Research Article

BASRAH JOURNAL OF VETERINARY RESEARCH, 2022, 21(3):31-41
https://bjvr.uobasrah.edu.iq/

The effects of crude alkaloid extract of *Matricaria chamomilla* L. on convulsions induced by pentylenetetrazole in chicks

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Received: July 20, 2022; Accepted: September 15, 2022.

Abstract: The present research was designed to evaluate the protective effect of crude alkaloid extract of *Matricaria chamomilla* L. (MC) against pentylenetetrazole (PTZ) induced convulsions in chicks. A total of seventy-two chicks were randomly divided into six groups each one consisting of 12 chicks. The first group was considered to be a negative group that received subcutaneous injection of normal saline, the second group (positive control) received subcutaneous injection of PTZ (90 mg/kg), and the third group received sodium valproate (200 mg/kg) orally for six days. The other groups (fourth, fifth and sixth) were treated with single intraperitoneal injection of crude alkaloids extract of MC (20, 40, 80 mg/kg) respectively. After 30 minutes of the treatment, the groups (third to sixth) received PTZ and observed the convulsion signs for the next 30 minutes. At the end of the experiment, various biochemical parameters were assessed including, brain gamma-aminobutyric acid (GABA) and glutamate, serum catalase (CAT), glutathione reductase (GR), malondialdehyde (MDA), and 8-isoprostane, and some electrolytes (potassium K^+, sodium Na^+, chloride Cl^-, ionized calcium iCa^{2+}, total calcium TCa^{2+}), pH, and glucose levels in serum. The results indicated that the group treated with crude alkaloids extract at higher dose (80 mg/kg) showed a significant increase in CAT and significantly decreased in 8-isoprostane, and an increase in Na^+ ions concentration in serum. It was concluded that pretreatment of crude alkaloids extract (80 mg/kg) of MC offers anticonvulsant activity by reducing the mortality rate induced by PTZ, as well as it plays an important role in inhibiting oxidative stress.

Keywords: Crude Alkaloids, *Matricaria chamomilla*, Pentylenetetrazole, Anticonvulsant, Antioxidant

Introduction

Epileptic seizures are well known to induce convulsion signs as a result of abnormal electrical activity in the brain (1). The main pathophysiological mechanism involved in the development of epileptic seizures is imbalance between excitatory glutamate and inhibitory GABA neurotransmitters in the brain (2).

Moreover, epileptic seizures have been shown to induce oxidative stress by excessive formation of reactive oxygen species in the brain, which then contribute to brain damage and further epileptic seizures (3). It has been proved the substances that possess anticonvulsant and antioxidant activities may provide a neuroprotective effect by modulating the development of convulsions and reducing oxidative stress induced during epilepsy (4). PTZ, a well-known convulsant agent, is often used in experimental animals to induce epileptic convulsions, and oxidative stress, as well as is used to evaluate the efficacy of antiepileptic substances (5).

The experimental studies focused on the use of natural compounds in the plant extract and finding the therapies for relieving neurodegenerative diseases including epileptic convulsions (6). MC belongs to the Asteraceae family and is widely grown all around the world (7). The dried flower parts of the plant are largely used in traditional medicine to treat different kinds of diseases, including neurodegenerative, gastrointestinal, cardiovascular and skin diseases (8). In addition, it has been reported that the extract of MC contains a wide range of biological activities in the CNS including, spasmolytic, anti-sedative, anti-depressant and antioxidant properties (9). Recent studies showed that MC contains numerous bioactive compounds including alkaloids, flavonoids, cardiac glycosides, saponin, and tannins (10,11).

Alkaloids are naturally nitrogen-containing compounds primarily found in 20% of plants, and contain various positive and negative biological effects (12). The plant-derived alkaloids possess positive roles in ameliorating the pathophysiology of neurodegenerative diseases such as epilepsy, depression, anxiety, and psychological disorders (13,14). The neuroprotective activities of the alkaloids have been shown through the vast mode of actions such as by increasing the level of GABA, N-methyl-D-aspartate (NMDA) antagonist, monoamine oxidase B (MAO-B) inhibitors, and antioxidant activity (15,16). The anticonvulsant efficacy of alkaloids extract of MC has not been evaluated yet. Hence, the present study was carried out to assess the efficacy of crude alkaloids extract of MC against convulsions and oxidative stress induced by PTZ in chicks as a model of convulsions.

Materials and Methods

Collection of the plant samples and authentication.

The flower parts of MC were harvested in the Duhok province of Iraq in the flowering period in April 2020. The plant specimen was taxonomically identified by the botanists in the Department of Forestry, College of Agricultural Engineering Sciences, University of Duhok, Iraq. Then the flower part was air-dried under shade for 14 days, then milled into coarse powder and stored in paper sacks in a dark place at room temperature until use (17).

