The Journal of Medicinal Plants and By-products

Original Article

Echium Amoenum Hydroalcoholic Extract Effect on Oxidative Stress due to Heat Stress in Rats

Running Title: *Echium Amoenum* Effect on Oxidative Stress due to Heat Stress

Ameneh Khoshvaghti[1*], Zahra Panahi [2] and Jafar Rahmani Kahnamoei[3]

Department of Clinical Sciences, Kaz.C., Islamic Azad University, Kazerun, Iran
Department of Biochemistry, Agriculture and New Technologies, Shiraz Branch, Islamic Azad University, Shiraz, Iran
Department of Clinical Sciences, TAMS.C., Islamic Azad University, Tabriz, Iran

[*]Corresponding author: Email: Am.khoshvaghti@iau.ac.ir , akhoshvaghti2004@gmail.com

Article History: Received 26 February 2025/Accepted in revised form 05 June 2025

ABSTRACT

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*, are known for their antioxidant properties. In this study, the protective effect of *Echium 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 *Echium 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

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, *Echium 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 *Echium 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 *Echium 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 *Echium amoenum* and

the results showed that *Echium amoenum* possesses high antioxidant power. Sadeghara et al. (2013) also expressed the hydroalcoholic extract of *Echium amoenum* has high antioxidant activity [9].

The phenolic compounds in the ethanolic extract of *Echium 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 Aantioxidant 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 *Echium 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, *Echium 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

Echium 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 T_1): rats exposed to 38±1$^{\circ C}$ temperature daily for one hour for 21 days. Acute heat stress control group (group T_2).

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

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

DISCUSSON

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 *Echium 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 *Echium 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 T_3 and T_4).

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 *Echium 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 *Echium 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 *Echium amoenum* has significant antioxidant and cellular protection impacts [16].

Considering that for *Echium 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 *Echium amoenum* extract may be related to the compounds mentioned in *Echium amoenum* extract. Previous researchers have stated, that *Echium 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-kB 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 *Echium 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 *Echium 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, *Echium amoenum* extract's effect on reducing oxidative stress caused by heat can be due to the phenolic compounds and RA in *Echium amoenum*.

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

A 2020 study by Abbasi Larki *et al.* emphasized *Echium amoenum* effects on permethrin-induced oxidative stress (PMN) in rats and SK-Hep-1 cells. The findings of this study showed that *Echium amoenum* has significant antioxidant, gene-regulating, and cellular protective properties [16].

Abd *et al*. reported that *Echium 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 *Echium 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 *Echium amoenum* and related this antioxidant property to the presence of bioactive components of *Echium 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 Kazeminia 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 *Echium 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 *Echium 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 *Echium 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.

REFERENCES

1. Bhardwaj S., Verma R., Gupta J. Challenges and future prospects of herbal medicine. International Journal of Medical Research and Health Sciences. 2018; 1(1): 12-15.
2. Naderi Haji B., Candi M., Rezaee M.J. IJOM., Research A.P. Primory phytochemical investigation of *Echium amoenium*. Iranian Journal of Medicinal and Aromatic Plants Research. 2004; 20(3): 377-383.
3. Nouri M., Farajdokht F., Torbati M., Ranjbar F., Hamedyazdan S., Araj-Khodaei M., Sadigh-Eteghad S.A. Close look at *Echium amoenum* processing, neuroactive components, and effects on neuropsychiatric disorders. Galen Medical Journal. 2019; 8: e1559.
4. Farahani M., Azad I., Branch Q. Antiviral effect assay of aqueous extract of *Echium amoenum*-L against HSV-1. Zahedan Journal of Research in Medical Sciences. 2013; 15(8): (In Persian)
5. Sabaeifard P. Antibacterial activity of *Echium amoenum* aqueous and ethanolic extracts. Third Int. Stud. Biotech. Cong. 2015.
6. Mahmoudi M., Shahidi S., Golmohammadi H., Mohammadi S .The effect of *Echium amoenum* hydroalcoholic extract on blood glucose level, lipid profile and lipoproteins in streptozotocin-induced diabetic male rats. Journal of Advances in Medical and Biomedical Research. 2015; 23(97): 72-81.
7. Patocka J., Navratilova Z.J.P. Bioactivity of *Echium amoenum*: A mini review. Biomed. Biomedical Journal of Scientific & Technical research. 2019; 20(2):14915-14917. DOI:10.26717/BJSTR.2019.20.003429.

