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# PROTECTIVE EFFECTS OF CRUDE FLAVONOIDS AND ALKALOIDS EXTRACTS OF *MATRICARIA CHAMOMILLA* L. IN CHICKS AGAINST PENTYLENETETRAZOLE-INDUCED CONVULSIONS

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## ABSTRACT

**The current study was carried out to assess the anticonvulsant and antioxidant effects of crude flavonoids and alkaloids extracts of *Matricaria chamomilla* L. against pentylenetetrazol (PTZ) induced convulsions in chicks. Seventy-two chicks of either sex were randomly divided into 6 groups (each group n=12). The groups were treated with normal saline, PTZ (90 mg/kg, subcutaneous injection, sodium valproate (200 mg/kg, orally), oral administration of crude flavonoids and intraperitoneal injection of crude alkaloids extracts (20, 40, 80 mg/kg) respectively. After treatment with sodium valproate and the crude extracts, the animals were treated with PTZ and observed for 30 minutes for convulsion signs. Subsequently, the brain and blood samples were collected for biochemical estimation of gamma-aminobutyric acid (GABA) and glutamate in the brain tissue, as well as catalase, glutathione reductase, malondialdehyde, 8-isoprostane, some electrolytes (potassium, sodium, chloride, ionized calcium, total calcium), pH, and glucose levels in the serum. The group pretreated with the highest doses (80 mg/kg) showed a significant increase in GABA, and a decrease in glutamate in the brain tissue. Additionally, there was a significant increase in catalase, and a significant decrease in malondialdehyde, 8-isoprostane, and an increase in sodium and ionized calcium ions concentration in the serum. It was concluded that a combination treatment of crude flavonoids extract with crude alkaloids extract attenuated the convulsions and oxidative stress induced by PTZ in chicks.**

**KEY *WORLDS*:** Anticonvulsant, Antioxidant, Flavonoids, Alkaloids, Matricaria chamomilla, Pentylenetetrazol.

## INTRODUCTION

A convulsive seizure is often used as a symptom of generalized tonic-clonic seizure which characterized by rapid rhythmical movements as a result of abnormal electrical activity in the brain (Vitaliti *et al*., 2019). However, not all convulsions are associated with epileptic seizures, and not all epileptic seizures cause convulsions, therefore the term convulsions are sometime used as synonym for seizures (Herrera-Calderon *et al*., 2018). The primary pathophysiological mechanism of convulsive seizure is a sudden imbalance between gamma-aminobutyric acid (GABA)-mediated inhibition and glutamate- mediated excitation (Vitaliti *et al*., 2019). Any disruption of this balance that either suppresses the inhibitory or enhances the excitatory can produce seizures. In contrast, a blockade of excitatory glutamate or enhancement of inhibitory GABA usually prevents seizures development (Sarlo and Holton, 2021).

In recent years, medicinal plants have been used to cure neurological disorders like convulsive seizure diseases. *Matricaria chamomilla* L., also known as German chamomile, is a traditional herbal medicine belonging to the family Asteraceae, and it is considered as miracle plant and consumed around the world as herbal tea (El Mihyaoui *et al*., 2022). Experimental studies have demonstrated that the chamomile extracts possess several biological properties, such as anticonvulsant (Numan *et al*., 2014), antioxidant (Al-Dabbagh *et al*., 2019), antidepressant (Namjou *et al*., 2018), anxiolytic (Ionita *et al*., 2019), as the plant extracts are rich in different bioactive compounds, such as flavonoids, alkaloids, terpenoids, phenolic compounds, tannins, saponins, glycosides, and cardiac glycosides (El Mihyaoui *et al*., 2022).

