An Experimental Evaluation of Anti-stress Effects of *Terminalia chebula*

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Abstract

Background: The fruit of *Terminalia chebula* (Combretaceae) has been used as traditional medicine against various human ailments, and has been extensively used in Ayurved, Uanai and Homeopathic medicine. The fruit was one of the Ayurvedic herbs used for the adaptogenic/anti-stress potential. The objective of the study was to investigate anti-stress activity of ethanolic extract of *T. chebula* fruits.

Methods: Anti-stress activity was evaluated in various animal models, namely anoxia stress tolerance and forced swimming test in mice, as well as cold resistant stress and immobilization test in rats. Adult male Wistar rats (200-250 g) and Swiss albino mice (25-30 g) were used in the study. *Withania somnifera* powder was taken as reference drug. The vehicle (1 ml/100 g), *W. somnifera* (100 mg/kg) and *T. chebula* (200 and 500 mg/kg, respectively) were administered orally 1 hour prior to study.

Results: The ethanolic extracts of *T. chebula* significantly increased the swim endurance and anoxia stress tolerance time. Cold resistant stress and immobilization stress altered the various biochemical parameters like glucose, cholesterol, triglycerides, blood urea nitrogen (BUN), plasma corticosterone, blood cell count (RBC and WBC) and weight of organs like liver, spleen, testis, and adrenal glands. The extract reduced stress-induced elevated levels of serum biochemical parameters, blood cell count, prevented alterations in the weight of the liver, adrenal gland and increased the weight of the spleen.

Conclusion: *T. chebula* exhibited anti-stress activity by preventing stress-induced elevated levels of biochemical and hematological changes and the alteration in organ weights.

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Keywords: *anti- stress, biochemical parameters, plasma corticosterone level, Terminalia chebula*

An adaptogen is an herb product that is a plant derivative. They are said to increase resistance to stress, trauma, anxiety and fatigue. The term is used mainly by herbalists who also refer to adaptogens as rejuvenating herbs, tonics, rasayanas, or restoratives. One specific characteristic of adaptogen action is that its effect is believed to help the body return to a balanced state. *Adaptogenic herbs* are distinct from other substances in their ability to balance endocrine hormones and the immune system, and that they help the body to maintain optimal homeostasis.[1] The constituents of the adaptogens contains triterpenoid saponins (dammarane triterpene saponins, cucurbitacins), phytosterols (beta-sitosterol), phytoecdysteroids (20-ecdysone, turkesterone), flavonoids (glucopyranosides, prenylated flavonoids, flavan glycosides), hydroxylated fatty acids (octadecadienoic acid).[2,3] Biological stress is a response to physical, chemical, biological and emotional changes, consisting of a pattern of metabolic and behavioral reactions that helps strengthen the organism.[4] During stressful situations, the energy requirement of the organism is increased, resulting in enhanced generation of free radicals.[5] Chronic stress can significantly affect many of the body's immune systems, as can an individual's perceptions of, and reactions to, stress. Chronic stress is seen to affect parts of the brain where memories are processed through and stored. When people feel stressed, stress hormones get over-secreted, which affects the brain. This secretion is made up of glucocorticoids, also known as cortisol, which are steroid hormones that the adrenal glands release.[6,7]

Terminalia chebula (Retz) *(T. chebula)* possesses antioxidant, hepatoprotective, antiviral and antibacterial, laxative, hypolipidemic[8,9] and anti-ulcer activities.[10] The plant is an important constituent of an herbal formulation with the name Triphala®, a very popular traditional medicine for chronic disorders like diabetes.[11,12] *T. chebula* is one of the ingredients in a polyherbal formulation, "Geriforte," an Ayurvedic Rasayana that is known to promote physical and mental health and improve immune power of the organism so that the body can tolerate any nature of stress.[8,13]

Fruits of *T. chebula* are a rich source of gallic acid-based secondary metabolites (20-36%). Major constituents are chebulagic acid, chebulinic acid, and chebulic acid; other constituents are tannic acid, gallic acid, ethyl gallate, ellagic acid. Other compounds identified in the fruits are: shikimic acid and related compounds (quinnic acid, dihydroshikimic acid, 5-dehydroshikimic acid), sugar

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(arabinose, fructose, sucrose), triterpenoids (chebupentol, terminoic acid, arjugenin), and steroids (β-sitosterol, daucosterol).[12]

However, detailed investigations of anti-stress activity of *T. chebula* had not been carried out so far. Hence this leads us to study the anti-stress activity of *T. chebula* in different stress-induced animal models.

