ASSESSMENT OF ANTICONVULSANT, MEMORY ENHANCING AND FEAR-REDUCING EFFECT OF NYCTANTHES ARBOR-TRISTIS L. IN ANIMAL-MODELS

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ABSTRACT

The prevailing study was deliberated to explore the effect of *Nyctanthes arbor-tristis L.* extract (Na.Cr) pertaining to anticonvulsant, memory-enhancement and anxiolytic in rodents. Methanolic extract of Na.Cr phytochemically screened for presence of possible major chemical constituents. After assessing toxicity, rodents (rats and mice) were treated with various doses of Na. Cr i.e. 100, 200 and 400mg/kg for assessing anticonvulsant activity in strychnine induced convulsions ($P > 0.05$), notable effect was observed in picrotoxin induced convulsions ($P < 0.05$), pentylenetetrazole induced acute convulsions ($P < 0.05$) and lithium pilocarpine induced status epilepticus ($P < 0.05$). Furthermore, Na.Cr also reverse scopolamine-induced memory deficit ($P < 0.05$) in Morris-water maze. Moreover, dose dependent anxiolytic effect was observed in various anxiety models including Elevated-plus-maze($P < 0.05$), light and dark-test($P < 0.05$) and open-field test($P < 0.05$). The current study validation supported the use of *N. arbor-tristis L.* in anticonvulsant, memory enhancing and anxiolytic in animal models. Presence of major chemical constituent like flavonoid and terpenoids and various constituent might have imparted vital neuroprotective role.

Keywords: Strychnine induced convulsions, picrotoxin induced convulsions, Scopolamine-induced memory loss, anxiety

INTRODUCTION

Epilepsy is the common neurological disorder affecting both genders and all age groups equally (Milligan, 2021). Epilepsy accounts for affecting about 70 million people yearly (Sen et al., 2020). Occurrence of seizure are due to abnormal synchronized neural firing either in a part of brain or throughout the brain arising due to metabolic, infectious, genetic and structural disturbances. While, tendency of unprovoked and recurrent seizure accompanying transient clinical manifestations is termed as epilepsy. Clinical manifestations include cognitive, neurobiological, social, psychological consequences of condition (Falco-Walter, 2020).

Alzheimer's-disease (AD), affected 24 million population globally, is the rampant disease presenting memory deficit in older persons (DeTure & Dickson, 2019). This condition is characterized by neurodegeneration leading to dementia with disorientation and behavior changes. Elevated oxidative stress is one of the leading etiologies of memory loss, beside this, amyloid beta, mitochondria dysfunction and mutation in tau protein are also some of the causes of Alzheimer's disease (DeTure & Dickson, 2019; Mintun et al., 2021). The depreciation Cholinergic neurotransmission plays a crucial role in AD. The prime pharmacological approach is to the inhibition of acetylcholinesterase enzyme by some drugs to augment cholinergic connection. But owing to shorter half life and various adverse effects, limit the use of these drugs (Javaid et al., 2021).

At some point in their lifetime, approximately 20% of the worldwide population turn out to be the target of these psychological-challenges. Anxiety and depression are considered to be leading psychic illness (Harro, 2018; Kalin, 2020). These illnesses were managed distinctly for decades using benzodiazepines for anxiety and amine reuptake inhibitors for depression. Benzodiazepines have certainly valuable but their property of being muscle relaxant and sedative along with physical-dependence drive them less-fancied drugs (Bandelow et al., 2017).

Numerous natural compounds are synthesized by plant, contributing to satisfy daily needs to humans. Amid them *N. arbor-tristis L.* belongs to Oleaceae-family, which is commonly known as coral jasmine, har-singar and parijaat. It is an ornamental plant with a pleasant fragrance (Sharma et al., 2021). It is a small tree or shrub with a height upto 10 m woody perennial terrestrial plant having a life span upto two decades. It has a pH of 5.6–7.5 and adaptive to barren and steppe climatic conditions (Majumder et al., 2023). The leaves of *N. arbor-tristis L.* are simple, hairy,

decussately opposite, and rough. The arrangement of flowers is at the tip of branches, present usually in clusters of 2-7 together. Acapsulated fruit with a diameter of 1-2 cm obcordate orbicular, long and broad are present in NAT. Presence of rough and firm, dark gray or brown bark, which is dippled due to scaling-off of circular bark and seems patchy due to gray brown colored regions. Seeds are thick testa, exalbuminous, the outer layer of heavily vascularized large transparent cells (Jain & Pandey, n.d.; Pandrangi et al., 2022).

N. arbor-tristis L. has a extensive range of pharmacological activities. Since the ancient times, it accounts for prevention of biliousness, gynecological-troubles, and hepato-protective activity in folk-medicine. Tribal people use it to relieve dysentery, sores, cough, snakebite, and hiccup (Parekh & Soni, 2020). Additionally, it has been screened by researchers for anticancer, antiviral, antihistaminic, analgesic, anthelmintic, antipyretic, anti-inflammatory, anti-depressant, CNS and antioxidant activities (Agrawal & Pal, 2013). The current study aimed to facilitate the understanding the effect of crude extract of Na.Cr in various seizure model, memory enhancing and anxiolytic activity for exploration of antiepileptic effect of Na.Cr.

MATERIAL AND METHOD

Animal

Rats (Sprague-Dawley) weighing 150 to 250 g and BALB/c mice (25-40 g) were harbored under controlled conditions including humidity (50%) and temperature (23-25 C). Animals were placed in polycarbonate cages with 12hours light and dark cycle was maintained in the animal-room of the FoP, B.Z University Multan, Pakistan. Animals were provided with standard gnawer feed and water, ad-libitum. Behavioral tests were performed from 8:00am to 5:00pm.

Extract (Na.Cr) Preparation

For preparation of extract, the powder (previously dried and ground) was macerated in the hydroalcoholic mixture (methanol and distilled water) with a ratio of 80:20 v/v respectively in an amber colored bottle. The macerated mixture was shaken frequently. Kept at standard-temperature for a period of 1 week (Loha et al., 2019). After culmination of required time, macerated plant was filtered using a muslin cloth to remove contaminants. Further filtration was done to obtain filtrate by using Whatmann # I filter-paper, for the separation and filtration of residue and filtrate

exclusively contain the chemical constituents present in the plant. The filtrate was dried-out in a rotary-evaporator to get concentrated crude drug-extract (Devi et al., 2020).

Phytochemical analysis

Flavonoids

Na.Cr extract (1g) was heated with 20ml H_2SO_4 until it boils and the solution was filtered. In filtrate 2-3 drops of NH_3 solution (1%) was added. The yellow color is the indicator of presence of flavonoids (Tepal, 2016).