Several recent studies have shown that flavonoids play significant role in the chamomile medicinal effects, protecting the brain against the free radical damage and excitability (Chauhan and Aishwarya, 2018). The most important flavonoids present in chamomile extracts including flavones (apigenin, apigenin-7-O-glucoside, and luteolin) and flavonols (quercetin, isoquercitrin, and rutin) (El Mihyaoui *et al*., 2022). It has reported that apigenin, a major flavonoid in chamomile flower, showed an anticonvulsant effect by modulating the $GABA_A$ receptor (Salehi *et al*., 2019). Moreover, apigenin has been reported to show many pharmacological properties such as neuroprotective, anticonvulsant, antioxidant, anti-inflammatory, and a therapeutic agent that overcome several types of diseases (Ali *et al*., 2017).

Alkaloids are a group of naturally occurring nitrogenous compounds mainly found in 20% of plants species (Jabbar *et al*., 2019). Although alkaloids are extremely toxic, yet they are considered useful as therapeutic agent in small quantities (Roy, 2017). The plant-derived alkaloids exert numerous activities in ameliorating neurological disorders such as seizures, anxiety, and depression (Aryal *et al*., 2022). The alkaloids have been shown to attenuate neurodegenerative disorders through their vast mode of actions such as increasing the activity of GABA, antagonist of N-methyl-D-aspartate (NMDA) receptor, inhibition of monoamine oxidase B (MAO-B), and antioxidant activities (Hussain *et al*., 2018). Seedo (2015) reported that the aqueous extract of chamomile flower exerted full anticonvulsant effect, while its ethanolic extract did not exhibit full anticonvulsant effect. Furthermore, the phytochemical analysis of the chamomile flower extracts showed the presence of alkaloids in aqueous extract of chamomile, and absence in the ethanolic extract (Seedo, 2015).

The anticonvulsant efficacy of crude flavonoids and alkaloids extracts of the chamomile flower in combination has not been evaluated yet. Hence, the present study was carried out to evaluate the combination efficacy of crude flavonoids and alkaloids extracts of chamomile flower against PTZ-induced convulsions in chicks.

## MATERIALS AND METHODS

### Collection of the plant samples and authentication

The flower parts of chamomile flower were collected from Duhok province, Kurdistan region, Iraq. The plant was identified and authenticated by a botanist in the college of agricultural engineering sciences, University of Duhok, Kurdistan region, Iraq. Then the flower parts of the plant were air-dried under shade for a period of two weeks. Then, they were milled to a fine powder, and stored in a dark place until use.

### Preparation of the crude flavonoids extract

A mixture of fine powder of the plant (50 gm) in ethanol 70% (250 ml) was kept for 24 hours. Then, the mixture was filtered using gauzes and filter papers. The filtrate was concentrated using rotary evaporator, and dried to yield 9.7 gram as alcoholic extract. Then 5 gm of the alcoholic extract was used to extract crude flavonoids extract by a method described by Andersen and Markham (2006). The obtained extract (0.8 gm) was light-reddish, and referred as crude flavonnoids extract. The desired concentrations of crude flavonoids extract (20, 40, and 80 mg/5ml) in distilled water were prepared and used within three days.

### Preparation of crude alkaloids extract

A mixture of fine powder of the plant (50 gm) in ethanolic acetic acid 10% (250 ml) was kept for 24 hours. The mixture was filtered using gauzes and filter papers. Then, the filtrate was concentrated to a quarter of its volume using rotary evaporator, and acidified with concentrated sulphuric acid (5 ml). The solution was adjusted at pH 9 by adding of concentrated ammonium hydroxide. Thereafter, the mixture was put in a separation funnel, and extracted with chloroform (3 × 20 ml). The alkaloids were separated in the organic layer, and concentrated using rotary evaporator, and then dried to obtain (1.15 gm) of crude alkaloids extract (Alsaadi, 2018). The desired concentrations of crude alkaloids extract (20, 40, and 80 mg/ml) in normal saline were prepared and used within three days.

### Phytochemical screening of the extracts

The phytochemical screening tests were carried out on the crude flavonoids and crude alkaloids extracts to investigate the presence of bioactive compounds, such as flavonoids, alkaloids, saponins and tannins in the extracts (Emara and Morsy, 2018; Rahmani and Ouahrane, 2022).

