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Antidepressant activity of *Nyctanthes arbor-tristis* in mice

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Abstract

The present study assesses the protective effect of *Nyctanthes arbor-tristis* (*Nyctaginaceae*) extracts and in combination with fluoxetine on stress-induced depression in mice. Leaves were extracted using different solvents (petroleum ether, chloroform and hydroethanol) and administered orally for 14 days. These extracts showed significant improvement in the mobility percentage but among these, hydroethanol extract showed better protective effect from day 1 to 14 in both forced swimming and tail suspension test model. Hydroethanol (100 mg/kg) and chloroform (100 mg/kg) extracts with fluoxetine showed synergistic effect when compared with fluoxetine treated group (10 mg/kg) alone at day 7 and 14. Among monoamine levels only hydroethanol extract (400 mg/kg) restored the 5-HT level near to level of fluoxetine-treated group. Hydroethanol extracts with two higher doses showed significant decrease in glucose and triglycerides levels. Clinically, it may useful as antidepressant drug.

Introduction

Depression is a common stress related mood disorders that can be precipitated at any time of life. However, it becomes a severe abnormal condition occur in behavior such as abnormalities in mood, development of neuro-vegetative functions, cognition and psychomotor activity (Moinuddin et al., 2012).

The exact pathomechanism behind this depression is still remains obscure, but the evidences suggest that a decline in the levels of neurotransmitter in the brain may leads to depression. Among the monoamines, decreased level of 5-hydroxytrptamine (5-HT) is report to play an important responsibility in the progress of depression (Gupta et al., 2011).

Traditional medicines as an extract form derived from plants have been widely used for the treatment of depression and other related ailments (Dubey et al., 2004; Aslam and Sultana, 2015). *Nyctanthes arbor-tristis* Linn (*Nyctaginaceae*) is one of the plants used extensively in the Ayurvedic system of medicine (Saxena et al., 1984; Rathee and Hassarajani, 2007). It is a night flowering sad tree commonly known as 'Harsinghar', 'Prajakta' or 'Night Jasmine'. Whole plant as well as the plant parts of *N. arbor-tristis* are richest source of phytosterols, phenolics, tannins, flavonoids, glycosides and saponins (Singh et al., 1995).

Several crude extracts of different plant part of *N. arbor-tristis* have been used to treat various diseases (Rathod et al., 2010). This plant is known to possess numerous pharmacological effects on the central nervous system activity (Das et al., 2008). Water-soluble portion of the ethanol extracts of flower, bark, seed and leaf have CNS depressant activity (Das et al., 2008).

Till now, no study has been reported to show behavioral parameters using antidepressant mice model. The present investigation was undertaken to assess the antidepressant potential of different extracts of *N. arbor-tristis* in mice using force swimming and tail suspension test model.

Materials and Methods

Drugs and chemicals

Pure salt of fluoxetine (standardized as per the Indian Pharmacopeia) was collected as gift sample from the Ind-Swift Pharmaceutical Ltd, India). Glucose and triglyceride estimation kits were purchased from Ecoline, Merck Ltd., India. All other reagents and the chemicals used in the study were of analytical grade.

Animals

Swiss Albino mice (30-40 g) were housed under standard conditions of temperature (24-28°C) and relative humidity (60-70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet (Lipton India Ltd.) and water was allowed *ad libitum.*

Collection of plants

N. arbor-tristis leaves were collected in March 2013 from the Government Botanical Garden of Khijrabad in Haryana, India. The specimen plant (NISCAIR/ RHMD/consult/-2013/2322/102) was identified with the help of literature and authenticated by Dr. Sunita Garg, Raw Materials Herbarium and Museum, NISCAIR, New Delhi, India. The fresh plant material was cleaned with distilled water in order to remove debris and dried at 35-40^{0}C for 10 days. Then it was pulverized in the electric grinder and the powder was passed through sieve No. 60.

Preparation of extracts

The dried powdered leaves (3 kg) were successively extracted in a soxhlet apparatus using petroleum ether first, then chloroform and lastly hydroethanol (80:20 v/ v) for 72 hours each. The last trace solvent was removed by vacuum drying method. The percentage yield was about 2.0% w/w of petroleum ether extract, 2.1% w/w of chloroform extract and 4.6% w/w of hydroethanol extract. The extracts were stored below 4°C until further used.