Anthraquinone

The filtrate, obtained during test of flavonoid, 10ml chloroform was mixed and vigorously shaken, two layers appeared. Upper layer which is of chloroform was pipetted out and 2ml dil. Ammonia solution was added. Appearance of violet color shows the presence of anthraquinone (Tepal, 2016).

Saponins

1g of extract was taken in a test-tube, and distilled water (10ml) was added together. Shaken vigorously so that froth may appear. Furthermore, few drops of olive oil were added. If froth or emulsion formed, then this shows the presence of saponins (Ajuru et al., 2017).

Tannins

Na.Cr extract (1g) was added to 20ml water. Subsequently, the solution was filtered and few drops of 1% $Fecl_3$ were mixed with the filtrate. Change in color was observed i.e. bluish-black or brownish-green shows presence of tannins (R. Yadav et al., 2017).

Reducing sugar test

Na.Cr extract (1g) was added to 10ml water. Fehling solution was added with it and heated. Observe the precipitates of brick red color, if they appeared that shows the presence of reducing sugar. This test was for detection of reducing sugar in glycosides (Labiad et al., 2017).

Deoxy-sugar in glycosides

Extract (5mg) was mixed with 1ml chloroform. H_2SO_4 was added along the wall of the test tube. At the interface, the brown color ring was observed. Presence of ring shows the presence of deoxy-sugar glycosides in it. This test is known as Keller killiani test (Rattana et al., 2010).

Alkaloids

Extract (0.1g) was added in 10ml of water and mixed. The solution was alienated into 4 test tubes in 4 different. Few drops of reagents(Mayer, Dragondorff, Hager and Wagner's) were solely added into four test-tubes. Observe the formation of precipitates or alteration in color. If precipitates or change in color appeared then it denotes the presence of alkaloids (Sonam et al., 2017)

Test for terpenoid (Salkowski test)

5ml of extract was added and mixed with chloroform (2ml). To form a layer there was careful addition of H_2SO_4 (3ml). if the color at interface was change to reddish brown, this indicates the presence of terpenoids (M. Yadav et al., 2014).

Test for phenols ($FeCl_3$)

Extract (2ml) was added with ferric-chloride (2ml). formation of blue colour indicate presence of phenols.

Acute-Toxicity Test

Acute-toxicity test was directed in accordance with guideline no. 425 (Up-and-Down method) established by Organization of Economic-Corporation Development (OECD) (Sachana et al., 2019). For acute toxicity test, 20 mice (20-30g) were taken and divided into 4 groups (n=5). One of these group was referred as control group and remaining groups were referred as treated group. Na.Cr was administered to all treated groups in the doses of 1000, 1500 and 2000 mg/kg intraperitoneally. Initially all groups were observed for 1 hour, then mice was observed at forth hour and then 48 hour after the administration of drugs for 2 weeks (Saleem et al., 2017). End-point toxicity was considered as mortality. However various behavioral changes were also monitored including irritability, tremors, responsiveness, diarrhea, salivation and lethargy. Furthermore, some serious complications including weight loss ≥15%, respiratory distress, immobility, oedema and ataxia were observed. According to the protocols, if animal reaches such serious complications then animal would be euthanized immediately by cervical dislocation (Khaliq et al., 2022).

Anti-epileptic activity

Strychnine (STY)induced convulsions

Mice of either gender were taken having a weight range of 25-40 grams. Plant extract (Na.Cr; i.p.) was administered 45min prior to strychnine. Na.Cr was given at 3 variant doses i.e. 100, 200 and 400mg/kg. Control-group was solely given strychnine while other groups i.e. standard or Na.Cr were also given strychnine (Baehr et al., 2023; Vasconcelos et al., 2007). Phenobarbitone(i.p) was taken as positive control. After STY administration, several parameters were analyzed.

Picrotoxin induced convulsions

Plant extract (Na.Cr;i.p) were given 45min before the administration of picrotoxin (i.p). Three different doses of Na.Cr were used. Picrotoxin and Extract were prepared freshly. Ethanol and distilled-water were mixed in a ratio of 0.1:0.9ml respectively to prepare solution of 1ml in order to dissolve picrotoxin. Diazepam(i.p) was taken as a standard-drug (Singh et al., 2023).

Pentylenetetrazole (PTZ) induced convulsions

Mice were carried to the experimental-room an hour before the experiment and fresh doses of Na.Cr and PTZ were prepared. Plant extract (Na.Cr; i.p) was given 45 min before PTZ. Control-group was solely given PTZ whereas in remaining groups standard or extract were also given PTZ (Yuskaitis et al., 2021). Standard-drug being Diazepam(i.p). After administration of PTZ, several parameters were analyzed.

Lithium-pilocarpine (L/Pi) induced-convulsions

Rats having a weight range of 200-300g were utilized in L/Pi induced-convulsions. Current model was gender-specific with utilization of only male rats. Lithium was administered 16-20hours before experiment (Imran et al., 2015). All doses were freshly prepared prior to the experiment. Na.Cr (i.p) was given 45min prior to administration of pilocarpine(s.c). Racine-scale was applied to analyze animals in order to categorize on the essence of symptoms severity (Imran et al., 2015). Status-epilepticus (SE) establish generally within 30min. If no SE appears within 3hours then rats was given diazepam at 10mg/kg (Goffin et al., 2009).

Water maze (WM)

Na.Cr and Piracetam was given for 8 days in order to study the effect of drugs on memory and learning (Othman et al., 2022). WM was designed for 6-days, out of which 2-days were assigned

for training, 3-days for experimental-trials and probe day would be the last day. In probe day platform was removed. Thirty-rats of either gender were taken randomly with 180-250g weight (Javaid et al., 2021). Rats were carried to the behavioral-room 60 minutes prior to the test. Room temperature was maintained at 22-25ºC. In the training session of two days, platform was retained 2-3cm above water while in experimental trials, platform was retained 2-3cm below water. Camera (Logitech c310) was used to capture videos and it was fixed at same place throughout the experiment. Scopolamine was given intra-peritoneally to the rats it was administered 45 min prior to trials. Na.Cr (i.p.) and Piracetam (i.p.) and was given 30min before the experiment. Plateform remained at the same quadrant (SW) throughout experiment and animals suspended at each poles i.e. South, North, East, West. Total time for the test was 2 min, if rat reaches in earlier than 2min the recording was stopped while if it fails to reach with given time then it was positioned on the platform for 1min (Lissner et al., 2021; Lu et al., 2020).