Materials and Methods

Collection of plant material

The fruits of *T. chebula* was collected from Nagaon district (Assam, India) and identified and authenticated by Dr. N. Shiddamallayya, Regional Research Institute (Ay.) Ashoka Pillar, Bangalore and preserved in the herbarium (specimen voucher No. 285-RRI/BNG/SMP/Drug auth/2007-08).

Preparation of extract

The fruits of *T. chebula* were separated, shade dried and coarsely powdered. The powdered plant material was then subjected to successive extraction with petroleum ether, chloroform and ethanol solvents (500 ml/100 g of dried powder) for 18 h in a Soxhlet extractor. After extraction, the dark green solution obtained was evaporated at 45°C under reduced pressure till a viscous mass material was obtained. The yield of the petroleum, chloroform and ethanolic extracts were found to be 5 %, 2.5 %, and 36 % (w/w), respectively. The dried ethanolic extract was stored in an airtight container and placed in a refrigerator. The test compound was prepared by dissolving the ethanolic extract of *T. chebula* powder in water before the experimental study.

Animals

Experimental protocols were approved by our Institutional Ethical Committee following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Swiss albino mice of both sexes (25-30 g) and adult male Wistar rats (200-250 g) were employed in the study. Mice and rats were housed five to six per cage at constant temperature (22 ± 2°C) and 12-h light/12-h dark cycle; they were fed standard laboratory food and water was given *ad libitum*.

Phytochemical analysis of the extract

The preliminary phytochemical screening was carried out on the petroleum ether, chloroform, and ethanolic extracts of the fruits of *T. chebula* for qualitative identification by the standard method described by Khandelwal.[14]

Acute toxicity studies

Acute toxicity studies were determined by using fixed dose method according to OECD guidelines.[15] Healthy adult Swiss albino mice, weighing 25-30 g, were used.

Stress procedures

Different stress procedures were used to evaluate the anti-stress activity of *T. chebula*

Swimming endurance test in mice

Albino mice were divided into four groups of six animals each. Stress was imposed on the mice by keeping them in cylindrical vessels (length 48 cm and width 30 cm) filled with water to a height of 25 cm and the total swimming time for individual mice was noted, the mice were allowed to swim daily until exhausted. Vehicle (Group I, stressed control), extract of *T. chebula* (200 and 500 mg/kg, orally for Group II and III) or *Withania somnifera* (Group IV) was given to mice once daily for 7 days. On the seventh day, one hour after treatment, all the mice were subjected to swimming endurance test. The mice were allowed to swim individually in a propylene tank, filled with water to a height of 25 cm maintained at room temperatures. The end point of the test was death of the mouse due to drowning, and swimming time of each mouse was noted.[16] The mean swimming time for each group was calculated.

Anoxia stress tolerance in mice

Swiss albino mice were divided into four groups of six animals each and treatment was given similar to the Swimming endurance test. Extract of *T. chebula* or standard drug was given to the mice once daily for 21 days. At the end of the 1st, 2nd and 3rd weeks, i.e. on the 7th, 14th and 21st days, one hour after the treatment, stress was induced in all mice by placing each animal individually in a hermetic vessel of 500 ml capacity to record anoxia tolerance time. The moment when the animal showed the first convulsions, it was immediately removed from the vessel and resuscitated if needed. The time duration between animal entry into the hermetic vessel and the appearance of the first convulsion was taken as the time of anoxia tolerance. Appearance of convulsion was a very sharp end point.[17]

Cold resistant stress

Group I animals served as unstressed control were administered vehicle (1 ml/100 g) and were not exposed to stress, Group II animals served as stressed control, Group III and IV animals were administered extract of *T. chebula* (200 and 500 mg/kg, p.o., respectively) once daily for 10 days and Group V received a standard drug *W. somnifera* (100 mg/kg/day for 10 days). The stress was induced by exposing animals to cold environment 4 ± 1°C for 4 h. This procedure was repeated for 10 days at a specific time period between 10:00 am to 2:00 pm. On the 11th day, blood was collected from retro-orbital plexus under light ether anesthesia. Serum and plasma was separated for estimation of serum glucose, cholesterol, triglycerides, blood urea nitrogen (BUN), blood cell count (RBC and WBC), and plasma corticosterone. Later animals were sacrificed by cervical dislocation at the end of a specified period. The weight of organs such as liver, spleen, testis and adrenal gland were recorded per 100 g body weight of the animal.[17,18]

