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Anticonvulsant Activity of *Glaucium vitellinum* Boiss & Buhse

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The anticonvulsant activities of *Glauciumvitellinum* extract and alkaloid sub-fraction were assessed in pentylenetetrazole (PTZ)-induced convulsion and maximal electroshock test (MEST) in mice, with Diazepam as standard drug. While mechanistic studies were conducted using flumazenil, a GABAA-benzodiazepine receptorcomplex site antagonist.The extract and the alkaloid sub-fractionproduced protectionagainst convulsion at100, 200, 400 mg/kg and 50 mg/kg respectively compared with protection with benzodiazepine.The mean onset and percentage protection against convulsion in extract/sub-fraction-treated mice werereduced by flumazenil. We also showed that *G. vitellinum* extract (200 mg/kg) exhibited inhibition of the tonic convulsion induced by MES and decreased mortality rate.These results suggest that *G. vitellinum*possesses biologically active constituent(s) mainly belong to isoquinoline alkaloid group that haveanticonvulsant activity which supports the ethnomedicinal claims of the use of the plant in the management of seizure.

Key words: *Glauciumvitellinum*, Anticonvulsant, Alkaloids.

Described as a chronic disorder of the central nervous system, epilepsy is a major medical and social problem which is characterized by recurrent seizures due to excessive discharge of cerebral neurons[1,2]. According to the WHO[3] around 450 million people in the world have affected mental, neurological, or behavioral problems at some time in their life of which about 50 million suffer epilepsy[4]. Extensive research on plants and their derivatives and investigations into natural sources of effective drugs that may be more readily accessible have taken place in recent years that could provide some new alternative treatments and therapeutic uses for diseases of the central nervous system (CNS) especially epilepsy and seizure. Interest in medicinal plants reflects the recognition of the validity of many traditional claims regarding the value of natural products in healthcare[5].

The medicinal use of plants has been known since the early times. Some are used in the control of emotions and mood, for their anticonvulsant properties and sedative, anxiolytic and antidepressant effects. Some studies suggest that they act by modulating the central neurotransmission[6].

The genus *Glaucium* (Papaveraceae) comprises about 25 species of annual, biennial or perennial herbaceous flowering plants in the world of which 11 species are found in different parts of Iran. Iran, particularly, is one of the centers of this genus origin with native and endemic species, described by the common Persian name of 'Shaghayegh'[7]. Several *Glauciem* species are used in folk medicine as laxative, hypnotic,

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antiseizure, antidiabetic, anti-inflammatory[8] and antispasmodic[9] agents, and for antiseptic and astringent properties as a topical remedy especially in dermatitis[10] and relieving warts[11]. The diversity, species richness and variation, as well as chemical properties have recently led to much research on the genus *Glaucium*. Phytochemical investigations have reported isoquinoline alkaloids including aporphines, protopines, protoberberines and proaporphines as the major constituents of *Glaucium* species[12].

G. vitellinum is one of the endemic perennial species distributed in central parts of Iran in rocky Mountains. It is an almost glabrous perennial herb with large yellow flowers and elongated capsule. Each of four yellow petals has little spot on the base. Blooms appear from April to July[13]. Literature survey revealed that *G. vitellinum* has just been phytochemical investigated and isocorydine, protopine, dicentrine, tetrahydropalmatine, muramine, bulbocapnine and glaucine have been identified in this plant[14].

Due to the folklore use of this plant in Iranian traditional medicine for relief and treatment of seizure, we prompted to evaluate the anticonvulsant activity of itstotal methanol extract and alkaloid sub-fractionand investigate the pharmacological basis for the folkloric use of it as an anticonvulsant agent. This study explores the anticonvulsant property of *G. vitellinum*by investigating their suppression of seizures induced by Pentylenetetrazole (PTZ) which enhances excitatory responses in the central nervous system by inhibiting inhibitory responses to glycine and gamma amino butyric acid respectively[15]. Maximal electroshock-induced convulsion model also employed to anticonvulsant activity evaluation of the extract.

MATERIALS AND METHODS

Plant material

Fresh flowering aerial parts of *G. vitellinum* were collected in June 2013 from the mountain areas of Khansar County, Isfahan Province, Iran: (33°152 N 50°202 E, 2600m). Specimen was identified by Dr. G.H. Amin and voucher was deposited in the Herbarium of Pharmaceutical Sciences Branch, Islamic Azad University (IAU), Tehran under code number 681.

Extraction procedure

2 kg of the air-dried grounded plant was extracted by percolator apparatus using methanol. The extraction was repeated for 3 times. The extract was concentrated by rotary evaporator apparatus and the solvent removed to produce a dark green gummy solid. An adequate part of the resulting extract was kept in a sterile vial in a dark and cool place for further tests. The remains were extracted for total alkaloids. 200 ml of acetic acid-water (50:50) was added to the residue and the mixture was filtered. The filtrate was extracted with petroleum ether (5x100 ml) to remove colored materials. The aqueous layer was then made alkaline with 25% ammonia and extracted with chloroform (5x150 ml). Evaporation of the solvent gave a crude mixture of alkaloids (alkaloid sub-fraction).

