbonate cages provided with bedding of husk. The temperature was maintained between 20-25° C in air conditioned room and relative humidity between 60-70% and 7:00-19:00 <i>i.e.</i> , 12 hours light dark cycle was maintained.

Route of Administration & Reason for Choice:

The oral route (gavage) was selected because, one of the propose route of pharmacological testing and the dose can be accurately administered.

Selection of Therapeutic Doses & Justification:

The present study was the first animal trial (Body Revival New Formulation) and there was no previous pharmacological data for its efficacy in animals. The doses were selected based on the following criteria:

Recommended human dose of Body Revival is 5 ml/day (Sponsor's Information).

Therefore, human dose per kg: $5 \div 60 = 0.083$ ml/kg/day

Hence, dose for rat: 0.083×6.2 (conversion factor) = 0.516 ml/kg/day

But, the animal dose was too low for any *in vivo* animal study (particularly, for herbal drug monitoring), hence, it has been considered to select three dose levels within and above human dose. The selected doses were 1 ml/kg, 2 ml/kg and 4 ml/kg body weight for rat. For this, 150 g rat was either taken 0.15 ml, or 0.3 ml or 0.6 ml.

Exercise Swim Stress Endurance:

Exercise or Stress was induced in rats by forced swim (physical & psychological) in a restricted chamber at fixed time (12:00 noon) for 15 minute everyday for 10 consecutive days. Individual rats were placed in a glass bath tub (45 cm X 20 cm X 45 cm) containing 20 cm height of water at 25°C. Rats were allowed to swim (acclimate) for 10 min, then their activity was continually monitored for next 5 minutes. The total period of immobility, characterized by complete cessation of swimming with the head floating above water level, were noted. This immobility period, after initial frenzied attempts to escape, was postulated to represent behavioral despair as an experimental model of endogenous depression and followed by anxiety and stress.^[10] The treatments were continued simultaneously with swim stress as follows:

Group	Treatment	Dose & Duration
1	Stress	Distilled water 5 ml/kg, oral, for 10 days
2	Stress + Body Revival	1 ml/kg, oral, for 10 days
3	Stress + Body Revival	2 ml/kg, oral, for 10 days
4	Stress + Body Revival	4 ml/kg, oral, for 10 days

The degree of physical performance (endurance) as also mental ability to overcome depression was considered by assessing the following parameters on day 10:

1. Swimming Time
2. Blood Lactic Acid
3. Blood Creatinine Kinase
4. Immobility Time
5. Depression Score

Analysis of Results:

The data were expressed as Mean \pm Standard Deviation. All data were compared with respective groups by soft-wares SPSS (Microsoft IBM, version 20). The data were mostly analyzed by appropriate Paired test. The p value was considered significant as $p < 0.05$.

Table 2. Body Revival on Physical and Mental Performances in Rats

	Control (Stress)	Stress + BR 1ml/kg	Stress + BR 2 ml/kg	Stress + BR 4 ml/kg
Physical Performance				
<i>Swimming Time (sec)</i>				
<i>Day 0</i>	75.6±5.01	76.5±3.61	76.5±4.08	76.3±5.46
<i>Day 28</i>	61.1±3.65	140.6±8.54*	125.8±4.44*	189±6.81*
<i>Blood Lactic acid (nM/L)</i>	5.55±0.13	4.48±0.12*	3.85±0.19*	3.41±0.17*
<i>Blood Creatinine kinase (U/L)</i>	387.6±7.39	314.5±5.85*	279±9.97*	255.5±9.89*
Mental Performance				
<i>Immobility Time (sec)</i>				
<i>Day 0</i>	224.3±5.02	223.5±3.61	223.3±4.08	223.6±5.46
<i>Day 28</i>	238.8±3.65	159.3±8.54*	125.8±4.45*	111.2±6.81*
<i>Depression Score</i>	6.48±1.90	-28.69±3.95*	-43.63±2.39*	-50.35±3.15*

N=6 in each group; Data are represented as Mean ± standard deviation; All data are statistically analyzed using Paired test by SPSS v.20 (IBM,USA) software and all data are compared to respective control within groups; * Denotes p is less than 0.001;