Preparation of the crude alkaloids extract

50 gm of air-dried flowers powder was macerated with 250 ml of (10 %) ethanolic acetic acid and mixed on a magnetic stirrer at room temperature for 24 hours, then the mixture was filtered and unwanted materials were removed. The filtrate was evaporated to a quarter of its volume and acidified with 5 ml of concentrated sulphuric acid. The acidic fraction was adjusted to pH 9 with concentrated ammonium hydroxide. After that the mixture was put in a separation funnel and extracted with chloroform (3 × 20 ml). The alkaloids

were isolated in the organic layer by evaporating the solvent, and afford (1.15 gm) of crude alkaloids extract (18, 19). The alkaloid was detected in the extract using modified Dragendorff`s reagent (Potassium bismuth iodide solution) (20). The crude alkaloids extract was dissolved in normal saline to give the desired concentrations (20, 40, and 80 mg/ml), and used within three days.

Animals and experimental design

The present study was conducted on 72 broiler chicks (one-day old) of both sexes, which were purchased from the hatchery of Jeen, Duhok, Iraq.

The chicks were reared for two weeks before being used in this study in the animal house at the College of Veterinary Medicine, University of Duhok, Duhok, Iraq at the temperature of 28–34 ºC with constant lighting, and feed and water were given *ad libitum*. The chicks were randomly divided into six groups of 12 chicks each. The first group (negative control) received subcutaneous (s.c.) injection of normal saline 1ml/kg, the second group (positive control) injected with PTZ (Sigma, USA) 90 mg/ml/kg s.c. (21), the third group was treated with sodium valproate 200 mg/5ml/kg (SANOFI, France) orally for six successive days (10).

The fourth, fifth and sixth groups were injected with single dose of crude alkaloids extract of MC at the dose of 20, 40, and 80 mg/kg intraperitoneal (i.p) respectively (22). Thirty minutes post-treatment in groups (3–6), the chicks were injected with PTZ and then behavioral activities were observed and recorded by a digital video camera for the next 30 minutes.

Collection of blood samples and analysis of serum biochemical parameters

Blood samples were collected by heart puncture in the overnight fasting animals after 3 hours from PTZ injection. The blood was kept in a plain tube to obtain serum by centrifuging it at 5000 rpm for 10 minutes, and then stored at -20 ºC until analysis (10). The level of CAT, GR, MDA and 8-isoprostane levels in the serum were measured by using commercially available Chicken CAT, GR, MDA and 8-isoprostane ELISA kits (BioAssay Technology Laboratory, China) according to the manufacturer's instructions. The serum electrolytes (K^+, Na^+, Cl^-, iCa^{2+}, and TCa^{2+}), and pH of the serum were measured using automatic electrolyte analyzer (fortress diagnostics, UK). Serum glucose level was measured colorimetrically using a diagnostic kit (Biolabo/France).

Collection of brain samples and measurement of neurotransmitters

At the end of the experiment, the animals were sacrificed by decapitation and the brain was isolated and stored in a plastic container at -20 ºC until use it for neurotransmitters analysis (10).

Then, the frozen brain tissues were thawed and 100 mg were minced and homogenized with 0.9 ml of cold phosphate buffered saline (pH 7.4) with an electrical homogenizer (Coyote, China) on ice, and centrifuged at 7250 rpm for 5 minutes at 4 °C according to the kits manufacturer's instructions. The supernatants were stored at -20 °C for neurotransmitters analysis. The brain GABA and glutamate levels were measured using Chicks GABA and Glutamate ELISA kits (BioAssay Technology Laboratory, China).

Statistical analysis

The data obtained were expressed as mean ± standard error of mean (SEM). The results were analyzed by one-way analysis of variance (ANOVA) using SPSS software, version 23. The comparison between means were accomplished by using the Duncan test and $P<0.05$ was considered to be significant.

Results

Single subcutaneous injection of PTZ (90 mg/kg) in chicks elicited obvious convulsive behaviors within 6–23 minutes including, restlessness, defecation, spasm and extension of the legs, involuntary contractions of the skeletal muscle and stiffness of the body (tonic convulsions), and inability to stand, vocalize violent sounds, involuntary movement of the body (myoclonic convulsions), uncontrolled wing flapping (tonic-clonic convulsions), loss of consciousness, and mortality from asphyxia in about (41%) of the animals as compared to the negative control group. The standard antiepileptic drug used sodium valproate (200 mg/kg) offered 100% protection when compared to the positive control group.
The single pretreatment of crude alkaloids extracts intraperitoneally at the doses of 20, 40, and 80 mg/kg caused a delay in the onset of convulsive behavior and reduced the mortality to about 33%, 25%, 16% respectively in convulsive chicks induced by PTZ.

