ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 7, Issue 2, 2014

ISSN - 0974-2441

Research Article

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

SONI HARDIK[1*], RANA DIVYA[2], ZAVERI MAITREYI[2], PATEL SONAL[2], PATEL GHANASHYAM[1]

[1] Vasu Research Centre, A Division of Vasu Healthcare Pvt. Ltd., 896/A, G.I.D.C., Makarpura, Vadodara – 390 010, Gujarat, India, [2] Department of Pharmacognosy and Phytochemistry, K.B. Institute of Pharmaceutical Education and Research, Sector - 23, Near GH - 6, Gandhinagar - 382 023, Gujarat, India.Email: hsoni@vasuresearch.com ; hardik_ayurved@yahoo.com

Received: 21 November 2013, Revised and Accepted: 29 January 2014

ABSTRACT

Stress and stress-related disorders are a significant cause of disease in modern times, perhaps contributing to 75% of illnesses. 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 become unable to scavenge these free radicals sufficiently. Ranger Syrup is a polyherbal Ayurvedic medicine which was taken up in the present study to evaluate acute toxicity, *in-vitro* anti-oxidant property and *in-vivo* anti-stress activity. Acute toxicity study was carried out on wistar rats as per OECD Guidelines 423. To evaluate anti-stress activity, forced swimming endurance test was performed. 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. Anti-oxidant property of Ranger Syrup was determined by the i*n-vitro* ferric reducing anti-oxidant power (FRAP) assay at various concentration. Study data revealed that Ranger syrup didn't produced any toxic effect even up to the dose level of 5000mg/kg hence was proven as safe. The pre-treatment of Ranger Syrup prevented the fall of rectal temperature in highly significant manner and increased swimming time in comparison to stress control group. Ranger Syrup treated animals showed significant restriction in elevation of serum cortisol and serum glucose level as compared to stress control group. In addition, it has significant potential to scavenge free radicals. From the results of these studies, it can be concluded that Ranger Syrup is safe, capable to increase tolerance against non-specific stress in experimental animals and having significant anti-oxidant activity.

Keywords: Ranger Syrup, acute toxicity, *in-vivo* anti-stress activity, *in-vitro* anti-oxidant property

INTRODUCTION

Pathologic conditions related to stress have been a subject of science since 1911 when Walter Cannon applied the engineering concept of stress to a physiologic context, suggesting that emotional stimuli were capable of causing physical damage to the body. Stress and stress-related disorders are a significant cause of disease in modern times, contributing to perhaps 75% of illnesses [1]. Stress has been postulated to be involved in the etiopathogenesis of a 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 functions. 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 cannot be able to scavenge these free radicals [3].

The benzodiazepine, despite having significant anti-stress activity against models of acute stress, not proved effective against chronic stress induced adverse effects on immunity, hypertension and peptic ulcer [2]. Furthermore, these drugs have adverse effects on the fetus during pregnancy and on the neonate during lactation [4]. It has been reported that some plant derived agents could induce a state of non-specific increase of resistance to restore internal homeostasis. These agents, named adaptogens, improve the response to stress. They help body to adapt by normalizing physiological processes in times of increased stress. Adaptogens can be viewed as tonics, prescribed to enhance vitality and are indicated when stress levels are high, during convalescence or difficult life challenging event [5].

Ranger Syrup is a polyherbal Ayurvedic proprietary formulation which contains extract of *Pyrus malus* (Apple) fruit [6], *Withania somnifera* (Ashwagandha) root [7,8], *Asparagus racemosus* (Shatavari) root tuber [9], *Vitis vinifera* (Draksha) fruit [10], *Mucuna pruriens* (Kauncha) seed [11], *Glycyrrhiza glabra* (Yashtimadhu) root [12], *Emblica officinalis* (Amalaki) fruit [13], *Centella asiatica* (Mandukparni) whole plant [14], *Terminalia arjuna* (Arjun) stem bark [8], *Boerhaavia diffusa* (Punarnava) root [15], *Tribulus terrestris* (Gokshur) fruit [16,17], *Zingiber officinale* (Shunthi) rhizome [18], *Myristica fragrans* (Jatiphal) kernel [19], *Nardostachys jatamansi* (Jatamansi) root [20], *Leptadenia reticulata* (Jivanti) aril [21] and *Tinospora cordifolia* (Guduchi) stem [22]. It is manufactured and marketed by Vasu Healthcare Pvt. Ltd., Vadodara. All ingredients of Ranger Syrup are well reported in Ayurvedic texts and scientific research publications for the treatment of general debility, physical and mental stress and convalescence. They 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, *in-vitro* anti-oxidant property and *in-vivo* anti-stress activity of Ranger Syrup.

