

Echium amoenum Hydroalcoholic Extract Effect on Oxidative Stress Due to Heat Stress in Rats

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Original Article

Article History

Received: 26 February 2025

Accepted: 05 June 2025

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Among the biggest problems facing the world today are climate change and heat stress in the environment. It is crucial to look into these effects on various organisms. One of the most critical consequences of global warming is heat stress, which significantly disrupts the oxidative balance in living organisms by increasing reactive oxygen species (ROS) and reducing antioxidant capacity. This imbalance leads to cellular and tissue damage. Therefore, identifying effective strategies to reduce oxidative damage caused by heat stress is essential. One of the top priorities is to eradicate heat stress in living things. Plant extracts can significantly reduce heat stress by enhancing the immune system, improving effectiveness, and having few side effects. Residual medicinal plant extracts, such as *Echium amoenum* Fisch. & C.A.Mey., are known for their antioxidant properties. In this study, the protective effect of *E. amoenum* hydroalcoholic extract on oxidative stress induced by acute and chronic heat exposure in rats was investigated. Six groups of sixty rats were prepared. These groups include healthy control, sham, acute and chronic heat stress control stress groups (groups T1 and T2), group T3: chronic heat stress conditions with daily injection of 400 mg/kg body weight of the extract for 21 days, and group T4: acute heat stress conditions with daily injection of 400 mg/kg body weight of the extract for 21 days. Blood was taken from the rats under sterile conditions at the end of the study. The investigated parameters were measured using conventional techniques in the resultant sera. The findings demonstrated that prolonged exposure to heat significantly lowers serum catalase. All of the indicators that were examined catalase, vitamin D, superoxide dismutase, glutathione peroxidase, malondialdehyde, and total antioxidant capacity showed a noteworthy variation in response to acute heat stress in comparison to the control group. However, following the extract injection, a comparison of two heat stress groups and the control group demonstrated the extract's beneficial effects on stress reduction. In conclusion, the findings of this study showed that treatment with *E. amoenum* notably improves oxidative markers, especially under acute heat stress. This extract can alleviate oxidative damage caused by heat stress in rats.

Keywords: *Echium amoenum* flower, Heat stress, Hydroalcoholic extract, Rat**How to cite this paper**Khoshvaghti, A., Panahi, Z., Rahmani Kahnamoiei, J. *Echium Amoenum* Hydroalcoholic Extract Effect on Oxidative Stress due to Heat Stress in Rats. Journal of Medicinal plants and By-Products, 2026;15(1): 14-19. doi: 10.22034/jmpb.2025.368747.1910**INTRODUCTION**

The utilization of plants for healing and medicinal purposes has been a widespread practice globally for many centuries. In recent years, the use of herbal medicines has probably attracted more attention due to many beneficial properties and few adverse effects. A large number of people around the world trust herbal medicines for early health care [1]. The borage plant with the scientific name of *Echium amoenum*, which belongs to the Boraginaceae family, is a medicinal, automobile, and herbaceous plant. Borage is a biennial or perennial plant covered with soft, thin, or long, silky fluffs that flower in mid-spring and summer [2]. At present, this plant is widely distributed in some European, Asian, and North American countries in mountainous and non-cultivable areas [3]. In traditional medicine, *E. amoenum* flowers are used as analgesics, diaphoretics, and blood pressure reducers. Other medicinal uses of the flowers and leaves of this plant include anticoagulant, anti-inflammatory, antibacterial properties [4,5], treatment of depression, uplifting, sedative and anti-stress, prevention of inflammation and irritation of the kidneys and urinary tract,

diuretics (due to potassium nitrate), laxatives, treatment of rheumatism, diabetes [6], cardiovascular diseases, colds, coughs, bronchitis and immune system strengthening [7], analgesic [8,9] etc., which are mainly consumed in the form of infusions, cooked or powdered.

Phytochemical research in the field of effective compounds has shown that *E. amoenum* has many various combinations such as rosmarinic acid (RA), flavonoids, palmitic acid, n-triclosan, linoleic acid, n-pentacosane, and pulegone [10,11].