Elevated plus maze

Na.Cr (i.p) was given for 20days, in direction to evaluate presence of anxiolytic effect in the plant (Sotoudeh et al., 2020). Rats (n=25) with either gender were used. Dosing was started 45 min prior to the experiment. Control group was given normal saline and placed in the middle facing open arm. The rats were allowed to move on EPM for 5min. Time-spent in open-arm denotes anxiolytic behavior whereas time-spent in the closed-arm denotes anxiety (Knight et al., 2021).

Light/dark chambers (L/D)

This test comprises of two compartments, a light-compartment and a dark-compartment. The dark-compartment is enclosed with a small door by which rat can pass to other side (Pitsikas et al., 2008). Apparatus was placed in the isolated chamber and lifted at a height of 2 -feet. Diazepam (i.p) was used as standard drug. Dosed of Na.cr (i.p) 45 min before the experiment and after that placed along the wall of the light-compartment. It was allowed to walk around the apparatus for 5 minutes (Renczés et al., 2020).

Open field chamber

It is a square shaped behavioural apparatus, enclosed by walls (Gould et al., 2009). Area of the apparatus was 80x80x45cm. Apparatus was separated into 64 small squares and each square is equal to10x10cm. Four middle squares were assigned as center-zone and each four corner -squares were designated as corner-zones. After assigning rats in each group, treatments were given in

respective groups. Rats were placed in the middle of the apparatus and allowed to walk around for 5min (Kraeuter et al., 2019).

Statistical analysis

Results conferred as the mean±SEM. The statistical-analysis was executed using Graph-pad Prism 7. Statistical-significance was evaluated by student's t-test. For multiple-comparison, one-way ANOVA followed by Dunnett test and the 2-way ANOVA followed by a Tukey-multiple comparison test was used to evaluate statistical-significance.

RESULTS

Phytochemical-analysis

Qualitative analysis of *Nyctanthes arbor-tristis L.*was performed for the absence or presence of various phytochemical constituents. Chemical constituents were shown in the table;

Chemical constituent	Presence or absence
Alkaloids	+
Anthraquinones	+
Flavonoids	+
Glycosides	+
Saponins	+
Tannins	+
Terpenoids	+
Phenols	+

Table 1: Phytochemical determination of Na.Cr, Absence (-) and Presence (+) are shown in the table.

Acute Toxicity test:

Methanolic Extract of *Nyctanthes arbor-tristis L.* does not cause any behavioral, toxicological or psychological abnormality in mice over period of 14 days. The current investigation revealed no changes in physical, neurological or behavioral status. This investigation also reveals that the lethal dose of Na.Cr is considered to be more than 2000 mg/kg.

Epilepsy

Strychnine-induced-convulsion

STY-induced-seizures was notably delayed in the positive control (Phenobarbitone-30mg/kg) and only 20% death was observed. Animals were observed for several parameters including onset of clonic seizure [F (4, 18) = 3677, P<0.0001], duration of clonic seizures [F (4, 18) = 18.35,P<0.0001], tonic seizures duration [F (4, 18) = 3.954, P<0.05] and time of death [F (4, 16) = 2756, P<0.0001]. Mice that were pre-treated with various doses of Na.Cr and then subjected to strychnine did not showed a pronounced effect. Statistical-analysis represent value of P>0.05 for the delay in and no protection seizure at all treatment doses of Na. Cr.

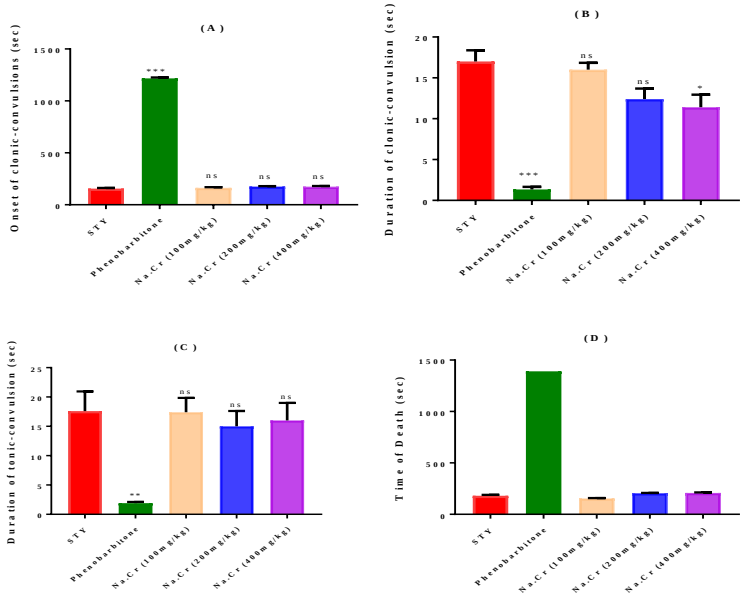

Figure 1: Effect of Na.Cr on the evaluation of antiepileptic activity in mice.

*The mice were administered with 100, 200 and 400 mg/kg of Na.Cr were observed for Antiepileptic activity. The (A) Onset of clonic-convulsions, (B) Duration of Clonic-convulsion, (C) Duration of tonic-convulsion and (D) time of death, were monitored and compared to control. Using Phenobarbitone as standard-drug. Data is evaluated by one-way ANOVA followed by Dunnett's-test and whole data is expressed as mean±SEM (n= 5). ns p> 0.05, *P≤ 0.05, **P≤ 0.01, ***P≤ 0.001*

Picrotoxin-induced convulsion

Diazepam abates convulsions with 100% protection against PTX induced-convulsions. Mice were observed for onset of seizure [F (4, 20) = 7339, P<0.0001], Duration of seizures [F (4, 20) = 90.98, P<0.0001], time of death [F (4, 13) = 35841, P<0.0001] and percentage protection. Mice imperiled to PTX after Na.Cr treatment at doses of 100, 200, 400mg/kg. Onset-of-convulsion was notably delayed in all doses of Na.Cr but much pronounced at a dose of 200 and 400mg/kg (P≤0.001) and in 100mg/kg (P≤0.01), on comparison with control. Duration-of-seizures lessen at 200mg/kg(P≤0.01) and 400mg/kg(P≤0.0001) and it gives 80% protection while other doses 200mg/kg 60% protection was observed and at 100 mg/kg, no protection was observed.

