Vol. 8/1 (2008) 82 - 88

JOURNAL OF NATURAL REMEDIES

Antioxidant and adaptogenic effect of an herbal preparation, Triphala

H. N. Shivaprasad [1]*, M. D. Kharya [2], A. C. Rana [3]

1. Natural Products Research Laboratory, Department of Pharmaceutical Sciences, Dr. H. S. Gour Vishwavidyalaya, Sagar - 470 003, Madhya Pradesh, India

2. Head, Department of Pharmaceutical Sciences, Dr. H. S. Gour Vishwavidyalaya, Sagar - 470 003, Madhya Pradesh, India

3. Director and Head, Pharmacology Division, B.N. College of Pharmacy, Udaipur - 313 001, Rajasthan, India

Abstract

Objective: To investigate the antioxidant and adaptogenic activities of Triphala, an Indian Ayurvedic medicinal preparation. Materials and Methods: Antioxidant activity of Triphala was determined by hydroxyl and nitric oxide radical scavenging methods. Adaptogenic activity was studied using swim endurance, anoxic stress tolerance and chronic stress induced behavioral despair test models. Triphala was administered at the dosage levels of 100 to 500 mg/kg b.w.p.o. Results and Discussion: Triphala was found to scavenge hydroxyl and nitric oxide radicals *in vitro*. The IC_{50} value for hydroxyl radical scavenging was 40.5 µg/mL and that for nitric oxide radical scavenging was found to be 40 µg/mL, respectively. Oral administration of Triphala formulation significantly improved the stress tolerance by increasing the swim duration (762.28 ± 7.17 minute), anoxic stress tolerance duration (39.11 ± 1.05 minute) and reduced the stress induced increase in the immobility period (61.11 ± 3.42 seconds) in chronic shock induced stress. Conclusion: Triphala has been found to be an excellent scavenger of hydroxyl radicals and nitric oxide radicals, whose excessive formation is implicated in oxidative stress. Triphala is capable of increasing the capacity to tolerate non-specific stress in experimental animals as evident from the restoration of parameters studied during different types of stress models.

Keyword: Triphala, antioxidant, adaptogenic

1. Introduction

Free radicals have been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes, etc. and compounds that can scavenge free radicals have great potential in ameliorating these disease processes [1]. Antioxidants thus play an important role to protect the human body against damage by reactive oxygen species. An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. The mechanism involves significant inhibition or delay in the oxidative process [2]. As per biochemist and epidemiologists,

* Corresponding author
Email: shivaguru100@gmail.com

antioxidants neutralize free radicals by binding their lonely electrons and rendering them harmless [3].

Despite the tremendous studies and intensive research by medical scientists, therapy to build up non-specific resistance to aging, various ailments, recovery after illness and environmental changes is till as elusive as it was decades ago. Adaptogens are pharmacological agents that induce a non-specific increase of resistance of the organism (SNIR) to aversive stimuli that threaten to perturb internal homeostasis [4]. The response to stress is non-specific and independent of the nature of the stress, as the stress induced state produced by diverse stresses is indistinguishable [5].

The corroborative and tonic plants, generally known as "Rasayana" drugs in the Ayurvedic literature, are used for remedies for various ailments in folklore medicines in different parts of the world. The present scientific inquiries have led to several drugs being reported as "Rasayana" [6]. Triphala is one such Ayurvedic "Rasayana", commonly prescribed by most healthcare practitioners in India. It is an equiproportional mixture of fruits of three medicinal herbs, amalaki (*Emblica officinalis*), haritaki (*Terminalia chebula*) and vibhitaki (*Terminalia bellerica*). It is mild, non-habit forming and rejuvenative, and hence is recommended for all. According to the Ayurveda, Triphala strengthens the different tissues of the body, prevents ageing, promotes health and immunity. It corrects constipation, cleanses and tonifies the gastrointestinal tract and also detoxifies the whole body and improves digestion and assimilation. It exhibits anti-viral, anti-bacterial, anti-fungal and anti-allergic properties [7]. In the present study, we have evaluated Triphala for its antioxidant and adaptogenic activity.

2. Materials and Methods

2.1 Triphala formulation

Fruits of *Terminalia chebula* Retz. (Combretaceae), *Terminalia bellerica* Roxb. (Combretaceae) and *Emblica officinalis* Gaertn. (Euphorbiaceae) were collected from Sagar District, Madhya Pradesh and were authenticated by Dr. T. N. Shivananda, Senior Scientist, Indian Institute of Horticulture Research, Bangalore, India. Seeds from individual fruits were removed and the dried fruit pulp was crushed to powder using a grinder and extracted using 70 % alcohol. Triphala was prepared from these alcoholic extracts by mixing them in equal proportions (1:1:1) based on formula of Ayurvedic Formulary of India [8].