Drugs and dosage

Extracts were administered orally at different doses (100, 200 and 400 mg/kg) in the form of suspension prepared using 2% w/v carboxymethyl cellulose. Fluoxetine hydrochloride (20 mg/kg p.o.) was also administered orally in suspension form.

Experimental design for forced swimming and tail suspension test model

Experiment No.: 1 (Forced swimming test)

Mice were divided into 11 different groups (6 mice each) and treated as follows: Group I: Vehicle-treated control group received 2% carboxymethyl cellulose; Group II: Received fluoxetine hydrochloride (20 mg/ kg); Group III, IV and V: Received petroleum ether extract (100, 200 and 400 mg/kg) of *N. arbor-tristis;* Group VI, VII and VIII: Received hydroethanol extract (100, 200 and 400 mg/kg) of *N. arbor-tristis;* Group IX, X and XI: Received chloroform extract (100, 200 and 400 mg/kg) of *N. arbor-tristis.*

Experiment No. 2 (Tail suspension test model)

Mice were divided into 11 different groups (6 mice each) and treated as follows: Group I: Vehicle-treated control group received 2% carboxymethyl cellulose, Group II: Received fluoxetine hydrochloride (10 mg/ kg); Group III, IV and V: Received fluoxetine hydrochloride (10 mg/kg) with petroleum ether extract (100, 200 and 400 mg/kg) of *N. arbor-tristis;* Group VI, VII and VIII: Received fluoxetine hydrochloride (10 mg/kg) with hydroethanol extract (100, 200 and 400 mg/kg) of *N. arbor-tristis;* Group IX, X and XI): Received fluoxetine hydrochloride (10 mg/kg) with chloroform extract (100, 200 and 400 mg/kg) of *N. arbor-tristis.*

The treatment period was 14 days each for both the experiments. On day 1, 7 and 14, immobility period was recorded for 90 min after drug administration. All treatments were done using an oral gavage. The doses were selected from an earlier report (Das et al., 2008).

Forced swimming test (FST)

In order to assess the antidepressant activity of plant extracts, the modified forced swim test was conducted (Porsolt et al., 1979). Immobility time, swimming and climbing time were measured by observing the motor activity of the mice placed in a pool of water. Two sessions were conducted: Pretest session followed 24 hours later by 6 min test session. In the pretest session, the mice without treatment were forced to swim in a glass aquarium (25 cm in diameter, 40 cm in height) containing fresh water up to the height of 12 cm. The temperature of the water in the aquarium was maintained at 23 ± 1°C. The level of water was filled at this stage so that the mouse was not able to support themselves by touching the bottom or the side walls of the chamber. Water in the aquarium was replaced at every time after subjecting each mouse for the test as water had been shown to alter the behavior (Abel and Bilitzke, 1990). During the test session, the immobility, swimming and climbing times were observed by a trained observer. The total duration of immobility was measured during the total 6 min testing period (David et al., 2001). This immobility was reduced by antidepressant drug administration. The behavior of the mouse was judged as follows: a) immobility- when the mouse remained floating in water without struggling, making only those movements that are necessary for it to keep its head over the water level; b) swimming- when the mouse was making active movements for swimming more than that required to maintain the head above water; c) climbing- When the mouse was making active movements with its forepaws in and out of water, directed against the walls.

Upon removal from water, mouse was towel dried and

Box 1: Tail suspension test (TST)

Principle

The tail suspension test, commonly employed as behavioral model for screening anti-depressant activity in mouse model. The method is based on the principle that a mouse suspended by tail above a fixed height of 50 cm from the ground shows alternate periods of agitation and immobility.

Requirements

Mice, material for suspension of mouse, adhesive tape

Procedure

Step 1: Mouse was allowed to acclimatize to the experimental room for 1-2 hours before the behavioral procedure

Step 2: After 90 min of the drug administration and vehicle to defined group, mouse was individually suspended vertically by the tail from a horizontal bar at a distance of 50 cm from floor using adhesive tape (distance from tip of tail = 2 cm)

Step 3: A 6 min test session was employed. Each mouse was visually isolated from the other mice during the test

Step 4: The behavioral parameter recorded were the number of seconds spent in a completely immobile posture, expressed as immobility.