Table 1 showed the effects of crude alkaloids extract of MC on the brain tissue neurotransmitters in chicks after PTZ injection. The result revealed that the group treated with PTZ showed a significant decrease in the level of brain GABA, while significantly increasing in the level of brain glutamate compared to the negative control group. In contrast, in the group pretreated with sodium valproate and PTZ, there was a significant elevation in the level of brain GABA, and a significant reduction in the glutamate level when compared with the positive control group. The groups pretreated with crude alkaloids extract in all three doses showed slight insignificant elevation of GABA, and insignificant reduction of glutamate levels in the brain tissue when compared with the positive control group.

The data in table 2 showed the effects of crude alkaloids extract of MC on serum antioxidant enzyme and oxidative stress biomarkers in convulsive chicks induced by PTZ. The results explained that the positive control group treated with PTZ exhibited a significant decrease in antioxidant enzyme CAT and GR, while lipid peroxidation markers MDA and 8-isoprostane increased significantly compared to the negative control group. On the other hand, the group pretreated with sodium valproate showed a significant increase in CAT, and no significant change was recorded in GR as compared to the positive control group. The MDA and 8-isoprostane significantly reduced as compared to the positive control group. The groups pretreated with crude alkaloid extracts (20 and 40 mg/kg) showed a slight insignificant elevation in the serum antioxidant enzymes, and a slight insignificant reduction in lipid peroxidation biomarkers as compared with the positive control group. In contrast, the group pretreated with the highest dose of crude alkaloid extract (80 mg/kg) revealed a significant increase in the level of CAT, while significantly decreasing in the level of 8-isoprostane compared to the positive control group.

Table 1: Effects of crude alkaloids extract of MC on brain tissue neurotransmitters in convulsions induced chicks treated by PTZ as compared with control groups.

Groups	GABA (ng/gm)	Glutamate (ng/gm)
NC	26.12±1.63 [a]	24.69±1.65 [b]
PC	15.04±0.90 [b]	45.04±2.17 [a]
SV 200 mg/kg	25.78±1.91 [a]	24.75±1.80 [b]
A 20 mg/kg	17.49±1.51 [b]	43.58±2.07 [a]
F 40mg/kg	17.80±0.83 [b]	42.27±2.81 [a]
F 80mg/kg	18.75±0.81 [b]	39.67±2.11 [a]

NC: Negative control, PC: Positive control, SV: Sodium Valproate, A: Alkaloid, , n =12 chicks per group, Values are expressed as mean ± SE. Different superscript letters within each column mean differ significantly at $p< 0.05$ according to the Duncan test.

Table 2: Effects of crude alkaloids extract of MC on serum antioxidant enzymes and oxidative stress biomarkers in convulsions induced chicks treated by PTZ in comparison with control groups.

Groups	CAT (ng/ml)	GR (ng/ml)	MDA (nmol/ml)	8-Isoprostane (ng/L)
NC	50.88±6.46 [a]	3.34±0.39 [a]	7.20±0.73 [c]	674.26±33.33 [b]
PC	27.13±3.73 [b]	1.83±0.18 [b]	15.51±2.12 [a]	881.40±88.76 [a]
SV 200 mg/kg	47.50±6.18 [a]	2.95±0.24 [ab]	8.90±0.82 [bc]	675.61±21.98 [b]
A 20 mg/kg	35.21±3.81 [ab]	1.91±0.19 [b]	13.87±2.42 [a]	764.36±53.76 [ab]
F 40mg/kg	41.50±2.60 [ab]	2.16±0.34 [b]	12.04±1.09 [ab]	776.88±48.67 [ab]
F 80mg/kg	44.03±5.29 [a]	2.48±0.55 [ab]	11.37±1.31 [ab]	680.09±50.23 [b]

NC: Negative control, PC: Positive control, SV: Sodium Valproate, A: Alkaloid, n =12 chicks per group, Values are expressed as mean ± SE., Different superscript letters within each column mean differ significantly at $p< 0.05$ according to the Duncan test.

The effects of crude alkaloids extract of MC on serum electrolytes, pH and glucose in chicks was illustrated in table 3. The results exhibited the control group that received PTZ showed a significant increase in K^+ ions, while the level of Na^+, iCa^{2+} and TCa^{2+} ions decreased significantly compared to the negative control group. In contrast, the group pretreated with sodium valproate showed a significant reduction in K^+ ions, and significant elevation in Na^+ and iCa^{2+} ions compared to the positive control group. The groups pretreated with crude alkaloids extract at the dose of 20 and 40 mg/kg revealed insignificant changes in the electrolyte, pH, and glucose parameters compared to the positive control group. On the other hand, the group pretreated with the highest dose of crude alkaloid extract (80 mg/kg) showed a significant increase in the level of Na^+ ion as compared with the positive control group.

