



Anticonvulsant and antioxidant activities of crude flavonoid extract of *Matricaria chamomilla* L. against convulsions induced by pentylenetetrazole in chicks

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Abstract

In the current study, crude flavonoid extract of *Matricaria chamomilla* L. (MC) was used to evaluate anticonvulsant, and antioxidant activities on pentylenetetrazole (PTZ) induced convulsion in chicks. The biochemical estimation was done by measuring brain tissue neurotransmitters (gamma-aminobutyric acid GABA and glutamate), oxidative stress biomarkers in serum (catalase CAT, glutathione reductase GR, malondialdehyde MDA, and 8-isoprostane), serum electrolytes (potassium Ka^+ , sodium Na^+ , chloride Cl^- , ionized calcium iCa^{2+} , total calcium TCa^{2+}), pH of serum, and glucose level in serum. Seventy-two broiler chicks (2 weeks old) were randomly divided into six groups (n=12): The first group (negative control) received the normal saline subcutaneous injection, the second group (positive control) received PTZ 90 mg/kg subcutaneous injection, and the third group treated with sodium valproate (SV) 200mg/kg orally. The fourth, fifth and sixth groups treated with 20, 40, 80 mg/kg of crude flavonoid extract of MC respectively orally for six days before PTZ injection. Thirty minutes post-treatment of the last dose, the chicks in the (third to sixth) groups received PTZ. The results showed that the crude flavonoid extract of MC attenuated the convulsion signs and mortality dose-dependently. The pre-treated crude flavonoid extract at the dose of 80mg/kg showed a significant increase in the serum level of Na^+ and iCa^{2+} , and a decrease in 8-isoprostane. In conclusion: the crude flavonoid extract of MC 80mg/kg possesses mild to moderate anticonvulsant and antioxidant effects.

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Introduction

Convulsion is often described as a symptom of an epileptic seizure characterized by sudden recurrent spontaneous seizures due to abnormal excessive electrical activity in the brain. Although not all convulsions have resulted from epileptic seizures, and not all epileptic seizures lead to convulsions, sometimes the term convulsion is used as a synonym for seizure (1,2). The pathophysiological mechanism related to the seizure is an imbalance between inhibitory GABA (γ -aminobutyric acid) mediated and

excitatory (glutamate) mediated neurotransmission pathways that are located at different parts of the brain (3). Furthermore, it was found that the seizure reduces the antioxidant defense mechanism and increases the reactive oxygen species (ROS) production in the brain, which further induces oxidative stress, lipid peroxidation, and neurodegeneration of the brain tissue and predisposes the brain to spontaneous recurrent seizure (4,5). Pentylenetetrazole is a well-known convulsant and is widely used in experimental studies to discover and evaluate the antiepileptic substances that are effective in attenuating the

convulsion induced by PTZ (6). In recent years have been more interested in using medicinal plants to find therapies for neurodegenerative diseases. *Matricaria chamomilla* L. (MC), commonly known as German chamomile, is a traditional medicinal plant belonging to the Asteraceae family (7). Experimental studies have shown that the plant extracts possess a diverse range of medicinal properties such as anticonvulsant (8), antioxidant (9), antidepressant (10), anxiolytic (11), treatment of upper respiratory tract infection (12), and dermatitis (13), as it is rich in different bioactive compounds such as flavonoids (apigenin, quercetin, and luteolin), chamazulene, bisabolol, coumarins, and terpenoids (14).

Experimental studies have demonstrated that flavonoids can protect the brain from oxidative stress-induced damage and prevent brain excitability (15). Previous studies have reported that apigenin, a major flavone-type molecule in MC, shows an anticonvulsant effect by modulating the GABA_A receptor (16,17). Moreover, it has been reported that apigenin also has many pharmacological properties such as neurodegenerative therapy, antioxidant, anti-inflammatory, and therapeutic agent to overcome several types of cancers (18). Therefore, the present study evaluates the anticonvulsant and antioxidant effects of crude flavonoid extract of MC on convulsion models chicks induced by PTZ.

