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Aqueous Extract of *Echium amoenum* Elevate CSF Serotonin and Dopamine Level in Depression rat

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ABSTRACT

Based on a traditional belief, *Echium amoenum* (Boraginaceae) dried flowers are used in Iran as an anxiolytic remedy and also as a mood enhancer. But interfering mechanisms unknown, In this study, efficacy of an aqueous extract of *E.amoenum* in depressive rat was evaluated, using measurement the changes level CSF serotonin and dopamine as indicator mood. Four groups of male wistar rats each group of seven rats were enrolled in this study. Depressive induced with intraperitoneal injection of reserpine (5mg/kg) and assessment depression by swimming test, the next two weeks of oral performing aqueous extract Echium amoenum, CSF serotonin and dopamine concentration of rats were measured using ELISA kit. Our finding indicated, performing aqueous extract Echium amoenum increased CSF serotonin and dopamine concentration in depression rats. The level of CSF serotonin enhanced in control group receiving Echium amoenum compared control group alone. These results showed the serious changes of neurotransmitters due to Echium amoenum which can be translated as mood enhancer. It can be concluded, Echium amoenum induce antidepressant effective in part by increasing level CSF serotonin and dopamine.

Key words: Echium amoenum**,** Depression, Serotonin, Dopamine, GABA.

INTRODUCTION

Depression disorder is one of the most common mental illness in the world and became a very important area of research interest in psychopharmacology. Major depressive disorder (MDD) is characterized by a lowered mood. The working mechanism of AD is believed to be either by increased neurotransmission by increased synaptic levels of serotonin, norepinephrine (NE) and dopamine (DA) (monoamines)[1]. The increased levels of monoamines were discoveredin the late fifties, when the TCAs and Monoamine oxidase A inhibitors (MAO-I) appeared to effectively treat MDD. This discovery resulted to the monoamine hypothesis: MDD might etiologically be explainedby a deficiency in monoamine neurotransmitters: serotonin (5-HT), NE or DA[2,3]. Desire in alternative medicine and plant-derived medications that affect the 'mind' is developing. Self administration of herbal medicines was the most popular alternative treatments to the official medicine. The application of herbal medications is becoming very common by physicians in Europe and Asia and researchers are exploring the traditional remedies to find a fit cure for these 'mind affecting diseases'.Moreover, Iranian climate

and favored geographical location have contributed to the diversity of medicinal plants.Borage (Echium amoenum) is a wild annual herb that belongs to Boraginaceae family which grows in large parts of Europe, Mediterranean region, and also in parts of Iran[4]. The flowers and the leaves of borage are used medicinally in the West and iran for the treatment of Stress, analgesic, anxiolytic, sedative, circulatory heart diseases, pulmonary complaints and other psychiatric illnesses[4-7]. Volatile constituents of E.amoenum was extracted by Ghassemi et al, These compounds include octadecane, heptadecane, viridiflorol, alpha cadinen etc[8]. Toxic pyrolizidine alkaloids of *E. amoenum* were separated by Mehrabani *et al.,*[9]. It has been shown that flavonoids possess mild sedative and anxiolytic effects, the naturally occurring flavonoids and their synthetic derivatives have been revealed to bind selectively to the central benzodiazepine receptors and to us anxiolytic and other benzodiazepine like effects in animals[10].The anxiolytic and antidepressant effect of the flower of Echium amoenum was demonstrated in several experimental studies in mice and human[9,11]. Despite the widespread use of *E. amoenum* as an antidepressant , there are no pharmacological data of antidepressant mechanism of *E. amoenum* . The aim of the present study was to evaluate effect the aqueous extract of E. amoenum on changes the level of CSF serotonin and dopamine as indicator mood in depression rat.

EXPERIMENTAL

Plant material an. Extraction

An aqueous extract (pH=6) of dried flowers was used in this study. *E.amoenum* flowers were collected from Ilam district, Iran. Flowers of this plant were separated and dried in roomtemperature (22-24 °C). The plant materials were powdered and exhaustively extracted with distilled water in a Soxhlet apparatus under reduced pressure. After evaporation of the solvent in rotary evaporator and then in oven at 40 °C, the residue was diluted with salinefor obtain the desire concentration (125 mg/ kg).

Animals

The experiment was conducted,using four groups (seven rat each)weighed 250-300g. The animals were kept in a 25±2 °C temperature with a 12 hr light /dark cycle and fed with standarddiet and tap drinking water. Animals were randomly divided into 4 groups; control ,depression, control + Echium amoenum and depression+ Echium amoenum,The rats were acclimatized to the laboratory for at least 1 h before oral performing of aqueous extractEchium amoenum(125mg/kg)and were administrated extraction CSF after two weeks of treatment,The experiments were carried out between 9.00 and 14.00 h. The experimental protocol was approved byof Animal house and Ethical Committee at Ilam University of Medical Sciences (IUMS).