Immobilization stress

The groups were categorized similar to the cold resistant stress model; vehicle, extract or standard drug were administered orally, once daily, for seven days. The stress was produced by restraining the naive animals inside an

adjustable acrylic hemi-cylindrical plastic tube (4.5 cm diameter, 12 cm long). The rats were confined individually and exposed continuously for a period of 150 minutes once daily for seven consecutive days. On the 7th day, immediately after the last exposure to stress, blood was collected from retro-orbital plexus under light ether anesthesia and serum and plasma were separated for biochemical estimation. The animals were sacrificed at the end of a specified period and the weight of organs were noted.[19]

Statistical Analysis

All the values are expressed as mean ± SEM. Statistical differences were determined by one-way ANOVA followed by Dunnett's post hoc test. $P < 0.05$ was considered significant. The statistical analysis was performed using Instat® software (GraphPad Software, La Jolla, CA, USA).

Results

Preliminary phytochemical screening revealed that petroleum ether extract showed positive for phytosterols and fixed oils. The chloroform extract showed positive for the tannins. Ethanolic extract showed positive for the presence of carbohydrates, anthraquinone glycosides, saponin glycosides, triterpenoids, saponins, tannins, polyphenols, proteins, amino acids and flavonoids. Acute oral toxicity studies of the ethanolic extract of *T. chebula* did not exhibit any sign of toxicity up to 2,000 mg/kg body weight. Since there was no mortality of the animals found at the highest dose, hence 200 (1/10th of 2,000) and 500 (1/4th of 2,000) mg/kg doses of extract were selected for evaluation of anti-stress activity.

Effect of *T. chebula* on swimming endurance test in mice

Ethanolic extract of *T. chebula* induced a striking increase in swimming time in mice in a dose dependant manner. The extract significantly enhanced the percentage increase in swimming endurance time over stressed control animals (Figure 1) by 26.63 % and 51.09 % in 200 and 500 mg/kg extract treated groups respectively.

Effect of *T. chebula* on anoxia stress tolerance in mice

The results are represented in Figure 2.The effect of *T. chebula* on anoxia stress tolerance in mice was found to be dose dependant. Pretreatment with ethanolic extracts had significantly increased the anoxia stress tolerance time at the end of 1st (7th day), 2nd (14th day) and 3rd (21st day) weeks of extract treatment with 200 and 500 mg/kg when compared to stressed control animals.

Effect of *T. chebula* on cold resistant stress

Results of anti-stress effect of *T. chebula* against cold resistant stress in animals are shown in Table 1. The biochemical parameters, e.g. glucose, cholesterol, triglycerides, BUN, and hematological parameters like WBC and RBC counts were found to be increased in stressed group compared to unstressed group. Ethanolic extract at doses of 200 and 500 mg/kg showed significant

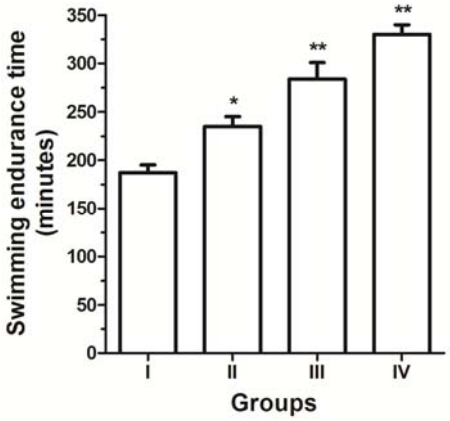

Figure 1 Effect of ethanolic extract of *T. chebula* on swimming endurance test in mice. Results are mean ± SEM (n = 6). Group I, stressed control (vehicle 1 ml/100 g); Group II, ethanolic extract of *T. chebula* (200 mg/kg); Group III, ethanolic extract of *T. chebula* (500 mg/kg); Group IV *W. somnifera* (100 mg/kg). *$P < 0.05$ and **$P < 0.01$, compared to stressed control, one-way ANOVA followed by Dunnett's post hoc test.