2.2 In vitro antioxidant activity

2.2.1 Hydroxyl radical scavenging activity

Hydroxyl radicals formed by the oxidation is made to react with DMSO (dimethyl sulphoxide) to yield formaldehyde. Formaldehyde formed can be estimated by Nash reagent [9]. The capacity of the Triphala formulation to scavenge nitric oxide radicals to 50 % was measured in terms of IC_{50}. Various concentrations of Triphala formulation dissolved in water were taken in test tubes. To these, 1 mL of Iron-EDTA solution, 0.5 mL of EDTA and 1 mL of DMSO were added and the reaction was initiated by adding 0.5 mL of ascorbic acid to each of the test tubes. Test tubes were capped tightly and heated on water bath at 80-90°C for 15 minutes. Then the reaction was terminated by the addition of 1 mL of ice-cold trichloro acetic acid to all the test tubes, kept aside for 2 minutes and the formaldehyde formed was determined by adding 3 mL of Nash reagent (2M ammonium acetate, 0.05M acetic acid and 0.02M acetyl acetone) which was left for 10-15 minutes for

colour development. Intensity of yellow colour formed was measured spectrophotometrically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity was calculated according to the following equation.

$$\% \text{ inhibition} = ((A_0-A_t) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (blank, without Triphala) and A_t was the absorbance in the presence of the Triphala.

2.2.2 Nitric oxide radical scavenging activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess reagent [10]. The capacity of the Triphala formulation to scavenge nitric oxide radicals to 50 % was measured in terms of IC_{50}. Sodium nitroprusside (10 mM) in phosphate-buffered saline was mixed with different concentrations of the Triphala formulation dissolved in water and incubated at 25°C for 150 min. The samples from the above were reacted with Griess reagent (1% sulphanilamide, 5% H_3PO_4 and 0.1% N-1-napthylethylene-diamine dihydrochloride).The absorbance of the chromaphore formed was read at 546 nm against control. The % nitric oxide radicals scavenging activity was calculated according to the following equation.

$$\% \text{ inhibition} = ((A_0-A_t) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (blank, without Triphala) and A_t was the absorbance in the presence of the Triphala.

2.3 Animals

Wistar albino rats of the either sex weighing 180-200 g and six to eight week old healthy, laboratory breed Swiss albino mice of either sex, weighing 25 ± 2 g were used for the present study. They were maintained under standard environmental conditions with 12 hr light and dark cycle. Rats were housed in groups of 4 in polypropylene cages and mice were housed in groups of 6 per cage. They were fed with standard pellet diet supplied by Hindustan Lever Ltd. Kolkata, India, and water *ad libitum*. Paddy husk was provided as bedding material, which was changed everyday. The cages were maintained clean.

2.4 Acute toxicity

The study was performed according to the acute toxic classic method (as per OECD guidelines) [11]. Wistar albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the Triphala formulation dissolved in water was administered orally at the dose of 1500 mg/kg and observed for 14 days. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection urinary incontinence, and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion).

2.5 Adaptogenic activity

2.5.1 Swim endurance test

Swiss albino mice (25 ± 2 g) of either sex were used for swim endurance. The normal animals were treated with normal saline (10 mL/kg, p.o.) and other five groups were treated with Triphala formulation (100-500 mg/kg, b.w. p.o. for 14 days), on day 14 mice were allowed to swim in cylindrical container filled with water maintained at 25 ± 2°C till they got exhausted and the moment they drowned was considered as the

endpoint ("Swimming Time") [12]. The time was noted and the data obtained were subjected to statistical analysis.

2.5.2 Anoxic stress tolerance test

Swiss albino mice (25 ± 2 g) of either sex were used for evaluating anoxic stress tolerance test [13]. The normal animals were treated with normal saline (10 mL/kg, p.o.) and other five groups were treated with Triphala formulation (100-500 mg/kg, b.w. p.o. for 14 days). Conical flasks of 250 mL capacity were used for the study. These flasks were made airtight using rubber cork before beginning the experiment. On day 14, 1 hr after the treatment, each animal was kept in the airtight vessel and time was noted using a stopwatch. The moment animal showed first convulsion, it was removed immediately from the vessel and resuscitated if needed. The time duration from the entry of the animal in the hermetic (conical flask) vessel to the appearance of the first convulsion was taken as the time of "Anoxic stress tolerance". The data obtained were subjected to statistical analysis.