The mouse was considered immobile when it was passive, completely motionless and did not show any body movements. In modified TST test model for mice, the observer recorded the immobility time (last 3 min) on kymograph paper. The test period was videotaped.

Click the **Video clip**

References

Steru et al., 1985

David et al., 2001

finally returned to the cage. The entire test session was videotaped and scoring was done by a trained rater.

Biochemical estimations

Blood samples were taken from the retro orbital plexus under anesthetized condition on day 14 after noting down the immobility period. Serum was separated by centrifugation at 3,000 rpm for 10 min. The glucose (Giordano et al., 1989) and triglyceride levels (Kaur and Kulkarni, 2000) were estimated on day 14 using double beam spectrophotometer (UV-1800) at wavelength (500-550 nm).

Estimation of neurotransmitters in the brain region at day 14

Levels of noradrenaline, dopamine and 5-hydroxy tryptamine were estimated in the entire brain region of forced swimming stressed mice in each group. Estimation of monoamines in the brain was based upon the method described previously (Singh et al., 2013). The brain tissue samples were homogenized in 0.17M perchloric acid using a glass homogenizer. Homogenates were then centrifuged at 33,000 rpm (Biofuge stratos) at 4°C. The samples were analyzed using high performance liquid chromatography (Waters, USA). The HPLC system consisted of 515 binary pumps (Waters, USA) and 2475 Fluorescent detector (Waters, USA). The chromatographic separation was achieved on reversed-phase analytical column (150 mm, 4.6 mm, 5 mm; Agilent, USA). The data were acquired and processed in Empower pros operating system (Waters, USA). Mobile phase comprised of sodium acetate (0.02M), EDTA (0.2 mM), methanol (16%), di-*n*-butylamine (0.01%) and heptane sulfonic acid (0.055%). The pH of the mobile phase was adjusted to 3.92 using phosphoric acid. The flow rate was 1 mL/min.

GC-MS analysis

GC-MS analysis of hydroethanol extract and chloroform extract were performed using a Perkin-Elmer GC Clarus 500 system and gas chromatograph interfaced to a mass spectrometer (GC-MS) Elite-I, fused silica capillary column (30 mm X 0.25 mm 1D X 1 µM df, composed of 100% dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min and an injection volume of 2 µL was employed (split ratio of 10:1). Injector temperature was maintained at 250°C. Ion-source temperature was 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with 9 min isothermal at 280°C. Mass spectra were taken at 70 eV a scan interval of 0.5 sec and fragments from 45 to 450 Da.

Total GC running time was 36 min. The relative % amount of each component was calculated by comparing its average peak area to the total areas, using turbo mass software. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Pharmaceutical Education and Research, India that has more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the library. The name, molecular weight and structure of the components of the test materials were ascertained (Devi et al., 2012) .

Statistical analysis

The data were expressed as mean ± standard error of mean (SEM). The statistical significance between mean was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A $p<0.05$ was considered statistically significant.

Results

The qualitative results of hydroethanol extract of *N. arbor-tristis* showed the presence of flavonoids, triterpenoids, phenols, tannins, fatty acids and alkaloids while the chloroform extract did not show any positive indication for the presence of phenols and alkaloids. The petroleum ether extract had only fatty acids and triterpenoids. The presence of various active constituents was confirmed by Rf value, which was found in the range of 0.7-0.9, indicated the presence of triterpenoids, flavonoids and alkaloids probably in these extracts (data not shown).