### Animals and experimental design

The current study was conducted on 72 broiler chicks of either sex (one day old), which were purchased from a local hatchery. The chicks were raised in the poultry hall in the College of Veterinary Medicine/University of Duhok for two weeks prior to be used in this study. They were kept at 28-33 $^{o}$C, with constant lighting. The

animals fed with a basal diet and water *ad libitum*. The chicks were randomly distributed into six groups (for each group n=12). Group 1: considered as negative control, was treated with subcutaneous (s.c.) injection of normal saline (1ml/kg). Group 2: served as positive control, was treated with s.c. injection of PTZ (90 mg/ml/kg) Group 3: treated with sodium valproate (200 mg/5ml/kg, orally) for six successive days (Seedo, 2015). Group 4, 5 and 6: treated with the crude flavonoids extracts (20, 40, and 80 mg/5ml/kg, orally) for six days respectively (Ibrahim *et al*., 2008), and followed by a single injection of the crude alkaloids extract (20, 40, and 80 mg/ml/kg, i.p.) respectively (Cruz *et al*., 2013). Thirty minutes post-treatment, the chicks in groups (3–6) were injected with PTZ (90 mg/ml/kg, s.c.), and observed for the convulsive signs using digital video camera for 30 minutes.

**Collection of blood samples**

Three hours afterward of PTZ treatment, the blood sample was collected by heart puncture. The blood was kept in a plain tube, and blood serum extracted after centrifuging at 5000 rpm for 10 minutes, and then stored it at -18 °C until used for biochemical analysis.

**Collection of brain samples**

After the animals were sacrificed by decapitation, the brain was removed and stored in a plastic container, and kept at -18 °C until will be used for biochemical analysis.

**Measurement of brain tissue neurotransmitters**

The thawed brain tissue (0.1 gm) was minced and homogenized with of cold phosphate-buffered saline (0.9 ml) using a homogenizer (Coyote/China) on ice, afterward centrifuged at 7250 RPM at 4 °C for 5 minutes. The supernatants were separated and stored at −20 °C for brain neurotransmitters analysis. The brain tissue concentration of GABA and glutamate were measured using corresponding ELISA kits (BT LAB/China).

**Analysis of serum biochemical parameters**

The serum level of catalase, glutathione reductase, malondialdehyde, and 8-isoprostane were measured using corresponding ELISA kits (BT LAB/China). The serum electrolytes concentration, such as potassium ion ($K^+$), sodium ion ($Na^+$), chloride ion ($Cl^-$), ionized calcium ion ($iCa^{2+}$), and total calcium ion ($TCa^{2+}$), and serum pH were measured using electrolyte analyzer (Fortress diagnostics, UK). The concentration of glucose in the serum was measured colorimetrically using 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 by SPSS using one-way ANOVA, and Duncan test was used to compare of differences between means, and determine the level of significant difference at $P<0.05$. (Permanasari *et al*., 2010).

## RESULTS

**Phytochemical screening of crude flavonoids and alkaloids extracts**

The crude flavonoids and alkaloids extract were subjected to a qualitative phytochemical screening for the presence of various phytochemicals, such as flavonoids, alkaloids, tannins and saponins as shown in Table 1. The results showed the presence of flavonoids in the crude flavonoids extract, and the absence of alkaloids, tannins, and saponin. The results also showed that the crude alkaloids extract contains only alkaloids, while flavonoids, tannins, and saponins were absent.

