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Antidepressant, anxiolytic sedative and muscle relaxant activities of *Nicotiana plumaginifolia* in mice

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ABSTRACT: *Nicotiana plumbaginifolia* is conventionally used as tranquilizer, diuretic, mucolytic, insecticide and a vomitive. In order to pharmacologically validate the sedative, anxiolytic, antidepressant and muscle relaxant activity of crude extract *N. plumbaginifolia* (NPCE). For the assessment of antidepressant effects, Forced Swim Test (FST), Tail Suspension Test (TST) was carried out. NPCE significantly (300-500mg/kg) decreases immobility time in both FST and TST. Pretreatment with NPCE at 300 mg/kg to 500 mg/kg significantly increased entries of animals to the open spaces and significantly reduced the time spent in the closed spaces in elevated plus maze (EPM). For assessment of sedative effect, NPCE was administered to experimental mice in open field test (OFT). The overall effective was highly significant and dose dependent and reduced the number of ambulation and rearing. Similarly, muscle relaxant effect was validated in traction test, where NPCE at doses of 300 mg/kg to 500 mg/kg did not show any effect on muscle co-ordination in comparison to standard. Altogether, outcomes of the current data revealed potent antidepressant, sedative and anxiolytic like activities of *N. plumbaginifolia* in in-vivo experimental animal models. Further studies are needed to identify and isolate pharmacological active compounds behind these effects and the possible mechanisms involved.

Keywords: *Nicotiana plumbaginifolia*; antidepressant; sedative; anxiolytic; muscle relaxant

1. Introduction

Currently millions of the world population suffer from psychological illnesses, placing neurological disorders among the main source of global disease burden throughout the world (World Health Organization, 2017). Some of common ailments include Parkinsonism, epileptic seizures, anxiety, depression, brain attack, inability to sleep, meningitis, sinus headache and disseminated sclerosis. Due to the said reason psycho-neurological ailments are leading causes of life ailments (Organization, 2006). The agents acting on central nervous system (CNS) were among the first evaluated drugs by prehistoric people and still are the most widely used class of medicines. Many medicinal plants have been unearthed and found active against CNS disorders and hence are of great use in treating of human disorders (Yadav et al., 2010).

The genus Nicotiana belongs to Solanaceae family which includes petunia, tomato, tobacco and potato. The plants of Solanaceae family are mostly known for their anxiolytic potential (Edewor-Kuponiyi, 2013; Giorgetti and Negri, 2011). In more than 60 species of nicotiana, there are 3-pyridyl derivatives with nicotine as a major alkaloid. Alkaloids in nicotiana exists majorly in the leaves of all species, Nicotine, Nornicotine, Anatabine, Anatalline and Anabasine are the major alakloids. Alkaloids that exists in minor quantities include Nicotine-1′-N-oxide, Nicotyrine, Myosmine, N′- methylanabasine, N - formylanabasine, N′- methylanatabine, N-formylanatabine, N′ - isopropylnornicotine, N′-carbomethoxynornicotineetc (Bush and Crowe, 1989). The leaves of some species of Nicotiana also contain glucosides, iso-quercitrin, tachacinin, tahacillin, 1-quinic acid, caffeic acid and oxalic acid (Rawat and Mali, 2013). The present study was designed to evaluate the phytochemical analysis of extract of whole plant *N. plumbaginifolia* followed by various neurological studies including sedative, hypnotic, muscle relaxant, anxiolytic and antidepressant like effects in *in-vivo* models.

Table 1: Compounds isolated from *N. plumbaginifolia* and their pharmacological effects.

