



Evaluation of Phytochemical and Antistress Activity of *Averrhoa carambola* L.

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Article History	Abstract
Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 20 Oct 2023	<p><i>In India, the medicinal plant Averrhoa carambola L. has been utilised for centuries to cure a wide range of illnesses and problems. The goal of the current study was to assess the ethanolic seed extract of Averrhoa carambola L. (EEAC) for its phytochemical and anti-stress properties. Flavonoids were identified as the main phytoconstituents by EEAC. Studies on acute toxicity were conducted on albino mice. At a level of 2000 mg/kg body weight, the ethanolic extract did not have a deadly effect, and no abnormalities or deaths were seen for a 14-day period. The EEAC effect and the gold standard Ashwagandha were evaluated. The anti-stress potential of this plant was examined using swimming endurance tests, anoxic stress tolerance tests and immobilisation stress models. The findings show that 100 mg/kg, 200 mg/kg, and 400 mg/kg dosages of an ethanolic extract of Averrhoa carambola L. seed significantly reduced stress levels. Based on the findings, it was determined that Averrhoa carambola L. has strong anti-stress properties.</i></p>
CC License CC-BY-NC-SA 4.0	<p>Keywords: Anoxia stress tolerance test, Swimming endurance test model, Immobilization stress, Averrhoa carambola L.</p>

1. Introduction

Stress disorders are on the rise in modern society, and the average person is constantly exposed to potentially stressful situations. ¹ Currently, stress is a frequent medical condition with major societal consequences that calls for logical pharmacotherapeutic treatment. Increased blood levels of corticosteroids and adrenaline are two of the most important pathogenic mechanisms of stress. The hypothalamic-pituitary-adrenal and sympathoadrenal systems are also activated. Stress impairs the function of proteins, nucleic acids, and other cell components, which may cause apoptosis. It also disrupts the metabolism of lipids as a free radical created by lipid peroxidation. ²

Stress overload causes free radicals to proliferate and damages various organs, including neural receptors. Agents that scavenge free radicals may be very effective in treating various illnesses and diseases. Stress is essentially the body and mind's response to a disturbance in equilibrium. ³

Stress is harmful to the body when it becomes life-threatening, thus it needs to be handled. Psychiatric illnesses like Disruption in thought processes, erectile dysfunction in men, stomach ulcers, diabetes, mellitus, and ulcerative colitis are all endocrine illnesses, as are depression, immunosuppression, anxiety, and hypertension are just a few of the diseases that are complicatedly triggered by stress. ⁴ The endocrine system's ability to release a lot of glucocorticoids and catecholamines is enhanced by stress. Continuous stress causes aberrant physiological changes that result in cognitive and psychological impairment. ⁵

A significant advancement in chemical and pharmacological research in recent years has added to our understanding of novel therapeutically active molecules derived from natural sources. These substances can be employed as direct leads for the creation of novel medications or as pharmacological resources for the identification of fresh active ingredients. Protection of the cell membrane from lipid peroxidation helps to minimize several pathologies such as aging, diabetes, atherosclerosis, and ischemic heart diseases. In traditional systems of medicine like Ayurveda, Siddha, Unani and Homeopathy, plant parts have been used to promote the general health of human being. Many medicinal plants have shown a

state of nonspecific increase of resistance against stress in experimental animals. *Panax ginseng* is the most prominent plant as the antistress agent.⁶

For the management of stress, a number of plants are employed, including *Aegle marmelos* (*Rutaceae*), *Allium sativum* (*Allicaceae*), *Hibiscus cannabinus* (*Malvaceae*), *Eugenia caryophyllus*, etc. Herbal remedies are safer and less expensive than pharmaceuticals, and some believe they could be effective anti-stress agents because of their ability to mitigate stress without altering physiological processes.⁷

In the current work, based on its traditional use in Ayurveda, the dried seed of *Averrhoa carambola* Linn is being studied here for its phytochemical and anti-stress activity using validated mouse models.

2. Materials And Methods

Collection of Plant Materials:

The seeds of *Averrhoa carambola* L. were isolated from their fruit capsules which was collected from local area of Raebareilly, Uttar Pradesh in mid-September and were authenticated from Department of Pharmacognosy & Phytochemistry, Integral University, Lucknow (Ref. No-IU/PHAR/HRB/19/13). All relevant departmental herbaria now have the voucher specimen on file.