Table 3: Effects of crude alkaloids extract of MC on serum electrolytes, pH and glucose in convulsion induced chicks by PTZ as compared with the 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	6.77 ± 0.19 ^{b}	145.89 ± 0.81 ^{a}	111.03 ± 0.81 ^{a}	1.05 ± 0.03 ^{a}	1.93 ± 0.07 ^{a}	7.62 ± 0.004 ^{a}	283.99 ± 6.80 ^{a}
PC	8.16 ± 0.34 ^{a}	141.79 ± 0.88 ^{b}	110.07 ± 0.97 ^{a}	0.86 ± 0.05 ^{c}	1.64 ± 0.10 ^{b}	7.61 ± 0.008 ^{a}	266.62 ± 6.80 ^{a}
SV 200 mg/kg	7.00 ± 0.28 ^{b}	144.96 ± 0.98 ^{a}	112.88 ± 0.85 ^{a}	1.01 ± 0.03 ^{ab}	1.88 ± 0.05 ^{ab}	7.61 ± 0.005 ^{a}	279.99 ± 6.36 ^{a}
A 20 mg/kg	7.32 ± 0.29 ^{ab}	143.25 ± 0.91 ^{ab}	111.22 ± 0.94 ^{a}	0.91 ± 0.04 ^{bc}	1.69 ± 0.08 ^{ab}	7.61 ± 0.004 ^{a}	277.53 ± 10.45 ^{a}
A 40 mg/kg	7.37 ± 0.36 ^{ab}	144.53 ± 1.08 ^{ab}	112.93 ± 1.47 ^{a}	0.92 ± 0.05 ^{bc}	1.75 ± 0.09 ^{ab}	7.61 ± 0.003 ^{a}	281.81 ± 7.01 ^{a}
A 80 mg/kg	7.27 ± 0.33 ^{ab}	144.70 ± 0.86 ^{a}	111.36 ± 0.56 ^{a}	0.94 ± 0.03 ^{abc}	1.80 ± 0.06 ^{ab}	7.61 ± 0.003 ^{a}	285.85 ± 11.98 ^{a}

NC: Negative control, PC: Positive control, SV: Sodium Valproate, A: Alkaloid, n =12 chicks per group, Values are expressed as mean ± SE., Different superscript letters within each column mean differ significantly at $p< 0.05$ according to the Duncan test.

Discussion

This study was concerned with the effect of crude alkaloids extract of MC on the behavior alteration and the level of brain neurotransmitters GABA and glutamate in chicks PTZ-induced convulsions. PTZ is often used to induce the development and study of convulsions, as well as, it is used to evaluate the potential efficacy of antiepileptic substances in experimental animals (23). The results of the study showed that the treatment of chicks by PTZ exhibited obvious convulsive behaviors. The biochemical results confirmed this event, as PTZ caused a significant decrease in the brain GABA level, while increasing in brain glutamate level. Meanwhile, the disturbance in the brain neurotransmitters caused by PTZ led to an increase in the brain neural activity and development of convulsions (24). PTZ is a well-known convulsant agent which exerts its effect by blocking the $GABA_A$ receptors in the brain tissue, and reducing the level of GABA in the brain tissue (25). So, establishing the disturbance in the brain neurotransmitter by decreasing inhibitory GABA and increasing excitatory glutamate, resulted in lowering the threshold level for the development of convulsions (26,27). The present results are in the same line with Taiwe *et al*. (28), who found that PTZ-induced convulsions by significantly decreasing GABA content in mice brain.

The pretreatment of animals with standard anticonvulsant drug, sodium valproate was expected to abolish the convulsions induced by PTZ. The present study found that sodium valproate led to a significant increase in brain GABA level while decreasing in glutamate level. Sodium valproate elevates the brain GABA level by inhibiting GABA transaminase (GABA-T) which is responsible for catabolism of GABA, and eventually increasing the availability of the GABA and enhancing the $GABA_A$ receptor neurotransmission (29). In addition, the sodium valproate reduces the brain glutamate level and inhibits the glutamate neurotransmission, so it blocks the neural firing and the development of convulsions by PTZ (30). Also, these results are consistent with Taiwe *et al*. (28), who reported that sodium

valproate inhibited PTZ-induced convulsions in mice by increasing the availability of GABA in the brain. The present study showed that the crude alkaloids extract of MC caused delay in the appearance of convulsion signs, and reduced the mortality rate resulted from PTZ. Moreover, the biochemical parameters revealed that the crude alkaloids extract of CM caused an insignificant elevation in the brain GABA level, and insignificant reduction in the brain glutamate level. The anticonvulsant activity of the crude alkaloids extract might be attributed to the presence of alkaloid substances in the extract (15). It has been reported that piperidine alkaloids provide anticonvulsant activity by increasing the level of brain GABA level in mice pilocarpine-induced convulsions (22). In addition, Seedo and Ali (10) reported that chicks pretreated with aqueous extract of CM and PTZ showed insignificant elevation in the brain GABA level.