Nature	Constituents	Pharmacology activities	References
Flavonoids	3,3′,5,6,7,8-hexamethoxy-4′,5′-methylenedioxyflavone	Antinociceptive, anxiolytic-like activity	(Nadipelly et al., 2016)
	3,3′,4′,5′,5,6,7,8-octamethoxyflavone (exoticin)		(Nadipelly et al., 2016)
	6,7,4′,5′-dimethylenedioxy-3,5,3′-trimethoxyflavone	Antinociceptive, anxiolytic-like activity	(Nadipelly et al., 2016)
	3,3′,4′,5,5′,8-hexamethoxy-6,7-methylenedioxyflavone	Antinoiceptive, anxiolytic-like activity	(Nadipelly et al., 2016)
	5 - hydroxy-3,3',6,7,8-pentamethoxy - 4',5'-methylenedioxy flavone	No activity reported yet	(Nadipelly et al., 2016)
Alkaloids	Nornicotine	No activity reported yet	(Sims and Bacic, 1995)
Carbohydrates	Arabinoxyloglucan	No activity reported yet	(Sims and Bacic, 1995)
	Galactoglucomannan	No activity reported yet	(Sims and Bacic, 1995)
	Arabinoglucuronomannan	No activity reported yet	(Sims and Bacic, 1995)
	Galacturonan	No activity reported yet	(Sims and Bacic, 1995)
Acids	Xylan	No activity reported yet	(Sims and Bacic, 1995)

2. Materials and Methods

2.1 Drugs and Chemicals

Drugs and chemicals used in the study includes Diazepam (Valium Injection 10mg/2ml manufactured by Martin Dow), Bupropion, methanol, n-Hexane and normal saline (25,100,500 ml).

2.2 Equipment's / Items

Open Field Test (OFT) apparatus**,** Traction test apparatus, Elevated plus maze (EPM) apparatus, Tail Suspension test (TST) apparatus, Force Swim Test (FST) apparatus, vertex mixer, analytical balance, animal weighing balance, disposable syringes, different size beakers, China

dish, Petri dish, digital video camera, different color markers, disposable latex gloves.

2.3 Experimental Animals

Healthy albino mice of either gender, weighing 20-40 g were purchased from Animal House, Veterinary hospital, Peshawar. A stainless-steel cage having saw dust as bedding at 24±1 Celsius in a 12-hours light/dark cycle was used for keeping mice. Adequate amount of food and water were available as libitum. Mice were divided into groups of 6 mice each for different tests. Animals were naive on experimental conditions. All experiments were conducted in a quiet room between 9:00 am and 4:00 pm.

2.4. Collection, Extraction and Fractionation

Whole plant was collected during the month of May and June 2018. Distilled water was used to thoroughly wash the plant, and then dried under room temperature. The dried plant was then grinded to powder form. The crude extract was obtained using a rotary evaporator under vacuum at around 45°C.

2.5. Phytochemical Identification Tests

In order to screen for various constituents of the crude extract, the following tests were performed. For the assessment of carbohydrates, the Fehling's, Benedict's, and Molisch's tests were performed. Proteins and/or amino acids were assessed using the Ninhydrin test. Mayer's, Hager's, and Wagner's tests were used to ascertain the presence of alkaloids in crude extract. Sterols and triterpenoids were identified using the Salkowski's tests. Gelatin and ferric chloride tests were used for the identification of tannins. Whereas, ferric chloride test, alkaline reagent test, and frothing test were used to identify the presence of phenolic compounds, flavonoids, and saponins, respectively.

2.5.1 Test for Carbohydrates

a. Molisch's test

For this test, 0.2ml concentrated sulphuric acid and few drops of α-napthol (alcoholic solution) were added to the crude extract. The appearance of violet to purple indicates the presence of carbohydrates (Evans, 2009).

b. Fehling's test

A few drops of crude extract was boiled with equal quantity of aqueous solution of copper sulphate (Fehling solution A) and aqueous solution of potassium tartrate and sodium hydroxide (Fehling solution B). The appearance of brick red precipitate (cuprous oxide) confirms the presence of carbohydrates (Evans, 2009).

c. Benedict's test

In this test, a few drops of Benedict's reagent was added to the crude extract and boiled. The appearance of reddish brown precipitates confirms the presence of carbohydrates (Evans, 2009).