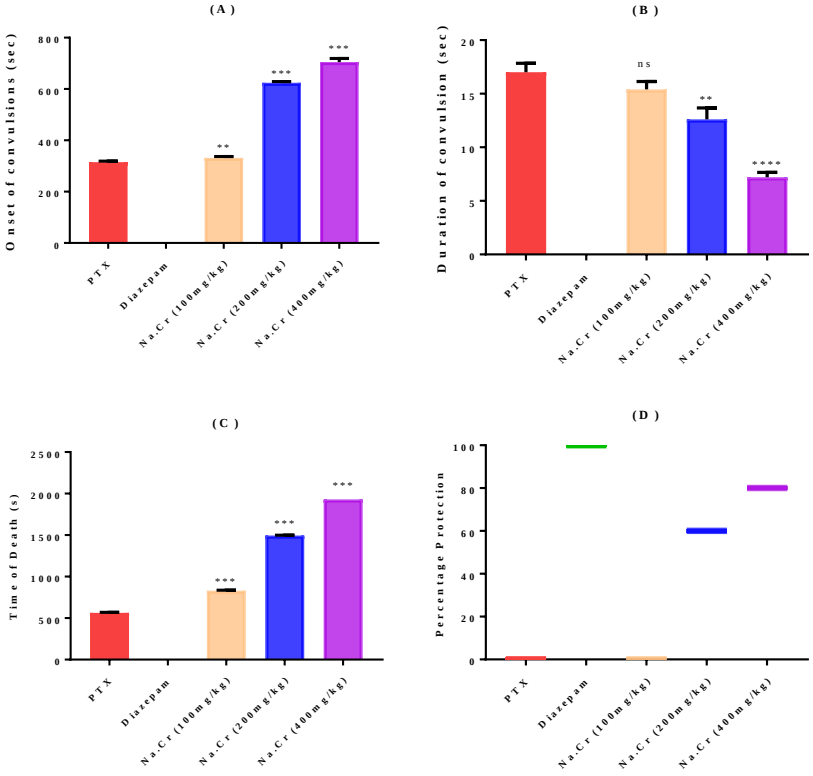

Figure 2: Result of Na.Cr on the evaluation of Picrotoxin induced convulsion in mice.

*The mice treated with 100, 200 and 400 mg/kg of Na.Cr were observed for Antiepileptic activity. The (A) Onset of convulsions, (B) Duration of convulsion, (C) time of death and (D) percentage protection, were monitored and compared to control, using Diazepam as standard. Data is evaluated by one-way ANOVA followed by Dunnett test and whole data is expressed as mean±SEM (n= 5). ns p>0.05, *P≤ 0.05, **P≤ 0.01, ***P≤ 0.001, ****P≤ 0.0001*

Pentylenetetrazole-induced-convulsion

Convulsions were induced using PTZ in mice with diazepam taken as standard drug. Mice were observed for onset of clonic-convulsions [F (4, 20) = 494.9, P<0.0001], duration of clonic-convulsions [F (4, 14) = 5901, P<0.0001], onset of tonic-convulsions [F (4, 20) = 33.13, P<0.0001] and duration of tonic convulsions[F (4, 17) = 18.69, P<0.0001]. Onset of convulsion was significantly increased and duration of convulsion was significantly decreased in 200(P≤0.01) and 400mg/kg (P≤0.001)

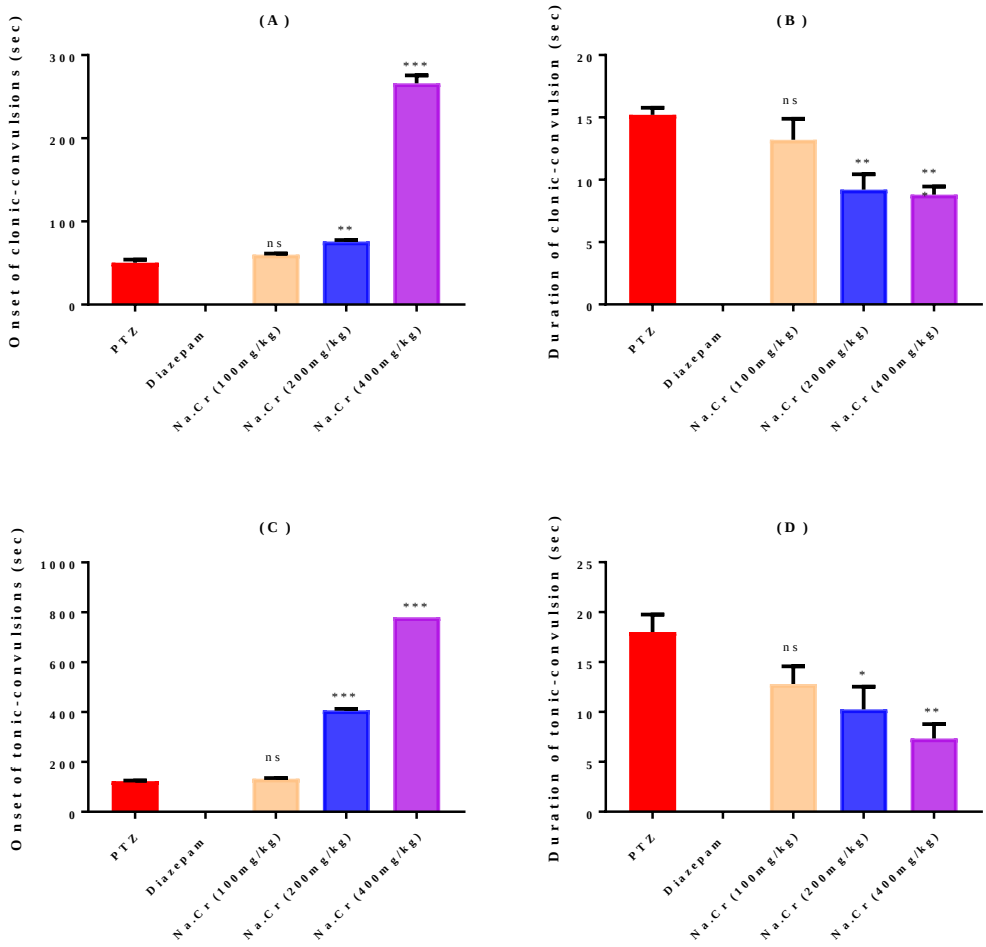

Figure 3: Result of Na.Cr on the evaluation of PTZ induced convulsion in mice.