The results showed that PTZ caused oxidative damages by decreasing in the level of antioxidant enzymes CAT and GR, while increasing lipid peroxidation markers MDA and 8-isoprostane. It was shown that PTZ induced oxidative stress by increasing the generation of reactive oxygen species (5). Furthermore, the free radical can induce further development of convulsions by direct inactivation glutamine synthase, and increasing the abnormal availability of excitatory neurotransmitter glutamate (4, 31). The result also showed that the pretreatment with sodium valproate and PTZ, led to a significant elevation in CAT and a reduction in MDA and 8-isoprostane in the serum. The antioxidant activity of sodium valproate might be linked to its anticonvulsant effect. As, it was reported by Taiwe *et al*. (28), the sodium valproate caused a significant decrease in MDA and a significant increase in reduced glutathione enzyme in the mice PTZ-induced seizures. The results also demonstrated that the crude alkaloids extract caused insignificant dose-dependent elevation in the CAT and GR and reduction in MDA and 8-isoprostane levels. The crude alkaloid extract of MC at higher dose (80 mg/kg) caused a significant increase in CAT and decrease in 8-isoprostane, and so the extract potentially protected the lipid peroxidation generated by PTZ injection. The antioxidant activity of the crude alkaloids extract could be related to the presence of alkaloids in the extract. It has been reported that piperine alkaloids possess a diversity of pharmacological effects such as neuroprotective properties through antioxidant and anticonvulsant pathways (15). The present findings were in accordance with Seedo and Ali (10), who reported that aqueous extract of CM caused a significant increase in the serum total antioxidant capacity (T-AOC) in the chicks treated with PTZ.

Electrolytes play an important role in the generation and transmission of action potentials, and therefore the electrolyte imbalances enabling the development of seizures, so the routine laboratory estimation of serum electrolytes is essential for management of the epileptic convulsions (32). In the present study, PTZ caused a significant increase in K^+ ions and decrease in Na^+, iCa^{2+} and TCa^{2+} ions. The mechanism by which PTZ elicited the electrolyte disturbances could be as a result of increasing the activity of excitatory neurotransmitters glutamate in the brain due to convulsions (33). As well as, it had been demonstrated that epileptic convulsions more frequently observed during hypocalcemia and hyponatremia, as they caused increased the neuromuscular excitability and membrane potential moved closer to firing threshold resulted in convulsions (34). The present findings are compatible with Seedo and Ali (10), who reported that the chicks treated with PTZ showed an increase in K^+ ions and decrease in Na^+ ions. Similarly, Hozayen *et al*. (35), also found that the epilepsy induced by pilocarpine resulted in increasing K^+ ions and decreasing Na^+ ions in the rats. On the other hand, Abdul Wahid (36) reported that the Na^+ ions increased while K^+ ions decreased in untreated epileptic patients.

In the results the chicks pretreated with sodium valproate and PTZ showed a decrease in K^+ ions and significantly increasing in Na^+

and iCa^{2+} concentration in the serum. Sodium valproate exerted its anticonvulsant effects by many mechanisms including blockage of voltage-gated sodium channels and enhancing the inhibitory GABA neurotransmission (30). These results are consistent with Seedo and Ali (10), who reported that chicks pretreated with sodium valproate and PTZ revealed an increase in Na^{+} ions concentration in the serum. Also, Abdul Wahid (36), who found that epileptic patients treated with sodium valproate had an increase in Ca^{2+} ions. In contrast Ali (37), who reported that sodium valproate caused a decrease in Na^{+} and Ca^{2+} ions in convulsive chicks induced by PTZ. The results also showed that the chicks pretreated with crude alkaloid extracts of MC at lower doses (20 and 40 mg/kg) did not cause significant changes in the electrolytes, while the higher dose (80 mg/kg) caused significant elevation in Na^{+} ions. These results also could be attributed to the presence of alkaloids in the extract that effective in preventing the neural excitability by increasing Na^{+} ions concentration, and increasing the threshold level for convulsions (15). The present finding is consistent with Seedo and Ali (10), who showed that Na^{+} ions significantly increased in chicks pretreated with aqueous extract of CM and PTZ. The results also showed that the glucose content and pH in the serum not significantly changed in the treated groups compared to the control groups. This finding agrees with Seedo and Ali (10), who found that the glucose did not change in the chicks PTZ-induced convulsions, and also in the chicks pretreated with sodium valproate, and aqueous extract of MC. On the other hand, Fadel and Mohammed (38), who reported that the glucose level increased in the chicks treated with PTZ.

Conclusion

It was concluded from this study that the use of crude alkaloids extract of MC at the higher dose (80 mg/kg) has an important role in ameliorating the convulsion signs, and oxidative stress induced by PTZ. This effect of the extract could be attributed to the presence of some kind of alkaloids in the extract. Hence, the isolation of biologically active alkaloid substances from the extract could be the next stage to continue with the research.

Acknowledgments

We would like to gratefully acknowledge Duhok Research Center, College of Veterinary Medicine, University of Duhok, for providing necessary facilities to carry out this research.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

1- Vitaliti, G., Pavone, P., Marino, S., Saporito, M. A., Corsello, G., Falsaperla, R. (2019). Molecular mechanism involved in the pathogenesis of early-onset epileptic encephalopathy. *Front. Mol. Neurosci*, 12(May), 1-10. DOI: 10.3389/fnmol.2019.00118.