2.5.3 Chronic stress induced behavioral despair test

Wistar albino rats (180-200 g) of either sex were used for the study [14]. The Animals were divided in seven groups of six rats each. Group I animals served as control (normal saline 10 mL/kg, p.o.) group II served as stress control (saline 10 mL/kg + chronic stress) and group III-VII animals treated with Triphala formulation (100-500 mg/kg, b.w. p.o. for 14 days). The animals of all the groups except vehicle control were subjected to chronic stress (foot shock), every day for 14 days through a grid floor in a standard conditioning chamber, after one hour of drug administration. The duration of each shock (4mA) was 6 seconds at an interval of 90 seconds for 15 minutes. Rats were forced to swim individually in a cylindrical container (60 x 40 cm, h x d) containing water (maintained at 25°C ± 1°C) upto 30 cm height, which ensured that Rat's feet did not touch the floor of the vessel. The rat was allowed to swim for 10 minutes. Thereafter next 5 minute; the total period of immobility characterized by complete cessation of swimming with the head floating

Table 1. Scavenging effects of Triphala formulation on Hydroxyl radical and Nitric oxide radical formation.

Concentration of sample (µg/mL) % inhibition	Hydroxyl radical scavenging	Nitric oxide radical scavenging
20	35.16	40.12
40	49.12	49.64
50	53.86	52.16
100	59.87	60.60
200	66.66	64.45
300	71.04	69.15
400	76.16	74.48
500	81.06	80.06
IC_{50} value (µg/mL)	40.5	40

Table 2. Effect of Triphala formulation on swim endurance and anoxic stress tolerance test in mice.

Treatment	Dose (mg/kg, p.o.)	Swimming survival time (minutes) Mean ± SEM	Anoxic stress tolerance time (minutes) Mean ± SEM
Control (Normal saline)	10 mL/kg	376.8 ± 7.76	24.30 ± 1.12
Triphala	100	436.14 ± 4.57*	33.00 ± 1.18**
Triphala	200	488.86 ± 4.88**	35.16 ± 1.09**
Triphala	300	549.18 ± 11.16**	37.20 ± 1.30**
Triphala	400	660.42 ± 2.87**	39.11 ± 1.05**
Triphala	500	762.28 ± 7.17**	38.20 ± 1.32**

Significance * $p < 0.01$, ** $p < 0.001$

Table 3. Effect of Triphala formulation on chronic stress induced behavioral despair test in rats.

Treatment	Dose (mg/kg, p.o.)	Time spent in immobility (seconds) Mean ± SEM
Control	10 mL/kg (Normal saline)	52.14 ± 5.58
Control + Chronic stress	10 mL/kg (Normal saline)	144.56 ± 4.43
Triphala	100	102.16 ± 5.32*
Triphala	200	90.26 ± 3.24**
Triphala	300	81.23 ± 1.15**
Triphala	400	66.24 ± 2.15**
Triphala	500	61.11 ± 3.42**

Significance * $p < 0.01$, ** $p < 0.001$

above water level was noted. The mean immobility period of treatment group was compared with that of stress control group. The data obtained were subjected to statistical analysis.

2.6 Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) followed by student's '*t*'-test.

3. Results and Discussion

3.1 In vitro antioxidant activity

3.1.1 Hydroxyl radical scavenging activity

Hydroxyl radicals have been implicated as highly damaging species in free radical pathology. This radical has the capacity to join nucleotides in DNA, cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. Hydroxyl radical scavenging capacity of an

extract is directly related to its antioxidant activity [15]. In the present study, Triphala formulation exhibited potent inhibition of hydroxyl radicals. **Table 1** illustrates the percentage inhibition of hydroxyl radicals by Triphala formulation. The IC_{50} value of Triphala formulation was found to be 40.5 µg/mL.

3.1.2 Nitric oxide radical scavenging activity

Nitric oxide is a free radical generated by endothelial cells, macrophages, neurons etc., involved in the regulation of various physiological processes [16]. Excess concentration of nitric oxide is associated with several diseases [17]. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxy nitrite anions, which act as free radicals. In vitro inhibition of nitric oxide radical is also a measure of antioxidant activity. In the present study, Triphala formulation competes with oxygen to react with nitric oxide and thus inhibits generation of the anions. **Table 1** illustrates the percentage inhibition of nitric oxide generation by Triphala formulation. The IC_{50} value of Triphala formulation was found to be 40 µg/mL.

3.2 Acute toxicity

Triphala formulation did not cause any mortality at the dose tested. None of the rats exhibited any abnormal behavioural responses. No change in skin and fur, eyes and mucous membrane (nasal), respiratory rate, circulatory, autonomic and central nervous system activity has been observed in the treatment group. Further there was no significant difference in the food and water consumption between the treatment group and the control.

3.3 Adaptogenic activity

3.3.1 Swim endurance test

Greater swimming endurance has been reported in mice when pretreated with adaptogenic agents [18] and the test has been utilized to investigate the adaptogenic activity of different agents, based on the fact that swim endurance reflects physical endurance [19]. In our results oral administration of Triphala formulation caused a dose dependent increase **(Table 2)** in swimming performance (762.28 ± 7.17 minute) *($p<0.001$)*.

