Available online through

www.jbsoweb.com

ISSN 2321 - 6328

Research Article

PHARMACOLOGICAL INVESTIGATION OF RANGER CAPSULE BY STUDYING ACUTE TOXICITY, *IN VITRO* ANTI OXIDANT PROPERTY AND *IN VIVO* ANTI-STRESS ACTIVITY

Soni Hardik^{1*}, Rana Divya², Zaveri Maitreyi³, Patel Sonal⁴, Patel Ghanashyam⁵

¹Assistant Manager – R and D, Vasu Research Centre (A Division of Vasu Healthcare Pvt. Ltd.), Makarpura, Vadodara, Gujarat, India

²Research Scholar, Department of Pharmacognosy and Phytochemistry, K.B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India

³Professor and Head, Department of Pharmacognosy and Phytochemistry, K.B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India

⁴Assistant Professor, Department of Pharmacognosy and Phytochemistry, K.B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India

⁵Senior Manager – R and D, Vasu Research Centre (A Division of Vasu Healthcare Pvt. Ltd.), Makarpura, Vadodara, Gujarat, India

	ABSTRACT
<p>*Correspondence</p> <p>Soni Hardik Assistant Manager – R and D, Vasu Research Centre (A Division of Vasu Healthcare Pvt. Ltd.), Makarpura, Vadodara, Gujarat, India</p> <p>DOI: 10.7897/2321-6328.02119</p> <p>Article Received on: 02/01/14 Accepted on: 10/02/14</p>	<p>The stress is generally considered as the functional adaptation of the organism in order to cope up with changing and challenging environment. Exposure to stressful conditions is among the most common human experience. It has been reported that exposure to physical and mental stress situations can stimulate numerous pathways, leading to increased production of oxygen free radicals. In such situation, the antioxidant defense systems of body may become insufficient to scavenge all free radicals. In the present study, an attempt was made to evaluate acute toxicity of a herbo-mineral Ayurvedic formulation, Ranger capsule along with its <i>in-vitro</i> anti-oxidant property and <i>in-vivo</i> anti-stress activity. Acute toxicity study was carried out on wistar rats as per OECD Guidelines 423. For anti-stress activity forced swimming endurance test was carried out on wistar rats. During this study, Difference in rectal temperature and swimming time was noted. Blood samples were collected for estimation of serum cortisol and serum glucose level. The reduction potential of Ranger Capsule was determined by the <i>in-vitro</i> ferric reducing anti-oxidant power (FRAP) assay at various concentration. Study data revealed that Ranger Capsule didn't produced any toxic effect even up to the dose level of 5000 mg/kg hence was proven as safe. The pre-treatment of Ranger Capsule prevented the fall of rectal temperature in highly significant manner and increased swimming time in comparison to stress control group. Ranger Capsule treated animals showed significant restriction in elevation of serum cortisol and serum glucose level as compared to stress control group. From the available results, it can be concluded that pre-treatment of Ranger Capsule is safe, capable to increase tolerance against non-specific stress in experimental animals and having significant anti-oxidant activity.</p> <p>Keywords: Ranger Capsule, acute toxicity study, <i>in-vivo</i> anti-stress activity, <i>in-vitro</i> anti-oxidant property, serum cortisol</p>

INTRODUCTION

The stress, generally regarded as the functional adaptation of the organism, is developed to cope up with a changing and challenging environment. Exposure to stressful conditions is one of the most common human experiences. In response stressor, a series of behavioral, neurochemical and immunological changes occur that ought to serve in an adaptive capacity.¹ Prolong exposure to stressful situations

may produce diverse variety of diseases ranging from psychiatric disorder such as anxiety and depression, immune-suppression, endocrine disorders including diabetes mellitus, male sexual dysfunction, cognitive dysfunctions, peptic ulcer, hypertension and ulcerative colitis.² It has been reported that exposure to physical and mental stress situations can stimulate numerous pathways, leading to increased production of oxygen free radicals. Free radicals cause