2- Roganovic, M., Pantovic, S., Dizdarevic, S. (2019). Role of the oxidative stress in the pathogenesis of epilepsy. *Neurol. Sci. Neurophysiol*, 36(1), 1-8. DOI: 10.5152/NSN.2019.11632.

3- Olowe, R., Sandouka, S., Saadi, A., Shekh-ahmad, T. (2020). Approaches for reactive oxygen species and oxidative stress quantification in epilepsy. *Antioxidants*, 9(10), 1-26. DOI: 10.3390/antiox9100990.

4- Parsons, A. L., Bucknor, E. M, Castroflorio, E., Soares, T. R., Oliver, P. L., Rial, D. (2022). The Interconnected Mechanisms of Oxidative Stress and Neuroinflammation in Epilepsy. *Antioxidants*, 11(1), 1-17. DOI: 10.3390/antiox11010157.

5- Samokhina, E., Samokhin, A. (2018). Neuropathological profile of the pentylenetetrazol (PTZ) kindling model. *Int. J. Neurosci*, 128(11), 1086-1096. DOI: 10.1080/00207454.2018.1481064.

6- He, L. Y., Hu, M. B., Li, R. L., Zhao, R., Fan, L. H., He, L., Lu, F., Ye, X., Huang, Y., Wu, C. J. (2021). Natural Medicines for the Treatment of Epilepsy: Bioactive Components, Pharmacology and Mechanism. *Front. Pharmacol*, 12(March), 1-28. DOI: 10.3389/fphar.2021.604040

7- El Mihyaoui, A., Esteves, J. C., Charfi, S., Candela, M. E., Lamarti, A., Arnao, M. B. (2022). Chamomile (*Matricaria chamomilla* L.): A Review of Ethnomedicinal Use, Phytochemistry and Pharmacological Uses. *Life*, 12(4), 1-41. DOI: 10.3390/life12040479

8- Bayati, Z. J., Moradi, K. N., Moradi, K. Z. (2014). Chamomile (*Matricaria recutita*) as a Valuable Medicinal Plant. *Int J Adv Biol Biomed Res*, 2(3), 823-829.

9- Ionita, R., Postu, P. A., Cioanca, O., Mircea, C., Hancianu, M., Hritcu, L. (2019). Anxiolytic and antidepressant effects of *Matricaria chamomilla* hydroalcoholic extract in a rat model of scopolamine. *Farmacia*, 67(1), 68–72. DOI: 10.31925/farmacia.2019.1.9

10- Seedo, S. K., Hassan, J. S. (2016). Phytochemical Analysis and Evaluation of Anticonvulsant Effect of Chamomile (*Matricaria chamomilla* L.) Flower Extract in Chicks. *J. Zankoy Sulaimani - Part A*, 19(1), 35-50. DOI: 10.17656/jzs.10583

11- Cvetanovic, A., Svarc-Gaji, J., Maskovic, P., Savic, S., Nikolic, L. (2014). Antioxidant and biological activity of chamomile extracts obtained by different techniques: perspective of using superheated water for isolation of biologically active compounds. *Ind. Crops Prod*, 11(1), 1-3.

12. Al-Saeed, A. H. M. (2019). Study the effect of isolated alkaloid from *Haloxylon salicornium* plant on some hematological and biochemical parameters in gastric ulceration rabbits induced by indomethacin. *Iraq Basrah J. Vet. Res*, 18(1), 36-55.

13- Cui, X., Lin, Q., Liang, Y. (2020). Plant-Derived Antioxidants Protect the Nervous System From Aging by Inhibiting Oxidative Stress. *Front. Aging Neurosci*, 12(July), 1-12. DOI: 10.3389/fnagi.2020.00209.

14- Aryal, B., Raut, B. K., Bhattarai, S., Bhandari, S., Tandan, P., Gyawali, K., Sharma, K., Ranabhat, D., Thapa, R., Aryal, D., Ojha, A., Devkota, H. P., Parajuli, N. (2022). Potential Therapeutic Applications of Plant-Derived Alkaloids against Inflammatory and Neurodegenerative Diseases. Evidence-based Complement. *Altern. Med*, 2022(March), 1-18. DOI: 10.1155/2022/7299778.

15- Hussain, G., Rasul, A., Anwar, H., Aziz, N., Razzaq, A., Wei, W., Ali, M., Li, J., Li, X. (2018). Role of plant derived alkaloids and their mechanism in neurodegenerative disorders. *Int. J. Biol. Sci*, 14(3), 341-357. DOI: 10.7150/ijbs.23247.