*The mice treated with 100, 200 and 400 mg/kg of Na.Cr were observed for Antiepileptic activity. The (A) Onset of clonic-convulsions, (B) Duration of clonic-convulsion, (C) Onset of tonic-convulsions and (D) Duration of tonic-convulsion, were monitored and compared to control. Diazepam was the standard. Data is evaluated by one-way ANOVA followed by Dunnett test and whole data is expressed as mean±SEM (n= 5). ns p 0.05, *P≤ 0.05, **P≤ 0.01, ***P≤ 0.001*

Lithium-pilocarpine (L/Pi) induced Status-epilepticus

Effect of L/Pi post treatment of Na.Cr was assessed using racine-scale. Figure 4 shows (A) comparison at control, standard and Na.Cr 100mg/kg [F (18, 120) = 13.1, P<0.0001], (B) comparison at control, standard and Na.Cr 200mg/kg [F (18, 120) = 16.06, P<0.0001] (C) comparison at control, standard and Na.Cr 400mg/kg [F (18, 120) = 7.052, P<0.0001]. In control-group shows stage-4 within 45min and within 60min all the rats show stage-5. The observations were dissimilar in the standard (diazepam-2mg/kg). The rats remained in stage-0 till 15min. Stage-1 was witnessed at half an hour in few rats. Till 120min, rats remained in stage-2 and revert back to stage-1 after 120min. In Na.Cr stage-1 was observed within 15min in all doses. Na.cr at 100mg/kg shows rearing and falling at 90min. Rearing and falling at 200mg/kg shows stage-4 starts at 60min while at 400mg/kg dose in 90min, stage-4 was observed while stage-5 was not witnessed.

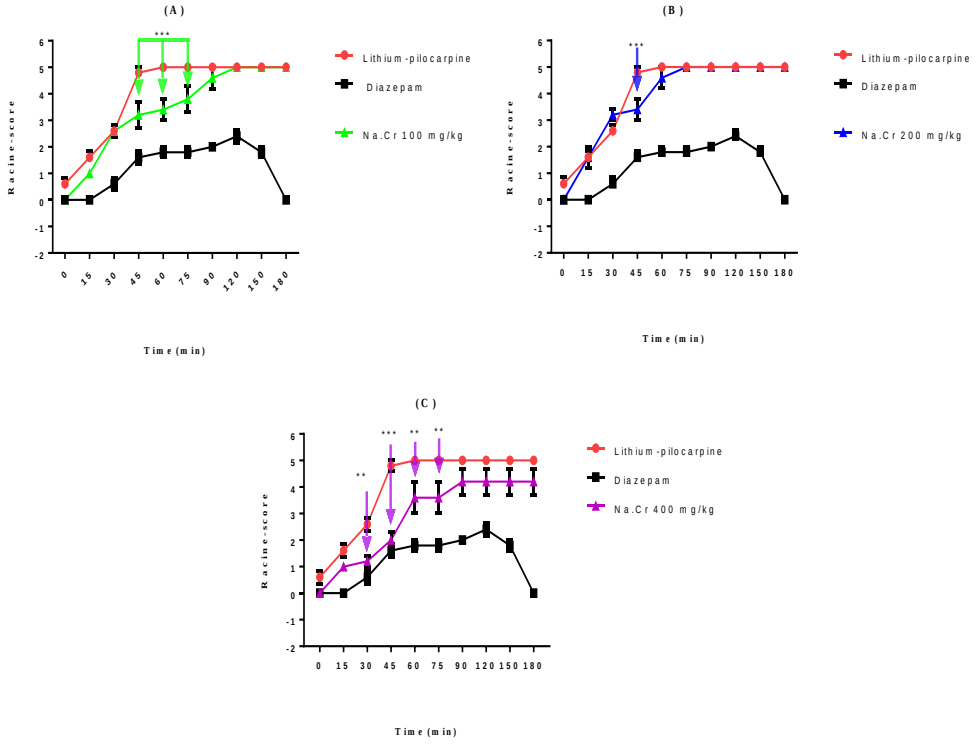

Figure 4: Result of Na.Cr on the assessment of L/Pi induced status epilepticus in rats.

The rats treated with 100, 200 and 400 mg/kg of Na.Cr were observed for Antiepileptic activity. The (A) Racine Score for control, standard and Na.Cr at 100 mg/kg, (B) Racine Score for control, standard and Na.Cr at 100 mg/kg,, (C) Racine Score for control, standard and Na.Cr at 100 mg/kg, were monitored and compared with control. Diazepam

as standard. Data was analysed by two-way ANOVA followed by Tukey's-multiple comparison and whole data is stated as mean±SEM (n= 5).

Neuro-behavioral test analysis

Water-maze

In order to assess memory-enhancing effect of Na.Cr, scopolamine-induced amnesia was established in rats. Subsequently, Na.Cr in several doses i.e. 100,200 and 400mg/kg was given to the amnesic-rats. The comparison was established between scopolamine-group with other treatment groups. One-way-ANOVA demonstrates decrease in escape latency on comparison with negative control group on each day i.e. day 1. [$F(5, 114) = 21.93$, $P<0.0001$], day 2 [$F(5, 114) = 15.56$, $P<0.0001$] and day 3 [$F(5, 114) = 22.81$, $P<0.0001$]. Results were much significant at all doses ($P\leq0.001$).

On final day, which is the probe day, platform was removed and time spent at SW (where platform was placed in experimental days) was observed. Significant results were observed in all treatment groups [$F(5, 24) = 2.953$, $P<0.05$]. Result demonstrate significant increase in time spent at SW zone at 200 ,($P<0.05$) and 400 mg/kg ,($P<0.05$) on comparison with negative control.