2.5.2 Test for Proteins and Amino Acids

a. Ninhydrin Test

The appearance of violet color following the mixing and boiling of crude sample with few drops of ninhydrin solution (0.2%) indicated the presence of proteins and/or amino acids (Kumar et al., 2009).

2.5.3 Test for Alkaloids

a. Mayer's test

The addition of a few drops of Mayer's reagent to the crude extract, followed by the appearance of creamy white precipitate was indicative of the presence alkaloids (Khandelwal, 2008).

b. Wagner's test

The appearance of reddish brown precipitate following the addition of few drops of Wagner's reagent to a sample of the crude extract confirms the presence of alkaloids (Khandelwal, 2008).

c. **Hager's test**

Alkaloids were detected following the appearance of yellow precipitate upon addition of few drops of Hager's reagent to the crude sample (Khandelwal, 2008).

2.5.4 Tests for Sterols and Triterpenoids

a. Salkowski's test

The appearance of red and yellow color indicates the presence of sterol and triterpenoids, respectively, following treating various solutions of crude extract in chloroform and few drops of conc. H_2SO_4. Whereas, no color in the upper layer shows the absence of triterpenoids and phytosterols (Harborne, 1998).

b. Ferric chloride test

The presence of deep bluish green color in indicative of the phenols in the sample extract following the addition of $FeCl_3$ (2ml) to the extract solution (Dahiru et al., 2006).

2.5.5 Flavonoids detection

a. Alkali reagent test:

In this test, the formation of yellow to red precipitation in the extract solution following the addition of NaOH solution (Kokate, 1994).

2.5.6 Test for Tannins

a. Gelatin test

In this test, the formation of white precipitate confirms the presence of tannins when the extract solution is treated with gelatin solution (1%) containing sodium chloride (Kokate, 1994).

b. Ferric chloride test

The presence of tannins is confirmed by the formation of blue green color following the mixing of $FeCl_3$ with extract sample solution (Kokate, 1994).

2.5.7 Saponin detection test

a. Frothing test.

The appearance of froth for some time in the test tube after shaking 5 ml extract solution shows the presence of saponins (Chaouche et al., 2011).

2.6 Behavioral Tests

2.6.1 Open Field Test

The open field test aims to assess the exploratory and locomotor behavior of the mice. In this test, a 40cm x 60cm x 50cm rectangular box, divided in to 12 equal rectangles was used as shown in Figure. In this test, locomotor activity was considered if the animal cross (four legs) the line inside the rectangular box. Whereas, standing on hind limbs (one rearing) for five minutes indicates the exploratory behavior of the animals (Felipe et al., 2007). In order to eliminate the olfactory sign of an animal, an ethanol 10% was used to clean the open field (Mbiantcha et al., 2019; Porsolt et al., 1977).

2.6.2 Forced Swimming Test

This test aims to assess the antidepressant activity of the test drug. In this test, a 10cm wide and 25cm high container filled with water having a temperature of 25 ± 1 ^{o}C up to a height of 19cm was used as shown in Figure. Each mouse was individually placed in the container for five minutes and the duration of immobility – when the mouse stops struggling and only moves to keep the head out of water – was recorded (Felipe et al., 2007).

2.6.3 Tail Suspension Test

This test is also used to assess the antidepressant activity of drugs. In this test, a mouse is suspended by its tail (1cm from the end of the tail) at height of 50cm above the ground with the help of a fixed tape for five minutes and the duration of immobility was recorded for each mouse as illustrated in Figure (Mbiantcha et al., 2019; Steru et al., 1985).

2.6.4 Traction test

This test comprises a metallic rod coated with rubber, which is rigidly supported at both ends at a height of 60cm. Various groups of mice (n=6) were treated with isotonic saline (0.9%), and diazepam (1 mg/kg). Following 30 minutes of treatment, the group of mice were exposed to the test. Typically, the mouse catches the line with the forepaws and places at least one rear foot within 5 seconds when the freedom of suspension is allowed. Failure to hold up at least one of the rear legs is considered a failure of the traction test (Muhammad et al., 2013).