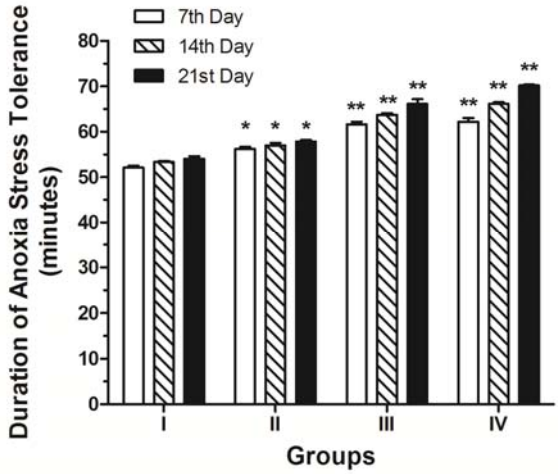

Figure 2 Effect of ethanolic extract of *T. chebula* on anoxia stress tolerance test in mice. Results are mean ± SEM (n = 6). Group I, stressed control (vehicle 1 ml/100 g); Group II, ethanolic extract of *T. chebula* (200 mg/kg); Group III, ethanolic extract of *T. chebula* (500 mg/kg); Group IV *W. somnifera* (100 mg/kg). *$P < 0.05$ and **$P < 0.01$, compared to stressed control, one-way ANOVA followed by Dunnett's post hoc test.

decrease ($P < 0.05$ and $P < 0.01$) in the elevated levels of glucose, cholesterol, triglycerides and BUN on pretreatment with *T. chebula*. Exposure to cold resistant stress resulted in a significant increase ($P < 0.01$) in the plasma corticosterone level in comparison to the unstressed control group of rats. Pretreatment with *T. chebula* (500 mg/kg) resulted in a significant ($P < 0.01$) decrease in the plasma corticosterone level in comparison to the stressed control group, whereas no significant change was observed with *T. chebula* (200 mg/kg) pretreatment (Figure 3). Weight of liver and adrenal gland, was increased, while weight of spleen and testis was reduced in stressed group compared to unstressed group. Pretreatment with extract significantly ($P < 0.01$) reduced the weight of the liver, adrenal gland and increased the weight of the spleen and testis (Table 3).

Table 1 Effect of ethanolic extracts of *T. chebula* on biochemical and hematological parameters in cold resistant stress rats.

Treatment	Biochemical parameters (mg/dl)				Hematological parameters	
	Glucose	Cholesterol	Triglycerides	BUN	Total WBC ($/mm^3$)	Total RBC ($10^6/mm^3$)
Unstressed control, vehicle (1 ml/100 g)	93.34 ± 2.37	54.66 ± 2.11	63.30 ± 1.93	23.50 ± 1.04	7,141 ± 254	4.14 ± 0.17
Stressed control, vehicle (1 ml/100 g)	158.69 ± 3.61	76.02 ± 1.61	97.94 ± 2.81	47.92 ± 0.99	12,491 ± 213	6.94 ± 0.05
T. chebula (200 mg/kg)	114.25 ± 1.21**	68.37 ± 1.25**	79.20 ± 0.35**	33.06 ± 0.92*	9,550 ± 163**	5.93 ± 0.01*
T. chebula (500 mg/kg)	109.33 ± 4.78**	63.26 ± 1.12**	77.70 ± 1.14**	30.41 ± 1.20**	8,916 ± 116**	5.46 ± 0.06**
W. somnifera (100 mg/kg)	105.70 ± 0.98**	60.63 ± 0.81**	71.48 ± 0.55**	28.66 ± 0.50**	7,966 ± 85**	5.03 ± 0.02**

Results are mean ± SEM (n = 6). *P < 0.05 and **P < 0.01, compared to stressed control; one-way ANOVA with Dunnett's post hoc test.

Table 2 Effect of ethanolic extracts of *T. chebula* on biochemical and hematological parameters in immobilization stress rats.