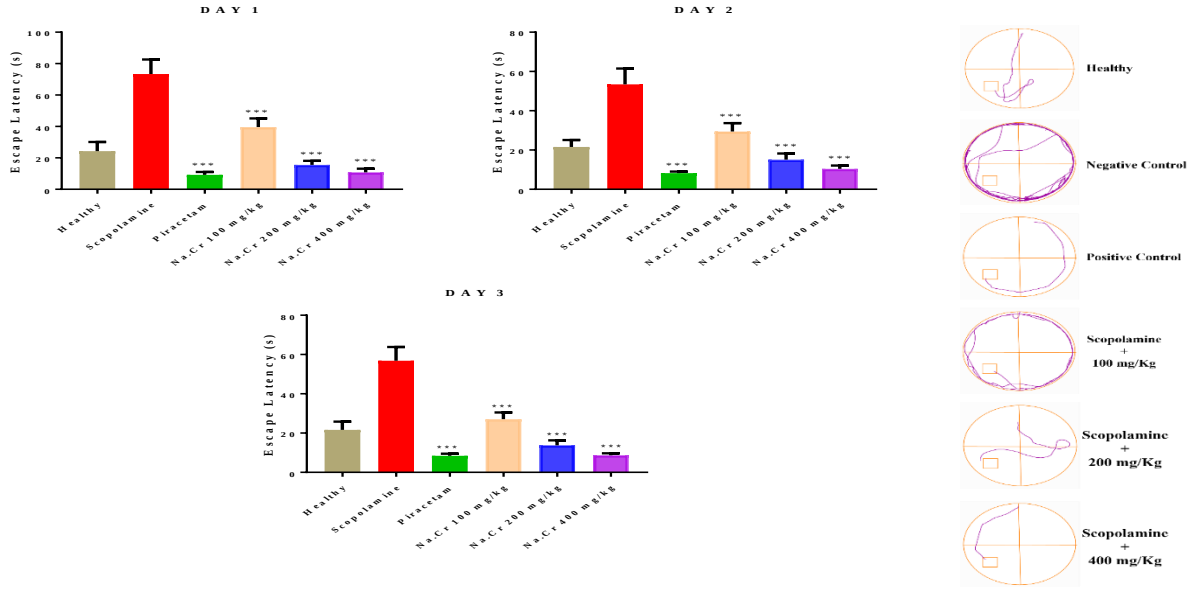

Figure 5: Result of Na.Cr on the evaluation of memory enhancing activity in rats using Morris water maze.

*The rats treated with 100, 200 and 400 mg/kg of Na.Cr were seen for memory enhancing activity along with tracing. The (Day 1) Experimental first day, (Day2) Experimental second day, (Day 3) Experimental third day , were observed and compared to control (scopolamine). Taking Piracetam as standard. mean±SEM (n= 5). ns p>0.05, *P≤0.05, **P≤0.01, ***P≤0.001*

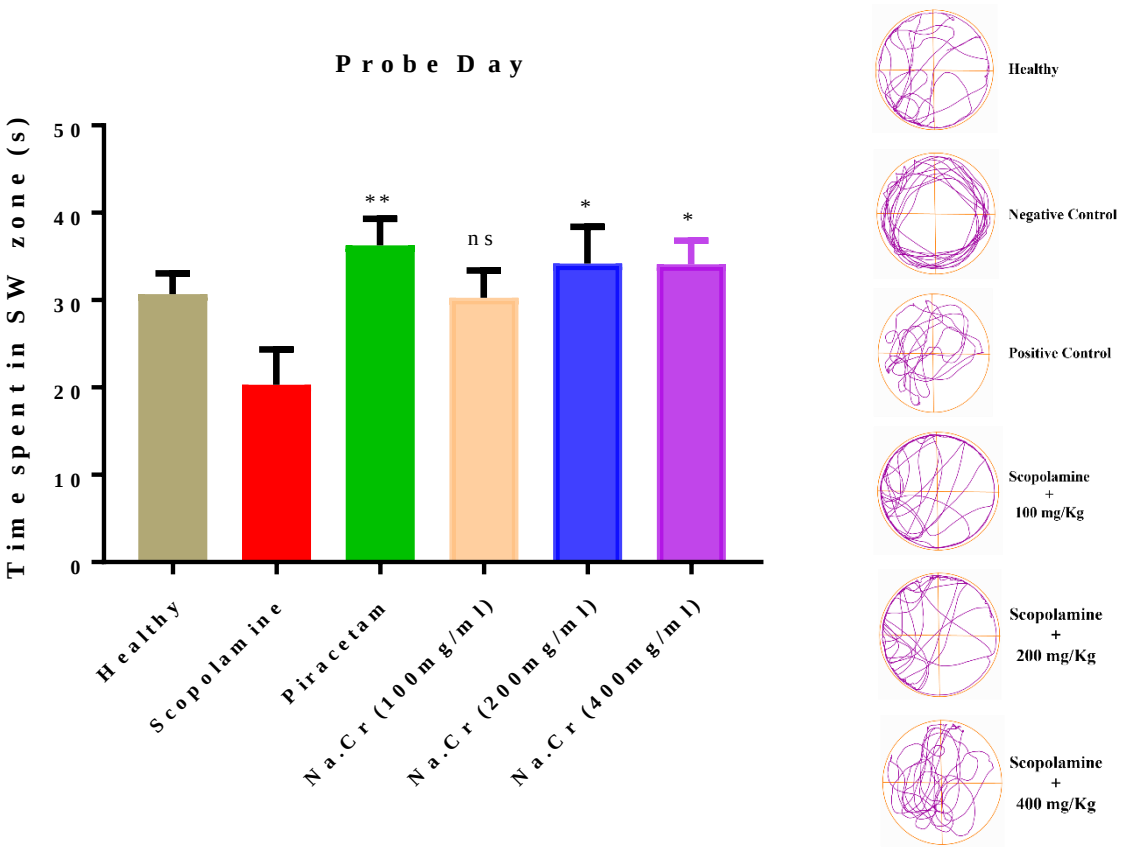

Figure 6: Effect of Na.Cr on the evaluated of memory enhancing activity in rats using morris water maze

*. The rats treated with 100, 200 and 400mg/kg of Na.Cr were witnessed for memory enhancing activity along with tracing of probe day (final day) and graph of time-spent in SW zone, were monitored and compared to control (scopolamine. Data is analysed by one-way ANOVA-followed by Dunnett-test and whole data is represented as mean±SEM (n= 5). ns p>0.05, *P≤ 0.05, **P≤ 0.01,*

Elevated-plus-maze

Anxiolytic-behaviour of Na.Cr was assessed using EPM. Animals were testing 45minutes after administration of doses at 100,200 and 400 mg/kg and single standard dose of diazepam. Significant differences were seen among all groups for spending time in open-arm [F (4, 20) = 36.24, P<0.0001] and number of entries in open arm [F (4, 20) = 17.72, P<0.0001]. Dose dependent increase in time-spent in open-arm i.e. at 200 mg/kg (P≤0.01) and 400 mg/kg (P≤0.001) was observed. Number of visits in open-arm at 400 mg/kg (P≤0.001) were much pronounced as compared to control.