oxidation of nucleic acids and proteins. Free radicals also damage bio-membranes, reflected by increased lipid peroxidation, thereby compromising cell integrity and function. Free radicals are natural by-products of our own metabolism but in stressful condition production of free radicals exaggerated. In such situation, the antioxidant defense systems of body becomes insufficient to scavenge all free radicals.³ A long term use of conventional anti-anxiety drugs like benzodiazepine has adverse effects on the fetus during pregnancy, on the neonate during lactation and produced peptic ulcer.^{2,4} It has been reported that some plant derived agents could induce a state of non-specific increase of resistance to affect internal homeostasis. These agents are called adaptogens which help improve the response to stress. They help the body to adapt by normalizing physiological processes in times of increased stress. Adaptogens can be viewed as tonics, prescribed to enhance vitality and when stress levels are high, during convalescence or difficult life challenging event.⁵ Forced swimming in small laboratory has been widely used for studying the physiological changes and the capacity of the animals in response to stress.⁶ Ranger Capsule is an Ayurvedic herbo-mineral formulation which contains extract of *Withania somnifera* (Ashwagandha) root^{7,8}, *Asparagus racemosus* (Shatavari) root tuber⁹, *Mucuna pruriens* (Kauncha) seed¹⁰, *Emblica officinalis* (Amalaki) fruit¹¹, *Centella asiatica* (Mandukparni) whole plant¹², *Vitis vinifera* (Draksha) fruit¹³, *Nardostachys jatamansi* (Jatamansi) root¹⁴, *Tribulus terrestris* (Gokshur) fruit^{15,16}, *Zingiber officinale* (Shunthi) rhizome¹⁷, *Tinospora cordifolia* (Guduchi) stem¹⁸, *Terminalia arjuna* (Arjun) bark⁸; powder of Swarnamakshik Bhasma¹⁹, Shuddha Shilajit^{20,21} and Godanti Bhasma²² processed in *Pyrus malus* (Apple) fruit juice²³. It is manufactured and marketed by Vasu Healthcare Pvt. Ltd., Vadodara, India. All ingredients of Ranger Capsule are well reported in Ayurvedic texts and scientific research publications for the treatment of general debility, physical and mental stress and convalescence. Ingredients of Ranger Capsule are also reported for having anti-oxidant property and anti-stress activity. However, no such evidence was found which proves safety and efficacy of their combination. In the present study, an attempt was made to evaluate acute toxicity study, *in-vitro* anti-oxidant property and *in-vivo* anti-stress activity of Ranger Capsule.

MATERIALS AND METHODS

Test drug and experimental dose

Ranger Capsule (Herbo-mineral Ayurvedic proprietary formulation) was received from Vasu Healthcare Pvt. Ltd., Vadodara, Gujarat, India and used for evaluation of acute toxicity study, anti-stress activity and *in-vitro* anti-oxidant property. For acute toxicity study 2000 mg/kg and 5000 mg/kg single dose was administered orally. For anti-stress activity, dose of the test drug was fixed by extrapolating the human dose to laboratory animals, based on body surface area ration as per the table of Paget and Barnes [23]. Test drug was administered at 100 mg/kg/day (p.o) in form of suspension by mixing with distilled water.

Experimental animals

Healthy Wistar albino rats, weighing 180-230 g of either sex were used for the acute toxicity study and anti-stress activity. The animals were housed in a three rats per polypropylene cages, maintained under controlled temperature ($22 \pm 2^\circ\text{C}$) and humidity ($55 \pm 5\%$) with 12:12 h light and dark cycle.