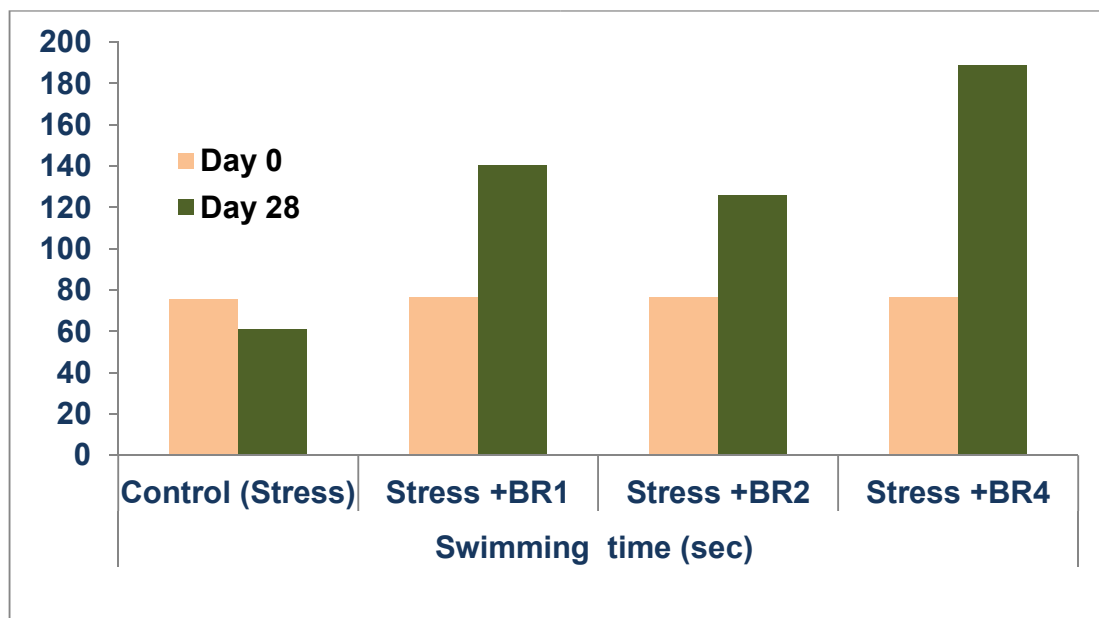


Fig. 1:

Comment: Body Revival Improves Swimming Time Significantly and Dose Dependently.

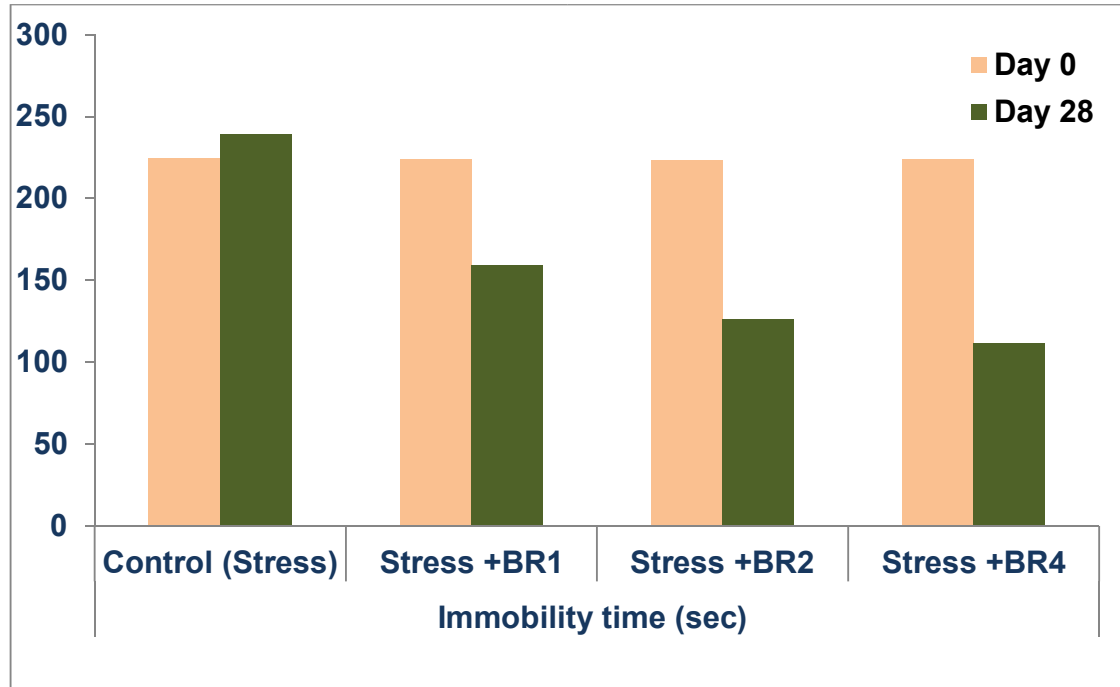


Fig. 2:

Comment: Body Revival Lowers Immobility Time Significantly and Dose Dependently.

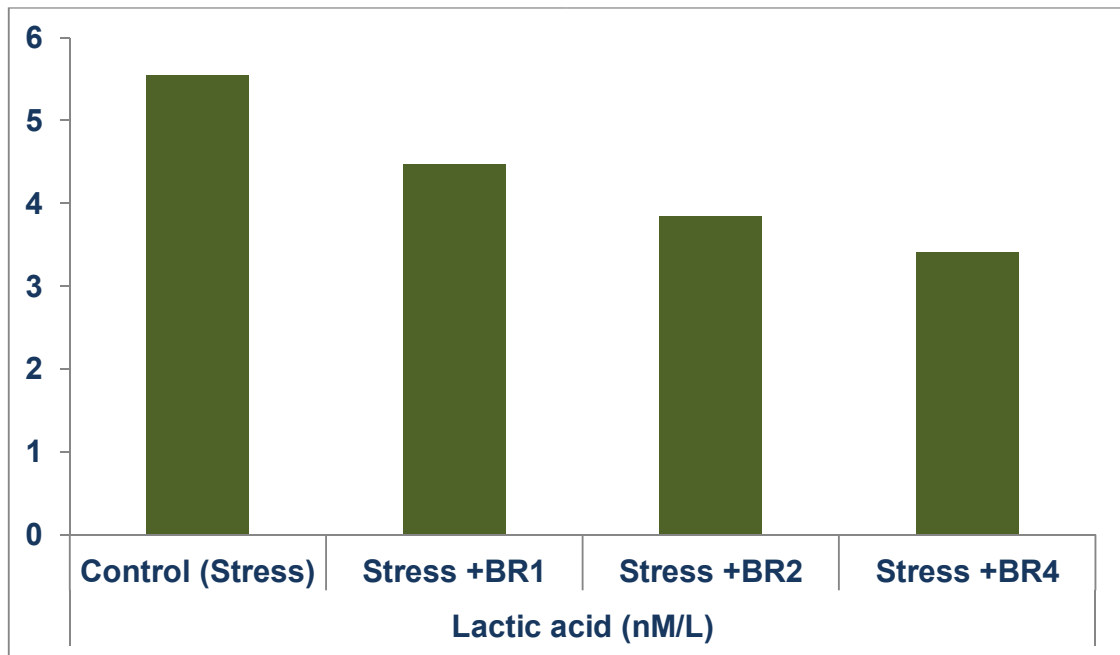


Fig. 3:

Comment: Body Revival Reduces Lactic Acid Significantly and Dose Dependently.