Materials and methods

Ethical approve

This experiment was performed with the approval of the Local Ethics Committee of College of Veterinary Medicine, University of Duhok, Iraq. The issue number was CVM2021027UD at 7/2/2021.

Collection of plant samples and authentication

The flower parts of MC were collected during the flowering period in April 2020 in Duhok province of Iraq. The plant was identified and authenticated by a botanical specialist in the Department of Forestry, College of Agricultural Engineering Sciences, University of Duhok, Iraq. Then the flower part was washed with tap water and air-dried under shade for two weeks. After that, finely ground and stored in paper sacks in a dark place and kept at room temperature until use (19).

Preparation of the crude flavonoids extract

50 gm of chamomile flower were mixed with 250 ml of 70% ethanol using a magnetic stirrer overnight. Then, gauzes and filter papers removed the residue. The filtered solution was concentrated under reduced pressure at 40 °C using a rotary evaporator to obtain alcoholic extract 9.7 gm. Then 5 gm of 70% ethanolic extract was used to extract the crude flavonoid present in it by methods described by (20,21). The yield extract was light reddish, referred to as crude flavonoid

extract 0.8 gm. The extract was stored in a will-closed glass container in the refrigerator to be used within three days.

Laboratory animals

This study obtained a 72-mixed breed of day-old broiler chicks of both sexes from a local hatchery. The birds were reared in an animal house at the college of veterinary medicine for two weeks before being used in this study and reach the weight 250–310 gm. They were maintained at 33 °C in the first week and gradually decreased the temperature to reach 28 °C on day 19 with persistent lighting. A basal diet of chick was fed to the animals and water *ad libitum*.

Experimental design and induction of convulsion

Seventy-two chicks were randomly divided into six groups of 12 chicks/group. The first group (negative control) received a subcutaneous (SC) injection of normal saline. The second group (positive control) injected pentylenetetrazole (PTZ) (Sigma, USA) subcutaneously at the neck region at the dose of 90 mg/ml/kg body weight (22), and the third group was treated with standard anticonvulsant drug sodium valproate at the dose of 200 mg/5ml/kg b.w. (SANOFI, France) orally for six successive days, and the fourth, fifth and sixth groups received 20, 40, and 80 mg/5ml/kg b.w. crude flavonoid extracts respectively orally for six days (23). Thirty minutes post-treatment of the last dose in groups 3-6, the chicks received PTZ SC at the dose of 90 mg/ml/kg b.w.

Observation and recording the signs of convulsions

Behavioral activities were observed and recorded by digital video camera for 30 minutes after PTZ injection to record the onset and duration of convulsion. The anticonvulsant activity of the flavonoids was considered by prolonging the latency period or preventing the convulsion (19).

Collection of blood specimens

At the end of the experiment, after 3 hours from PTZ injection, the blood sample was collected by heart puncture in overnight fasting for 12 hours. The blood was kept in a plain tube to obtain serum by centrifuging it at 5000 rpm for 10 minutes, and then kept at -18°C until use for some electrolytes and biochemical parameters analysis (24).

Collection of brain samples

At the end of the experiment, the animals were sacrificed by decapitation, and the brain was removed. The brain tissue was stored in a plastic container and kept at -18 °C until use for neurotransmitters analysis (19).

Measurement of neurotransmitters in the brain

The frozen brain tissues were thawed. The brain tissues 100 mg were minced and homogenized with 0.9 mL of cold phosphate-buffered saline (pH 7.4) with a homogenizer

(Coyote, China) on ice, and centrifuged at 7250 rpm for 5 minutes at 4 °C according to the instruction of the manufacture kits. The supernatants were stored at -20 °C for neurotransmitters analysis. According to the manufacturer's instructions, the brain GABA and glutamate levels were measured using Chicks GABA and Glutamate ELISA kits (BioAssay Technology Laboratory, China).

Analysis of oxidative stress parameters in the serum

The antioxidant enzymes, catalase (CAT) and glutathione reductase (GR), and the lipid peroxidation markers, malondialdehyde (MDA) and 8-isoprostane levels in the serum were measured by using commercially available chicken catalase, glutathione reductase, MDA and 8-isoprostane Chicken ELISA kits (BioAssay Technology Laboratory, China) according to the manufacturer's instructions.