Medicinal herbs can be used to reduce oxidative stress [12]. Biologically, reactive oxygen species (ROS) are formed as a natural by-product of natural oxygen metabolism. Under certain conditions such as heat or exposure to UV rays, their levels increase which causes damage to the cell structure and predisposes them to the occurrence of various diseases [13].

Factors such as tobacco, drugs, and radiation are also effective in increasing ROS production. Free radicals can induce many dangerous diseases due to damage cells and intra cellular organelles [14,15].

Previous research has shown that the ethanolic extract of *E. amoenum* has antioxidant activity due to its high content of phenolic and flavonoid compounds [16]. Sadeghara *et al.* (2013) evaluated the antioxidant capacity of the alcoholic extract of *E. amoenum* and the results showed that *E. amoenum* possesses high antioxidant power. Sadeghara *et al.* (2013) also expressed the hydroalcoholic extract of *E. amoenum* has high antioxidant activity [9].

The phenolic compounds in the ethanolic extract of *E. amoenum* have less flavor and aroma than other aromatic plants, and for this reason, it is more widely used in the food industry and is a suitable option in the production of edible coatings with antioxidant properties [17].

The antioxidant potential of this plant may be due to its bioactive antioxidant compounds, mainly polyphenols as rosmarinic acid and flavonoids. In fact, antioxidants chemically or naturally neutralize the effects of reactive oxygen species and free radicals [9,17].

The Antioxidant activity of plants has been given special attention [6]

The climate changes have important short and long term effects on people's health. The increase in ambient temperature is one of the main climate changes [18]. Temperatures globally are projected to rise between 1.4 and 5.8 °C. Such an increasing trend is positively correlated with the increase in hyper heat and heat wave deaths in many countries, both globally and across Africa [19].

The problems caused by heat stress and related to global warming are increasing with the impact on biological systems. Heat stress as a factor that causes oxidative stress and affects heat stress naturally exists in the environment and has become a social hazard. Oxidative stress is caused by heat stress when the oxidative agent levels exceed the body's defense mechanism. In this case, it causes the destruction of vital macromolecules in the body such as proteins, DNA, and lipids.

Unfortunately, the daily lifestyle has caused many organisms to endure very high and abnormally high levels of oxidative stress, which can increase the decrease in the body's efficiency [20].

Given the increasing incidence of heat stress due to climate change and its serious implications on oxidative balance in biological systems, it is vital to explore natural interventions to prevent or reduce its harmful effects [21] Oxidative stress occurs when reactive oxygen species (ROS) exceed the capacity of the body's antioxidant defenses, leading to damage of cellular macromolecules [22] In this context, the present study was designed to examine the potential of hydroalcoholic extract of *E. amoenum* a medicinal plant with known antioxidant compounds to protect against oxidative stress caused by both acute and chronic heat exposure in rats. This research specifically aims to determine whether this extract can restore antioxidant enzyme levels and mitigate the oxidative damage typically associated with heat stress.

MATERIALS and METHODS

Plant Collection and Extraction

Echium amoenum (PM 1570, *E. amineum* Boraginaceae) was obtained from a reputable herbal medicine center in Shiraz. The plant's identity was confirmed by experienced professors from the Department of Plant Biology at Kazerun Islamic Azad University. After ensuring that the *Echium amoenum* was completely dry, the plant was turned into a powder by an electric mill. Each 10 grams of *Echium amoenum* was added to Erlen containing 200 ml of 70% alcohol and gently mixed on an agitator for about 48 hours at 25 °C temperature.

Then, the solvent and plant mixture were separated by Wattman filter paper and the primary extracts were collected. The initial extracts were fed into a rotary distillation machine and evaporated to the dry border. At the end of the process, it was spilled into a clean glass container and was laid in a 40 °C oven for about 24 hours until the dry extract was obtained [16,23].

Analysis of the Extract

E. amoenum extract was analyzed by GC-MS for the identification of chemical compounds. The major compounds of this extract were palmitic acid, n-triclosan, n-pentacosane, and flavonoids [24, 25].

Experimental Animals

Studied rats were kept according to the instructions set by the National Research Council of the National Academies to maintain and work with laboratory animals. Animals were kept in standard conditions for one week. During the research, standard conditions of temperature (25±2 °C) and humidity (50±10%) were provided for studied rats. In this study, the duration of the day and night in the rat storage area was 12 hours.

