International Journal for Pharmaceutical Research Scholars (IJPRS)
ISSN No: 2277-7873
RESEARCH ARTICLE
V-2, I-1, 2013

Anti-fatigue Activity of Extracts of *Syzygium cumini* Leaves

Bhanumathy M*[1], Shivaprasad HN[2], Manohar D[2], Nargund LVG[1]
[1]*Dept. of Pharmacology, Nargund College of Pharmacy, Bangalore, Karnataka, India*
[2] *R&D Centre, Olive Lifesciences Pvt. Ltd., Bangalore, Karnataka, India*
Manuscript No: IJPRS/V2/I1/00005, Received On: 07/01/2013, Accepted On: 09/01/2013

ABSTRACT

In the present study, the anti-fatigue effect of extracts (aqueous, methanolic and ethylacetate) of *Syzygium cumini* leaves was evaluated against swimming endurance followed by post swimming muscle coordination (rota-rod test) and spontaneous motor activity using actophotometer in rats. Animals were treated for 21 days at doses of 200 and 400 mg/kg leaves extracts and these activities were tested using *Withania somnifera* as a standard drug. At the end of the treatment all animals were individually subjected to stress stimuli. Pretreatment rats with test extracts showed dose dependant significant enhancement in swimming endurance time and antifatigue effect in post swimming muscle coordination and spontaneous motor activity. In addition, the test extracts was found to possess normalizing activity against physical stress induced changes in norepinephrine, dopamine and 5-hydroxy tryptamine. The results obtained provide biochemical evidence for antifatigue activity of the tested extracts. Gallic acid was identified by TLC and estimation of total phenolic content in terms gallic acid equivalent was one of the active priniciples responsible for the anti-fatigue activity.

KEYWORDS

Antifatigue, Actophotometer, Physical stress, Rota-rod, Syzygium cumini.

INTRODUCTION

Stress occurs in response to physical, chemical, biological and emotional changes; and is a pattern of metabolic and behavioural reactions. It triggers a wide range of the body changes called General Adaptation Syndrome (GAS) and induce a marked rise in the brain levels of biogenic amines such as adrenaline and nor-adrenaline which assist the organisms to cope with stress. If the stress is extreme, the homeostatic mechanisms of organism become deficit and the survival of organism is threatened. However, increased and prolonged severe stress is responsible for fatigue, reduced stamina and lowered mood; and in the etiopathogenesis of variety of diseases like depression, anxiety, immunosuppression, endocrine disorders, male impotency, cognitive dysfunction, peptic ulcer, hypertension and ulcerative colitis.[1]

Fatigue is a complex phenomenon that can be described as a time-dependent exercise-induced reduction in the maximal force generating capacity of a muscle. Alteration in performance tends to vary across sports that are influenced more or less by factors like decreased muscular power and endurance, decreased motor skill performance and mental lapses.[2] Drugs having antistress properties induce a state of non-specific resistance against stressful conditions. Routinely people use the drugs like benzodiazepines, certain central nervous system stimulants such as amphetamines and caffeine as well as some anabolic steroids to combat stress. The incidence of toxicity and dependence has limited the therapeutic usefulness of above mentioned drugs.[3]

***Address for Correspondence:**
Bhanumathy M.
Department of Pharmacology,
Nargund College of Pharmacy,
Banashankari, Bangalore 560 085, India.
E-Mail Id: bhanugcp@gmail.com

Herbal drugs have been in use for many years not only in Asian countries but also globally for human well-being. The herbal drugs claimed to enhance physical endurance; mental functions and non-specific resistance of the body have been termed as adaptogens. The potential utility of safer and cheaper herbal medicines as antistress agents have been reported as they can withstand stress without altering the physiological functions of the body. Various herbs like *Withania somnifera, Emblica officinalis, Asparagus racemosus, Ocimum sanctum, Tribulus terrestris* and *Piper longum* are claimed to have immunomodulatory, adaptogenic, anabolic effects and the ability to improve vital energy.[3]