Preparation of Extract:

Use the seeds after drying them in the shade. *Averrhoa carambola* L. seeds were coarsely crushed and macerated for 72 hours with ethanol. The spent solvent was replaced with fresh solvent every 24 hours. During the extraction process, there was some shaking. Using a rotary evaporator, the obtained extract was filtered, pooled, and concentrated under vacuum.⁸

Preliminary phytochemical screening:

Test extract underwent preliminary phytochemical screening to detect phytoconstituents using standard procedures described in the literature.⁹

Chemicals and reagents: All the chemicals and reagents utilised were of the highest grade available for analytical study.

Experimental Animals:

Swiss Albino mice in the experiment, either weight about 20 - 30 gm of each sex were used. Animals were acclimated for ten days under regular husbandry conditions after being randomly divided into different groups.

- Room temperature: $27 \pm 3^\circ$
- Relative humidity: $65 \pm 10\%$,
- 12 hr light/dark cycle

All of the animals were kept in strict hygienic conditions and fed a rodent pellet diet along with unlimited access to water. The Institutional Animal Ethics Committee (IAEC) approved the study procedure before the experiment was conducted. The Institutional Animal Ethics Committee gave its previous clearance for the animal experimentation with the approval number ACPR/IAEC/025/2020.

Acute Toxicity Study (LD₅₀):

Averrhoa carambola L. ethanolic seed extract's acute toxicity was assessed in accordance with OECD Guideline No. 423. (Organization for Economic Cooperation and Development). Even at a dose of 2000 mg/kg body weight, it was found that the test extract was not lethal. As a result, the doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg were chosen for additional research. Albino mice were fasted for 12h. Toxicity was assessed by observing mortality within 48 hours and delayed toxicity for further 14 days.

Evaluation of anti-stress activity of ethanolic seed extract of *Averrhoa carambola* Linn

Swimming Endurance Test :

Male and female Swiss albino mice weighing 20-30 gm were separated into five groups of six mice each for the experiment.

Group I - Control,(CMC)

Group II - Std. (Ashwagandha 100mg/kg, p.o.)

Group III - EEAC (100 mg/kg, p.o.)

Group IV – EEAC (200 mg/kg, p.o.)

Group V – EEAC (400mg/kg, p.o.)

The seven-day ethanolic extract of *Averrhoa carambola* L. (EEAC) (100 mg/kg, 200 mg/400 kg, p.o.) pre-treatment regimen was administered to the control groups. On day 7, 1 hour after the administration of the extracts, all the animals were subjected to swimming stress by being housed in a polypropylene vessel measuring 45 x 40 x 30 cm with a water level of 20 cm, and the immobility period of each mice was measured for 30 minutes.¹⁰

Anoxic Stress Tolerance Test:

The mice were all Swiss albino strains and ranged in weight from 20-30 gm. were separated into five groups of six mice each for the experiment.

Group I -Control, (CMC)

Group II - Std. (Ashwagandha 100mg/kg, p.o.)

Group III - EEAC (100 mg/kg, p.o.)

Group IV – EEAC (200 mg/kg, p.o.)

Group V – EEAC (400mg/kg, p.o.)

The pre-treatment regimen for the treatment groups included EEAC (100 mg/kg, 200 mg/400 kg, p.o.). Each mouse was separately treated to anoxic stress on the seventh day 1 hr after the administration of the extracts, by being kept in a small, sealed 500 ml glass jar. At the end point, it was determined how long it took the mice to experience their first clonic convulsion. The animals were taken out of the boat right away for recovery and, if necessary, resuscitation.¹¹

Immobilization Stress Test:

For this study, we chose Swiss albino mice (20-30 g) of either sex and randomly assigned them to into six groups of six mice each for the experiment.

Group I - Normal control (CMC)

Group II - Stress control (CMC)

Group III - Std. (Ashwagandha 100mg/kg, p.o.)

Group IV - EEAC (100 mg/kg, p.o.)

Group V – EEAC (200 mg/kg, p.o.)

Group VI – EEAC (400mg/kg, p.o.)

The therapy was administered as described above for 12 days. On day 12, one hour after the last treatment, the forelimbs and hind limbs of the mice were tied with adhesive tape thereby immobilizing them for 2 hrs. After the induction of stress for 2 hrs, the adhesive tapes were removed and blood was collected from retro orbital plexus of the stressed and non-stressed rat.¹²

Statistical analysis:

ANOVA and the Tuckey Test were used in the statistical analysis of the findings from the current experiment. The results are given as Mean ± SEM.

3. Results and Discussion

Preliminary Phytochemical Screening:

Initial phytochemical testing showed the presence of a variety of bioactive substances, including alkaloids, carbohydrates, phenolic compounds, and flavonoids.

