

## 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 27<sup>th</sup> Dec., 2001).<sup>[1]</sup>

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 ( $\mu$ l/100g)	Log dose	Dead/Total				Dead%	LD <sub>50</sub>
			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 LD<sub>50</sub> 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.<sup>[2-3]</sup> 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.<sup>[3]</sup> Studies of swimming in rats have shown acclimations similar to those observed in humans.<sup>[4-5]</sup> 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.<sup>[6-8]</sup> This conclusion has been reified by the findings that the immobility response observed during the forced swim test is potently diminished by administration of common anti-depressant agents.<sup>[6]</sup> As a result, the forced swim test has been used extensively as a diagnostic for the screening of compounds with potential anti-depressant activity.<sup>[9]</sup> 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
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:  $0.083 \times 6.2$  (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.<sup>[10]</sup>

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

n=6 in each group; Data are represented as Mean  $\pm$  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; <sup>a</sup> Denotes p is less than 0.001;

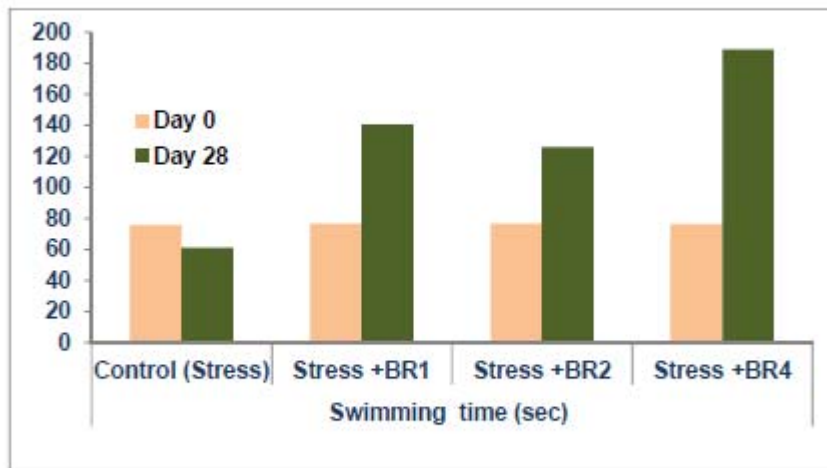


Fig. 1:

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

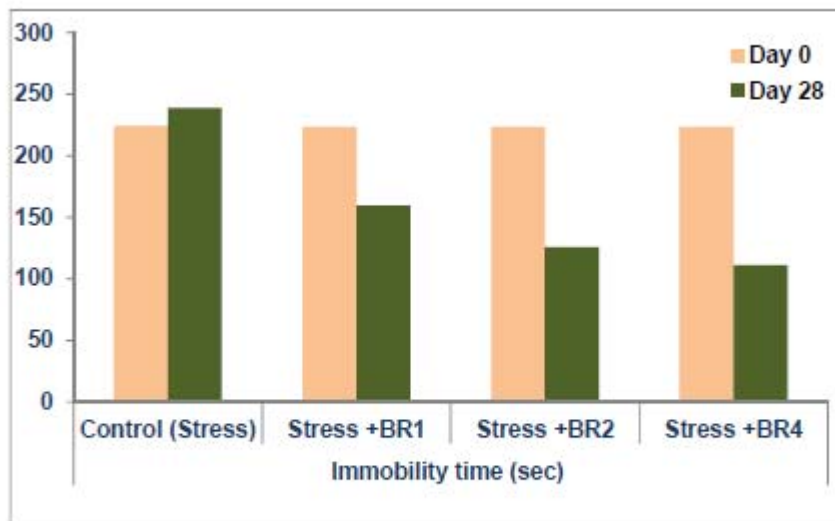


Fig. 2:

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

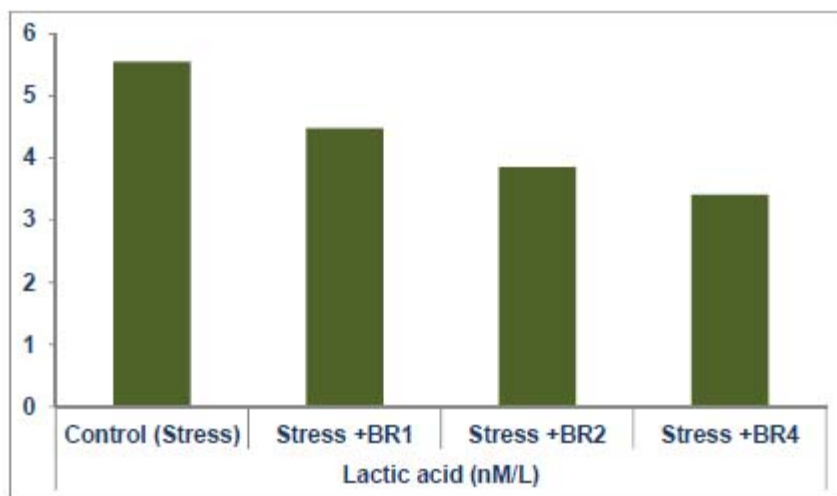


Fig. 3:

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

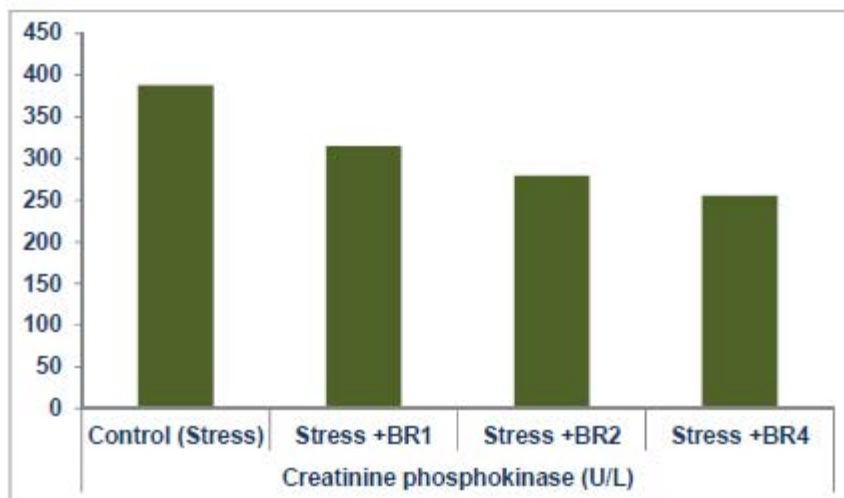


Fig. 4:

Comment: Body Revival Reduces CPK Significantly and Dose Dependently.



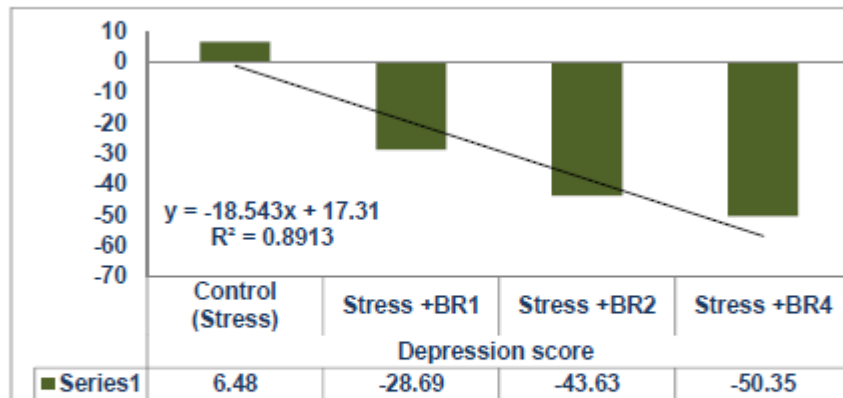


Fig. 5:

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.<sup>[11]</sup>
- ✓ 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.

## References

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