Syzygium cumini (*S. cumini*) (L.) Skeels has been attributed in the Indian folklore medicine system to possess several medicinal properties. It is commonly known as jambolan, black plum, jamun and Indian blackberry. All parts of the jambolan can be used medicinally and it has a long tradition in alternative medicine. The bark of the plant is astringent, sweet, refrigerant, carminative, diuretic, digestive, anthelmintic, febrifuge, constipating, stomachic and antibacterial. The fruits and seeds are used to treat diabetes, pharyngitis, spleenopathy, urethrorrhea and ringworm infection. The leaves have been extensively used to treat diabetes, constipation, leucorrhoea, stomachalgia, fever, gastropathy, strangury and dermopathy.[4, 5] People from different parts of the world used *S. cumini* leaves for the treatment of different ailments. But there is lack of literature on antistress activity of *S. cumini* leaves. Hence the study was aimed to evaluate the antifatigue activity of different solvent extracts of *S. cumini* leaves in experimental models.

MATERIAL AND METHODS

Plant Material

The leaves of *S. cumini* were collected in December 2010 from Bangalore, Karnataka, India. Taxonomic identification was conducted by Dr. T.N. Shivananda, Senior Scientist, Indian Institute of Horticulture Research, Bangalore, India. A voucher specimen was deposited in the herbarium of our laboratory under number NCP/65.

Preparation of Plant Extracts from the Dried Leaves of *S. cumini*

Coarse ground leaves of *S. cumini* were charged into three different extractors fitted with a reflux condenser. Solvent (water/methanol/ethylacetate) was added to the extractor and the contents were refluxed for 3 h by maintaining temperature at 75-80^{o} C. The liquid extract was drained from the extractor into a separate vessel and fresh solvent (water/methanol/ethylacetate) was added to the extractor containing the marc. The extraction procedure was carried out for three times as described above and the liquid extracts from each extraction step was subjected to distillation under vacuum (at <75^{o} C) until an extract with a total solid content of 15-20% w/w was obtained. 15-20% w/w total solid solution was then spray dried and sieved (# 40) to get different powder extracts of *S. cumini* leaves.

Thin Layer Chromatography Analysis

Thin layer chromatography (TLC) was performed to identify the presence of phytochemical compounds in aqueous, methanol and ethyl acetate extracts of *S. cumini* leaves. Solvent system was prepared by using toluene, ethyl acetate and formic acid in a proportion of 4.5:4.5:1, respectively.[6] The spots obtained from all the three extracts were examined under ultra violet light of wave length 254 nm.

Track 1: Standard solution (Quercetin/gallic acid)

Track 2: Ethylacetate extract of *S. cumini* leaves

Track 3: Methanol extract of *S. cumini* leaves

Track 4: Aqueous extract of *S. cumini* leaves

Solvent system -Toluene:Ethyl acetate:Formic acid (4.5:4.5:1)

Stationary phase: Silica gel G

Visualization: Under ultra violet light of wave length 254nm.

Estimation of Total Phenolic Content

Total phenolic content of the leaves was estimated based on the Folin-Ciocalteau's method.[7] Gallic acid served as the reference standard and different concentrations of the standard and extracts were prepared in methanol. Then 0.5 ml of the sample was mixed with 2.5 ml of ten-fold diluted Folin-Ciocalteau's reagent and 2 ml of 7.5% sodium carbonate. The mixture was allowed to stand for 30 mins at room temperature before the absorbance was read at 760 nm. The total phenolic contents were expressed as gallic acid equivalents (GAE).

Animals

Wistar albino rats of either sex weighing 125–150 g were used. They were fed standard diet and water ad libitum and housed in cages at room temperature (30±2°C) with a 12 h light and dark cycle.

Dosing Schedule

The animals were divided into eight groups of six animals each. Group I served as vehicle control. Group II served as standard (diazepam 4 mg/kg, b.w.) treatment. Group III – VIII treated with *S.cumini* leaves extracts (aqueous, methanol and ethylacetate extracts) at two different dose levels (200 and 400 mg/kg, b.w.) respectively for 21 days.