Determination of some electrolytes, pH, and glucose levels in the serum

Potassium (K⁺), sodium (Na⁺), chloride (Cl⁻), ionized calcium (iCa²⁺), total calcium (TCa²⁺), and pH of the serum were measured by an automatic electrolyte analyzer (fortress diagnostics, UK). The serum glucose level was measured calorimetrically by a commercially available kit (Biolabo/France).

Statistical analysis

Results were presented and described as mean±standard error of the mean (SEM). The statistical analysis of the data was carried out using one-way analysis of variance (ANOVA) and then subjected to the Duncan test for comparison of differences between means. The results were considered statistically significant at P<0.05. The statistical analysis was performed by SPSS software, version 23.

Results

A Single subcutaneous injection of PTZ 90 mg/kg b.w. induced obvious convulsion signs in animals within 7–25 minutes. The convulsive behaviors were observed, including restlessness, defecation, spasm and extension of the legs (Figure 1A), inability to stand (Figure 1B), vocalizing violent sounds, the uncontrolled flapping of wings (tonic-clonic convulsions) (Figure 1C), loss of consciousness, asphyxia and mortality in about (33%) as compared to the negative control group. The standard anticonvulsant drug, sodium valproate at dose 200 mg/kg b.w. orally provided 100% protection compared to the positive control group, as shown in (Figure 1D). The crude flavonoid extract showed dose-dependent protection on PTZ-induced convulsion in chicks by delaying the onset of convulsion signs and decreasing mortality by about 25%, 16%, and 10% in chicks

pretreated with crude flavonoid extract at doses 20, 40, 80 mg/kg respectively.

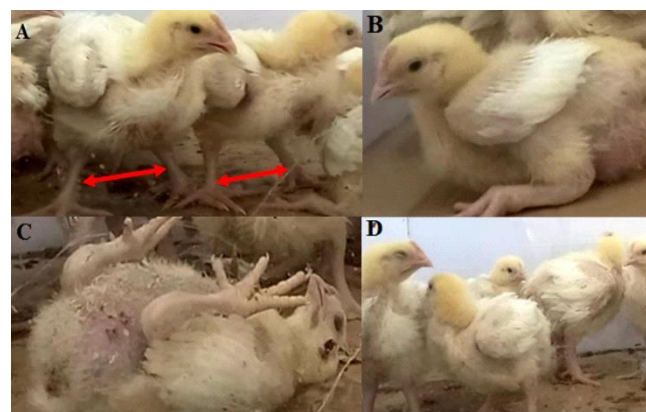


Figure 1: Clinical signs of convulsion induced by PTZ in chicks pretreated with sodium valproate and crude flavonoid extracts of MC. (A- legs extension and muscle contraction, B- unable to stand, C- tonic-clonic convulsions, D- no obvious convulsion signs observed).

The effect of crude flavonoid extracts on brain tissue neurotransmitters was shown in table 1. The present study showed that the group treated with PTZ caused a significant decrease in the level of GABA and increased glutamate in brain tissue compared with the negative control group that received normal saline. The group pretreated with sodium valproate and PTZ showed that GABA significantly increased, while the glutamate level decreased significantly in brain tissue compared with the positive control group. The groups pretreated with crude flavonoid extract in all three doses showed a slight elevation in the GABA level. Also, the glutamate level in brain tissue was insignificantly reduced compared with the positive control group.

Table 1: Effect of crude flavonoid extract of MC on brain tissue neurotransmitters in convulsions induced chicks treated by PTZ compared to control groups

Groups	GABA (ng/gm)	Glutamate (ng/gm)
NC	31.27±4.24 ^a	28.63±1.61 ^b
PC	12.03±0.42 ^b	42.41±1.62 ^a
SV 200mg/kg	28.02±1.54 ^a	27.04±1.67 ^b
F 20mg/kg	16.05±2.11 ^b	40.48±1.68 ^a
F 40mg/kg	17.66±2.19 ^b	37.92±0.92 ^a
F 80mg/kg	17.41±1.14 ^b	40.04±1.10 ^a

NC Negative control, PC positive control, SV Sodium Valproate, F Flavonoids, values are expressed as mean±SE, n=12 chicks per group, Different letters within each column differ significantly at P<0.05 according to the Duncan test.