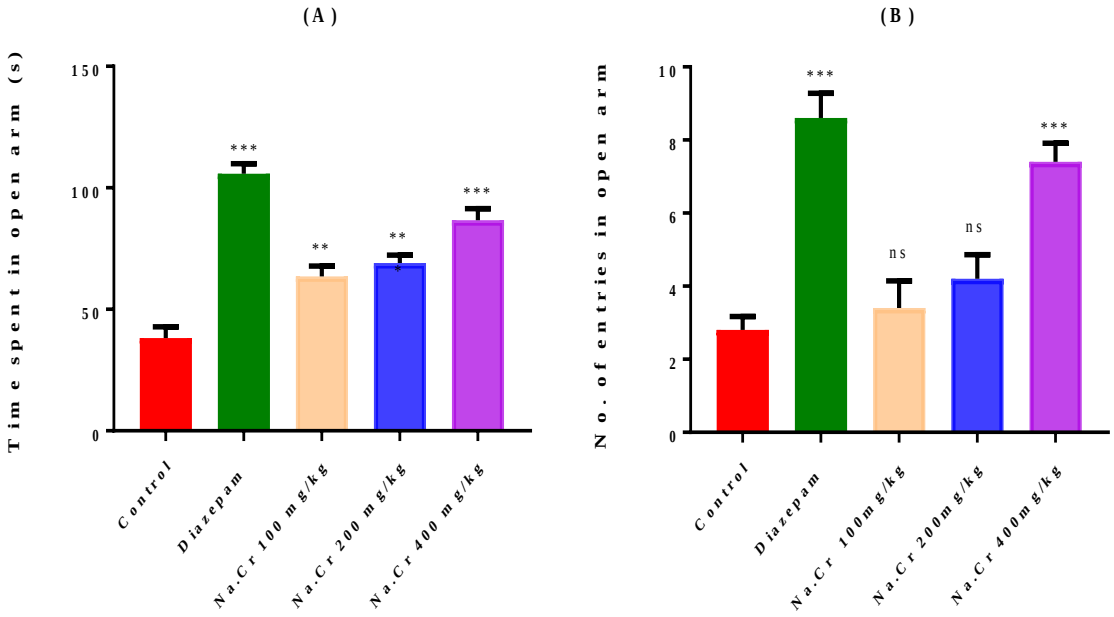

Figure 7: Result of Na.Cr on the evaluated of anxiolytic activity in rats using Elevated Plus Maze.

*The rats treated with 100, 200 and 400 mg/kg of Na.Cr were observed for anxiolytic activity. The (A) Time-Spent in Open-arm, (B) Number-of entries in open-arm, were monitored and compared to control. Data is analysed by one-way-ANOVA followed by Dunnett-test and whole data is expressed as mean + SEM (n= 5). ns p> 0.05, *P≤ 0.05, **P≤ 0.01, ***P≤ 0.001*

Light-and dark test (L/D)

Anxiolytic behavior was evaluated by means of L/D. Na.Cr at various doses were administered to rats i.e. 100, 200, 400mg/kg and single-dose diazepam used as standard. Results demonstrate, comparison with control, that there was a dose dependent increase in time of stay in light chamber [F (4, 20) = 81.85, P<0.0001] and number of time animal visited the light chamber [F (4, 20) = 21.15, P<0.0001]. Na.Cr at 200 mg/kg(P≤0.01) and 400 mg/kg (P≤0.01) shows a pronounced effect.

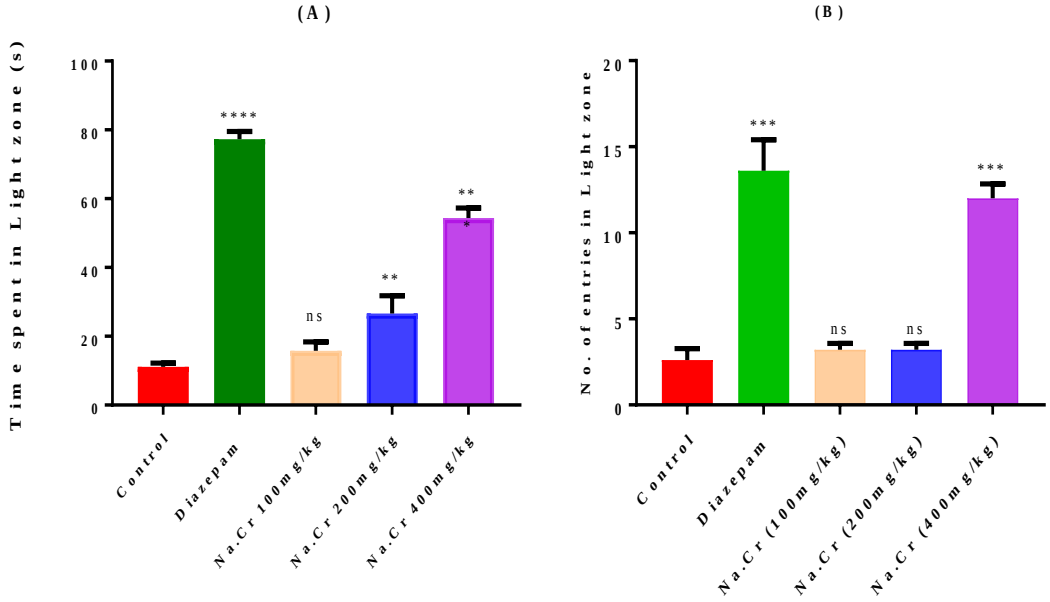

Figure 8: Result of Na.Cr on the valuation of anxiolytic activity in rats using Light and dark test.

*The rats treated with 100, 200 and 400 mg/kg of Na.Cr were observed for anxiolytic activity. The (A) Time Spent in Light zone, (B) Number of entries in Light Zone, were monitored and compared to control. Data is assessed by one-way ANOVA followed by Dunnett test and whole data is expressed as mean±SEM (n= 5). ns p> 0.05, *P≤ 0.05, **P≤ 0.01, ***P≤ 0.001*

Open-field-test

Animals were tested using open-field for the evaluation of anxiolytic activity of Na. Cr with various doses (100mg/kg, 200mg/kg and 400mg/kg) and diazepam was taken as a positive control at a single dose. Result shows dose dependent induction of anxiolytic activity in animals as well as notable variation between groups for time-spent in centre-zone [F (4, 20) = 15.2, P<0.0001] and number of entries in centre zone [F (4, 20) = 7.379, P<0.0001]. Result demonstrates increase time in centre zone at 200mg/kg (P≤0.05) and 400mg/kg (P≤0.001), on comparison with the control group