Animals had free access to 'Sabardan' pelleted diet and purified drinking water *ad libitum*. All protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) (Approval No.: KB/11/239) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Acute toxicity study

Healthy Wistar albino rats (180 - 230 g) were divided into 2 groups of 3 animals each. The animals had free access to water and food throughout the experiment, except for the fasting period before the oral administration of the single dose of Ranger Capsule. The Ranger Capsule was administered as it is by gavages (orally) at single dose of 2000 mg/kg to 1st group and single dose of 5000 mg/kg to 2nd group. The general behavior and mortality of the rats was continuously monitored for 1 h after dosing periodically during first 24 h (with special attention given during the first 4 h.) and then daily for a total of the 14 days. Changes in the normal activity of rats, sign and symptoms of toxicity and mortality were monitored and recorded. Acute toxicity study was carried out as per OECD Guidelines 423.²⁴

Forced swimming endurance test

The selected animals were divided into three groups where each group consisted of six animals.

Group-I (NC): Served as normal control and received distilled water

Group-II (SC): Served as stress control and received distilled water + forced swimming induced stress

Group-III (TD): Served as test drug (Ranger Capsule) treated group and received Ranger Capsule (100 mg/kg/day, p.o.) + forced swimming induced stress

Test drug (Ranger Capsule) was given for seven consecutive days in Group-III. On 7th day the rats were kept in metabolic cage for overnight fasting with free access to distilled water. For experiment, tank with dimension of $37 \times 37 \times 30$ cm was filled with water to a height of 25 cm and temperature was maintained $22^\circ\text{C} \pm 2$. On 8th day the rats of group II and III were subjected to swimming stress by keeping them in tank till complete exhaust. The endpoint was taken when the animal started drowning and the mean swimming time for each group was calculated. The initial rectal temperature of individual rats of all groups was noted prior to exposure of swimming stress. The final rectal temperature was noted and difference in rectal temperature was calculated. Blood samples were collected from retro-orbital plexuses. Samples were allowed to clot for 30 minutes at room temperature. Sera from the samples were obtained by centrifugation after 30 minutes at 4000 rpm. Serum cortisol²⁵ and serum glucose²⁶ were estimated.

Statistical analysis

Analysis was done with the help of standard statistical software, Graph pad prism version 5. Results were expressed as Mean $\pm$ Standard Error of Mean (SEM). Different groups were compared by analysis of variance (ANOVA) followed by post hoc Dunnett's test. A $p < 0.05$ was considered as statistically significant.

In-vitro* Ferric reducing anti-oxidant power (FRAP) assay*Preparation of standard solution**

Stock solution of ascorbic acid (10 mg/mL) was prepared in distilled water. Aliquots of 0.1, 0.15, 0.2, 0.25, 0.3 mL were taken from stock solution and diluted up to 10 mL with phosphate buffer to get the concentrations of 100, 150, 200, 250, 300 µg/mL respectively.

Preparation of sample solution

Stock solution of Ranger Capsule (10 mg/mL) was prepared in distilled water. Aliquots of 0.1, 0.15, 0.2, 0.25, 0.3 mL were taken from stock solution and diluted up to 10 mL with phosphate buffer to get the concentrations of 100, 150, 200, 250, 300 µg/mL respectively.

Procedure

Various concentrations of sample and standard solution were taken in test tubes (1 mL each). 2.5 mL of 1 % potassium ferricyanide solution was added in each test tubes and mixture was kept at 50°C on water bath for 30 minutes. After cooling, 2.5 mL of 10 % tri-chloro acetic acid was added to these mixtures and centrifuged for 10 minutes at 3000 rpm. 2.5 mL of supernatant was diluted with 2.5 mL of distilled water. 0.5 mL of freshly prepared 0.1 % ferric chloride solution was added and incubated for 10 minutes at room temperature. Control was prepared in similar manner excluding sample. The absorbance was measured at 700 nm. The absorbance of samples and standard were compared by plotting graph of concentration (µg/mL) versus absorbance.²⁷

RESULTS**Acute toxicity study**

The animals were observed for mortality and other toxic symptoms for 14 days of observation period. No toxic symptoms and mortality were found at both the dose level during this study.