MATERIALS AND METHODS

Test drug and experimental dose

Ranger Syrup (Polyherbal Ayurvedic proprietary formulation) was received from Vasu Healthcare Pvt. Ltd., Vadodara, Gujarat and used for evaluation of acute toxicity, anti-stress activity and *in-vitro* anti-oxidant property. For acute toxicity study 2000mg/kg and 5000mg/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 0.9 mL/kg/day (p.o).

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±2°C) and humidity (55±5%) with 12:12 h light and dark cycle. Animals had free access to 'Sabardan' pelleted diet and purified drinking water *ad libitium*. 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 Syrup. The Ranger Syrup 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 (0.9 mL/kg/day, p.o.)

Group-II (SC): Served as stress control and received distilled water (0.9 mL/kg/day, p.o.) + induced stress by forced swimming

Group-III (TD): Served as test drug (Ranger Syrup) treated group and received Ranger Syrup (0.9 mL/kg/day, p.o.) + induced stress by forced swimming

Test drug (Ranger Syrup) 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 37X37X30 cm was filled with water to a height of 25cm and temperature was maintained 22°C ± 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 min at room temperature. Sera from the samples were

obtained by centrifugation after 30 min at 4000 rpm. Serum cortisol [25] and serum glucose [26] were estimated.

Statistical analysis

Analysis was done with the help of standard statistical software, Graph pad prism version 5. Results were expressed as Mean ± 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 Syrup (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 min. 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 min. 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 Syrup on parameters of forced swimming endurance test

Stress control showed 5.32±0.81 difference in rectal temperature. Fall in rectal temperature during stressful condition was significantly prevented by pre-treatment of Ranger Syrup. 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 Syrup significantly increased swimming time in comparison of SC group (Table 1).

Stress induced elevation in level of serum cortisol and serum glucose were significantly ($p<0.001$) arrested by Ranger Syrup treated group (Table 1).

Table 1: Effect of Ranger Syrup 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±0.26###	174.30±5.59###
Ranger Syrup treated (TD)	3.45±0.64**	153.30±19.50***	1.47±0.75***	129.00±11.97***

All the values are expressed as mean ± SEM (n=6). ###p<0.001 when compared to normal control (NC) group. **p< 0.01, ***p< 0.001when compared to stress control (SC) group. NA: Not applicable.

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

The reduction potential of Ranger Syrup was determined by the i*n-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.

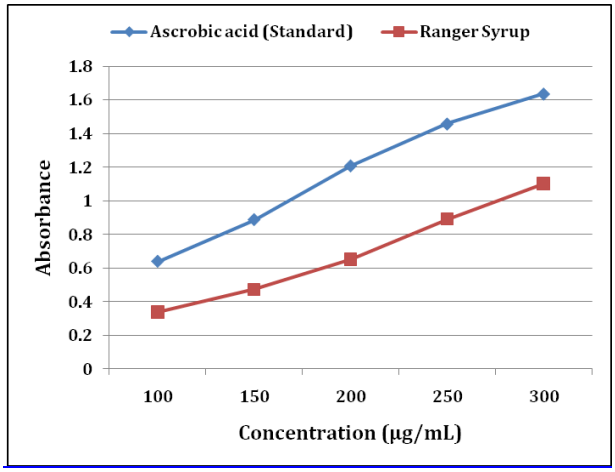

Fig.1: FRAP assay at various concentration of the Ranger Syrup against ascorbic acid standard

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 are having adaptogenic and ant-stress activity reverse the hypothermia in stress conditions [28]. Present study data revealed that pre-treatment of Ranger Syrup prevented the fall of rectal temperature in highly significant manner in comparison to stress control group. It also increased swimming time significantly 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 Syrup 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 Syrup on physical and mental stress. In addition Ranger Syrup also showed prominent anti-oxidant activity which supports its free radicals scavenging and thus the anti-aging activity (Figure 1).