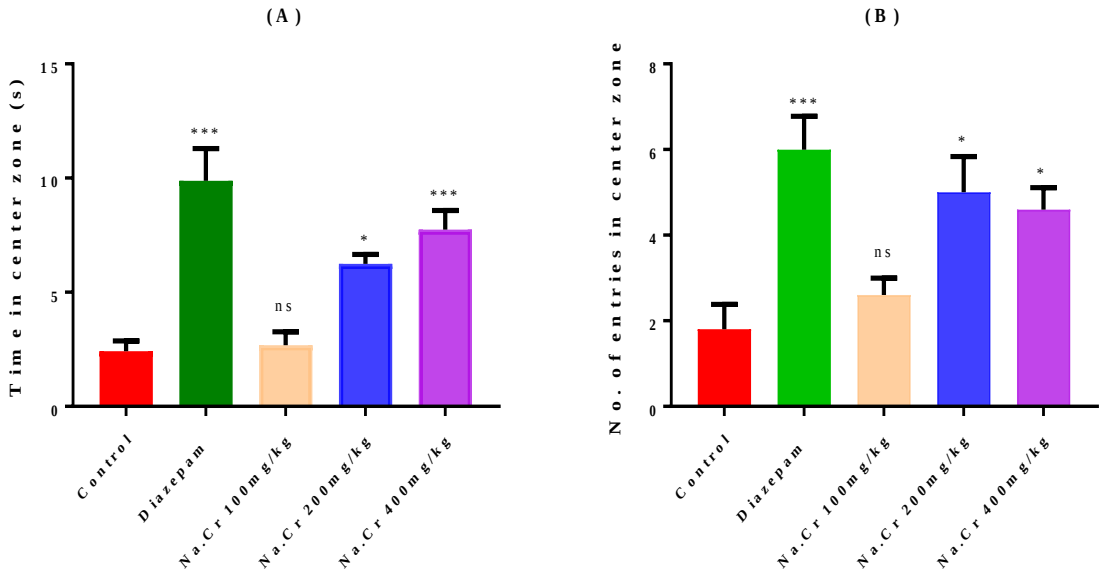

Figure 9: Outcome of Na.Cr on the assessment of anxiolytic activity in rats using Elevated Plus Maze.

*The rats treated with 100, 200 and 400 mg/kg of Na.Cr were observed for anxiolytic activity. The (A) Time Spent in center zone, (B) Number of entries in center zone, were monitored and compared to control. Data is evaluated by one-way-ANOVA followed by Dunnett-test and whole data is expressed as mean±SEM (n= 5). ns p> 0.05, *P≤0.05, ***P≤ 0.001*

DISCUSSION

Around 50 million people are being affected globally by epilepsy and out of them 80% of which accounts for the population living in developing countries like Pakistan. This is due to high cost and unavailability of appropriate treatment (Kaur et al., 2021). Despite of availability of some antiepileptic drugs for the treatment, there were many cases of drug interaction and adverse-effect was observed. So, the use of traditional medication based on plant source is increasing day by day (Baradaran Rahimi et al., 2019). Seizures occur due to a imbalance between inhibitory and excitatory neurotransmitters in brain such as GABA and Glutamate, respectively (Vollenweider et al., 2006).

In the current study, methanol-extract of arial part of plant of *Nyctanthes arbor-tristis* was initially screened for phytochemical constituent with one of the major components comes out to be terpenoids. Terpenoids are previously demonstrated to have neurological properties including anticonvulsant activity due to presence of antioxidant property (Chauhan et al., 1988). Medicinal Plants having antioxidant activity are useful in such diseases in which there is involvement of free redicals such as neurological illnesses and some other diseases. Various studies reveals that the presence of antioxidant and free-radical scavenging activities are due to presence of flavonoids and phenols (Asuntha, Prasannaraju, & Prasad, 2010).

The present study uses strychnine for seizure induction in mice. Strychnine is considered as neurotoxin obtained from seed of Strychnos nux vomica. In inhibit the binding of glycine to the glycine-gated-chloride channel (Ghaderkhani et al., 2014). If the glycine receptor binds to glycine, it causes chloride ion influx which leads to hyperpolarization of cell imparting its inhibitory activity. Strychnine will result in conclusion, tetany and ultimately death of animal caused by respiratory paralysis. Na.Cr at various doses do not impart effect on STY-induced seizure. Onset was not delayed significant and no protection was observed.

PTX and PTZ is considered to be a well-recognized $GABA_A$ receptor antagonist that results in convulsions. It gives a rapid screening of anticonvulsant effect of new investigational drug. PTZ was taken up by the brain after intraperitoneal injection to initiate a sequence of epileptic events including clonic and tonic seizures. The current study demonstrate a dose-dependent anticonvulsant property of Na.Cr was observed with a significant delay in onset of seizure or

mortality rate. This may be due to the increase in GABAergic effect (González-Trujano et al., 2021; Randrianarivo et al., 2016).

For inducing temporal lobe epilepsy, L/Pi induced seizures are one of the most frequently used model. Lithium was co-administered with pilocarpine. Administration of combination of lithium and pilocarpine result in accumulation of Ach, inositol monophosphate with a reduction in inositol cortically (Asuntha et al., 2015). This produce 10 times greater effect than pilocarpine alone. In current study there was dose dependent reduction in severity of the disease. L/Pi induced seizures re associated with neuropathological and electrophysiological characteristic of temporal lobe epilepsy (Asuntha et al., 2010). Therefore, active constituent in Na.Cr is useful in dealing this this seizure type .

To demonstrate the effect of Na.Cr on learning and memory, Morris-water maze was performed using scopolamine-induced-memory deficit. Phenols possess anticholinesterase activity that may irradicate deficiency of Ach (Sulaiman & Balachandran, 2012). Phenols and flavonoids, being the component of Na.Cr, are reported to treat memory deficit. They modulate cerebral blood flow that influence synaptic plasticity and cognition (Sathya et al., 2022). They also exert neuroprotective activity by activating caspase-3. Caspase-3 reverse the apoptotic effect induced by various factor (Kelotra et al., 2015).

The phytochemical screening also reveals the presence of flavonoid and phenols in Na. Cr. It recognized the link between these constituents and the antioxidant activity (Foyet et al., 2012). For neuromodulation, rats were administered chronically with various doses of Na. Cr for testing anxiety-related behavior. Animals were tested on EPM, L/D and OFT, which revels dose dependent anxiolytic effect of Na. Cr. Animals treated with Na. Cr at dose of 200 and 400 mg/kg shows more time-spent in open-arm in EPM, light chamber and center zone comparison to control. Although, diazepam as a standard anxiolytic drug impact better result comparing to control. Anxiolytic drugs show their effect by modulating GABAergic effect. Flavonoids also impart their effect by modulate GABA level in brain (Hanrahan et al., 2011). So the major phytochemical constituent possessed by *N. arbor-tristis* might be responsible for neuropharmacological activity which were observed under the current study.

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