**Table (1):-** Phytochemical screening of the crude flavonoids and alkaloids extracts.

| Phytochemical | Crude flavonoids extract | Crude alkaloids extract |
|---|---|---|
| Flavonoids | Present | Absent |
| Alkaloids | Absent | Present |
| Tannins | Absent | Absent |
| Saponins | Absent | Absent |

**Effects of crude flavonoids with alkaloids extracts on convulsive behaviors**

A single subcutaneous injection of PTZ, administrated at the dose of 90 mg/kg, elicited pronounced convulsive seizures in chicks within 6–24 minutes. The present study documented various convulsive seizures, comprising of restlessness, defecation, extension of the legs, stiffening of the body (tonic convulsions), failure to maintain an upright posture, involuntary contractions and motion of the skeletal muscle (myoclonic convulsions), uncoordinated wing flapping (tonic-clonic convulsions), loss of consciousness, and mortality from asphyxia in about one-third of the animals as compared to the negative control group. The administration of

standard anticonvulsant drug, sodium valproate, at a dose of 200 mg/kg, resulted in complete protection against convulsion, as evidenced by comparison with the positive control group. The oral administration of crude flavonoids extracts orally at the doses of (20, 40, and 80 mg/kg) over a period of six days, coupled with a subsequent single intraperitoneal injection of crude alkaloids extracts (20, 40, and 80 mg/kg), yielded a dose-dependently delay in the onset of convulsive seizures and a corresponding reduction in the mortality rate 25%, 16%, 8% respectively in chicks PTZ-induced convulsions.

**Effects of crude flavonoids with alkaloids extracts on brain tissue neurotransmitters**

Table (2) displayed the influence of six days pretreatment with crude flavonoids extracts followed by a single intraperitoneal injection of crude alkaloids extract on brain tissue neurotransmitters in chicks PTZ-induced convulsions. The experimental findings indicated that the control group, which was treated with PTZ revealed a significant reduction in the concentration of GABA, and significant elevation in the glutamate level in brain tissue compared with the negative control group. In comparison, 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, as compared to the positive control group. The results demonstrated that the groups pretreated with crude flavonoids and crude alkaloids extracts at the doses 20 and 40 mg/kg showed a slight insignificant elevation of GABA, and insignificant reduction of glutamate levels in the brain tissue compared to the positive control group. In contrast, the group pretreated with crude flavonoids and crude alkaloids extracts at the doses 80 mg/kg showed a marked elevation in GABA level, and reduction in glutamate level in the brain tissue compared with the positive control group.

**Table (2):-** Effects of crude flavonoids combined with alkaloids extracts of chamomile flower on brain tissue neurotransmitters in chicks PTZ-induced convulsions in comparison to the control group.

| Groups | GABA (ng/gm) | Glutamate (ng/gm) |
|---|---|---|
| NC | 30.73±1.75 $^{A}$ | 28.36±1.32 $^{C}$ |
| PC | 17.21±2.11 $^{C}$ | 47.95±1.19 $^{A}$ |
| SV 200 mg/kg | 29.36±2.52 $^{A}$ | 28.47±2.51 $^{C}$ |
| F+A 20 mg/kg | 19.93±1.55 $^{BC}$ | 46.19±2.07 $^{A}$ |
| F+A 40mg/kg | 21.23±1.88 $^{BC}$ | 43.69±2.26 $^{AB}$ |
| F+A 80mg/kg | 25.76±1.93 $^{AB}$ | 39.69±1.42 $^{B}$ |

Negative control (NC), Positive control (PC), Sodium Valproate (SV), Flavonoid (F), Alkaloid (A), n =12 chicks per group. The values are presented as mean ± SE. Different superscript letters within each column indicate statistical significance at $p< 0.05$ according to the Duncan test.

**Antioxidant effects of crude flavonoids combined with alkaloids extracts**

The data in Table (3) showed the impact of pretreatment of crude flavonoids, followed by crude alkaloids extract on the antioxidant enzymes and oxidative stress biomarkers in the serum of PTZ-induced convulsions chicks. The positive control group treated with PTZ showed a significant reduction in antioxidant enzymes CAT and GR, while noticeable elevation in the level of lipid peroxidation markers, MDA and 8-isoprostane as compared with the negative control group. However, the group that was pretreated with sodium valproate exhibited a significant increase in CAT, and insignificant increase in GR. The MDA and 8-isoprostane were significantly reduced as compared with the positive control. The results revealed that the groups pretreated with crude flavonoids with crude alkaloids extracts (20 and 40 mg/kg) showed a slight insignificant elevation in the concentration of CAT and GR, and an insignificant reduction in MDA when compared with the positive control group. The groups received 40 mg/kg of crude flavonoids and crude alkaloids extracts showed a significant decrease in the level of 8-isoprostane compared with the control group that received PTZ. In contrast, the group that received the highest dose of crude flavonoids and crude alkaloids extracts (80 mg/kg) revealed a significant raise in the level of CAT, as well as a significant reduction in MDA and 8-isoprostane levels.