Effect of Ranger Capsule on parameters of forced swimming endurance test

Stress control showed 5.32 ± 0.81 differences in rectal temperature. Fall in rectal temperature during stressful condition was significantly prevented by pre-treatment of Ranger Capsule. Difference in rectal temperature was found significantly decreased in TD group when compared to SC group (Table 1). The study data revealed that pre-treatment of Ranger Capsule significantly increased swimming time in comparison of SC group (Table 1). Stress induced elevation in level of serum cortisol and serum glucose were significantly ($p < 0.01$) arrested by Ranger Capsule treated group (Table 1).

***In-vitro* Ferric reducing anti-oxidant power (FRAP) assay**

The reduction potential of Ranger Capsule was determined by the *in-vitro* ferric reducing anti-oxidant power (FRAP) assay at various concentration. Ascorbic acid was taken as a standard. The results were summarized in Figure 1.

Table 1: Effect of Ranger Capsule on parameters of forced swimming endurance test

Groups	Difference in rectal temperature (°C)	Duration of swimming (Min.)	Serum cortisol (µg/dL)	Serum glucose (mg/dL)
Normal control (NC)	0.20 ± 0.01	NA	0.27 ± 0.06	92.50 ± 5.54
Stress control (SC)	5.32 ± 0.81	100.60 ± 12.07	$3.01 \pm 0.26^{###}$	$174.30 \pm 5.59^{###}$
Ranger Capsule treated (TD)	$4.00 \pm 0.59^*$	$132.80 \pm 16.95^{**}$	$2.08 \pm 0.06^{**}$	$140.71 \pm 6.35^{**}$

All the values are expressed as mean $\pm$ SEM (n = 6). ^{###}p < 0.001 when compared to normal control (NC) group, *p < 0.05 and **p < 0.01 when compared to stress control (SC) group. NA: Not applicable

Figure 1: FRAP assay at various concentration of the Ranger Capsule against ascorbic acid standard

DISCUSSION

The forced swimming endurance test is the most widely used experimental model for evaluation of anti-stress and adaptogenic activity. Reduction in rectal temperature (hypothermia) was observed in rats subjected to forced swimming stress. Drugs which having adaptogenic and anti-stress activity reverse the hypothermia in stress conditions.²⁸ In the present study, data revealed that pre-treatment of Ranger Capsule prevented the fall of rectal temperature in significant manner in comparison to stress control group. Pre-treatment of Ranger Capsule also increased swimming time in comparison to stress control group which is suggestive of its anti-stress and adaptogenic activity (Table 1). The long-term effects of stress alter our ability to maintain a healthy balance and harmony. This internal shift is due to a greater demand for stress hormones, namely cortisol, which is a major contributing factor that leads to the development of chronic illnesses, and hastens the aging process. All illnesses, to some extent, are a byproduct of our inability to adapt to changes and challenges of our life.⁵ The fast pace of life in modern times contributes to an increase in the production and sustained release of the stress hormones adrenaline and cortisol. Chronic activation of these stress hormones can cause deterioration of vital organs. Research has shown a close connection between high cortisol levels and serious health problems such as obesity, diabetes, hypertension, depression and osteoporosis.⁵ Stresses, both physical and emotional, act via neural pathways to hypothalamus and lead to increase in corticotrophin releasing hormone (CRH) secretion. Increased plasma cortisol influences the mobilization of stored fat and carbohydrate, which increases level of blood glucose.²⁹ In stress control animals, the level of serum cortisol and serum glucose was found to be significantly increased. Ranger Capsule treated animals showed significant restriction on elevation of serum cortisol and serum glucose level when compared with stress control group (Table 1). It indicates positive effect of Ranger Capsule on physical and mental stress. In addition Ranger Capsule also showed prominent anti-oxidant property which supports its free radicals scavenging and thus the anti-aging activity (Figure 1).

CONCLUSION

From the available results, it can be concluded that pre-treatment of Ranger Capsule is capable to increase tolerance against non-specific stress in experimental animals. In addition, it has potential to scavenge free radicals. It can be a safe and effective therapy for long term treatment of physical and mental fatigue and general debility.