The data in table 2 showed the effect of the flavonoid extract on the antioxidant enzyme and oxidative stress biomarkers in the serum. It was found that the positive control group treated with PTZ showed a significantly decreasing in CAT and GR, while lipid peroxidation markers MDA and 8-isoprostane increased significantly as compared with the negative control group. The group pretreated with sodium valproate showed an insignificant increase in CAT and a significant increase in GR compared to the positive control received PTZ. The MDA and 8-isoprostane significantly reduced as compared with the positive control. The groups pretreated with crude flavonoid extract (20, 40 and 80 mg/kg b.w.) led to an insignificantly increase in the level of CAT and GR and a decrease in MDA compared to the positive control group. The groups pretreated with crude flavonoid extract (40 and 80 mg/kg b.w.) caused a significant decrease in the level of 8-isoprostane compared to the control group that received PTZ.

The effect of the flavonoid extract on some serum electrolytes concentrations, serum pH, and serum glucose in PTZ-induced convulsions in chicks was summarized in table 3. The results showed that the control group that received PTZ showed a significant increase in K⁺ ions, and a decrease in Na⁺ and iCa²⁺ ions compared with the negative control group. The group pretreated with sodium valproate showed a significant reduction in K⁺ ions, and a significant elevation in Na⁺ and iCa²⁺ ions compared to the positive control group. The group pretreated with crude flavonoid extract at the dose of 40 mg/kg showed a significant increase in Na⁺ ions, and the extract at a dose of 80 mg/kg showed a significant increase in Na⁺ and iCa²⁺ ions when compared with the positive control group. No statistically significant differences were observed in Cl⁻ ion, TCa²⁺, pH, and serum glucose levels among all groups.

Table 2: Effect of crude flavonoid extract of MC on serum antioxidant enzymes and oxidative stress biomarkers in convulsions induced chicks treated by PTZ compared with control groups

Groups	CAT (ng/ml)	GR (ng/ml)	MDA (nmol/ml)	8-Isoprostane (ng/L)
NC	45.95±6.74 ^a	2.62±0.24 ^a	5.01±0.55 ^b	478.93±26.92 ^c
PC	26.75±3.15 ^b	1.63±0.16 ^b	10.02±1.79 ^a	834.34±54.33 ^a
SV 200mg/kg	40.29±4.59 ^{ab}	2.47±0.16 ^a	5.84±1.08 ^b	659.88±42.93 ^b
F 20mg/kg	26.60±2.90 ^b	1.86±0.35 ^{ab}	8.52±1.47 ^{ab}	715.45±35.97 ^{ab}
F 40mg/kg	28.51±3.18 ^b	2.02±0.28 ^{ab}	7.16±1.29 ^{ab}	676.51±47.05 ^b
F 80mg/kg	35.07±4.04 ^{ab}	2.22±0.21 ^{ab}	6.67±0.94 ^{ab}	651.53±57.60 ^b

NC Negative control, PC positive control, SV Sodium Valproate, F Flavonoids, n =12 chicks per group, Values are expressed as mean±SE. According to the Duncan test, different letters within each column differ significantly at P<0.05.

Table 3: Effect of crude flavonoid extract of MC on serum electrolyte parameters, serum pH and serum glucose in convulsion induced chicks by PTZ compared with control groups