In order to conduct this study, 60 Wistar healthy male rats weighing about 200±20 gr were prepared from the laboratory animal breeding and maintenance center of Shiraz Medicinal Sciences University.

There were 6 groups of rats under study: the healthy control group, which was kept at normal temperature for 21 days and fed normally; the sham group received 1 cc of distilled water intraperitoneally in addition to normal feeding and was kept at normal temperature for 21 days. Chronic heat stress control group (group T1): rats exposed to 38±1 °C temperature daily for one hour for 21 days. Acute heat stress control group (group T2).

Group T3: rats that were exposed to 38±1 °C temperature daily for one hour during 21 days (chronic heat stress) and during this period received 400 mg/kg B.W of *E. amoenum* hydroalcoholic extract intraperitoneally. Group T4: rats were exposed to normal temperature for 20 days and day 21, were exposed to 38±1 °C temperature for 4 hours (acute heat stress) during this period of 21 days, they received 400 mg/kg B.W of *E. amoenum* hydroalcoholic extract intraperitoneally daily [20,26].

Blood Sampling

In this study, after 21 days, the rats were anesthetized with ketamine and xylazine. The chest was split from the bottom, and then the inferior vein was removed from the rats' hearts, and their blood was collected with the help of a syringe. The blood was transferred to a test tube, and after clotting, the samples were centrifuged at 2500 rpm for 15 minutes. The sera were transferred to clean Eppendorf tubes. The samples were stored in a freezer at -20 degrees Celsius until the laboratory tests were performed. Serum total antioxidants [27], superoxide dismutase [28], glutathione peroxidase [29] were measured with ELISA methods by RANDOX diagnostic kits (Randox Laboratories Ltd., Crumlin, Country Antrim, UK), catalase [30] and malondialdehyde [31] were assayed by diagnostic ELABSCIENCE kits (Elabscience Laboratories, USA), by with colorimetric method and vitamin D was measured by HPLC method and was carried out using a Shimadzu system (Columbia, MD) [32].

Statistical Analysis

In the current study, the data were analyzed by SPSS software version 25. For this purpose, the data were entered into the software, and the obtained data were analyzed using independent t-test, one-way ANOVA, and Duncan's post hoc test. It is worth

mentioning that for all the statistical tests used in the study, $P < 0.05$ was the significance level.

RESULTS

At first, to determine the type of statistical test, the distribution of the data obtained from the research was evaluated by the Kolmogorov-Smirnov test. This evaluation showed that the distribution of data was normal and therefore parametric tests including independent t-test and one-way ANOVA test were used to analyze the data and the results are presented in tables.

The findings of the statistical analysis derived from the gathered data are presented in Tables 1 to 4.

Table 1 shows the mean and standard deviation of all of the studied parameters.

The statistically independent T-test demonstrated that the results of all studied parameters were comparable between the healthy control group and the other groups, as illustrated in Table 2. Therefore, the findings from the healthy control group will serve as a basis for further comparisons. Numerous studies have examined the impact of heat stress on oxidative damage within mammalian tissues [33].

Increasing the activity of the body's antioxidant system can be one way to deal with heat stress [34]. Therefore, in the current research, the effect of hydroalcoholic extract of *E. amoenum* effect on oxidative stress due to heat stress was investigated.

The statistical analysis of this study revealed a significant difference in blood serum catalase levels between rats exposed to chronic heat stress (T_1) and the healthy control group, as illustrated in Table 2. Notably, chronic heat stress significantly reduced catalase activity. Furthermore, the administration of the hydroalcoholic extract of *Echium amoenum* (group T_3) did not result in a statistically significant increase in catalase levels sufficient to match those observed in the healthy control group. Additionally, chronic heat stress did not exert a significant impact on the other evaluated parameters, as detailed in Table 2.

In contrast, statistical tests showed that acute heat stress was able to make a significant difference in all indices including serum total antioxidant capacity, catalase, vitamin D, glutathione peroxidase, malondialdehyde, and superoxide dismutase levels in comparison to the healthy control group (Table 2). This difference was due to a decrease in the amount of antioxidants and an increase in the amount of malondialdehyde in rats under acute heat stress (group T_2) compared to the healthy control group.