ACKNOWLEDGEMENT

Authors are sincerely thankful to the management of Vasu Healthcare Pvt. Ltd. for providing test drug samples and K.B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India for providing the necessary facilities to conduct the study.

REFERENCES

- Anisman H. Understanding stress: Characteristics and caveats. *Alcohol Res Health* 1999; 23: 241-9.
- Elliot GR, Eisdorfer C. *Stress and Human Health*. New York; Springer Publishing; 1982.
- Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. *World Allergy Organ J* 2012; 5(1): 9-19. <http://dx.doi.org/10.1097/VOX.0b013e3182439613>
- Trevor AJ, Way WL. Sedative-hypnotic drugs. Katzung BG, editor. *Basic and clinical pharmacology*. New York: Lange Medical; 2001. p. 364-81.
- Pawar Vinod S, Hungar Shivakumar. A current status of adaptogens: natural remedy to stress. *Asian Pac J Trop Disease* 2012; 2(suppl 1): S480-S490.
- Lakshmi BV, Sudhakar M. Adaptogenic activity of *Lagenaria siceraria*: An experimental study using acute stress models on rats. *J Pharmacol Toxicol* 2009; 4: 300-6. <http://dx.doi.org/10.3923/jpt.2009.300.306>
- Malik F, Singh J, Khajuria A, Suri KA, Satti NK *et al*. A standardized root extract of *Withania somnifera* and its major constituent withanolide-A elicit humoral and cell-mediated immune responses by up regulation of Th1-dominant polarization in BALB/c mice. *Life Sci* 2007; 80(16): 1525-38. <http://dx.doi.org/10.1016/j.lfs.2007.01.029>
- Sandhu J, Shah B, Shenoy S, Chauhan S, Lavekar G, Padhi M. Effects of *Withania somnifera* (Ashwagandha) and *Terminalia arjuna* (Arjuna) on physical performance and cardiovascular endurance in healthy young adults. *Int J Ayurveda Res* 2010; 1(3): 144-9. <http://dx.doi.org/10.4103/0974-7788.72485>
- Gautam M, Saha S, Bani S, Kaul A, Mishra S, Patil D *et al*. Immunomodulatory activity of *Asparagus racemosus* on systemic Th1/Th2 immunity: implications for immune adjuvant potential. *J Ethno pharmacol* 2009; 121(2): 241-7. <http://dx.doi.org/10.1016/j.jep.2008.10.028>
- http://www.allayurveda.com/topic_month_july2011.asp; 2012.
- Sampath KP, Debjit B, Dutta A, Yadav A, Paswan S, Srivastava S *et al*. Recent trends in potential traditional Indian herbs *Embllica officinalis* and its medicinal importance. *J Pharmacog Phytochem* 2012; 1(1): 24-32.
- Gupta YK, Veerendrakumar MH, Srivastava AK. Effect of *Centella asiatica* on pentylenetetrazole-induced kindling, cognition and oxidative stress in rats. *Pharmacol Bio chem Behavior* 2003; 74: 579-85. [http://dx.doi.org/10.1016/S0091-3057\(02\)01044-4](http://dx.doi.org/10.1016/S0091-3057(02)01044-4)
- Pari L, Suresh A. Effect of grape (*Vitis vinifera* L.) leaf extract on alcohol induced oxidative stress in rats. *Food Chem Toxicol* 2008; 46(5): 1627-34. <http://dx.doi.org/10.1016/j.fct.2008.01.003>
- Damodara K, Shetty L, Krishna AP, Suchetha N, Sanjeev G, Naveen P. The efficacy of *Nardostachys jatamansi* against radiation induced hematological damage in rats. *J Clin Diagn Res* 2013; 7(6): 982-6.