Porsolt swim test for assessment depression

The procedure for the Porsoltforced swim test was as previously reported (Porsolt, LePichon, and Jalfre, 1977). By administrating depression rats with intraperitoneal injection of reserpine-sigma(5mg/kg) (12) and assessment depression by swimming test, Briefly, rats were placed in a cylindrical container (40 cm deep, 27 cm in diameter) filled with 30 cm of 30°C water. The amount of time the rats spent swimming or immobile was recorded in a 10-min test. Swimming was defined as movement of the forelimbs and hindlimbs without the front paws breaking the surface of the water. Immobility was recorded when there was an absence of any movement other than that necessary to keep the head andnose above the water (e.g., when rats were floating in a vertical position).

CSF sampling

The rats were anesthetized with 40 mg kg of sodium thiopental, intrapritonal andplaced in a stereotaxic frameand implanted with a guide cannula which was an insulin syringe (27 gauge 31/ 20 length by direct puncture of the cisterna magna was drawn CSF (40–60 micro liter per rat), CSF was collected from each rat (13).

Neurotransmitters analysis

The level serotonin and dopamine of CSF samples were measured using the IBL international GMBH ELISA kit (Germany) and LDN GMBH Kit according to the manufacturer instruction (14, 15, 16). The procedures for each neurotransmitter is summarized as follows.

Serotonin ELISA

20 µL of each Control and sample was

pipetted into glass test tubes the 100 µL of diluted Assay Buffer was added to each tube. Vortex.Then 25 µL of Acylation Reagent 1 (3 %) Pipetted into each tube. Vortex each tube immediately after pipe ting. Cover tubesIncubated 15 min at 37°C in a water bath, Then 4 mL of diluted Assay Buffer was Pipetted into each tube. Vortex. The all tubes Centrifuged for 10 min at 1500 x g. immediately Prepared samples assayed, The supernatant was stabled for only 1 h at 18-25°C.

Dopamine ELISA

In the first 25 µl of the Enzyme Solution was pipetted into all wells of the Dopamine Microtiter Strips. Then was added 100 µl of the standards, controls and samples into the appropriate wells incubated for 30 min at RT on a shaker (approx. 600 rpm) and also 50 µl Dopamine antiserum was added to the wells and the plate covered with adhesive Foil, then incubated for 2 hours at RT on a shaker. 3 times washing content of the wells and discarding followed by using 300 µl washing buffer. Next stage was added 100 µl of enzyme conjugate into all wells incubating 30 min at RT on a shaker. After washing, 100 µl of the substrate was added into all wells and incubated for 20-30 min at RT followed by adding 100 µl of the stop solution to each well and reading the absorbance of the solution in the wells within 10 minutes, using a micro plate reader set to 450 nm and a reference wavelength between 620 nm and 650 nm.

Statistical analysis

Results are presented as mean±S.E.M. ANOVA was used for compare serotonin and dopamine in each group of 7 rat. Normality of data in each group was checked using one sample kolmogroror- simirnov test, Dennett test was performed as a post hoc multiple comparison analysis,P- value <0.05 was significantstatistically.

RESULTS

Effect of Echium amoenum aqueousextract on changes level CSF serotonin

Figure 1 display, the effect of aqueous extract of Echium amoenumon on changes level CSF serotonin in different groups, ANOVA analysis indicated the differences between various groups (P<0.05), the following analysis with Dunnett- test (Table 1) showed that changes level serotonin in the control group with the depression group and depression group receiving Echium amoenum was statically significant(P<0.05). However, the difference between depression group with depression group receiving Echium amoenum was statically significant (P<0.05). whereas for the other cases, it was not. In tables and figures con equal control, dep (depression) and Ech (Echium amoenum).

Effects of Echium amoenum aqueous extract on changes level CSF dopamine

Figure 2 indicate, the effect of aqueous extract of Echium amoenumon on changes level CSF dopamine in different groups, ANOVA analysis indicated the differences between various groups (P<0.05), the following analysis with Dunnett- test (Table2) showed that changes level dopamine in the control group with the depression group was statically significant(P<0.05). However, the difference between depression group with depression group receiving Echium amoenum was statically significant (P<0.05). Whereas for the other cases, it was not.