Also, statistically significant differences between CAT and MDA were observed in the chronic heat stress-treated group in comparison to the healthy control group. Also, there was a statistically significant difference between CAT in the acute heat stress-treated group compared to the healthy control group (Table 2). However, there were statistically significant differences between all of the studied parameters in the acute heat stress control group compared to the healthy control group.

Table 1 Mean and standard deviation of studied parameters in different groups

Parameters groups	Mean \pm SD					
	TAC (mM/ml)	SOD (u/ml)	GPX (mu/ml)	CAT (μ M/ml)	D Vit. (ng/ml)	MAD (μ M/ml)
Healthy control	0.810 \pm 0.464	1.5773 \pm 0.420	104.49 \pm 53.74	7.713 \pm 1.205	28.62 \pm 16.86	9.033 \pm 3.71
Sham	0.741 \pm 0.373	1.6374 \pm 0.332	85.16 \pm 55.25	7.170 \pm 2.788	22.98 \pm 20.80	12.00 \pm 5.22
Chronic heat stress control (T_1)	0.755 \pm 0.168	1.4354 \pm 0.522	90.62 \pm 35.85	4.218 \pm 1.947	24.73 \pm 14.50	12.86 \pm 7.075
Acute heat stress control (T_2)	0.450 \pm 0.187	1.0200 \pm 0.579	43.42 \pm 32.92	3.619 \pm 2.270	21.58 \pm 14.03	21.65 \pm 8.69
Chronic heat stress treated (T_3)	0.669 \pm 0.362	1.2700 \pm 0.343	92.15 \pm 89.90	5.600 \pm 1.530	28.20 \pm 15.42	17.60 \pm 1.32
Acute heat stress treated (T_4)	0.720 \pm 0.271	1.480 \pm 0.264	95.34 \pm 49.79	6.270 \pm 1.643	30.71 \pm 18.43	11.03 \pm 6.38

Table 2 Comparison of mean and standard deviation in studied parameters between healthy control group and other groups

Parameter groups	TAC	SOD	GPX	CAT	D Vit.	MAD
Sham	0.690	0.700	0.390	0.580	0.470	0.120
Chronic heat stress control (T_1)	0.703	0.470	0.465	0.007	0.550	0.112
Acute heat stress control (T_2)	0.024	0.014	0.003	0.003	0.037	0.001
Chronic heat stress treated (T_3)	0.40	0.071	0.680	0.001	0.940	0.021
Acute heat stress treated (T_4)	0.570	0.530	0.660	0.023	0.700	0.350

Table 3 Comparison of mean and standard deviation in studied parameters between acute and chronic heat stress control groups

TAC	SOD	GPX	CAT	D Vit.	MAD
0.001	0.080	0.003	0.490	0.590	0.010

Table 4 Comparison of mean and standard deviation in studied parameters between acute and chronic heat stress treated groups

TAC	SOD	GPX	CAT	D Vit.	MAD
0.690	0.100	0.900	0.309	0.700	0.090

DISCUSSION

The core problem addressed in this study was the oxidative damage caused by heat stress, which has become a major biological concern as global temperatures continue to rise. Exposure to high environmental temperatures disturbs the redox homeostasis in living organisms, primarily by elevating reactive oxygen species

(ROS) and diminishing antioxidant defenses [35]. This study confirms that heat stress, especially acute stress can significantly alter antioxidant indices such as catalase, superoxide dismutase, and glutathione peroxidase while increasing oxidative markers like malondialdehyde. Importantly, the hydroalcoholic extract of *E. amoenum* was able to mitigate these effects, particularly under acute stress conditions, highlighting its potential as a natural therapeutic agent for oxidative stress management.

In past research, some physiological and biochemical changes have been observed in many animals after heat stress. The imbalance of glucocorticoids, adrenocorticotropic hormone, growth hormone, and norepinephrine has been reported in heat stress conditions [36]. In addition, a series of compounds act as antioxidants, including catalase, glutathione peroxidase, and C-reactive protein, also body inflammatory response was affected by heat stress. The extensive

changes in gene expression due to heat stress have been demonstrated by advanced methods [37].