Groups	K ⁺ ion (mmol/L)	Na ⁺ ion (mmol/L)	Cl ⁻ ion (mmol/L)	iCa ²⁺ ion (mmol/L)	TCa ²⁺ (mmol/L)	pH	Glucose (mg/dl)
NC	7.60±0.36 ^b	146.25±1.2 ^a	111.92±0.7 ^a	1.07±0.1 ^a	1.97±0.1 ^a	7.65±0.06 ^a	268.45±5.1 ^a
PC	8.73±0.40 ^a	142.52±1.2 ^b	113.64±0.3 ^a	0.84±0.1 ^c	1.72±0.1 ^a	7.64±0.07 ^a	258.11±9.6 ^a
SV 200mg/kg	7.62±0.29 ^b	145.82±0.8 ^a	112.25±0.7 ^a	0.98±0.1 ^a	1.84±0.1 ^a	7.64±0.07 ^a	263.58±8.7 ^a
F 20mg/kg	8.39±0.33 ^{ab}	145.07±1.1 ^{ab}	113.90±0.6 ^a	0.93±0.1 ^{bc}	1.80±0.1 ^a	7.65±0.07 ^a	257.54±7.3 ^a
F 40mg/kg	7.86±0.19 ^{ab}	145.84±0.7 ^a	113.48±0.3 ^a	0.95±0.1 ^{abc}	1.82±0.1 ^a	7.65±0.06 ^a	269.12±6.4 ^a
F 80mg/kg	7.80±0.20 ^{ab}	146.20±0.9 ^a	113.66±0.8 ^a	0.99±0.1 ^{ab}	1.85±0.1 ^a	7.65±0.05 ^a	268.97±8.8 ^a

NC Negative control, PC positive control, SV Sodium Valproate, F Flavonoids, n =12 chicks per group, Values are expressed as mean±SE. Different letters within each column differ significantly at P<0.05 according to the Duncan test.

Discussion

The anticonvulsant effects of crude flavonoid extracts of MC were evaluated in PTZ treated chicks by monitoring the appearance of convulsion signs in the animals and the measurement of the major neurotransmitters (GABA and glutamate) in the brain that are involved in the development

of convulsion signs. PTZ is a well-known convulsant and is widely used in experimental studies to discover antiepileptic substances that effectively attenuate the convulsion induced by PTZ (6). The present study results showed that PTZ injection caused obvious convulsion signs in about 100% of the animals in the positive control group. Furthermore, the estimated biochemical parameters in brain tissue revealed

that PTZ exerted a perturbation in the balance between inhibitory (GABA) and excitatory (glutamate) neurotransmitters in the brain, as it induced a significant decrease in GABA and increased in glutamate in the brain tissue. The convulsant effects of PTZ result from suppressing the activity of GABA neurotransmitters at GABA_A receptors (22). It has long been considered that inhibition of GABA activities and or suppression of the GABA_A receptor contribute to the development of epilepsy. The elevated glutamate exerts its action by increasing the permeability of the membrane to sodium and calcium, resulting in neuronal depolarization and hyperexcitability (25). These results agreed with Wang *et al.* (26), who found that GABA levels were reduced and glutamate was elevated in the brain tissues of PTZ-challenged mice. In addition, it has been reported by Ali (27) that the concentration of GABA in the brain and especially in the serum decreased in PTZ-treated chicks.

The standard anticonvulsant drug sodium valproate offered 100% protection against convulsions induced by PTZ. Pretreatment with sodium valproate caused a significant elevation in the GABA levels, while there was a significant reduction in glutamate level in brain tissue. Sodium valproate exhibits its effect by increasing the concentrations of GABA in the brain, probably by inhibiting the enzymes responsible for its catabolism, and also it blocks neural excitability by inhibiting voltage-dependent Na⁺ channels (28). The elevated level of inhibitory neurotransmitter GABA in the brain caused neural hyperpolarization by Cl⁻ influx and thus inhibiting neural impulse transmission (29). The protection of chicks against PTZ-induced seizures by sodium valproate is expected. Kumar *et al.* (30) reported that the pretreatment with sodium valproate (200 mg/kg) exerted anticonvulsant activity by significantly increasing the GABA levels in brain tissue in mice. Also, it had been reported by Ali (27) the chicks pretreated with sodium valproate and convulsions induced by PTZ caused an insignificantly increase in GABA concentration in the brain, and also it increased significantly in serum. The crude flavonoid extracts offered protection against convulsion induced by PTZ by delay in the onset and frequency of convulsions. The result showed that the extract in all doses caused a slight elevation in the level of GABA and reduced the level of glutamate neurotransmitters in the brain tissue compared to the positive control group. It could be attributed to the flavonoid contents present in the extract, which can bind with the GABA_A receptor, and raise the seizure threshold in the brain (31). The present result was in agreement with Seedo and Hassan (19), reported that the chicks pretreated with aqueous extract of MC caused an insignificant increase in GABA concentration in brain tissue.