Table 1: Preliminary phytochemical screening of EEAC

S.N.	Chemical Test	Ethanolic Extract
1	Test for Alkaloid	
	a) Mayer’s reagent	+
	b) Wagner reagent	+
2	Test for Amino Acids	
	a) Ninhydrin test	+

3	Test for Carbohydrates	
	a) Molisch's test	+
	b) Fehling's Test	+
4	Test for Glycosides	
	a) Borntrager's test	+
	b) Legal test	+
5	Test for Phenolic compounds and Tannins	
	a) Ferric Chloride test	+
	b) Gelatin test	+
	c) Lead acetate test	+
	d) Alkaline reagent test	+
	c) Magnesium & Hydrochloric acid reduction	+
6	Test for Flavonoids	
	a) Shinoda Test	+
	b) Lead Acetate test	+
7	Test for phytosterols	
	a) Libermann-Burchard's test	+
8	Test for Proteins	
	a) Millon's test	+
	b) Biuret test	+
9	Test for Saponins	-

Note: (+) means present and (–) means absent

Swimming endurance test in mice:

Mice were given graded dosages of the test extract (100, 200, and 400 mg/kg) for seven days, on seventh day after dosing the animal were subjected to swim. There was a dose-dependent substantial decrease in the immobility time observed. It was discovered that swimming performance time decreased proportionately. However, it was discovered that the test extract's impact on immobility time was less powerful than that of the reference standard medication, Ashwagandha. Table -2 presents the results in tabular form.

Table 2: Effect of EEAC on Immobility time in Swimming Endurance Test

S. No.	Treatment	Duration of Immobility in Sec
1.	Control (CMC)	422.33±1.55
2.	Ashwagandha	241.33±2.29
3.	EEAC (100 mg/kg, p.o.)	322.33±1.55
4.	EEAC (200 mg/kg, p.o.)	298.50±2.29
5.	EEAC (400 mg/kg, p.o.)	258.33±1.57

Anoxic stress tolerance time in mice:

The test of mice's ability to tolerate hypoxia measured success based on how long their clonic convulsions lasted. On the seventh day, after dosing animals subjected to anoxic tolerance test. Clonic convulsions were significantly delayed in the graded doses of the test extract (100, 200, and 400mg/kg) compared to the control group. Table 3 presents the findings.

Table-3 Effect of EEAC on Latency of convulsion in Anoxic Stress Tolerance Test-

S.N.	Treatment	Time of Convulsion in min
1.	Control (CMC)	41.40±0.79
2.	Ashwagandha	81.67±1.54
3.	EEAC (100 mg/kg, p.o.)	45.54±1.42
4.	EEAC (200 mg/kg, p.o.)	59.17±1.07

5.	EEAC (400 mg/kg, p.o.)	66.54±1.82
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Immobilization Stress:

Effect on biochemical parameters:

The concentration of numerous biochemical indicators in serum was negatively impacted by immobilisation stress. Contrasted with the usual control group, the immobilisation stress-induced mice had significantly higher serum levels of glucose, triglycerides, and cholesterol as well as a cortisol level. Comparing stress control mice to animals pre-treated for 12 days with test extract at different dose levels (100, 200, and 400 mg/kg), all biochemical parameters noteworthy and dose-dependently decreased. Table-4 presents the findings.

Table-4 Effect of EEAC on biochemical parameters in Immobilization Stress test-

S.N.	Treatment	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Cortisol (µg/dl)
1.	Control (CMC)	84.25±1.42	60.37±1.47	45.07±1.01	11.00±0.62
2.	Stress Control (CMC)	169.00±1.82	138.00±1.25	102.17±3.07	18.17±0.91
3.	Ashwagandha	86.67±3.60	70.00±1.06	55.04±1.19	4.54±0.26
4.	EEAC (100 mg/kg, p.o.)	134.40±1.91	109.63±1.72	89.23±1.09	9.68±0.27
5.	EEAC (200 mg/kg, p.o.)	121.41±1.86	90.54±1.44	82.57±1.07	8.50±0.51
6.	EEAC (400 mg/kg, p.o.)	98.25±1.41	83.75±1.27	65.57±1.49	6.17±0.40

Stress is a significant issue that affects people's health and organ systems on a global scale. Anxiety, behavioural depression, cognitive impairment, elevated corticosterone and glucose levels, immunological suppression, stomach ulcers, and elevated oxidative stress have all been linked to prolonged stress. Short periods of stress have no negative effects on the body. Many therapeutic plants demonstrate efficacy against established stress, which led to harmful conditions in the body.