- Rogerson S, Riches CJ, Jennings C, Weatherby RP, Meir RA, Marshall Gradisnik SM. The effect of five weeks of *Tribulus terrestris* supplementation on muscle strength and body composition during pre-season training in elite rugby league players. *J Strength Cond Res* 2007; 21(2): 348-53. <http://dx.doi.org/10.1519/R-18395.1>
- Kamboj P, Aggarwal M, Puri S, Singla SK. Effect of aqueous extract of *Tribulus terrestris* on oxalate-induced oxidative stress in rats. *Indian J Nephrol* 2011; 21(3): 154-9. <http://dx.doi.org/10.4103/0971-4065.83727>
- Lakshmi BV, Sudhakar M. Attenuation of acute and chronic restraint stress-induced perturbations in experimental animals by *Zingiber officinale* Roscoe. *Food Chem Toxicol* 2010; 48(2): 530-5. <http://dx.doi.org/10.1016/j.fct.2009.11.026>
- Sankhala LN, Saini RK, Saini BS. A review on chemical and biological properties of *Tinospora cordifolia*. *Int J Med Arom Plants* 2012; 2(2): 340-4.
- Rastantrasar and Shiddhaprayog Sangrah. Published by Krishna Gopal Ayurved Bhavan, Ajmer; part-1; 1980. p. 138.
- Shashtri Shankar Dajipade: Aryabhishak Athva Hindustanano Vaidraj. Published by Sastu Sahitya Vardhak Karyalay, Ahmedabad, 25th edition; 2006. p. 698.
- Frawley, David, Lad V. *The Yoga of Herbs*. Lotus Press. Twin Lakes, WI, 2nd edition; 2001. p. 250.
- Rastantrasar and Shiddhaprayog Sangrah. Published by Krishna Gopal Ayurved Bhavan, Ajmer; part-1; 1980. p. 166.
- Mathur A, Verma S, Gupta V, Singh S, Singh S *et al*. Comparative studies on different varieties of apple (*Pyrus malus* L.) of kashmir (J and K) on the basis of PPO activity, total phenolic content (TPC) and *in-vitro* antioxidant activity. *Int J Pharm Sci* 2011; 2(3): 1-6.
- OECD 423. OECD guidelines for testing of chemicals - Acute Oral Toxicity Method. OECD 17th Dec; 2001. p. 1-14.
- Fowder AL, Mijoric J, Silver M. The effect of cortisol on hepatic and renal gluconeogenic enzyme activities in the sheep fetus during late gestation. *J Endocrinol* 1993; 137: 213-22. <http://dx.doi.org/10.1677/joe.0.1370213>
- Barham D, Trinder P. An improved color reagent for the determination of blood glucose by the oxidase system. *Analyst* 1972; 97: 142-5. <http://dx.doi.org/10.1039/an9729700142>
- Oyaizu M. Studies on products of browning reaction: Anti-oxidative activities products of browning reaction prepared from glucosamine. *Jpn J Nutr* 1986; 44: 307-15. <http://dx.doi.org/10.5264/eiyogakuzashi.44.307>
- Somarathna WK, Chandola HM, Ravishankar B, Pandya KN, Attanayake AMP, Ashok BK. Evaluation of adaptogenic and anti-stress effect of Ranahamsa Rasayanaya – A Sri Lankan classical Rasayana

- drug on experimental animals. Ayu 2010; 31(1): 88-92.
<http://dx.doi.org/10.4103/0974-8520.68201>
29. Lakshmi BV, Sudhakar M. Screening of *Psidium guajava* leaf extract for anti-stress activity in different experimental animal models. Pharmacog Res 2009; 1(6): 359-66.

Cite this article as:

Soni Hardik, Rana Divya, Zaveri Maitreyi, Patel Sonal, Patel Ghanashyam. Pharmacological investigation of ranger capsule by studying acute toxicity, In vitro Anti oxidant property and In vivo anti-stress activity. J Biol Sci Opin 2014;2(1):86-90 <http://dx.doi.org/10.7897/2321-6328.02119>

Source of support: Nil; Conflict of interest: None Declared