The antioxidant effect of crude flavonoid extract of MC on PTZ-induced convulsions was evaluated by measuring serum antioxidant enzymes and lipid peroxidation markers. The result showed that PTZ induced oxidative damages, as

the antioxidant parameters CAT and GR significantly reduced, and lipid peroxidation parameters MDA and 8-isoprostane significantly increased compared with the negative control group. This indicated that free radicals accumulated in the body due to recurrent convulsions induced by PTZ, which induced the development of convulsions and further oxidative stress (32,33). The present results agreed with Kumar *et al.* (30), who reported that PTZ had induced oxidative damages. Pretreatment of chicks with sodium valproate and PTZ provided an antioxidant effect, and it caused a significant increase in the level of GR and reduced lipid peroxidation markers MDA and 8-isoprostane in serum. Despite its anticonvulsant effect, it has been reported that sodium valproate also prevented the development of oxidative stress by increasing the availability of GABA in the brain tissue (34). The present findings were consistent with Wang *et al.* (26), who reported that sodium valproate prevents oxidative damage in PTZ-induced convulsions in mice. The ability of the crude flavonoid extract of MC to attenuate the oxidative stress induced by convulsions was investigated. The result showed that chicks pretreated with all doses of crude flavonoid extract exerted an insignificant increase in antioxidant parameters CAT and GR and an insignificant decrease in the MDA compared to the positive control group. Furthermore, the treated group with crude flavonoid extract (40 and 80 mg/kg) caused a significant decrease in 8-isoprostane levels compared to the positive control group. The antioxidant effect of the extract could be attributed to the presence of flavonoids in the crude extract that provides antioxidant effects (9). It has been proved that the treatment with antioxidant substances could reduce oxidative stress and epileptogenesis (35).

The electrolyte disturbance plays a critical role in enabling convulsions development, and diagnosis and treatment of convulsions rely on routine laboratory estimation of serum electrolytes (Na⁺, K⁺, and Ca²⁺) (36). This study showed that PTZ induced electrolyte imbalances in the serum, as it caused a significant increase in K⁺ ions and a decrease in Na⁺ and iCa²⁺ ions compared with the control group received saline. It has been demonstrated that repetitive neural activity during convulsions caused an increase in extracellular K⁺ concentration and a decrease in Na⁺ extracellular concentration (hyponatremia) due to change in neural ion channels and high influx of Na⁺ into the neurons (37). The present findings agreed with the finding reported by Seedo and Hassan (19), who reported that chicks treated with PTZ showed a significant increase in K⁺ ion and a significant decrease in the serum Na⁺ ion. In contrast, it has been reported by Ali (27), who found a significant increase in Na⁺ ions in chicks PTZ induced convulsions.

The group pretreated with sodium valproate showed a significant decrease in K⁺ level while a significant increase in Na⁺ and iCa²⁺ ions compared with the positive control group. The decrease in K⁺ level and increase in Na⁺ and iCa²⁺

levels could be attributed to the anticonvulsant effect of the drug sodium valproate, which reduces voltage-gated sodium channel activities (34). Present results were consistent with Seedo and Hassan (19), who reported that the Na⁺ level increased in chicks treated with sodium valproate and PTZ-induced convulsions. A similar result was also reported by Hamed *et al.* (38) that the serum level of Na⁺ and Ca²⁺ significantly increased in epileptic patients treated with valproate. On the other hand, it had been reported by Ali (27) found that the Na⁺ and Ca²⁺ levels decreased significantly in chicks treated with sodium valproate and PTZ-induced convulsions.