2.6.5 Elevated plus Maze (EPM) Model

In animal models, the anxiety-like effects are assessed using the EPM model. This model has two open arms, 30cm long and 5cm wide, two close arms (30cm long, 5cm wide) which are attached in such a way that they look like a plus (+), and a central square 5cm long, 5cm wide as shown in Figure. The EPM model stays at a height of around 50cm. The base and walls of EPM model were made from wood and painted black. During the experiment, the mice were placed individually in the center in such a way that its face is in the direction of the open arms. The number of entries to the open and closed arms and the time spent on the arms was recorded for 5 minutes (Nogueira and Vassilief, 1996).

2.7 Statistical Analysis

All the results are expressed as Mean ± S.E.M. and subjected to one way Analysis of Variance (ANOVA), n=6., * $P<0.05$, ** $P<0.01$*** $P<0.001$ using GraphPad Prism version 6.0.

3. Results

3.1 Effect of Phytochemical Analysis

Crude extract of *N. plumbaginifolia* contained all the basic compounds, such as carbohydrates, phenols, tannins, sterols and triterpenoids, flavonoids, alkaloids, sterols and triterpenoids as shown in the **table 2**.

Table 2: Results of preliminary test crude extract of *Nicotiana plumbaginifolia* for identification of different chemical groups.

S. No	Constituents	Test Name	Result
1	Alkaloids	Mayer 's test	+ve
		Wagner 's test	+ve
		Hager 's test	+ve
2	Proteins and amino acids	Ninhydrin Test	+ve
3	Carbohydrate	Molisch 's test	+ve
		Benedict test	+ve
		Fehling 's test	+ve
4	Flavinoid	Alkaline reagent test	+ve
5	Saponin	Frothing-test	+ve
6	Sterols and triterpenoids	Salkowski's test	+ve
7	Tannins	Gelatin test	+ve
		Ferric chloride test	+ve
8	Phenolic compounds	Ferric chloride test	+ve

3.2 Effect of NPCE in Antidepressant Assays

3.2.1 Force Swim Test

NPCE dose dependently (300-500mg/kg) decreases immobility time, while increased swimming time (**Figure 1**). The immobility time was 197.0±4.546 sec, in control group. In the groups treated with NPCE, the overall effect was dose dependent. It caused significant action at doses of 300mg per kg with immobility time 0f 127.8±14.43 sec, and 83.67±26.03, at the dose of 400mg per kg. Similarly, at the dose of 500mg/kg, crude extract reduced immobility time to

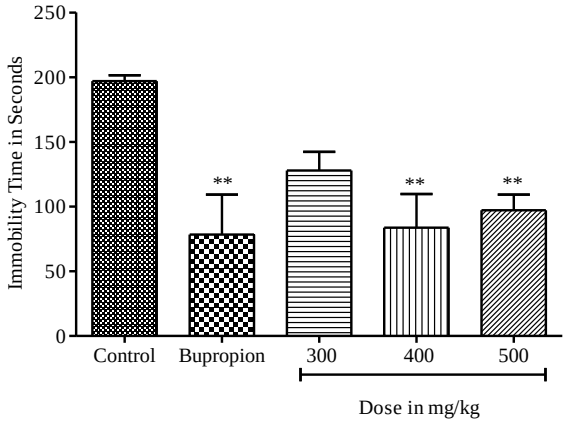

Figure 1. Antidepressant effects of *Nicotiana plumbaginifolia* crude extract and bupropion in forced swim test. Data are expressed as mean ± SEM, n=6. * p<0.05, **p<0.01, *** p<0.001 Vs saline group, one-way ANOVA with Dunnett's test.

97.17±12. saline. The standard Bupropion (10mg/kg) produced more pronounced effect with immobility time was 78.33±30.97 (P<0.01 vs. saline).