8. Sayyah M., Boostani H., Pakseresht S., Malaieri A.J.P.I.N.P., Psychiatry B. Efficacy of aqueous extract of *Echium amoenum* in treatment of obsessive–compulsive disorder. Progress in Neuro-Psychopharmacology & Biological Psychiatry. 2009; 33(8):1513-1516. doi: 10.1016/j.pnpbp.2009.08.021.
9.Sadighara P., Barin A., JAHED G., Farjadmand F. Assessment of antioxidant capacity and anti-inflammatory of alcoholic extraction of chamomile, morus, marshmallow, borage and rosemary. Knowledge and Healthy. 2013; 8(1):31-34.
10. Erdemoglu N., Kusmenoglu S., Vural M. γLinolenic acid content and fatty acid composition of Boraginaceae seed oils. European Journal of Lipid Science and Technology. 2004; 106(3):160-164.
11. Hajghanbari L., Alizadeh A., Honarvar M. Essential oil constituents and fatty acids in *Echium amoenum* grown wild in Iran. International Journal of Biosciences. 2015; 6(1): 156-161.
12. Chandran R. and Abrahamse H. Identifying plant-based natural medicine against oxidative stress and neurodegenerative disorders. Oxidative Medicine and Cellular Longevity. 2020;8648742. 10.115/2020/8648742.
13. Jomova K., Raptova R., Alomar S., Awasel S.Y., Nepovimova E., Kuca K., Valko M. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Archives of Toxicology. 2023; 97(10):2499–2574. doi: 10.1007/s00204-023-03562-9
14.Andrade-Cuvi M.J., Moreno C., Zaro M.J., Vicente A.R., Concellón A. Improvement of the antioxidant properties and postharvest life of three exotic Andean fruits by UV-C treatment. Food Quality. 2017. https://doi.org/10.1155/2017/4278795 .
15. Schumacker P.T. Reactive oxygen species in cancer: a dance with the devil. Cancer cell. 2015; 27(2):156-157.
16. Abbasi Larki R., Zayerzadeh E., Harzandi N., Anissian A. Protective effects of *Echium amoenum* on oxidative stress and gene expression induced by permethrin in Wistar rats. Hepatitis Monthly. 2020;20(6): 2103774. Doi: 10.5812/hepatmon.103774.
17. Ranjbar M and Motiee N. Comparative and combined effects of *Echium amoenum* and *Cichorium intybus* extracts on live of Wistar rats. Medicinal Plants By- products. 2023;4:495-503. https://doi.org/10.22092/jmpb.2022.356065.1403.
18. World Health Organization. COP24 special report: health and climate change.2018.
19. Ncongwane K.P., Botai J.O., Sivakumar V., Botai C.M.J.S.A literature review of the impacts of heat stress on human health across Africa .Iran J .Med .Arom. Plants Res. 2021; 13(9), 5312.
20. Shamuni A., Razmi N. Effects of heat stress on malondialdehyde levels and catalase, glutathione peroxidase and superoxide dismutase activity in Rattus. J. Anim. Environ. 2021; 13(2), 107-112.
21. Wasti S., Sah N., Mishra B. Impact of heat stress on poultry health and performances, and potential Mitigation strategies .Animals (Basel). 2020; 24; 10(8):1266. doi: 10.3390/ani10081266
22. Raptova R., Alomar S.Y., Alwasel S.H., Nepovimova E., Kuca K., Valko M. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging Klaudia Jomova1. Archives of Toxicology. 2023; 97:2499–2574 https://doi.org/10.1007/s00204-023-03562-9 .
23. Rabiei Z., Setorki M. Effect of hydroalcoholic *Echium amoenum* extract on scopolamine induced learning and memory impairment in rats. Pharmaceutical Biology. 2018; 12: 56(1):672-677.