The result showed the group that was pretreated with crude flavonoid extract 80 mg/kg, similar to the group that was pretreated with sodium valproate, caused a significant increase in Na⁺ and iCa²⁺ ions compared to the positive control group. The group pretreated with the extract at a dose of 40/kg caused only a significant increase in Na⁺ ion compared with the positive control. It could be attributed to the effect of flavonoids in the extract, reducing the voltage-gated sodium channels activity and neural depolarization (39). The present finding was in agreement with Seedo and Hassan (19), who found a significant increase in Na⁺ ions in chicks treated with aqueous extracts of MC and convulsions induced by PTZ. The present results demonstrated that the pH of the serum and serum glucose level was not changed significantly in all treated groups as compared with control groups. The present results were consistent with Seedo and Hassan (19), who showed the glucose level did not change significantly in chicks pretreated with ethanol extracts of MC and convulsion induced by PTZ. In contrast, it had been shown that the glucose level increased after 3, 24 hours after PTZ induced epilepsy in female rats (40).

Conclusion

The present study indicated that the crude flavonoid extract of MC at the dose of 80 mg/kg has mild to moderate anticonvulsant and antioxidant effects in convulsion-induced chicks by PTZ. This effect of the extract could be attributed to the flavonoids, the most important bioactive compounds present in the extract that attenuate the epileptic convulsion due to multiple mechanisms, including antioxidant potential.

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Conflict of interest

There is no conflict of interest.

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التأثير المضاد للاختلاجات العصبية والأكسدة لمستخلص الفلافونويدات الخام لزهرة البابونج على الاختلاجات العصبية المستحثة بالبنتلين تترازول في أفراخ الدجاج

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الخلاصة

صممت تجارب هذه الدراسة بهدف معرفة تأثير المستخلص الخام للفلافونويدات المعزول من زهرة البابونج كمضاد للاختلاجات العصبية (التشنجات) والأكسدة على الاختلاجات العصبية المستحثة تجريبياً بحقن البنتلين تترازول في أفراخ الدجاج. تم قياس المعايير الكيموحيوية في نسيج الدماغ (حمض الكاما امينو بيوتريك والكلوتامين)، ومؤشرات الإجهاد التأكسدي في مصل الدم (إنزيم الكاتاليز، إنزيم الكلوتاثيون المختزل، المالونديالدهيد والايذوبروستان- 8) والأيونات مثل البوتاسيوم، الصوديوم، الكلوريد، الكالسيوم المتأين، الكالسيوم الكلي، درجة حموضة الدم ومستوى الكلوكوز في المصل. اخذ ٧٢ فرخ بعمر أسبوعين وتم تقسيمهم عشوائياً الى ست مجاميع كل منها تضم ١٢ فرخاً كالاتي: المجموعة الأولى مجموعة السيطرة السليمة حقنت بالمحلول الملحي الفسلجي تحت الجلد، المجموعة الثانية استحدثت فيها المرض بحقنها بالبنتلين تترازول بتركيز ٩٠ ملغم/كغم من وزن الجسم تحت الجلد، المجموعة الثالثة تمت معالجتها بدواء الصوديوم فالبرويت بتركيز ٢٠٠ ملغم/كغم من وزن الجسم عن طريق الفم لمدة ستة أيام قبل إحداث المرض تجريبياً. المجموعة الرابعة، الخامسة والسادسة عولجت بمستخلص الفلافونويدات الخام بتركيز ٢٠، ٤٠، ٨٠ ملغم/كغم من وزن الجسم عن طريق الفم لمدة ستة أيام وتم حقن البنتلين تترازول بعد ٣٠ دقيقة من آخر جرعة. أظهرت النتائج أن مستخلص الفلافونويدات الخام خفف من اختلاجات العصبية ونسبة الهلاكات وهذا التأثير كان متغيراً اعتماداً على جرعة المستخلص. أظهر مستخلص الفلافونويدات الخام بجرعة ٨٠ ملغم/كغم زيادة معنوية في مستوى الأيونات الصوديوم والكالسيوم المتأين، كما أدى الى انخفاض مستوى الايذوبروستان-8. استناداً الى نتائج هذه الدراسة تم الوصول الى انه مستخلص الفلافونويدات الخام لزهرة البابونج بتركيز ٨٠ ملغم/كغم يمتلك تأثيرات خفيفة الى معتدلة كمضاد للاختلاجات العصبية وكمضاد للأكسدة.