**Table (3):-** Effects of crude flavonoids combined with alkaloids extracts of chamomile flower on the serum antioxidant enzymes and oxidative stress biomarkers in chicks PTZ-induced convulsions in comparison to the control group.

| Groups | CAT (ng/ml) | GR (ng/ml) | MDA (nmol/ml) | 8-Isoprostane (ng/L) |
|---|---|---|---|---|
| NC | 52.04±4.01 $^{A}$ | 2.63±0.36 $^{A}$ | 8.28±0.92 $^{C}$ | 691.51±35.47 $^{C}$ |
| PC | 28.59±4.15 $^{C}$ | 1.43±0.15 $^{B}$ | 13.62±1.35 $^{A}$ | 916.43±67.63 $^{A}$ |
| SV 200 mg/kg | 46.01±3.61 $^{AB}$ | 2.28±0.33 $^{AB}$ | 9.69±0.93 $^{BC}$ | 747.24±41.50 $^{BC}$ |
| F+A 20 mg/kg | 29.74±4.54 $^{C}$ | 1.64±0.18 $^{B}$ | 12.71±1.46 $^{AB}$ | 849.32±39.32 $^{AB}$ |
| F+A 40mg/kg | 38.99±3.01 $^{BC}$ | 1.88±0.23 $^{AB}$ | 11.94±0.56 $^{AB}$ | 768.65±37.49 $^{BC}$ |
| F+A 80mg/kg | 42.88±3.60 $^{AB}$ | 2.11±0.30 $^{AB}$ | 10.39±0.74 $^{BC}$ | 764.81±55.76 $^{BC}$ |

Negative control (NC), Positive control (PC), Sodium Valproate (SV), Flavonoid (F), Alkaloid (A), n =12 chicks per group. The values are presented as mean ± SE. Different superscript letters within each column indicate statistical significance at $p< 0.05$ according to the Duncan test.

**Effects of crude flavonoids combined with alkaloids extracts on some electrolytes, pH and glucose level in the serum**

Table (4) summarized the effect of the crude flavonoids along with alkaloids extracts on certain electrolytes, pH, and glucose levels in the serum in chicks PTZ-induced convulsions. The results showed the group that received PTZ showed a significant elevation in $K^+$ ions, while decline in $Na^+$, $iCa^{2+}$ and $TCa^{2+}$ ions when compared to the negative control group. The group pretreated with sodium valproate exhibited a significant reduction in $K^+$ ion, whereas an elevation in $Na^+$ and $iCa^{2+}$ ions in comparison to the positive control group. The groups that pretreated with crude flavonoids and alkaloids extracts at the dose of 20 and 40 mg/kg did not show any significant alterations in the electrolytes, pH, and glucose levels when compared to the positive control group. On the other hand, the group that pretreated with the highest dose of crude flavonoids with alkaloids extracts (80 mg/kg) showed a significant rise in the level of $Na^+$ and $iCa^{2+}$ ions when compared with the positive control group.