Experimental animals

Albino mice of either sex (20–25 g) were housed in groups of 5 and were allowed free access to food and water except for the short time that the animals were removed from their cages for testing. All experiments were conducted during the period between 10 a.m. and 13 p.m. with normal room light (12 h regular light/dark cycle) and temperature (22±1 °C). The procedures were carried out in accordance with the institutional guidelines for animal care and use (ethical approval number: 3183). Each mouse was used only once.

Anticonvulsant activity

Pentylenetetrazole (PTZ)-induced convulsion in mice

Myoclonic seizure induced by pentylenetetrazole (PTZ) is a standard experimental model of clinical myoclonic petit-mal seizures with both face and construct validity. To assess the seizure susceptibility, the more sensitive method of IV administration of PTZ that allows better detection of modulatory effects on convulsive tendency was used[16]. The threshold of PTZ was determined by infusion of PTZ (0.5%) at a constant rate of 0.5 mL/min into the tail vein of unrestrained freely moving mice. Infusion was halted when forelimb clonus followed by full clonus of the body was observed[17].

Maximal electroshock-induced convulsion in mice

The seizures were induced by maximal electroshock in male NMRI mice with the help

of electroconvulsiometer by passing current of 50 mA for 0.2 s using ear clip electrodes. The studied compounds or drugs were given 1 hour prior to seizure induction. Drops of saline were instilled in both eyes in order to ensure current transmission. The occurrence and latency of tonic convulsion, characterized by tonic hind-limb extension (THLE), and death were registered[18]. The ability to prevent this feature or prolong the latency and/or onset of the THLE was considered as an indication of anticonvulsant activity[19,20].

Treatments

The method of IV administration of PTZ to assess the seizure susceptibility was used. 55mice were divided into 11 groups each containing 5 mice. The first and second groups received the vehicles, salin(IP) and DMSO 10%, the third to tenth groups received 100, 200, 400, 800 mg/kg and 50, 100, 200 and 400 mg/kg IPof the extract and alkaloid sub-fraction respectively, while the eleventh group was injected with diazepam 0.025 mg/kg IP. Thirty minutes after treatment, the mice in all the groups received PTZ. Mice were placed into separate individual plastic cages for observation lasting 1 h. The onset of a general clonus was used as the endpoint. The general clonus was characterized by forelimb clonus followed by full clonus of the body[21]. We also studied the effects of flumazenil, a selective benzodiazepine receptor antagonist site in the GABAA-BZD receptor complex, on the anticonvulsant activity of *G. vitellinum* extract in order to elucidate the mechanism involved in extract/alkaloid sub-fraction-induced protection of mice from PTZinduced seizure. Flumazenil (0.5 mg/kg) was administered 15 min before diazepam and 20 min before the extract (400 mg/kg) and 80 min before i.v. administration of diazepam.

Statistical analysis

Data were expressed as mean±SEM. The oneway analysis of variance (ANOVA) followed by Tukey multiple comparisons were used to analyze the data of clonic seizures. P<0.05 was considered the significant level between the groups.

RESULTS AND DISCUSSION

The anticonvulsant activities of the extract and alkaloid sub-fraction of *G. vitellinum* were determined using chemically induced (PTZ) convulsion in mice.

Figure 1 shows the effect of acute IP administration of different doses of *G. vitellinum*extract (100, 200, 400 and 800 mg/kg) on the clonic seizure threshold induced by intravenous PTZ. Different doses of the extract were administered 30 min prior to PTZ to distinct groups of mice. One-way Anova revealed a significant effect of the extract in doses of 100, 200 and 400 mg/kg (P< 0.05) compared to the group which received just the vehicle.

Figure 2 demonstrates the effect of acute IP administration of different doses of *G. vitellinum* alkaloid sub-fraction (50, 100 and 200 mg/kg) on the clonic seizure threshold induced by intravenous PTZ. Different doses of the alkaloid sub-fraction were administered 30 min prior to PTZ to distinct

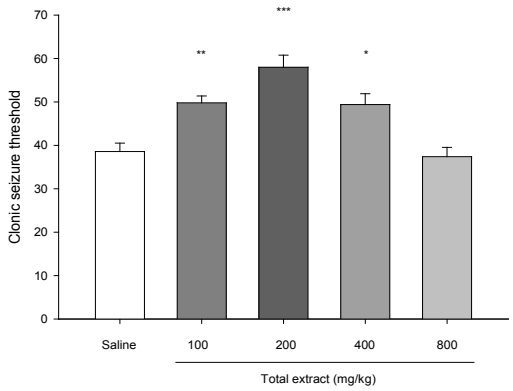

Fig. 1. The effect of different doses of *G. vitellinum* extract on PTZ-induced clonic seizure threshold in mice 30min prior to PTZ injection. * P<0.05 compared to vehiclecontrol group.