Forced swimming test

The hydroethanol extract in all tested doses showed a statistically significant ($p<0.001$) decrease in immobility time period when compared with control group from day 1 to 14. Overall percentage of improvement (13.4 to 38.8%) in the mobility period of hydroethanol extract (100, 200 and 400 mg/kg) showed a statistical significantly ($p<0.001$) increased from 193.8 ± 10.0 to 173.6 ± 8.6, 178.8 ± 18.5 to 163 ± 9.4 and 168 ± 17.0 to 141 ± 6.5. All the tested doses of other two extracts showed a statistical significantly decrease in immobility duration ($p<0.001$) after 7 days of treatment when compared with control group. However, both petroleum ether (200 and 400 mg/kg) and chloroform extracts (100 and 400 mg/kg) also showed a good effect after 14 days of treatment. Overall, the immobility time period in this experiment showed an extremely statistical significant difference ($p<0.0001$; f=66.9 to 132.5) (Figure 1). In case of climbing parameters (%mobility of mice), only petroleum ether extract (400 mg/kg) revealed statistically significant difference ($p<0.0001$; %mobility: 14.4 to 30.6) at all different time intervals when compared control group. Similar type of effect was obtained at day 14 with hydroethanol extract (400 mg/kg; %mobility: 32.7) and chloroform extract (200 mg/kg) on day 1 and 7 (Figure 2). The mobility was increased from 22.5% to 38.2%. Only petroleum ether extract (400 mg/kg) demonstrated a statistically non-significant effect when compared with fluoxetine-treated group. In swimming time period, all three different extracts at 400 mg/kg showed a statistically significant ($p<0.001$) improved in the percentage mobility at different intervals (petroleum ether 29.0 to 54.5; hydro ethanol extract 63.4 to 132.2 and chloroform 48.8 to 85.8) (Figure 3). No statistical significant difference was observed when compared between hydroethanol extracts (200 mg/kg) at all time intervals and fluoxetine-treated group. In both parameters (swimming and climbing), the significance of non-significant results shown by this experiment was similar type of effect as shown by fluoxetine-treated group.

Tail suspension test

In %immobility time period using tail suspension test,

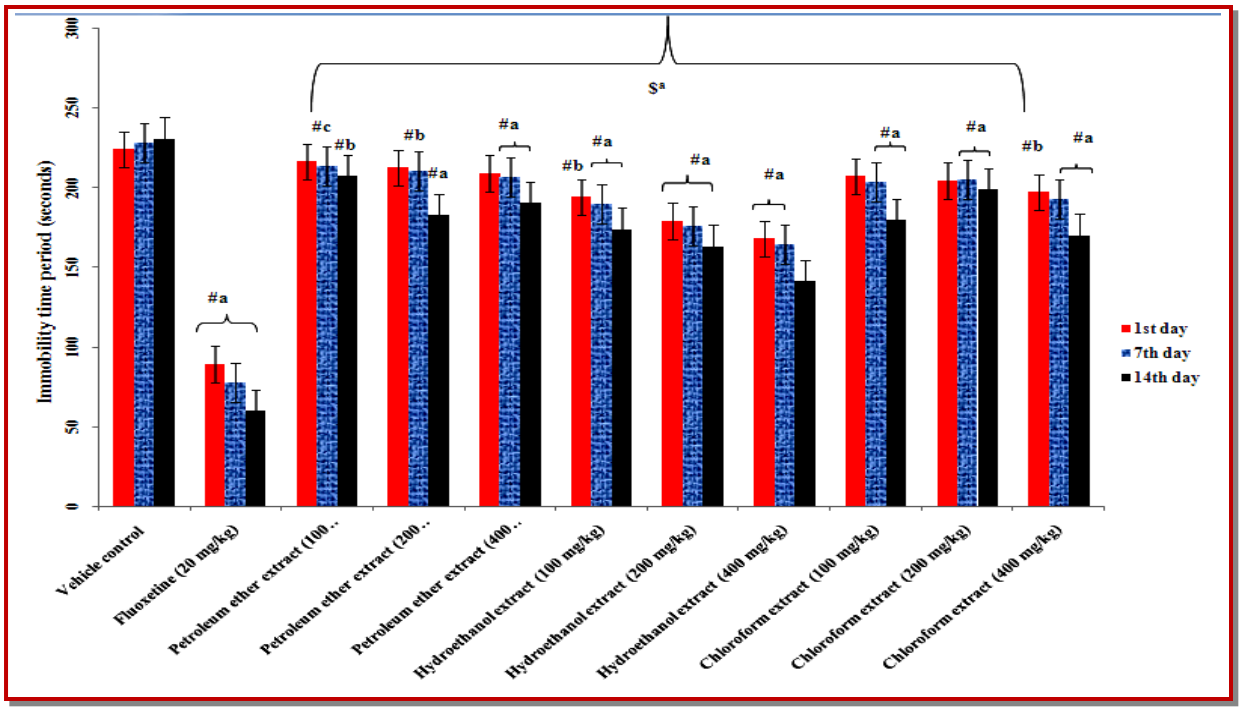

Figure: 1 Effect of different extracts of *Nyctanthes arbor-tristis* on immobility time in forced swimming test model (FST)