Assessment of Activity

Anti-fatigue Test

The animals treated with *S. cumini* extracts/std (*Withania somnifera)*/vehicle were made to swim in a water tank (140X60X45 cm) maintained at room temperature (30±2°C) until they sank. This was recorded as the swimming time. The animals were removed and allowed to recover and dry for about 5 min. The animals were subsequently tested for muscle coordination on a rota rod rotating at 15 rpm and the duration of stay on the rod was recorded. Immediately after that they were tested for spontaneous motor activity in a photoactometer for 05 min. Thereafter, the animals were sacrificed by cervical dislocation; whole brain was dissected to estimate the brain monoamine levels [norepinephrine (NE), dopamine (DA) and 5-hydroxytryptamine (5-HT)].[8]

Estimation of Brain Monoamine Levels in Rats

Brain monoamine levels were estimated spectrofluorimetrically by Das and Guha method.[9] Immediately after collection, brain tissues were washed in ice-cold saline and homogenized in 10 ml acidified butanol. Tissue homogenate (4 ml) was mixed with 10 ml of 10% heptane and 5 ml of 0.003 N HCl. Then the mixture was shaken for 5 minutes and centrifuged at 2000 rpm for 10 minutes. Acid layer (4.5 ml) was eluted and mixed with 100 mg alumina and 1 ml of 2 M sodium acetate. Then the mixture was shaken for 5 minutes and centrifuged at 2000 rpm for 10 minutes and the resultant supernatant was taken for the estimation of 5-HT and the precipitate was used for the estimation of NE and DA.

- ***Estimation of Serotonin (5-HT)***

To the supernatant obtained previously, 3 ml of 10% isobutanol and 2 ml of boric acid buffer (pH 10) was added. After shaking the mixture, 2 ml of 10% heptane was added to the butanol phase and 5 ml of 0.1 N HCL was added followed by shaking again and 1 ml of 0.3 N HCL was mixed. Then it was taken for spectrofluorometric reading of 5-HT.

- ***Estimation of Dopamine and Norepinephrine***

Cold distilled water (5 ml) was added to the precipitate, shaken well and centrifuged at 2000 rpm for 30 seconds. 3 ml of 0.33 N acetic acid was added and centrifuged at 2000 rpm for 3 minutes. Supernatant was transferred to glass stoppered centrifuge tube. 1.2 ml of freshly prepared ethylenediamine and ethylene diammonium dihydrochloride mixture (7:5) was added to it and incubated at 50°C for 40 mins. Mixture was cooled at room temperature and saturated with sodium chloride and 4 ml of 10% isobutanol was added. It was then centrifuged at 2000 rpm for 3 mins. Supernatant was taken for

the estimation of DA and the precipitate was mixed with 4 ml cold distilled water for the estimation of NE.

The fluorescence of 5-HT, DA and NE was measured in the Perkin Elmer MPF 44B Fluorescence spectrophotometer, USA with activation and emission wavelength set at 295 nm and 550 nm (for 5-HT), 320 nm and 370 nm (for DA) and 385 nm and 485 nm (for NE).[9]

STATISTICAL ANALYSIS

All the data were expressed as MEAN ± S.E.M and analyzed statistically by One-Way Analysis of Variance (ANOVA) followed by Dunnett's test. Difference below the probability level 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Mobile phase of TLC was toluene, ethyl acetate and formic acid in the proportion of 4.5:4.5:1, respectively. The spots produced by TLC were observed under UV light (Table 1). Standard solution (quercetin and gallic acid) showed spots with Rf value 0.6 and 0.48 respectively. Aqueous extract showed one spot with Rf value 0.44 which is matching with standard solution gallic acid (blue color). The other two extracts (ethylacetate and methanol) did not showed any spots in the plate. Thus aqueous extract of *S.cumini* leaves containing gallic acid.