Treatment	Biochemical parameters (mg/dl)				Hematological parameters	
	Glucose	Cholesterol	Triglycerides	BUN	Total WBC ($/mm^3$)	Total RBC ($10^6/mm^3$)
Unstressed control, vehicle (1 ml/100 g)	93.34 ± 2.37	54.66 ± 2.11	63.30 ± 1.93	23.50 ± 1.04	7,141 ± 254	4.14 ±0.17
Stressed control, vehicle (1 ml/100 g)	175.00 ± 2.43	78.94 ± 2.11	98.74 ± 1.55	41.08 ± 0.21	12,258 ± 224	6.88 ± 0.03
T. chebula (200 mg/kg)	117.50 ± 3.54**	70.71 ± 0.98**	81.53 ± 0.61**	37.32 ±0.53*	9,966* ± 145	5.86 ± 0.05*
T. chebula (500 mg/kg)	112.00 ± 2.69**	64.39 ± 1.52**	76.91± 1.12**	30.45 ±0.71**	8,441 ± 164**	5.41 ± 0.07**
W. somnifera (100 mg/kg)	109.61 ± 1.15**	63.28 ± 1.28**	71.46 ± 0.69**	29.39 ± 0.33**	8,091 ± 118**	4.99 ± 0.02**

Results are mean ± SEM (n = 6). *P < 0.05 and **P < 0.01, compared to stressed control; one-way ANOVA with Dunnett's post hoc test.

Table 3 Effect of ethanolic extracts of *T. chebula* on organ weights in cold resistant stress rats.

Treatment	Dose	Organ weight (g/100 g body weight)			
		Adrenal gland	Liver	Spleen	Testis
Unstressed control	Vehicle 1 ml/100 g	7.47 ± 0.25	3.20 ± 0.05	0.50 ± 0.02	0.53 ± 0.01
Stressed control	Vehicle 1 ml/100 g	13.24 ± 0.54	5.42 ± 0.15	0.38 ± 0.01	0.38 ± 0.007
T. chebula	200 mg/kg	9.33 ± 0.09**	4.09 ± 0.06*	0.43 ± 0.008*	0.46 ± 0.004*
T. chebula	500 mg/kg	8.90 ± 0.49**	3.89 ± 0.23**	0.47 ± 0.01**	0.50 ± 0.006**
W. somnifera	100 mg/kg	8.38 ± 0.08**	3.63 ± 0.06**	0.48 ± 0.005**	0.51 ± 0.005**

Results are mean ± SEM (n = 6). *P < 0.05 and **P < 0.01, compared to stressed control; one-way ANOVA with Dunnett's post hoc test.

Table 4 Effect of ethanolic extracts of *T. chebula* on organ weights in immobilization stress rats.

Treatment	Dose	Organ weight (g/100 g body weight)			
		Adrenal gland	Liver	Spleen	Testis
Unstressed control	Vehicle 1 ml/100 g	7.47 ± 0.25	3.20 ± 0.05	0.50 ± 0.02	0.53 ± 0.01
Stressed control	Vehicle 1 ml/100 g	11.77 ± 0.33	5.24 ± 0.17	0.31 ± 0.004	0.32 ± 0.01
T. chebula	200 mg/kg	9.14 ± 0.19**	3.99 ± 0.05**	0.33 ± 0.004*	0.39 ± 0.005*
T. chebula	500 mg/kg	8.55 ± 0.27**	3.58 ± 0.06**	0.35 ± 0.01**	0.44 ± 0.02**
W. somnifera	100 mg/kg	8.21 ± 0.13**	3.46 ± 0.03**	0.39 ± 0.009**	0.47 ± 0.009**

Results are mean ± SEM (n = 6). *P < 0.05 and **P < 0.01, compared to stressed control; one-way ANOVA with Dunnett's post hoc test.

Effect of *T. chebula* on immobilization stress

Table 2 and 4 summarize the results obtained in the experimental model of immobilization stress induced in rats. Serum glucose, cholesterol, triglycerides, BUN and hematological parameters like WBC, RBC count were found to be increased in stressed group compared to unstressed group in immobilization stress model, but were significantly reduced ($P < 0.01$) on pretreatment with extract and the standard drug. Plasma corticosterone were significantly increased by immobilization stress, while *T. chebula* extract reduced plasma corticosterone levels significantly (Figure 3). Organs weights were significantly changed in stressed group compared to unstressed group. Pretreatment with ethanolic extract of *T. chebula* significantly reduced ($P < 0.01$) the weight of the liver, adrenal gland and increased that of the spleen and testis.