3.2.2. Tail Suspension Test

NPCE dose-dependently (300-500 mg per kg) reduce immobility duration in TST (**figure 2**). The immobility time in control group was 175.0 ± 14.74 sec, while the immobility time was 120.7 ± 13.69 sec in NPCE group treated with 300 mg per kg with a p-value of <0.05. Whereas, in the NPCE group treated with 400 mg per kg, the immobility time was recorded as 103.5±24.74 sec with a p-value of <0.05. Furthermore, NPCE group treated with 500 mg per kg shows an immobility time of 73.83 ± 20.34 sec with a significant p-value of <0.01. In Bupropion group treated with 10mg per Kg, the immobility duration was decreased to 90.17 ± 11.77 seconds with a p-value < 0.01.

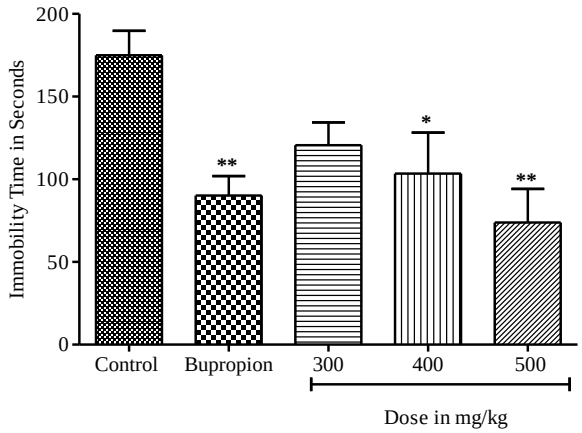

Figure 2. Antidepressant effects of *Nicotiana plumbaginifolia* crude extract and bupropion in tail suspension test. Data are expressed as mean ± SEM, n=6. * p<0.05, **p<0.01, *** p<0.001 Vs saline group, one-way ANOVA with Dunnett's test.

3.3 Effect of NPCE in Open Field Test

In control group, the line crossings were 123.7 ± 19.29, whereas in NPCE group treated with 300mg per kg, line crossings were 30.83±9.680 with a p-value of <0.01 which showed the sedative effect like the standard drug (diazepam 1 mg per kg) treated group, in which numbers of lines crossed were 29.50 ± 4.28 sec with a p-value < 0.01 (**table 3**). While, in NPCE group treated with 400 mg per kg, numbers of lines crossed were 86.0±25.67 with a p-value >0.05. Similarly, in NPCE group treated with 500 mg per kg, numbers of lines crossed were 95±25.74 with a p-value >0.05, revealing anxiolytic activity.

Table 3: Sedative effects of *Nicotiana Plumbaginifolia* crude extract in Open Field Test

Treated Group	Dose	Numbers of line crossed
Normal Saline	10 mL / kg	123.7±19.29
Diazepam	1mg/kg	29.50±4.28 **
NPCE	300mg/kg	30.83±9.68 **
	400mg/kg	86.0±25.67
	500mg/kg	95.17±25.74

Data are expressed as mean ± SEM, n=6. * p<0.05, **p<0.01, *** p<0.001 Vs saline group, one-way ANOVA with Dunnett's test.

3.4. Effect of NPCE in Elevated Plus Maze Model (EPM)

The effect of sample extract of *N. plumbaginifolia* in EPM is illustrated in **table 4**. NPCE treated animals at a dose of 300-500 mg per kg, spent increased time in the open arms and less in the closed arms compared with control group. Whereas, duration spent by animals treated with 1 mg per kg diazepam in open arms were higher than control and NPCE treated groups.