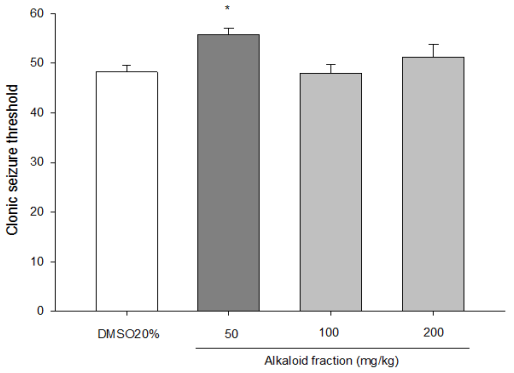

Fig. 2. The effect of different doses of *G. vitellinum* alkaloid sub-fraction on PTZ-induced clonic seizure threshold in mice 30min prior to PTZ injection. * P<0.05 compared to vehiclecontrol group.

groups of mice. One-way Anova revealed a significant effect of the sub-fraction in dose of 50 mg/kg (P< 0.05) compared to the group which received just the vehicle. All the mice received dose of 800 mg/kg of alkaloid sub-fraction died.

Clonic seizure was induced by ã-aminobutyric acid (GABA) transmission blocker PTZ (22).Regarding the possible contribution ofGABAergic system in the anticonvulsant activityof the extract, flumazenil, a benzodiazepinereceptor antagonist, was used (19). Flumazenildecreased the prolongation of seizure latencyinduced by the extract and it also antagonized theeffect of the extract on decreasing the duration ofclonic seizures in the PTZ model. Since theanticonvulsant effect of the extract was blocked by an antagonist of benzodiazepine receptor, itseffect seems to be related to benzodiazepinereceptor activation.

Figure 3 and 4 show the effect of the extraxt (100 and 200 mg/kg) and alkaloid sub-fraction (25 and 50 mg/kg) on tonic hindlimb etension duration respectively. Chi-aquare revealed a significant effect of neroli on the incidence of tonic seizure and death after electroshock (P<0.001 and P<0.01 respectively).

Recent studies on medicinal plants and their maincomponents have attracted the attention of manyscientists and encouraged them to screen thesenatural sources for their chemical andpharmacological aspects that might potentiallylead to the development of new anticonvulsantcompounds. The present study has investigatedthe anticonvulsant effect of *G. vitellinum*methanol extract and alkaloid sub-fraction using myoclonic seizureinduced by pentylenetetrazole (PTZ) modelwhich is a

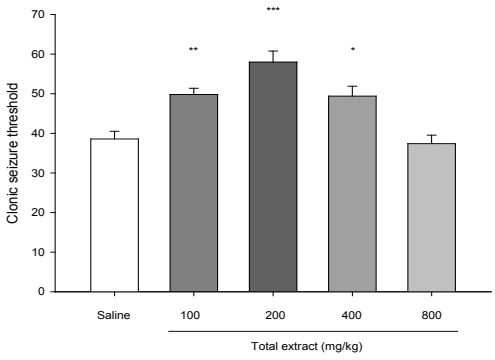

Fig. 3. The effect of different doses of *G. vitellinum* extract on tonic hind limb extension duration in Maximal electroshock-induced convulsion. * P<0.05 compared to vehiclecontrol group

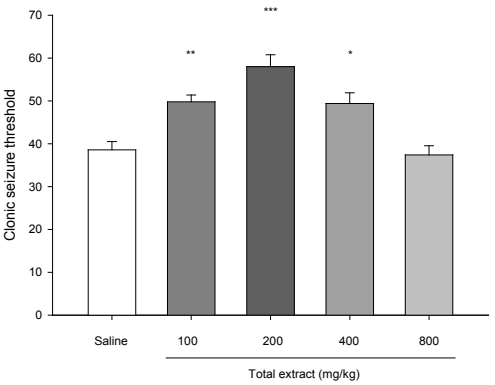

Fig. 4. The effect of different doses of *G. vitellinum* alkaloid sub-fraction on tonic hind limb extension duration in Maximal electroshock-induced convulsion. * P<0.05 compared to vehiclecontrol group

standard experimental model ofclinical myoclonic petit-mal seizures with bothface and constructs validity. Maximal electroshock-induced convulsion model also employed to anticonvulsant activity evaluation of the extract and the related sub-fraction. The results of thepresent study have demonstrated that *G. vitellinum* extract especially the related alakloid sub-fraction possessed anticonvulsantactivity.The major components of the aerial parts of the investigated plant have been reported asIsoquinolin alkaloids including isocorydine, protopine, dicentrine, tetrahydropalmatine, muramine, bulbocapnine and glaucine[14]. Psychopharmacological evaluation of isoquinoline alkaloids such as berberine have revealed that these compounds modulate several neurotransmitter systems like N-methyl-D-aspartate, nitric oxide and serotonin, which modulate convulsions[23]. As the results, *G. vitellinum* especially its alkaloid sub-fraction which is rich in isoquinoline alkalois could be useful in treatment of convulsion and epilepsy. The present results provide a rationale for *G. vitellinum* use in folkloremedicine for management of epilepsy.

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