**Table (4):** Effects of crude flavonoids combined with alkaloids extracts of chamomile flower on serum electrolytes, pH, and glucose in chicks PTZ-induced convulsions in comparison to the control group.

| 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 | 8.28 ± 0.20 $^{B}$ | 144.14 ± 0.89 $^{A}$ | 109.16 ± 0.66 $^{A}$ | 1.05 ± 0.06 $^{A}$ | 1.88 ± 0.08 $^{A}$ | 7.64 ± 0.009 $^{A}$ | 281.31 ± 4.02 $^{A}$ |
| PC | 9.42 ± 0.28 $^{A}$ | 138.70 ± 1.24 $^{C}$ | 110.18 ± 0.92 $^{A}$ | 0.81 ± 0.07 $^{C}$ | 1.56 ± 0.10 $^{B}$ | 7.62 ± 0.008 $^{A}$ | 265.10 ± 9.26 $^{A}$ |
| SV 200 mg/kg | 8.30 ± 0.29 $^{B}$ | 143.86 ± 0.74 $^{A}$ | 108.37 ± 0.67 $^{A}$ | 1.01 ± 0.05 $^{AB}$ | 1.83 ± 0.12 $^{AB}$ | 7.65 ± 0.007 $^{A}$ | 273.94 ± 8.23 $^{A}$ |
| F+A 20 mg/kg | 8.99 ± 0.25 $^{AB}$ | 139.82 ± 0.79 $^{C}$ | 109.78 ± 0.60 $^{A}$ | 0.85 ± 0.02 $^{BC}$ | 1.70 ± 0.05 $^{AB}$ | 7.62 ± 0.004 $^{A}$ | 276.14 ± 4.84 $^{A}$ |
| F+A 40 mg/kg | 8.89 ± 0.25 $^{AB}$ | 140.68 ± 0.70 $^{BC}$ | 109.58 ± 0.45 $^{A}$ | 0.91 ± 0.03 $^{ABC}$ | 1.75 ± 0.07 $^{AB}$ | 7.63 ± 0.005 $^{A}$ | 273.45 ± 5.37 $^{A}$ |
| F+A 80 mg/kg | 8.65 ± 0.22 $^{AB}$ | 143.26 ± 1.12 $^{AB}$ | 108.15 ± 1.01 $^{A}$ | 0.97 ± 0.03 $^{AB}$ | 1.79 ± 0.08 $^{AB}$ | 7.63 ± 0.019 $^{A}$ | 272.47 ± 6.45 $^{A}$ |

Negative control (NC), Positive control (PC), Sodium Valproate (SV), Flavonoid (F), Alkaloid (A), n =12 chicks per group. The values are presented as mean ± SE. Different superscript letters within each column indicate statistical significance at $p< 0.05$ according to th
e Duncan test.

## DISCUSSION

The present study was carried out to investigate the impact of the crude flavonoids extract administrated orally for six consecutive days followed by a single intraperitoneal injection of crude alkaloids in chicks PTZ-induced convulsions, and evaluating the effects of these extracts on the behavior alteration, the brain tissue neurotransmitters, and the antioxidant and oxidative stress parameters, as well as on some electrolyte parameters and glucose level in the serum.

**Phytochemical screening of crude flavonoids and alkaloids extracts**

In the present study, the preliminary phytochemical screening tests were carried out on the crude flavonoids and alkaloids extracts, and the phytochemical screening confirmed the presence of flavonoids in the crude flavonoids

extract, and presence of alkaloids in the crude alkaloids extract. The present result was consistence with Al-Maliki (2012) who reported the presence of alkaloids and the absence of other compounds in the alkaloid isolated from *M. chamomilla* L.

**The effects of crude flavonoids together with alkaloids extracts on convulsive behaviors and brain tissue neurotransmitters**

PTZ, a $GABA_A$ receptor antagonist, is widely used to induce convulsive seizures by lowering the seizure threshold, and to assess the efficacy of anticonvulsant substances in experimental animals (Asadi-Shekaari *et al*., 2014). The results of the present experiment showed that the PTZ induced obvious convulsive behaviors such as tonic, myclonic, clonic, and tonic-clonic convulsive seizures. These convulsive behaviors were linked to significant decreasing of GABA, while increasing of glutamate levels in the brain tissue. PTZ is a well-known convulsing used, exerting its effect by blockade of $GABA_A$ receptors, leading to decrease inhibitory GABA and increase excitatory glutamate levels in the brain tissue (Shimada and Yamagata, 2018). Thus, it brings the membrane potential closer to seizure threshold and firing action potentials (Asadi-Shekaari *et al*., 2014). The present results are in line with Wang *et al*. (2021), who found that PTZ provoked convulsions by reducing the level of GABA and elevating glutamate level in the brain tissue.