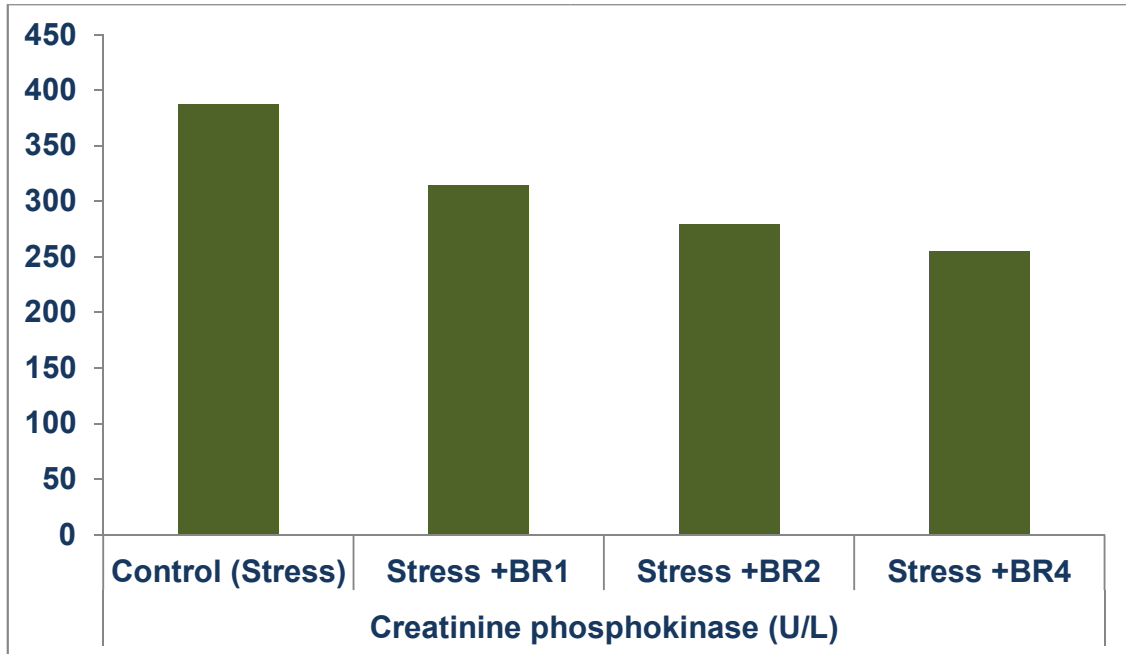


Fig. 4:

Comment: Body Revival Reduces CPK Significantly and Dose Dependently.

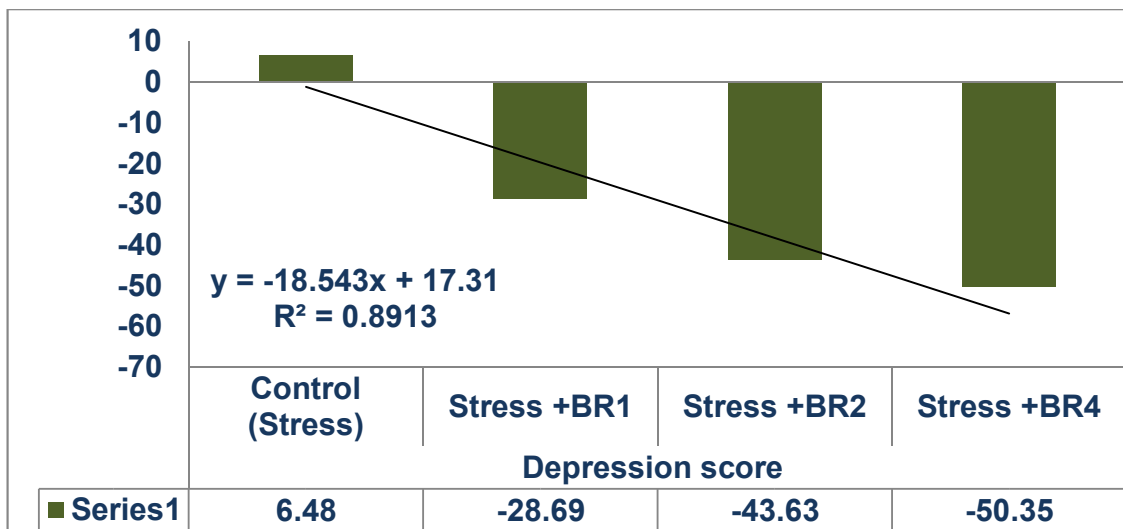


Fig. 5:

Comment: Body Revival Lowers Depression Score Significantly and Dose Dependently.

Interpretation of Results

- ✓ It is claim that Body Revival has herbal active ingredients which works as *Balya, Rasayana and Medhya* and useful in general debilities and life style related disorders etc.^[11]
- ✓ In this study, Body Revival ingestion improves exercise endurance power by enhancing to swimming time significantly and dose dependently within 10 days in rats.
- ✓ It also lowers immobility time or fatigue. Body Revival also enhances anaerobic capacity by lowering blood lactic acid.
- ✓ It also reduces muscle wasting as evidence by lowering blood CK level. Moreover, Body Revival improves mental strength or ability and thereby diminishes depressions.
- ✓ All these results suggest Body Revival has the capability to empower the physical as well as mental state form untoward conditions.

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