Statistical analysis of data was carried by one-way ANOVA followed by Tukey-Kramer multiple comparisons test. The values are Mean ± SEM for each group (n=6), P value less than 0.05 was considered as significant. # Vehicle treated control vs all groups, $ Fluoxetine vs all groups. [a]$p<0.001$, [b]$p<0.01$, [c]$p<0.05$

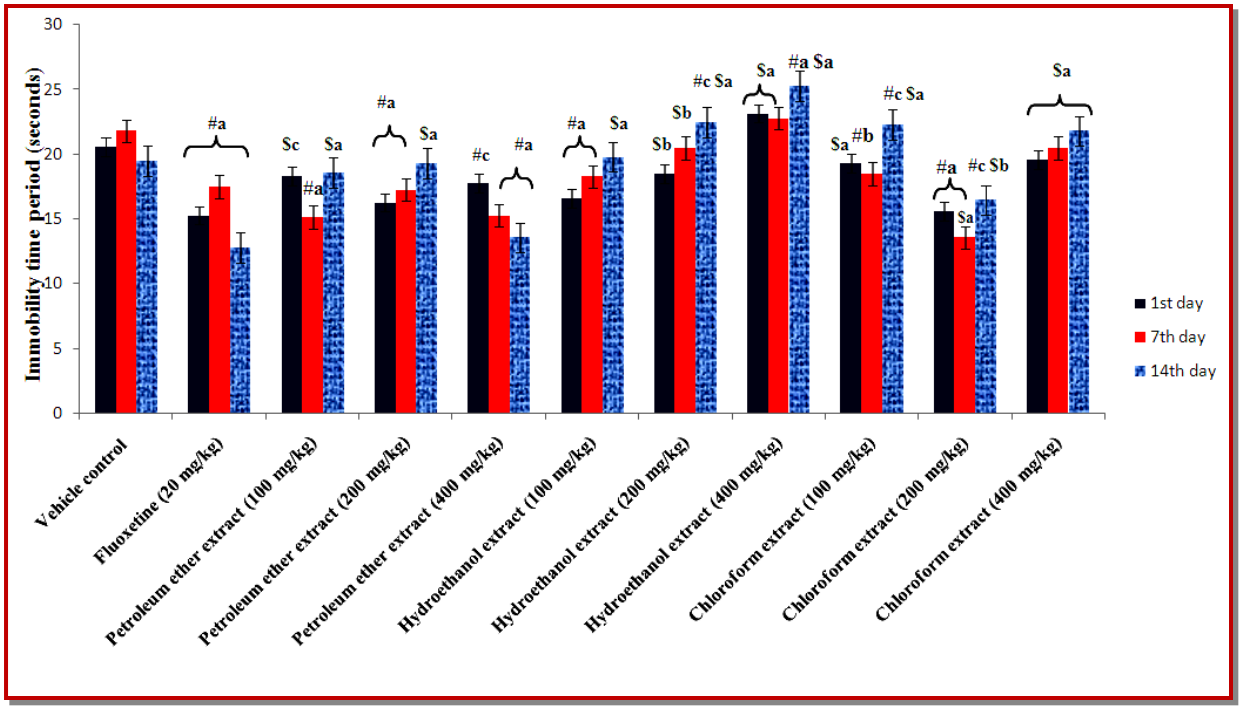

Figure: 2 Effect of different extracts of *Nyctanthes arbor-tristis* on climbing time in forced swimming test model (FST)

Statistical analysis of data was carried by one-way ANOVA followed by Tukey-Kramer multiple comparisons test. The values are Mean ± SEM for each group (n=6), P value less than 0.05 was considered as significant. # Vehicle treated control vs all groups, $ Fluoxetine vs all groups. [a]p<0.001, [b]p<0.01, [c]p<0.05