The protective effects of anticonvulsant drugs, sodium valproate was linked to the improving the GABA neurotransmission which abolished the convulsive seizures induced by PTZ. The result was found that the chicks pretreated with sodium valproate revealed a significant elevation in GABA level and suppression in glutamate level in the brain tissue. Sodium valproate inhibit GABA-transaminase, and leading to an increase the availability of GABA in the brain tissue, and thereby facilitating GABA mediated responses (Bourin, 2020). Furthermore, sodium valproate decreases the brain glutamate level by inhibition of voltage-gated sodium channels, and preventing action potential firing (Zawab and Carmody, 2014). The present results are consistent with Kumar *et al*., (2016), who reported that pretreatment with sodium valproate led to substantial rise the GABA level in brain tissue.

It was observed that the groups pretreated with crude flavonoids along with crude alkaloids extract resulted in delaying the onset of convulsive seizures as well as reduced in mortality rate. The groups pretreated with the higher doses of both crude flavonoids and alkaloids extract (80 mg/kg) showed significant increasing in brain GABA and decreasing in glutamate level in brain tissue. It has been demonstrated that flavonoids exert anticonvulsant activity by modulating the $GABA_A$ receptors channel, as they are structurally similar to benzodiazepines (Diniz *et al*., 2015). Furthermore, the alkaloids possess anticonvulsant activity by activating GABA neurotransmission and increasing the level of brain GABA (Hussain *et al*., 2018). In addition, Seedo (2015) reported that the aqueous extract of chamomile flower exerted complete anticonvulsant effect, whereas the ethanolic extract did not exhibited full anticonvulsant effect, and the phytochemical analysis revealed the presence of both flavonoids and alkaloids in aqueous extract while only presence of flavonoids in the ethanolic extract.

**Antioxidant effects of the crude flavonoids and alkaloids extracts**

The results showed that PTZ caused oxidative damage by reducing CAT and GR levels, and elevating MDA and 8-isoprostane levels. It was well-known that PTZ triggers the production of free radicals. In addition, free radicals have an important role in the development and pathogenesis of convulsive seizures (Kumar *et al*., 2016). The development of convulsive seizures induces decreasing the antioxidant defense mechanism and increasing the generation of free radicals, which further induces the oxidative stress (Borowicz-Reutt and Czuczwar, 2020). The free radical can lead to seizure activity by inhibition of glutamine synthase and glutamate decarboxylase, thereby causing an abnormal accumulation of excitatory neurotransmitter glutamate (Diniz *et al*., 2015). The present result consisting with Ullah *et al*., (2022) who reported that PTZ induced oxidative stress in mice by decreasing CAT and increasing of MDA levels.

The use of sodium valproate prior to convulsions induced by PTZ resulted in significantly reduction in CAT level, and increase in both MDA and 8-isoprostane. It has been demonstrated that antioxidant properties of sodium valproate can be linked to its anticonvulsant effects, and thereby enhance antioxidant enzymes and reducing oxidative damages (Abdulqader *et al*., 2021). Similarly, Bhati *et al*., (2018) reported that the pretreated of sodium valproate against convulsions induced by PTZ exhibited substantial elevation in CAT and reduction in MDA levels in albino mice. The results demonstrated that combination of crude flavonoids and alkaloids extracts (40 mg/kg)

caused significant reduction in 8-isoprostane, while at a higher dose (80 mg/kg) caused significant elevation in CAT and reduction in 8-isoprostane. Flavonoids provide antioxidant activity by several modes including, directly scavenging the ROS promptly by donating a hydrogen atom or single-electron transfer (Banjarnahor and Artanti, 2014). Furthermore, it has been reported that alkaloids also possess neuroprotective properties through antioxidant and anticonvulsant pathways (Hussain *et al*., 2018). The present findings were in accordance with the findings of Seedo and Hassan (2016) who reported that utilizing aqueous extract of chamomile flower, which contain both flavonoids and alkaloids caused a notable elevation in serum T-AOC in chicks treated with PTZ.