Table 4: Anxiolytic effects of *Nicotiana Plumbaginifolia* crude extract in Elevated-Plus-Maze

Treated Group	Dose	Time taken in Open Arm	Time taken in Closed Arm
Normal Saline	10 mL /kg	22.50 ± 4.680	279.2 ± 2.92
Diazepam	1mg/kg	62.33 ± 11.22 **	237.2 ± 12.61
NPCE	300mg/kg	40.33 ± 9.68 *	259.7 ± 9.68
	400mg/kg	46.75 ± 8.65 *	253.3 ± 8.65
	500mg/kg	55.80 ± 19.25 **	244.2 ± 19.25

Data are expressed as mean ± SEM, n=6. * p<0.05, **p<0.01, *** p<0.001 Vs saline group, one-way ANOVA with Dunnett's test.

3.5 Traction Test

The reestablishment time was not significantly changed among animals, treated with NPCE at a dose of 300-500 mg per kg, instead all animals showed 0% failure and performed reestablishment immediately (**Table 5**).

Table 5: Muscle relaxant effects of *Nicotiana Plumbaginifolia* crude extract in Traction Model.

Table 5: Muscle relaxant effects of *Nicotiana Plumbaginifolia* crude extract in Traction Model

Traction Test			
S.NO	Treated Group	Dose	Percent Failure
1	Control Group	1ml/kg	0.00%
2	Diazepam	1mg/kg	50%
3	NPCE	300mg/kg	0.00%
		400mg/kg	0.00%
		500mg/kg	0.00%

4. Discussion

This study was conducted to evaluate antidepressant, anxiolytic, sedative and muscle relaxant effects of *N. plumaginifolia*, in mice, using different pharmacological techniques. It is believed that stressful situation has an important role in depression (Ahmed et al., 2009). Experimental models like FST and TST models causes physical stress that leads to depression (Steru et al., 1987). These models of depression provide an efficient, authentic behavior and a screening test for compounds/agents having antidepressant like properties. A reduction of monoamine type of neurotransmitter in the brain i.e. norepinephrine, 5 HT and Dopamine leads to depressive disorder (Delgado, 2000).

It has been hypothesized that immobility reflects a mood of behavioral discomfort and an inability to adapt to stress (Płaźnik et al., 1988). Compounds that decreases the duration of immobility in FST and TST, while increases climbing or swimming activity in FST, have antidepressant effects (Borsini and Meli, 1988; Steru et al., 1987). Similarly, it has been suggested by Detke and his colleagues (1995) that swimming behavior depends on 5 Hydroxytryptamine, whereas climbing behaviour exhibit the involvement of noradrenergic neurotransmissions (Detke et al., 1995). The decrease in immobility with pretreatment of NPCE dose dependently increased the swimming behavior in FST, which is indicative of antidepressant effect. In FST, like fluoxetine, NPCE decreased immobility time while increased swimming time without significantly affecting climbing behavior. This may suggest that serotonergic neurotransmissions may be involved in antidepressant effect of NPCE. Similarly, in TST, it dose dependently reduced the immobility time indicating a strong antidepressant like effect. Among all the available models for assessment of anxiety, the most famous and acceptable available model is EPM (Dhonnchadha et al., 2003). It was based on the study of spontaneous and unlearned behavior. The most widely used tool to assess antianxiety like effects of test animals is elevated plus maze. The normal healthy animals in EPM choose to spend their time in closed arm. While the movement of animals towards open arms produced fear and anxiety in animals due to open spaces (Silva and Brandao, 2000). Moreover, it is accepted as a very adaptive and purpose built method for substances that exhibit their anxiolytic effect through benzodiazepine receptors and GABA type A receptors linked anxiolytic drugs (Silva and Brandao, 2000). In several studies it has been reported that GABAergic neurotransmission is involved in etiology, expression and the therapy of anxiety disorders (Avijit et al., 2010). Drugs that enhance open arms entries, such as those enhanced by diazepam, are considered to have anxiolytic effects. NPCE like diazepam shown a significant increase in the duration spent and number of entries to open arms while reduced the duration spent and number of entries in closed arm (Griebel et al., 1998).