CONCLUSION

From the results of these studies, it can be concluded that pre-treatment of Ranger Syrup 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 for providing the necessary facilities to conduct the study.

SOURCE OF SUPPORT

Nil

CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interest.

REFERENCES

1. Ward Dean. Role of novel Russian adaptogenic compounds in restoration of adrenal function. Townsend letter for doctors and patients, Nov 2001.
2. Elliot GR, Eisdorfer C. stress and human health. New York; Spinger Publishing; 1982.
3. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J 2012;5(1):9-19.
4. Trevor AJ, Way WL. Sedative-hypnotic drugs. Katzung BG, editor. Basic and clinical pharmacology. New York: Lange Medical; 2001:364-381.
5. Pawar Vinod S, Hungar Shivakumar. A current status of adaptogens: natural remedy to stress. Asian Pac J Trop Disease 2012: S480-S490.
6. 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&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.
7. 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.
8. 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.
9. 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 immunoadjuvant potential. J Ethnopharmacol 2009;121(2):241-7.
10. 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.
11. http://www.allayurveda.com/topic_month_july2011.asp accessed on dated 12-02-2012.
12. Moharana D. Shatavari, Yastimadhu and Aswagandha the Ayurvedic Therapy. Orissa Review 2008:72-77.
13. Sampath KP, Debjit B, Dutta A, Yadav A, Paswan S, Srivastava S *et al*. Recent trends in potential traditional Indian herbs *Emblica officinalis* and its medicinal importance. J Pharmacog Phytochem 2012;1(1):24-32.
14. Gupta YK, Veerendrakumar MH, Srivastava AK. Effect of *Centella asiatica* on pentylenetetrazole-induced kindling, cognition and oxidative stress in rats. Pharmacol Biochem Behavior 2003; 74:579-85.
15. AR Mahesh, Harishkumar, Ranganath MK, Devkar RA. Detail Study on *Boerhaavia diffusa* plant for its medicinal importance - A review. Res J Pharma Sci 2012;1(1):28-36.
16. 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 preseason training in elite rugby league players. J Strength Cond Res. 2007;21(2):348-53.
17. 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.
18. 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.

19. Mohd Abdul Rasheed Naikodi, Mohd Abdul Waheed, Mohammad Ataullah Shareef, Mushtaq Ahmad, Kommu Nagaiah. Standardization of the unani drugs – *Myristica fragrans* Houtt. (Javetri) with modern analytical techniques. Pharm Methods 2011; 2(2):76-82.
20. 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.
21. Girishkumar V, Sreepriya M, Praveenkumar S, Bali G, Jagadeesh MS. Modulating effect of *Leptadenia reticulata* (Retz) against chromate (VI)-induced immunosuppression and oxidative stress on mouse splenic lymphocytes and bone marrow derived macrophages. J Ethnopharmacol 2010;131(2):505-8.
22. 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.
23. Paget GE, Barnes JM. Evaluation of drug activities. In: Lawrence DR, Bacharach AL, editors. Pharmacometrics. Vol. I. New York: Academic Press; 1964. p.161.
24. OECD 423. OECD guidelines for testing of chemicals - Acute Oral Toxicity Method. OECD 17th Dec, 2001. p.1-14.
25. 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.
26. Barham D, Trinder P. An improved color reagent for the determination of blood glucose by the oxidase system. Analyst 1972;97:142-5.
27. 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.
28. 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.
29. Lakshmi BVS, Sudhakar M. Screening of *Psidium guajava* leaf extract for anti-stress activity in different experimental animal models. Pharmacog Res 2009;1(6):359-66.