Table 1: Results of TLC

Track	Visualization		
	No. of spots	**UV wavelength (254 nm)**	**Rf value**
Standard solution • Quercetin • Gallic acid	01 01	Brown color Blue color	0.6 0.48
Ethyl acetate extract	0	No spot	--
Methanol extract	0	No spot	--
Aqueous extract	01	Light blue	0.44

Total phenolic content in aqueous, methanolic and ethylacetate extracts of *S.cumini* leaves was found to be 10.7% w/w, 10.4% w/w and 9.14% w/w equivalent to gallic acid respectively. The phenolic content found in the extracts was compared with that of the standard gallic acid which may be attributed to poor specificity of the Folin-Ciocalteau method employed. In addition, phenolic compounds depending on the number of phenolic groups respond differently to the Folin-Ciocalteau reagent.[7]

Extracts of *S.cumini* leaves at doses 200 and 400 mg/kg exhibited significant increase in swimming performance time in rats in dose related manner. In post swimming muscle coordination (anti-fatigue) test, extracts of *S.cumini* leaves increased the duration (sec) of stay on rota-rod in rats which was also dose dependant. Actophotometer reading for spontaneous motor activity (anti-fatigue test) was increased in rats treated with extracts of *S.cumini* leaves which were also dose dependant (Table 2).

Stress alters the normal functioning of the body in special contrivance, when an animal forced to swim becomes immobile after an initial period of vigorous activity. This resembles a state of mental depression and causes severe fatigue.[10] The results of the present study showed that pre-treatment with extracts of *S.cumini* leaves increased labor efficiency as evident by the increase of swimming performance and also offered significant post swimming antifatigue effect in rats. Thus the study indicating anti-fatigue potential of extracts of *S.cumini* leaves.

Pretreatment with extracts of *S.cumini* leaves was found to prevent the stress induced depletion of norepinephrine and dopamine levels thus helping the animals to cope up better during stress (Table 3). Increase in brain norepinephrine and dopamine levels after treatment with extracts indicate stimulation of sympathetic outflow as a result of stress. Pretreatment with extracts was found to significantly reduce the stress induced rise in brain serotonin level by preventing the alarm

Table 2: Effect of *S. cumini* Leaves on Anti-fatigue Test in Rats

Groups	**Swimming time (sec)**	**Rota-rod test (falling of time in sec)**	**Spontaneous motor activity (Actophotometer score)**
Control	176.5±1.73	16.67±0.66	79.50±0.76
Std (*Withania somnifera* 50 mg/kg)	221.7±0.88***	34.50±0.76***	167.7±2.2***
Aqueous extract (200 mg/kg)	191.3±0.66***	24.33±1.63***	131.5±0.76***
Aqueous extract (400 mg/kg)	211.0±0.57***	27.0±0.57***	141.7±0.88***
Methanolic extract (200 mg/kg)	185.5±1.33***	20.83±0.94**	112.3±0.71***
Methanolic extract (400 mg/kg)	206.2±0.60***	24.17±0.79***	124.3±0.88***
Ethylacetate extract (200 mg/kg)	182.2±1.24***	19.17±0.60	80.33±0.88
Ethylacetate extract (400 mg/kg)	200.3±0.61***	21.83±0.60***	101.3±0.76***

Values are mean ± SEM (n=6). **p<0.01 and ***p<0.001 as compared to control

Table 3: Effect of *S. cumini* Leaves Pretreatment on Brain Monoamine Levels Determined After Physical Stress

Treatment	**Concentrations ng/g of brain tissue**		
	Nor-adrenaline	Dopamine	5-Hydroxy tryptamine
Normal	366.7±0.88	175.0±1.06	189.7±1.22
Stress control	306.5±0.99	130.5±0.99	259.3±0.88
Std (*Withania somnifera* 50 mg/kg)	464.2±1.11***	240.5±1.12***	164.0±0.96***
Aqueous extract (200 mg/kg)	350.7±0.88***	164.8±1.16***	210.3±0.88***
Aqueous extract (400 mg/kg)	364.7±1.05***	180.8±1.07***	194.2±0.94***
Methanolic extract (200 mg/kg)	334.5±0.76***	155.3±1.05***	199.0±2.55***
Methanolic extract (400 mg/kg)	355.5±0.76***	164.8±1.30***	190.5±0.76***
Ethylacetate extract (200 mg/kg)	325±0.853***	144.5±1.11***	195.5±0.99***
Ethylacetate extract (400 mg/kg)	344.8±1.16***	161.8±1.49***	174.8±1.16***