24. Nofouzi K., Mahmoudi R., Tahapour K., Amini E. *Verbascum speciosum* methanolic extract: phytochemical components and antibacterial properties. Journal of Essential Oil Bearing Plants. 2014; 19(2):499-505.
25. Chaichi M.J., Mehrzad J., Sedaghat R. GC-MS analysis of chemical compounds from aceton extract of *Echium amoenum* Fisch. Journal of Research in Pharmacy. (RIP) 4(Supplement). 2017: 9.
26. Hamidi E., Khaksari M., Hojabri K .The effects of aqueous extracts of *Echium amoenum* and *Citrus aurantiflia* on blood pressure and heart rate before and after phynelephrine injection in rat. Journal of Kerman University of Medical Sciences. 2011; 18: 349-357.
27. Miller N.J., Rice-Evans C., Davies M.J., Gopinathan V., Milner A.A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clinical Science. 1993; 84:407–12.
28. Arthur J.R., Boyne R. Superoxide dismutase and glutathione peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. Life Sciences. 1985; 22: 36(16):1569-75.
29. Paglia D.E., Valentine W.N. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical Medicine. 1967;70:158–169.
30.Góth L.A simple method for determination of serum catalase activity and revision of reference range. Clinica Chimica Acta. 1991; 196 (2-3):143-51.
31. Esterbauer H., Cheeseman K.H. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Meth. Enzym. 1990;186:407-21.
32. Norusis M.Y. Spss for windows base system user's guide release 6.0, 1st ed. SPSS Inc, Michigan. 1993; 281-290.
33. Gupta A., Chauhan N.R., Singh A., Chowdhury D., Meena R.C., Chakrabarti A., Singh S.B J.D.L.S.J. Heat induced oxidative stress and aberrations in liver function leading to hepatic injury in rats. Defence Life Science Journal. 2018; 4: 21-32. DOI:10.14429/dlsj.4.13174.
34. Gaykwad C.J., Garkhal G.E., Chethan S.N., De U.K.: Stress using N-acetylcysteine in canine parvoviral enteritis. Journal of Veterinary Pharmacology and Therapeutics. 2018; 41(1): 68–75.
35. Fortunato S .Redox signaling in plant heat stress response .Antioxidants. 2023; 12(3): 605; https://doi.org/10.3390/antiox12030605 .
36.Gonzalez-Rivas P.A., Chauhan S.S., Ha M., Fegan N., Dunshea F.R., Warner R.D. Effects of heat stress on animal physiology, metabolism, and meat quality: A review. Meat Science. 2019;162(2020): 108025. https://doi.org/10.1016/j.meatsci.2019.108025.
37. Dou J., Khan A., Khan M.Z., Mi S., Wang Y., Yu Y., Wang Y.J.G. Heat stress impairs the physiological responses and regulates genes coding for extracellular exosomal proteins in rat.Gen. 2020;11(3):306. doi: 10.3390/genes11030306.
38. Senturk E., Ustundag H.J.B.S. Heat Stress Alters oxidative and inflammatory responses in many tissues of male rats. Bezmialem Science. 2023; 11(4): 453-460.
39. Bhat S., Rao G., Murthy K.D., Bhat P.G. Seasonal variations in markers of stress and oxidative stress in rats. Indian Journal of Clinical Biochemistry. 2008; 23, 191-194.
40. Wang H.L., Xing G.D., Qian Y., Sun X.F., Zhong J.F., Chen K.L.J.E., Safety E. Dihydromyricetin attenuates heat stress-induced apoptosis in dairy cow mammary epithelial cells through suppressing mitochondrial dysfunction. Ecotoxicology and Environmental Safety. 2021; 214:112078. doi: 10.1016/j.ecoenv.2021.112078.
41. Jin J., Boersch M., Nagarajan A., Davey A.K., Zunk M.J.A. Antioxidant properties and reported ethnomedicinal use of the genus *Echium* (Boraginaceae). Antioxidants. 2020; 9 (8): 722. https://doi.org/10.3390/antiox9080722 .