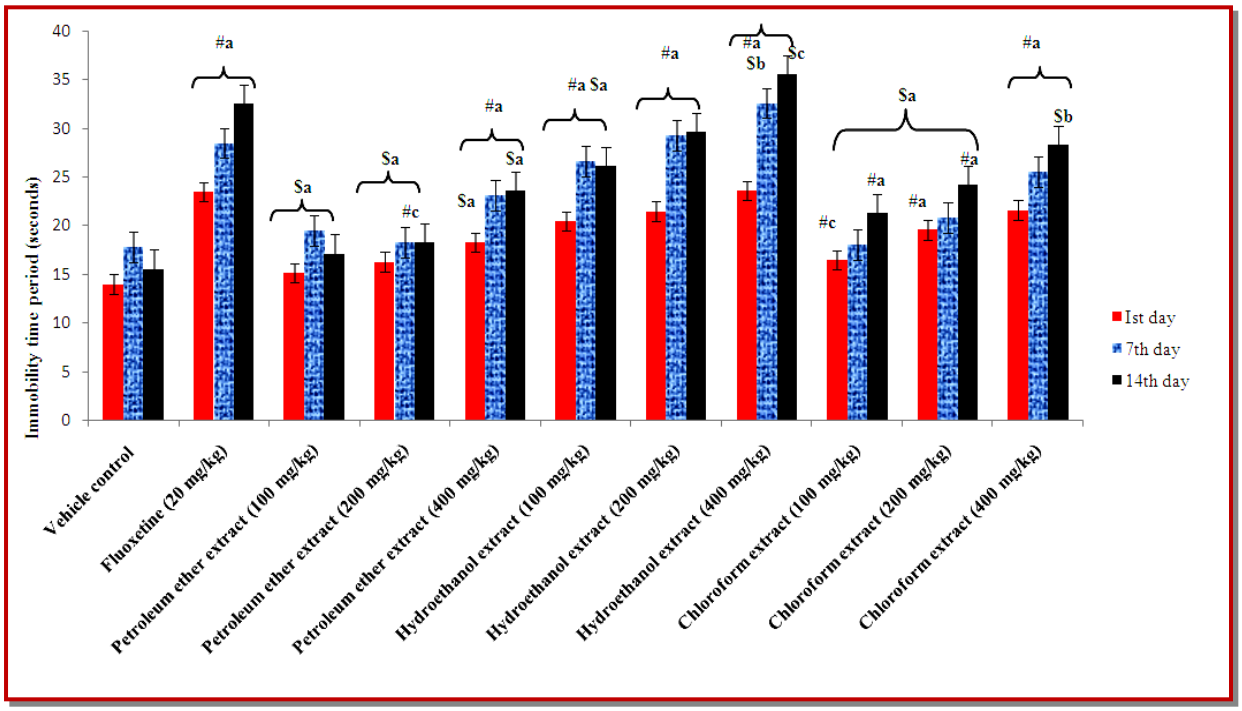

Figure 3: Effect of different extracts of *Nyctanthes arbor-tristis* on swimming time in forced swimming test model (FST)

Statistical analysis of data was carried by one-way ANOVA followed by Tukey-Kramer multiple comparisons test. The values are Mean ± SEM for each group (n=6), P value less than 0.05 was considered as significant. # Vehicle treated control vs all groups, $ Fluoxetine vs all groups. [a]p<0.001, [b]p<0.01, [c]p<0.05