Values are mean ± SEM (n=6). ***p<0.001 as compared to stress control

reaction, thereby arresting the genesis of stress related disorders (Table 3). Serotonin is widely distributed monoamine in brain and involved in mood and impulse control. Different stressors like immobilization or restrain stress, foot shock lead to increased synthesis/metabolism of serotonin in limbic regions.[11] Thus measurement of brain monoamines indicated that extracts of *S.cumini* leaves can possess antistress activity.

CONCLUSION

Extracts of *S.cumini* leaves displayed anti-fatigue potential against physical stress model followed by post-swimming muscle co-ordination and spontaneous motor activity on experimental animals. The results suggest that administration of extracts of *S.cumini* leaves is capable of increasing the capacity to tolerate non-specific stress in experimental animals as evident from the restoration of a large number of parameter studied during swimming endurance and post swimming anti-fatigue tests. Gallic acid was identified by TLC and estimation of total phenolic content in terms gallic acid equivalents was one of the active priniciples responsible for the anti-fatigue activity. Further studies may be carried out to identify and characterize the active principles responsible for the activity.

REFERENCES

1. Joshi T, Sah SP, Singh A, "Antistress activity of ethanolic extract of *Asparagus racemosus* Willd roots in mice", Indian Journal of Experimental Biology, 2012, 50(6), 419-424.
2. Zhang XL, Ren F, Huang W, Ding RT, Zhou QS, Liu XW, "Anti-fatigue activity of extracts of stem bark from *Acanthopanax senticosus*", Molecules, 2010, 16(1), 28-37.
3. Desai SK, Desai SM, Navdeep S, Arya P, Pooja T, "Antistress activity of *Boerhaavia diffusa* root extract and a polyherbal formulation containing *Boerhaavia diffusa* using cold restraint stress model", International Journal of Pharmacy and Pharmaceutical Sciences, 2011, 3(1), 130-132.
4. Ayyanar M, Subash babu P, "*Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses", Asian Pacific Journal of Tropical Biomedicine, 2012, 240-246.
5. Ruan ZP, Zhang LL, Lin YM, "Evaluation of the antioxidant activity of *Syzygium cumini* leaves", Molecules, 2008, 13(10), 2545-2556.
6. Ramachandran AP, Prasad SM, Samarakoon SM, Chandola HM, Harisha CR, Shukla VJ, "Pharmacognostical and phytochemical evaluation of Vara Asanadi Kwatha", Ayurveda, 2012, 33(1):130-35.
7. Omoregie ES, Osagie AU, Iruolaje EO, "In-vitro antioxidant activity and the effect of methanolic extracts of some local plants on nutritionally stressed rats", Pharmacologyonline, 2011, 1, 23-56.
8. Ahmad G, Amin KM, Khan NA, Tajuddin, "The anti-stress activity of a gem-containing Unani formulation against diverse stressors", Journal of Ethnopharmacology, 1998, 59(3), 187-193.
9. Das S, Guha D, "Spinacia oleracea retards the development of Amygdala kindled epilepsy in rats", Al Ameen Journal of Medical Sciences, 2011, 4(2), 175-190.
10. Nataraj GR, Nanjappaiah HM, Shivakumar Hugar, "Evaluation of adaptogenic potential of *Hibiscus cannabinus* in acute stress induced mice", Pharmacologyonline, 2011, 2, 508-513.
11. Nesari T, Parchure SN, Mhetre N, Ambavade S, Bodhankar SL, "Study of Shankhapushpi on spontaneous locomotor activity and anoxic stress in rodents", Pharmacologyonline, 2011, 2, 253-262.