It has previously been reported that the antioxidant system is affected by heat stress and glutathione levels (the oxidized and reduced state) change in response to heat stress. [33].

The results showed that heat stress negatively affects the oxidative/antioxidant status of the brain, heart, and testicular tissues of mice and the immune response of the brain, duodenum, heart, liver, and testicular tissues. It also showed that the negative effects of heat stress exposure were associated with stress intensity in the heart, liver, and testicular tissue [38].

In 2008, Bhatt *et al.* investigated seasonal changes and the effect of temperature increase on adult female Wistar rats and measured the oxidative degradation of red blood cells, cortisol levels, and plasma antioxidants. The results of their studies showed that thiobarbituric acid reactive substrates of erythrocytes and plasma cortisol levels increased significantly at high temperatures and humidity in warm seasons compared to cold seasons and the levels of glutathione, glutathione peroxidase and superoxide dismutase levels of erythrocytes decreased significantly [39].

In the present study, a comparison of the acute heat stress group received extract (group T4) with the healthy control group showed that these two groups just had a statistically significant difference in the serum catalase level (Table 2). This finding indicates that *E. amoenum* extract consumption in rats exposed to acute heat stress was able to improve all serum antioxidant parameters except catalase to the same level in the control group.

In line with the findings of the present research, the results of Wang *et al.*'s research proved that heat stress increases the activity of aspartate transaminase, alanine transferase, and serum malondialdehyde concentration in mice, and decreases the activity of serum catalase and superoxide dismutase. In addition, these researchers reported that *Macalea cordata* extract (MCE) was able to modulate changes in metabolic pathways induced by heat stress in the gut microbiota [40].

In the current study, there are significant differences between the mean serum concentration of glutathione peroxidase, malondialdehyde, and total antioxidant capacity in the chronic heat control group and the acute heat control group (Table 3). This statistically significant difference shows that acute heat stress has more effect on decreasing serum antioxidants and increasing malondialdehyde content in comparison to chronic heat stress.

Also, the statistical results show that there are no significant differences between the studied parameters in the two groups affected by acute and chronic heat stress after injection of the extract (groups T₃ and T₄).

Although chronic heat stress only reduces the amount of catalase in the present research, however, the reduction of this factor is so significant that even the consumption of *E. amoenum* extract doesn't put its amount in the control group. Due to the significant effect of acute heat stress on oxidative stress index (malondialdehyde) and antioxidants, and also due to the stable effect of both types of heat stress on the amount of catalase, it can be concluded that heat stress can have negative effects on the body's antioxidant system and alter oxidative indices.

In general, the present study showed that heat stress leads to a drastic reduction in serum catalase, but serum glutathione peroxidase, superoxide dismutase, vitamin D, total antioxidant capacity, and malondialdehyde affected by acute heat stress significantly, and injection of hydroalcoholic extract of *E. amoenum* with acute and chronic heat stress in rats, leads to

statistically significant increase in all these antioxidants (except catalase) and a decrease in the amount of serum malondialdehyde. A decrease in serum concentrations of glutathione peroxidase, catalase, total antioxidant capacity, and superoxide dismutase and an increase in malondialdehyde following heat stress can be indicative of heat-induced oxidative stress. In a study in line with the present study, Abbasi Larki *et al.*, 2020 evaluated important genes associated with oxidative stress and reported decreased mRNA expression of glutathione peroxidase and glutathione S-transferase genes in mice following exposure to permethrin-induced oxidative stress (PMN). Also, the results of their research showed that *E. amoenum* has significant antioxidant and cellular protection impacts [16].

Considering that for *E. amoenum* extract has various antioxidants including flavonoids, anthocyanins, tannins, coumarins, xanthines, and procyanidins that have been reported in some studies [41]. In the current research, the oxidative stress reduction after the use of *E. amoenum* extract may be related to the compounds mentioned in *E. amoenum* extract. Previous researchers have stated, that *E. amoenum* extract exerts its protective effects through mechanisms that depend on the presence of some bioactive compounds such as rosmarinic acid with anti-inflammatory effect and cyanidin 3-glucoside. These compounds prevent the activation and transfer of factors c-Jun and NF-κB into the cell nucleus. Suppression of cyclooxygenase-2 expression and reduction of intracellular reactive oxygen, species levels (ROS) through activation of the glutathione antioxidant system (GSH) may play a role in this process [9].