Table 1: The effect of aqueous extract Echium amoenum with dose 125 mg/kg after two weeks of oral performing on changes level CSF serotonin in different groups. Data are presented as mean values (“S.E.M.) from a group of 7 rat each. P-0.05 compared with control

Dependent Variable	J group	I group	Mean Difference (J-I)	Std. Error	Sig.
CSF serotonin	Control	Depression	-8.25714	1.06954	.000
	Control	Depression+Ech	-2.21429*	61994.	.004
	Control+Ech	Depression+Ech	-1.25714	61994.	.131
	Depression	Depression+Ech	-2.65714	.61994	.001

Table 2: The effect of aqueous extract Echium amoenum with dose 125 mg/kg after two weeks of oral performing on changes level CSF dopamine in different groups. Data are presented as mean values ("S.E.M.) from a group of 7 rat each. P-0.05 compared with control

Dependent Variable	J group	I group	Mean Difference (J-I)	Std. Error	Sig.
CSFdopamine	Control Depression		-8.25714	1.06954	.000
	Control Depression+Ech		.04286	.04041	.590
	Control +Ech Depression+Ech		.10000	.04041	.053
	Depression Depression+Ech		-.20000	.04041	.000

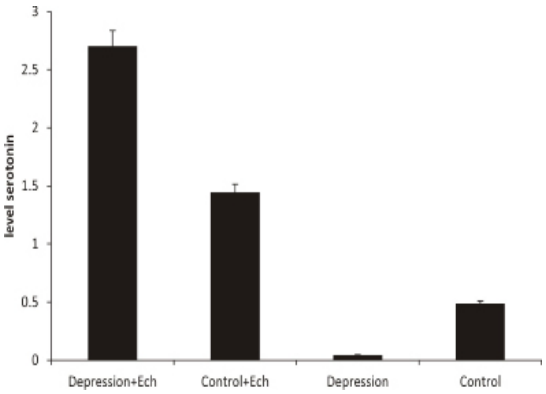

Fig. 1: The effect of Echium amoenum aqueous extract on changes level CSF serotonin in different groups

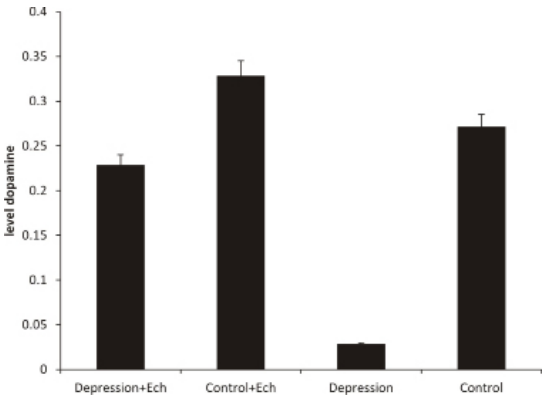

Fig. 2: The effect of Echium amoenum aqueous extract on changes level CSF dopamine in different groups

DISCUSSION

Impact of Echium amoenum aqueous extract on rat depression by evolution change level CSF serotonin

The lowering of in brain serotonin cause lower mood and enhancing in irritability or aggressive behaviors but the increasing concentration of serotonin possibly help to develop more constructive social interactions by reducing aggression and increasing dominance(17,18). Altered serotonin metabolism may be a highly magnify interfering factor aggression and suicide behavior in human, because reported earlier that lowering levels of cerebrospinal fluid 5-hydroxyindoleacetic acid (5-HIAA), a serotonin metabolite cause these behaviors (19). Our finding showed which the level of CSF serotonin increased in depression group and control group treatment by Echium amoenum (Figure 1).

Impact of Echium amoenum aqueous extract on rat depression by evaluation change level CSF dopamine

The studies revealed that depressive symptoms in schizophrenic patients are accossieted with a decrease in dopamine synthesis(20-22). The finding indicated that DA and DA D2 receptors play magnify role in the reinforcing responses to psycho stimulants in humans[23].The studies Electro physiological demonstrated dopamine responses that consist predominantly of depressions[24]. Direkvand-Moghadam, A., Khosravi, A.

The impact of a novel herbal Shirazi Thymus Vulgaris on primary dysmenorrhea in comparison to

the classical chemical ibuprofen (2012) Journal of Research in Medical Sciences, 17 (7), pp. 668-670. Cited 2 times.
http://www.scopus.com/inward/record.url?eid=2-s2.0-84866760275&partnerID=40& md5=6ba1 2cc6 0d7fad8a28bb322699275455

Document type: Article

SOURCE: Scopus).Our finding demonstrated that the level CSF dopamine increased in depression group treatment by Echium amoenum (Figure 2), the results showed that anxiolytic effect of the extract Echium amoenum was most evident in 125 mg/kg group (6). Petals of E. amoenum have been advocated for its anxiolytic, sedative, anti-inflammatory, demulcent and analgesic effects, particularly for common cold, in folk medicine of Iran (4-7).

Data analysis of the present study showed that the aqueous extract of Echium amoenum (125 mg/kg) significantly increased the level of CSF serotonin and dopamine. The results suggests that Echium amoenum has effect antidepressant and enhancer mood , in part active with elevating level of CSF serotonin and dopamine as indicators mood. Althught arenot clear contributing mechanisms we suggest the researches continue with investigate effect Echium amoenum on changes level norepinephrine, GABA and other neurotransmitters as mood disorders indicators.

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