3.3.2 Anoxic stress tolerance test

Anoxia is a very severe stressor. All the body functions, including cellular respiration depends on oxygen supply to them. Lack of this vital element will cause all the vital functions of the body to cease. Increase in adaptation during this stress by any drug could be considered as its adaptogenic effect [13]. When mice are exposed to a hypobaric environment for a specified period, the mitochondria of heart and brain cells of mice are seriously damaged and brain neurotransmitters, i.e. norepinephrine (NE), dopamine (DA), serotonin (5-HT) and acetylcholine (ACh), are significantly decreased. In our study, pretreatment with Triphala formulation significantly *($p<0.001$)* increased **(Table 2)** the anoxic stress tolerance time (39.11 ± 1.05 minute) in mice in a dose related manner. The effect is probably related to an increase in the cerebral resistance to anoxia and reducing the cerebral consumption of oxygen in anoxic stress. The protective action on anoxic stress mice may be due to the action on the pituitary–adrenal gland axis [6].

3.3.3 Chronic stress induced behavioral despair test

Chronic stressed rodents, when forced to swim in a restricted space become immobile after an initial period of vigorous activity. This immobility signifies behavioral despair, resembling a state of mental depression [20]. In our study, Triphala formulation was able to reverse chronic stress induced indices validated as animal model of depression. The chronic shock treatment caused significant increase in the immobility time,

indicative of depression. Triphala significantly *($p<0.001$)* reduced **(Table 3)** the stress induced increase in the immobility period (61.11 ± 3.42 seconds) in the forced swimming test in rats.

3.4 Conclusion

Our study disclosed the avenue properly for evaluating the therapeutic efficacy of Triphala and substantiated the therapeutic claims documented in ancient Ayurvedic texts by giving solid scientific support. Triphala is capable of increasing the capacity to tolerate non-specific stress in experimental animals as evident from the restoration of parameters studied during different types of stress models. In view of adaptogenic activity, Triphala may find a place for building up non-specific resistance against diverse type of stress. Furthermore, the antioxidant activity of Triphala provide mechanistic basis in relieving stress by way of combating oxidative damage.

On the basis of the results obtained in the present study it can be concluded that Triphala has the potential to scavenge free radicals and it may be a potential candidate in stress related conditions. However, clinical evidences are required to establish the possible correlation among the mentioned activities.

Reference

1. Sabu MC, Kuttan R. (2002) *J. Ethanopharmacol.* 81: 155-160.

2. Susan. (2000) *Metabolism.* 49: 160-62.

3. Larry M. (2000) *Technical Bulletin.* 38: 1-2.

4. Brekhman II, Dardymov IV (1969) *Ann. Rev. Pharmacol.* 9: 419-430.

5. Selye H. (1973) *American Science.* 61: 692.

6. Singh B, Saxena AK, Chandan BK, Gupta DK, Bhutani KK, Anand KK. (2001) *Phytother. Res.* 15: 311-318.

7. Naik GH, Priyadarsini KI, Bhagirathi RG, Mishra B, Mishra KP, Banavalikar MM, Mohan H. (2005) *Phytother. Res.* 19: 582-586.

8. The Ayurvedic Formulary of India Part II (2002). Department of Indian System of Medicine and Homoeopathy: New Delhi.

9. Babu PS, Stanely M, Prince P. (2004) *J. Pharm. Pharmacol.* 56 (11): 1435-1442.

10. Jagetia GC, Shaival KR, Manjeshar SB, Kiran SB. (2004) *Phytother. Res.* 18: 561–565.

11. OECD (2000). Acute oral Toxicity - Acute oral toxic class method. Guideline 423, adopted 23.03.1996. In: Eleventh Addendum to the OECD guidelines for the Testing of Chemicals. Organisation for Economic Co-Operation and Development, Paris.

12. Nimbakar SR, Patki VP, Patki MP. (2001) *Indian Drugs.* 38: 27–39.

13. Tomar VS, Singh SP, Kohli RP. (1984) *Indian Drugs.* 3: 233-235.

14. Kumar V, Singh PN, Bhattacharya SK. (2001) *Indian J. Exp. Biol.* 39: 344-349.

15. Babu BH, Shylesh BS, Padikkala J. (2001) *Fitoterapia.* 72: 272-277.

16. Lata H, Ahuja GK. (2000) *Indian J. Physio. Allied Sci.* 57: 124.

17. Ialenti A, Moncada S, Di-rosa M. (1993) *Br. J. Pharmacol.* 110: 701?706.

18. Singh RH. (1979) *Journal of Research in Indian Medicine, Yoga and Homeopathy.* 14: 49–59.

19. Bhargava KP, Singh N. (1981) *Indian J. Med. Res.* 73: 443-451.

20. Thiebot MH, Martin P, Puech AJ. (1992) *Br. J. Psychiatry Suppl.* 15: 44-50.