A laboratory study has shown that treatment by *E. amoenum* petals anthocyanin-rich extract reduces oxidative stress in human endothelial cell cultures [3, 42, 43].

In line with the present study, previous research has shown that the extracts of some plants can reduce heat stress due to their specific compounds.

Some research has shown that polyphenols can adjust antioxidant defense systems. RA, the caffeic ester of lactic acid is usually present in many plants such as Boraginaceae families. The more important phenolic compound of *E. amoenum* petal alcoholic extract is RA. RA has been widely used in traditional medicine in the treatment of many diseases and infections [44].

Research has shown that RA has more antioxidant characteristics than vitamin E [45, 46]. Zeng *et al.* proposed that astragalus polysaccharides affect the serum hormonal profiles in dairy cows under heat stress [47]. Kra *et al.* showed that resveratrol is a natural polyphenol that increases MDA concentrations and may increase lipid lipolysis and decrease lipogenesis under heat stress [48].

Previous research demonstrated that feeding dairy cows with bamboo leaf flavonoids reduces oxidative damage [49].

In this regard, the researchers have shown that heat stress increased lipids and significantly decreased serum LDL-C, TG, TC, and FFA levels, and HDL-C and LPS increased with serum PSPA consumption in Wenchang chickens [50].

According to the mentioned mechanism, in the current investigation, *E. amoenum* extract's effect on reducing oxidative stress caused by heat can be due to the phenolic compounds and RA in *E. amoenum*.

In various researches, the antioxidant effects of *E. amoenum* extract have been mentioned, some of which are mentioned below.

A 2020 study by Abbasi Larki *et al.* emphasized *E. amoenum* effects on permethrin-induced oxidative stress (PMN) in rats and SK-Hep-1 cells. The findings of this study showed that *E.*

amoenum has significant antioxidant, gene-regulating, and cellular protective properties [16].

Abd *et al.* reported that *E. amoenum* extract significantly reduced the inflammatory response of acute pancreatitis induced by improving pancreatic edema, serum levels of amylase and lipase, pro-inflammatory cytokines, myeloperoxidase activity, lipid peroxidation, and pathological changes. The researchers concluded that *E. amoenum* reduces the severity of acute pancreatitis caused by cereoliin with anti-inflammatory, immune-modulating, and antioxidant effects [51].

Ranjbar *et al.* stated a significant decrease in blood lipid peroxidation due to the antioxidant activity of aqueous extract of Persian *E. amoenum* and related this antioxidant property to the presence of bioactive components of *E. amoenum*, especially flavonoids [52]. Mehrabi *et al.* expressed a suitable inhibitory effect against pathogenic bacteria and also antioxidant properties for thyme essential oil [53].

Also, Mohammadzadeh *et al.* reported antimicrobial properties for *Froriepia subpinnta* essential oils from the Guilan region, especially after flowering [54]. The findings of Kazemini *et al.* revealed that plants of the Asteraceae family can be widely used in the food industry and medicine [55]. Nofouzi *et al.* reported antibacterial effects for methanolic extract of the aerial parts of *Verbascum speciosum* [24].

CONCLUSION

The findings of the current research revealed that chronic heat stress only reduces serum catalase levels but acute heat stress causes significant changes in the enzymatic and non-enzymatic antioxidant systems of serum and leads to oxidative stress with increasing the amount of malondialdehyde.

Consumption of hydroalcoholic extract of *E. amoenum* can modify all changes in the antioxidant system caused by heat stress, except the catalase level.

Therefore, it can be concluded although the decrease of catalase affected by acute and chronic heat stress is so much that *E. amoenum* extract with the dose used in this study cannot put its activity in the normal range, but other findings of this study show that *E. amoenum* extract can improve oxidative stress caused by heat stress and reduce the negative effects of heat stress, particularly oxidative stress in rats.

Conflict of Interests

The authors of this manuscript do not have any conflict of interest to declare.

Acknowledgement

Not applicable.

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