Chronic exposure of organism to stressful situation produces oxidative damage which results into lipid peroxidation and development of various human pathological states. Brain consumes high level of oxygen and contains high amount of PUFA, which all make it very susceptible to oxidative stress induced damage by oxygen free radicals.

The most popular technique for assessing a novel compound's antistress properties is the swimming endurance test. When forced to swim in a small space, mice show signs of stress by becoming motionless after a brief time of strong exercise. Mice that have been pre-treated with EEAC exhibit a notable reduction in immobility and an improvement in swimming time.

This method stems from the realisation that captive aquatic animals developed a recognisable, motionless stance over time. Idleness is a physical manifestation of mental states such as exhaustion, fatigue, low stamina, or depression (hopelessness). People who are under a great deal of pressure often display these signs.

The availability of oxygen is essential to every aspect of bodily physiology, including cellular respiration. When an essential component is missing, all physiological mechanisms will become unbalanced. Any medication that promotes tolerance can function as an adaptogenic agent, increasing adaptability during stress by depleting any critical components. It is thought that adaptogens increase non-specific resistance in order to have their therapeutic effects during stressful situations. In this study, animals go into convulsions when oxygen levels in hermetic vessels fall too low, and pre-treatment with EEAC enhanced stress tolerance, showing their anti-stress function.

The anti-stress property of EEAC was also assessed using the anoxic tolerance test based on the lengthening of the latency to anoxic convulsions in mice. Convulsive events during the anoxic tolerance test are a result of acute oxygen deprivation and carbon monoxide build-up in the brain. Because the brain cells are so susceptible to oxygen deprivation and elevated levels of carbon monoxide, these conditions can cause neuronal cell death and function loss. It is known that substances with adaptogenic activity cause mice to experience anoxic convulsions more slowly.

As evidenced by the longer anoxic stress tolerance period in mice, our findings demonstrated that EEAC has anti-stress activity. Increased cerebral resistance and effective oxygen utilisation during the acute hypoxic stress response in mice may be factors in the anti-anoxic action of EEAC. More experimental analysis and validation are required, nevertheless.

Hyperglycaemia developed in experimental rats subjected to immobilisation stress because the adrenal cortex secretes too much cortisol under stressful circumstances. The processes of gluconeogenesis and lipogenesis are kept in check by excessive cortisol release. The current study's findings showed that the EEAC had a positive impact on regulating hyperglycaemia, preventing changes to the adrenal cortex, and assisting in the maintenance of homeostasis.

Majorly the abnormal level of blood glucose and triglyceride were due to adrenal gland activity by increasing the activity of cortisol in the blood. The adrenal gland also increases the level of catecholamines consider as stress hormones which affect the concentration of blood sugar, cholesterol and triglycerides by gluconeogenesis and lipogenesis mechanism. Increase in the level of blood glucose, cholesterol and triglyceride were observed in the present study due to restraint stress. A similar effect also reported by many researchers in their study. Administration of EEAC significantly reduced the increased level of blood parameters as evidence of anti-stress activity.

Adrenocorticotrophic hormone (ACTH) is released in response to stress; it stimulates the adrenal cortex, leading to increased cortisol production and secretion. A rise in plasma cortisol causes an increase in blood glucose, total proteins, cholesterol, and triglycerides, all of which contribute to the mobilisation of fat and carbohydrate stores. The changes in these biochemical levels brought on by stress were greatly reduced by pre-treatment with the EEAC.

Most of the herbal treatments' effectiveness is credited to a variety of active principles working together. Seeds contained alkaloids, carbohydrates, phenolic chemicals, and flavonoids, according to the results of a phytochemical screening. So, it is likely that the constituents that are abundant in the extracts may help explain some of the antistress impact that has been noted.

4. Conclusion

The rapid industrial growth and the drastic increase in the world population has a direct impact on environment and society thus making the human being easily vulnerable to stress conditions. Global search is on, for the development of an effective anti-stress drug from natural source which can effectively tone up the disturbed physiological conditions of the human beings affected by such stress problem. The non-specifically increased resistance produced by the *Averrhoa carambola* Linn seeds extract may account for the observed antistress activity. This may have occurred because the extract inhibited central and peripheral hypothalamic-pituitary-adrenal axis desensitisation (HPA). *Averrhoa carambola* Linn. seed extract has a stress-reducing and antioxidant effect.

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