42. Gutierres J.M., Carvalho F.B., Schetinger M.R.C., Agostinho P., Marisco P.C., Vieira J.M., Rosa M.M., Bohnert C., Rubin M.A., Morsch V.M., Spanevello R., Mazzanti C.M. Neuroprotective effect of anthocyanins on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia in rats. International Journal of Developmental Neuroscience. 2014;33: 88-97.
43. Min J., Yu S.W., Baek S.H., Nair K.M., Bae O.N., Bhatt A., Majid A.J.N.l. Neuroprotective effect of cyanidin-3-O-glucoside anthocyanin in mice with focal cerebral ischemia. Neuroscience Letters. 2011 18; 500(3):157-61. doi: 10.1016/j.neulet.2011.05.048.
44.Mehrabani M., Ghassemi N., Ghannadi E.S.A., Shams-Ardakani M.J.D.J.O.P.S. Main phenolic compound of petals of *Echium amoenum* Fisch. and C.A. Mey., a famous medicinal plant of Iran. DARU Journal of Pharmaceutical Sciences. 2005; 13(2): 65-69.
45. Bekhradnia S., Ebrahimzadeh M.A.J.R.C.J. Antioxidant activity of *Echium amoenum*. Review Chemistry. 2016; 67(2): 223-226.
46. Friedman T.J.J.O.R.M. The effect of rosmarinic acid on immunological and neurological systems: A Basic Science and Clinical Research. 2015; 4(1): 50.
47. Zeng H., Xi Y., Li Y., Wang Z., Zhang L., Han Z.J.A. Analysis of astragalus polysaccharide intervention in heat-stressed dairy cows' serum metabolomics. Animals (Basel). 2020 29; 10(4):574. doi: 10.3390/ani10040574.
48. Kra G., Daddam J.R., Gabay H., Yosefi S., Zachut M.J.A. Antioxidant resveratrol increases lipolytic and reduces lipogenic gene expression under in vitro heat stress conditions in dedifferentiated adipocyte-derived progeny cells from dairy cows. Antioxidants (Basel). 2021; 10(6):905.
49. Wang M., Huang X., Liu Y., Zeng J. Effects of *Macleaya cordata* extract on blood biochemical indices and intestinal flora in heat-stressed mice. Animals. 2022; 12(19): 2589. https://doi.org/10.3390/ani12192589.
50. Fang X., Nong K., Qin X., Liu Z. Effect of purple sweet potato-derived anthocyanins on heat stress response in Wenchang chickens and preliminary mechanism study. Poultry Science. 2023; 102(97): 102861. DOI:10.1016/j.psj.2023.102861.
51. Abed A., Minaiyan M., Ghannadi A., Mahzouni P., Babavalian M.R. Effect of *Echium amoenum* Fisch. et Mey a traditional Iranian herbal remedy in an experimental model of acute pancreatitis. International Scholarly Research Notices (ISRN) Gastroenterol. 2012; 141548: 7. doi: 10.5402/2012/141548.
52. Ranjbar A., Khorami S., Safarabadi M., Shahmoradi A., Malekirad A.A., Vakilian K.Medicine A. Antioxidant activity of Iranian *Echium amoenum* Fisch & CA Mey flower decoction in humans: a cross-sectional before/after clinical trial. Evidence-Based Complementary and Alternative Medicine. 2006;3(4):469-473. doi: 10.1093/ecam/nel03.
53. Mehrabi A., Mahmoudi R., Khedmati Morasa H., Mosavi S., Kazeminia M., Attaran Rezaei F., Shahsavari S., Vahidi R. Study of chemical composition, antibacterial and antioxidant activity of thyme leaves and stems essential oil. Journal of Medicinal Plants and by-Prod. 2022; 2: 253-263.
54. Mohammadzadeh M., Mahmoudi R., Ghajarbeigy P. Evaluation of chemical composition and antibacterial properties of *Froriepia subpinnta* essential oils from Guilan region: before and after flowering. Journal of Essential Oil-Bearing Plants. 21(4):1119-1127.
55. Kazeminia M., Mehrabi A., Mahmoudi R. Chemical composition, biological activities, and nutritional application of Asteraceae family herbs: A systematic review. Trends in Phytochemical Research. 2022; 6(3):187-213.