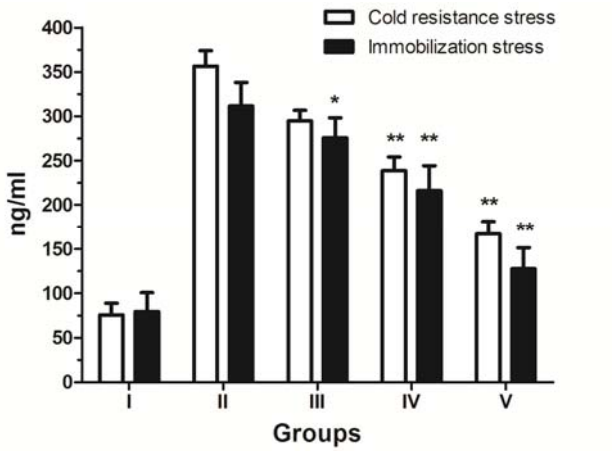

Figure 3 Effect of ethanolic extract of *T. chebula* on plasma corticosterone levels in cold resistant stress and immobilization stress rats. Results are mean ± SEM (n = 6). Group I, unstressed control (vehicle 1 ml/100 g); Group II, stressed control (vehicle 1 ml/100 g); Group III, ethanolic extract of *T. chebula* (200 mg/kg); Group IV, ethanolic extract of *T. chebula* (500 mg/kg); Group V *W. somnifera* (100 mg/kg). *$P < 0.05$ and **$P < 0.01$, compared to stressed control, one-way ANOVA with Dunnett's post hoc test.

Discussion

Stress is a global menace fortified by the advancement of industrialization and elicited by a variety of factors, viz., environmental, social or pathological phenomenon of life. Considerable evidence published in the last decade has focused on a constellation of neurochemical, biochemical and molecular effects caused by stress in the CNS, endocrine system, and immune system. Normally stress-induced changes are self-limiting and adaptive until events override "threshold" limits, becoming irreversible and pathological. Advancements in the understanding of processes leading to the pathogenesis of stress-induced disorders cannot obscure the simple fact that the exhaustion of energy supply is still the basis for triggering the disorders and collapse of energy metabolism following glucose deprivation in the circulation. The desire to augment the coping mechanism has led to the emergence of the science of adaptation that focuses on elucidating mechanisms that may help to counteract excessive and unnecessary responses to stress.[19]

Adaptogens are substances that push the organisms into a state of non-specifically heightened resistance, in order to better resist stressors and adapt to extraordinary challenges. They normalize the body functions, strengthen systems and functions that are compromised by stress and have a protective effect against a wide variety of environmental and emotional stress.[20]

There are reports that plasma levels of adrenaline and noradrenaline are enhanced during stress induced by swimming endurance test. In addition, monoamine oxidase (MAO) levels in the brain are reportedly decreased during stress.[21] The swim endurance test results indicate clearly that the extract has the properties whereby it increases the physical endurance as well as the overall performance in rats and possess significant anti-stress activity. It may be possibly normalizing the plasma level of catecholamine and MAO.[22] It may be due to increased utilization of the ATP-CP pathway, increased levels of muscle glycogen (a storage form of glucose that can provide energy for more prolonged activities), or decreased concentrations of muscle lactic acid and ammonia (two toxic by-products of muscular effort). It can be attributed to the anti-oxidant effect of plant extract which prevent the free radical-induced damage of the vital organs.[23]

All the body functions, including cellular respiration depends on oxygen supply. Lack of any vital element will play havoc on all body mechanisms. Increased adaptation due to the depletion of any vital elements during stress by any drug that increases the tolerance can act as adaptogenic agent. Adaptogens producing beneficial effects in stress are believed to act by increasing non-specific resistance. In the present study depletion of oxygen in hermetic vessel leads to convulsions in animals and pretreatment with ethanolic extract of *T. chebula* had increased the stress tolerance indicating their adaptogenic/anti-stress activity. This may be due to that during stress the extract of *T. chebula* was capable of increasing succinate dehydrogenase (SDH) in the brain. This enzyme is responsible for utilization and conservation of energy in the cellular system of the organism, which helps adaptive processes during stress.[24] The present study also investigated the ability of the extract of *T. chebula* to hold back stress-induced changes in biochemical, hematological parameters and organ weights in cold stress and immobilization stress models.