In 1934, a test was initially developed quantify emotionality in rodents which was later on named as Open field test (OFT). OFT has become the most widely used behavioral test for checking animal psychology. It has attained the status of being one of the most widely used measures of behavior in animal psychology. This test is an easy and quick assessment model for animal behavior. Further, this test requires either little or no specialized training for the human administering the test and also of the test subject. By the help of this test qualitative and quantitative general activity and exploratory behavior of the rodents can be assessed. The most common assessed parameter in open field test is ''movement''(Seibenhener and Wooten, 2015). Numerous variables can be measured in the open field test involving motor activity (movement) (Walsh and Cummins, 1976). The motor activity can be influenced by number of factors like freezing, motor output, explorative activity, defecation and other fear related behavior. Ambulation is the most common behavior studied but others such as latency or rearing can also be measured. The open field apparatus is basically a square, cylindrical or rectangular box in order to prevent the escape of animals from the boundaries of the apparatus. Diazepam being

sedative and anxiolytic reduces both exploratory and locomotor activity of the test animals respectively (Ramos, 2008).

At lower doses benzodiazepines have anxiolytic and sedative properties, while they act as muscle relaxants and anti-epileptic at higher doses (de Melo et al., 2006). For the assessment of sedative like properties of test articles, Open Field Test was. NPCE at 300 mg per kg elicited almost the same sedative effect as that of Diazepam. However, the effect NPCE was more prominent at higher doses used.

GABA-A receptors are central inhibitory receptors in the brain. GABA-A receptors are further classified as α1, α2, α3 and α5. Among them α1 is involved in the sedative activity while the other subclasses α2, α3 or α5 are involved in anxiolytics and muscle relaxant activities, but it is not necessary for compounds that it must block all subtypes of GABA-A receptors and produced both anxiolytics as well as sedative effect (Muhammad et al., 2013; Rudolf et al., 1999). Some compounds produced anxiolytics and sedative like effect but may not produce muscle relaxant like activity (Karim et al., 2012). Gilani and his colleague found the presence of combination of synergistic and neutralizing compounds in medicinal plants (Gilani, 2005).

The muscle relaxant properties of the crude extract were assessed using traction model test and the effect of NPCE on muscle coordination was compared with diazepam. Diazepam (standard) at a dose of 1mg per kg has increased the reestablishment time of the mice most significantly, when mouse unable to place their hind paws on the rubber-coated wire within 5 seconds, reflecting strong muscle relaxation nature. While NPCE did not increase the reestablishment time of the mice this might be probably because of the presence of neutralizing compounds in *N. Plumaginifolia* crude extract.

Phytochemically, NPCE has showed the presence of all the basic compounds, such as, flavonoids, alkaloids, sterols, triterpenoids, carbohydrates, phenols, and tannins. The different flavonoids isolated from the plant including 3,3′,5,6,7,8-hexamethoxy-4′,5′-methylenedioxyflavone, 6,7,4′,5′-dimethylenedioxy-3,5,3′-trimethoxyflavone and 3,3′,4′,5,5′,8-hexamethoxy-6,7-methylenedioxyflavone has already shown antinociceptive, anxiolytic-like activity (Nadipelly et al., 2016). Similarly, 3,3′,5,6,7,8-hexamethoxy-4′,5′-methylenedioxyflavone, 3,3′,4′,5′,5,6,7,8-octamethoxyflavone (exoticin), 6,7,4′,5′-dimethylenedioxy-3,5,3′-trimethoxyflavone, and 3,3′,4′,5,5′,8-hexamethoxy-6,7-methylenedioxyflavone has been reported from the plant anxiolytic effects (Shajib et al., 2018). Thus, it is assumed that these compounds might be responsible for the current show in part.

CONCLUSIONS

It is evident from the results that *N. plumbaginifolia* possesses potential sedative, anxiolytic, and antidepressant actions, which provided a scientific background for the use of the plant in treating neuropsychological ailments. Moreover, molecular level research is needed further to identify and isolate pharmacological active compounds behind these effects and the possible mechanisms involved.

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