16- Faggion, S. A., Cunha, A. O., Fachim, H. A., Gavin, A. S., Santos, W. F. (2011). Anticonvulsant profile of the alkaloids (+)-erythravine and (+)-11-α-hydroxy-erythravine isolated from the flowers of Erythrina mulungu Mart ex Benth (Leguminosae-Papilionaceae). *Epilepsy Behav*, 20(3), 441-446. DOI: 10.1016/j.yebeh.2010.12.037.

17- Sharafzadeh, S., Alizadeh, O. (2011). German and roman chamomile. *J. Appl. Pharm. Sci*, 1(10), 1-5.

18- Al–Maliki, A. D. (2012). Isolation and Identification of Phenols and an Alkaloidic Compound from *Matricaria chamomilla* Plant Flowers and Study of Their Medicinal Activity Against the Pathogenic Bacteria of Skin Infections. *J. Thi-Qar Univ*, 7(3), 1-16.

19. Harbi, J. and Alsaadi H. (2018). Extraction, Isolation and Identification of Some Alkaloids Compounds From Iraqi Medicinal Plant *Haloxylon Salicornicum* and Study Antihyperglycemic Effect in Alloxan-Induced Diabetic Rabbits. *Iraq Basrah J. Vet. Res*, 17(3), 81-100.

20- Rahmani, S. E., Ouahrani, M. R. (2022). Phytochemical investigation of Ajuga iva, *Matricaria chamomilla* and *Ruta chalepensis* from Algerian steppe (Djelfa

district). *GSC Biol. Pharm. Sci*, 19(1), 193-201. DOI: 10.30574/gscbps.2022.19.1.0145.

21- Erkec, O. E., Arihan, O. (2015). Pentylenetetrazol Kindling Epilepsy Model. *J. Turkish Epilepsi Soc*, 21(1), 6–12. DOI: 10.5505/epilepsi.2015.08108.

22- Cruz, G. M., Felipe, C. F., Scorza, F. A., Costa, M. A., Tavares, A. F., Menezes, M. L., Andrade, G. M. (2013). Piperine decreases pilocarpine-induced convulsions by GABAergic mechanisms. *Pharmacol. Biochem. Behav*, 104(1), 144-153. DOI: 10.1016/j.pbb.2013.01.002.

23- Yuskaitis, C. J., Rossitto, L. A., Groff, K. J., Dhamne, S. C., Zhang, B., Lalani, L. K., Singh, A. K., Rotenberg, A., Sahin, M. (2021). Factors influencing the acute pentylenetetrazole-induced seizure paradigm and a literature review. *Ann. Clin. Transl. Neurol*, 8(7), 1388-1397. DOI: 10.1002/acn3.51375.

24- Doi, T., Ueda, Y., Nagatomo, K., Willmore, L.J. (2009). Role of glutamate and GABA transporters in development of pentylenetetrazol-kindling. *Neurochem. Res*, 34(7), 1324-1331. DOI: 10.1007/s11064-009-9912-0.

25- Shimada, T., Yamagata, K. (2018). Pentylenetetrazole-induced kindling mouse model. *J. Vis. Exp*, 136(June), 1-10. DOI: 10.3791/56573.

26- Huang, R. Q., Bell-Horner, C. L., Dibas, M. I., Covey, D. F., Drewe, J. A., Dillon, G. H. (2001). Pentylenetetrazole-induced inhibition of recombinant γ-aminobutyric acid type A (GABAA) receptors: Mechanism and site of action. *J. Pharmacol. Exp. Ther*, 298(3), 986-995.

27- Asadi-Shekaari, M., Eslami, A., Kalantaripour, T., Joukar, S. (2014). Potential mechanisms involved in the anticonvulsant effect of walnut extract on pentylenetetrazole-induced seizure. *Med. Princ. Pract*, 23(6), 538-542. DOI: 10.1159/000365759.

28- Taiwe, G. S., Kouamou, A.L., Dabole, B., Ambassa, A.R., Mambou, H. M., Bila, R. B., Tchoya, T. B., Menanga, J. R., Dzeufiet, P. D., Bum, E. N. (2021). Protective Effects of Anthocleista djalonensis Extracts against Pentylenetetrazole-Induced Epileptic Seizures and Neuronal Cell Loss: Role of Antioxidant Defense System. *Altern. Med*, 2021(August):1-18. DOI: 10.1155/2021/5523705.

29- Kumar, V., Sharma, S. K., Nagarajan, K., Dixit, P. K. (2016). Effects of lycopene and sodium valproate on pentylenetetrazol-induced kindling in mice. *Iran J Med Sci*, 41(5), 430–436.

30- Zawab, A., Carmody, J. (2014). Safe use of sodium valproate. *Aust. Prescr*, 37(4), 124-127. DOI: 10.18773/austprescr.2014.048.