**Effects of crude flavonoids and alkaloids extracts on some electrolytes, pH and glucose levels**

The disruption of electrolytes is critical in the manifestation of convulsions, and assessment and management of convulsions depend on routine laboratory evaluation of serum electrolytes, including $Na^+$, $K^+$ and $Ca^{2+}$ (Nardone *et al*., 2016). The control group that received PTZ exhibited a significant elevation in $K^+$ ions and decline in $Na^+$, $iCa^{2+}$ and $TCa^{2+}$ ions. PTZ is a $GABA_A$ receptor antagonist, used to chemically induced convulsive seizures, and screening and evaluating the potential effects of anticonvulsant substances (Shimada and Yamagata, 2018). It caused convulsive seizures by impairing inhibitory GABA and enhancing the activity of excitatory glutamate neurotransmitters in the brain tissue (Asadi-Shekaari *et al*., 2014). Consequently, resulted in considerable metabolic stress and alteration of electrolyte gradients, which further increase the neural and neuromuscular excitability during seizures (Borowicz-Reutt and Czuczwar, 2020). Thus, convulsive seizures are more frequently observed during hypocalcemia and hyponatremia. This is because such deficiencies increase the excitability of neuromuscular, causing membrane potential to be closer to firing threshold, ultimately resulting in convulsions (Nardone *et al*., 2016). The current results are consistence with those of Seedo and Hassan (2016) who previously reported that PTZ treatment resulted in increasing $K^+$ ions and decreasing $Na^+$ ions level in chicks. In contrast, Abdul Wahid (2010) reported an elevation in $Na^+$ ions and reduction in $K^+$ ions in epileptic patients received no treatment.

The group of chicks pretreated with sodium valproate and subjected to convulsion by PTZ showed a decrease in $K^+$ ions, while the concentration of $Na^+$ and $iCa^{2+}$ increased. The potential connection between the impacts of sodium valproate and its anticonvulsant properties might from its ability to inhibit GABA-T and block the voltage-gated sodium channels, which can consequently enhance inhibitory GABA neurotransmission in the brain tissue (Zawab and Carmody, 2014; Bourin, 2020). These results are consistence with the Seedo and Hassan (2016) who reported that the treatment of chicks with sodium valproate and PTZ resulted in an raise in concentration of $Na^+$ ions in the serum. Abdul Wahid (2010) documented that the administration of sodium valproate in the epileptic patients resulted in increasing concentration of $Ca^{2+}$ ions.

The results of the present study indicated that the chicks pretreated with crude flavonoids extract along with crude alkaloids extract at a higher dose (80 mg/kg) showed significant elevation in $Na^+$ and $iCa^{2+}$ ions. These results also could be attributed to the effects of both flavonoids and alkaloids in the extracts, which led to a decrease neural excitability through increasing concentration of $Na^+$ and $iCa^{2+}$ ions. Moreover, they reduced the membrane depolarization, while raising the threshold level for convulsions (Hussain *et al*., 2018). The current findings align with Seedo and Hassan (2016), who demonstrated a raise in $Na^+$ ions in convulsive chicks pretreated with aqueous extract of chamomile flower. The results indicated that the treated groups did not exhibit any significant change in glucose and pH levels in the serum compared to the control groups. The present results are consistence with those of Seedo and Hassan (2016) wherein glucose level no changed in the groups of chicks pretreated with sodium valproate, and aqueous extract of chamomile and followed by the PTZ-induced convulsions.

## CONCLUSION

It was concluded that the combination of 80 mg/kg crude flavonoids extract along with crude alkaloids extract had mild to moderate anticonvulsant and antioxidant effects. The study suggests that combined administration of these extracts potentially reduce the seizure threshold and oxidative damage.

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