In the present study, a significant hyperglycemia was observed with both cold stress and immobilization stress models. Under stressful conditions, cortisol in human and corticosterone in rats will be secreted by adrenal cortex. Hypersecretion of cortisol helps the maintenance of internal homeostasis through the process of gluconeogenesis and lipogenesis.[19,25] Ethanolic extract of *T. chebula* significantly reduced the hyperglycemia by reducing the hyperactivity of adrenal cortex and also by maintenance of homeostatic mechanism in cold stress and immobilization stress animals.

The mechanism by which stress raises serum cholesterol is likely to be related to the enhanced activity of hypothalamo-hypophyseal axis, resulting in increased liberation of catecholamines and corticosteroids which lead to elevated

levels of serum cholesterol since adrenaline mobilizes the lipids from adipose tissues.[26] After treatment with *T. chebula* extract cholesterol was reduced in both cold stress and immobilization stress models.

The effect of stress on serum triglycerides has been shown to be variable; probably catecholamines mobilize lipids from adipose tissues. In the present study both cold stress and immobilization stress models showed an increase in triglyceride levels. However ethanolic extract of *T. chebula* were able to suppress the stress-induced increase in triglycerides levels. BUN levels were increased in both cold stress and immobilization stress models as these are the end products of protein metabolism. In excess adrenocortical activity, due to increased metabolism of protein increases urea excretion, in the present study a similar effect was observed.[25] However extract of *T. chebula* decreased the BUN levels as compared to stress control, indicating a diminished catabolism of protein under stressful conditions**.** Cold stress and immobilization typically increases total leukocyte and erythrocyte count, during stress heart rate, blood pressure, blood flow rate and oxygen demand increases, to meet these extra demands, RBC and WBC count increases.[17]

Plant adaptogens are smooth pro-stressors which reduce the reactivity of host defense system and decrease the damaging effects of various stressors due to increased basal levels of mediators involved in the stress response.[27] Pretreatment with ethanolic extract of *T. chebula* reduced the stress-induced elevated levels of hematological parameters in both stresses, Since the stress-induced increased total WBC and RBC count was decreased by extract of *T. chebula,* hence the plant possess anti-stress activity**.**

Adrenal glands and liver weights were significantly increased in both cold stress and immobilization stress models. Stress induces adreno-medullary response in man to release adrenaline which in turn stimulates β_2 receptors on the pituitary gland. It leads to greater release of ACTH that can stimulate the adrenal medulla as well as cortex resulting in further release of adrenaline and increase in weight of adrenal gland to a greater extent. The adrenal hypertrophy takes place in response to the secretion of ACTH from the pituitary for increased corticosterone from cortical cells to combat stress.[7] The level of corticosterone was found to be elevated during the cold resistant and immobilization stress in the experiment.

Spleen contracts during stress and releases more amount of blood (RBC) into circulation, hence its weight decreases.[25] The weight of testis decreases because there is suppression of spermatogenesis and decrease testosterone levels during stress.[28] Pretreatment with *T. chebula* prevented the stress-induced increase in weight of liver and adrenal glands and a decrease in spleen and testis weight, indicating the protective effect against stress. Interestingly, it can be inferred that the anti-stress activity of *T. chebula* extract (500 mg/kg) was equal to that of standard drug *W. somnifera.*

Ulcer is the one more common complication in the stress condition. Probably it may be mediated by histamine release with enhanced acid secretion, reduced mucous production, generation of free radicals, mast cell activation, alterations in prostaglandin generation, cytokine liberation and breakdown of normal cytoprotective mechanisms.[29] Ulcers due to cold stress are both due to physiological and psychological factors.[30] Our previous studies on *T. chebula* showed a significant anti-ulcer and cytoprotective activity against stress-induced ulcer models.[10] Those anti-ulcer results are also consistent with *T. chebula* showing significant anti-stress activity against stress-induced models. Flavanoids, tannins and phenolic glycosides were reported to possess a variety of biological activities including adaptogenic activity.[17,31]

Conclusion

Ethanolic extract of *T. chebula* exhibited anti-stress activity by preventing stress-induced elevated levels of biochemical and hematological changes and the alteration in organ weights. The anti-stress activity of fruits of *T. chebula* extract of may be due to the presence of flavonoids, glycosides, tannins and polyphenols.

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Conflict of Interest

The authors hereby declare that there was no conflict of interest and no source of funding for this project.

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