31- Borowicz-Reutt, K. K., Czuczwar, S. J. (2020). Role of oxidative stress in epileptogenesis and potential implications for therapy. *Pharmacol Reports*, 72(5), 1218–1226. DOI: 10.1007/s43440-020-00143-w

32- Nardone, R., Brigo, F., Trinka, E. (2016). Acute symptomatic seizures caused by electrolyte disturbances. *J Clin Neurol*, 12(1), 21–33. DOI: 10.3988/jcn.2016.12.1.21

33- Keshavarz, M., Yekzaman, B. (2018). Amelioration of pentylenetetrazole-induced seizures by modulators of sigma, n-methyl-d-aspartate, and ryanodine receptors in mice. *Iran. J. Med. Sci*, 43(2), 195-201.

34- DiNuzzo, M., Mangia, S., Maraviglia, B., Giove, F. (2014). Physiological bases of the K^+ and the glutamate/GABA hypotheses of epilepsy. *Epilepsy Res*, 108(6), 995-1012. DOI: 10.1016/j.eplepsyres.2014.04.001

35- Hozayen, W. G., Ahmed, R. R., Khalefa, A. K., Ahmed, M. B. (2012). Modulatory Effects of Fish Liver Oil on Pilocarpine-Model of Epilepsy in Rats Compared to Topirmate as a Common Antiepileptic Drug. *J. Am. Sci*, 8(4), 1-9.

36- Abdul Wahid, H. H. (2010). Evaluation of serum electrolytes and uric acid in Iraqi epileptic patients. *Iraqi Acad. Sci. J*, 9(1), 84-87.

37- Ali, J. S. H. (2019). *Syzyguim Aromaticum* I. and their effect on seizure chickens. *Basrah J Vet Res,* 18(1), 85–105.
38- Fadel, M. A., Mohammad, F. K. (2019). Effect of diphenhydramine and phenobarbital in the concentration of glutathione and malondialdehyde and glucose in plasma and brain of chicks treated with pentylenetetrazol. *Iraqi J. Vet. Sci,* 33(2), 97-104. DOI: 10.33899/ijvs.2019.163173.

تأثيرات المستخلص القلوى الخام لزهرة البابونج على التشنجات المستحدثة بالبنتلين تترازول في افراخ الدجاج

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الخلاصة

صممت الدراسة الحالية لتقييم التأثير الوقائي لمستخلص القلويات الخام لزهرة البابونج ضد التشنجات التي يسببها البنتلين تترازول في افراخ الدجاج. استخدم اثنين و سبعين فرخ دجاج قسمت بشكل عشوائي الى ست مجموعات كل مجموعة مكونة من ١٢ فرخ. المجموعة الأولى اعتبرت مجموعة سيطرة سليمة حقنت تحت الجلد بمحلول الملح الفسيولوجي، بينما المجموعة الثانية مجموعة سيطرة ايجابية حقنت تحت الجلد بالبنتلين تترازول (٩٠ ملغم/كغم)، المجموعة الثالثة تمت معالجتها بدواء الصوديوم فالبرويت (٢٠٠ ملغم/كغم) لمدة ستة ايام عن طريقة الفم. تمت معالجة المجاميع الرابعة و الخامسة و السادسة بحقن مستخلص القلويات الخام من زهرة البابونج داخل البريتون بتراكيز (٢٠، ٤٠، ٨٠ ملغم/كغم) على التوالي. بعد ٣٠ دقيقة من العلاج تم حقن المجاميع الثالثة الى السادسة بالبنتلين تترازول و تحت مراقبتها لمدة ٣٠ دقيقة لملاحظة الاعراض التشنجات عليها. في نهاية التجربة تم قياس العديد من المعايير الكيميوحيوية كمستوى حمض الكاما امينو بيوتريك والكلوتامين في الدماغ و مؤشرات الاجهاد التأكسدي في مصل الدم (انزيم الكاتاليز، انزيم الكلوتاثيون المختزل، المالونديالديهايد و الايزوبروستان-٨) و بعض الايونات مثل البوتاسيوم، الصوديوم، الكلورايد، الكالسيوم المتأين، الكالسيوم الكلي، و درجة حموضة الدم، و مستوى الكلوكوز في مصل الدم. اشارت النتائج الى أن المجموعة المعالجة بمستخلص القلويات الخام بأعلى جرعة (٨٠ ملغم/كغم) أظهرت زيادة معنوية في انزيم الكاتاليز و انخفاض معنوي في الايزوبروستان-٨، و زيادة تركيز أيونات الصوديوم في المصل الدم. استنتج من نتائج هذه الدراسة أن المعالجة المسبقة بمستخلص القلويات الخام (٨٠ ملغم/كغم) أدى نشاطا مضادا للاختلاج عن طريق تقليل معدل الوفيات الناتجة بحقن البنتلين تترازول، كما لعب دورا مهما في تثبيط أضرار الاجهاد التأكسدي.

الكلمات المفتاحية: المستخلص القلوى الخام، بنتلين تترازول، المضاد الاختلاجات العصبية، المضاد الأكسدة.