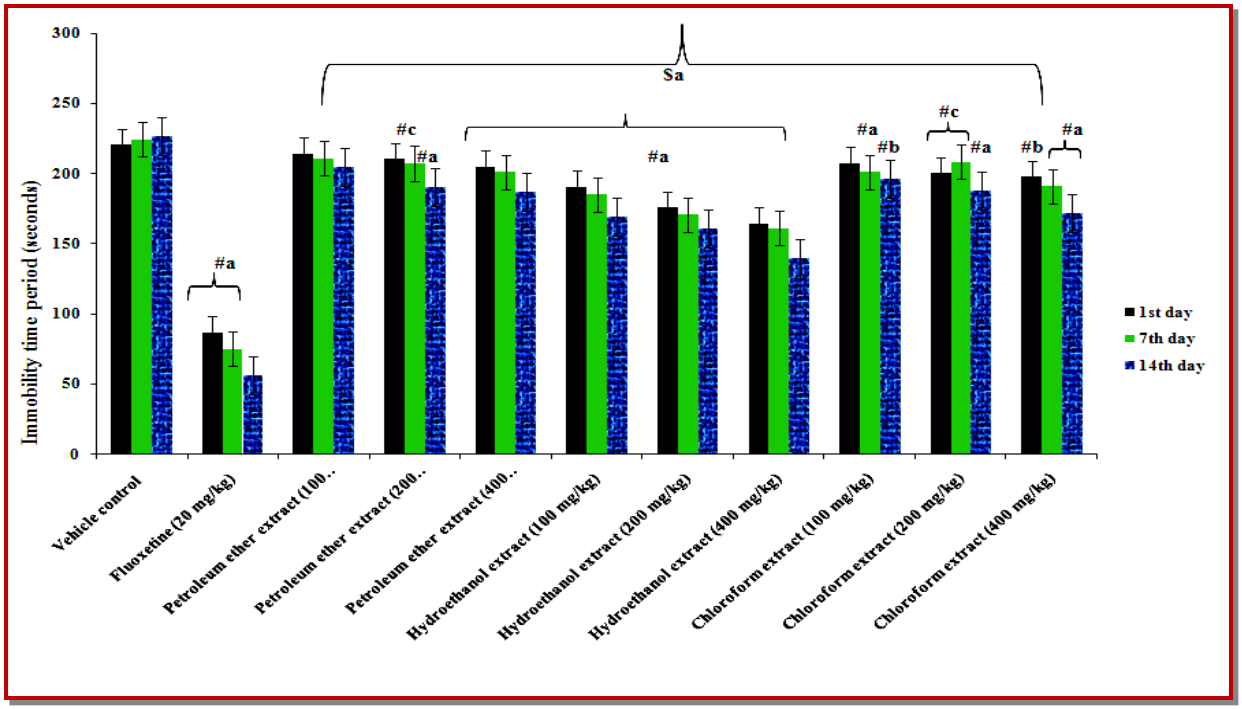

Figure 4: Effect of different extracts of *Nyctanthes arbor-tristis* on immobility time in tail suspension test

Statistical analysis of data was carried by one-way ANOVA followed by Tukey-Kramer multiple comparisons test. The values are Mean ± SEM for each group (n=6), P value less than 0.05 was considered as significant. # Vehicle treated control vs all groups, $ Fluoxetine vs all groups. [a]p<0.001, [b]p<0.01, [c]p<0.05

the effects of hydroethanol and petroleum ether extract were almost similar to those observed in forced swimming test (Figure 4). At different time intervals in mobility period, the highest percentage improvement was found at higher dose of each extract on day 14 includes petroleum ether (17.5%); hydroethanol (38.5%) and chloroform extract (24.0%). All doses of hydroethanol extract and higher dose of petroleum ether extract-treated group showed an excellent statistically significant (p<0.001) difference were observed when compared with control group. Fluoxetine type of response was not found in any type of the extracts at any given time interval which showed non-significant effect when compared between fluoxetine-treated group and all extracts-treated group. In modified TST model, the observer recorded the duration of immobility length (cm) by mice on kymograph paper for 3 min continuously. All three doses of hydroethanol, medium and higher dose of chloroform and higher dose of petroleum ether extract-treated group showed exceptionally statistical significant (p<0.001) difference when compared with control group (Figure 5). Non-significant results were observed between hydroethanol extract-treated group versus fluoxetine-treated group and last two higher doses of chloroform extract-treated group versus fluoxetine-treated group. In combinational treatment (Figure 6), hydroethanol extract (100 mg/kg) with fluoxetine-treated group (10 mg/kg) (55.1% on day 7) and chloroform extract (100 mg/kg) with fluoxetine-treated group (10 mg/kg) (58.2% on day 14) showed a synergistic effect in improvement of percentage mobility when compared with fluoxetine-treated group alone (56.9% on day 7 and 63.4% on day 14) while in case of hydroethanol extract (200 mg/kg) with fluoxetine-treated group (10 mg/kg) on day 7 (62.3%) and day 14 (70.5%) demonstrated additive type of effect showed a non-significant result when compared with fluoxetine-treated group alone (56.9% on day 7 and 63.4% on day 14).

Biochemical estimations

Among different monoamine levels (norepinephrine, dopamine and 5-hydroxytryptamine) in the brain after day 14 of treatment, fluoxetine treated-group and all doses of hydroethanol extracts-treated group showed a statistical significant (p<0.0001) improvement in their levels when compared with vehicle control-treated group. Similar type of effect was shown in higher dose of petroleum ether and chloroform extracts-treated groups. Lesser significant effect was shown only in 5-hydroxytrptamine level with hydroethanol extract (400 mg/kg)-treated group when compared with fluoxetine-treated group which means the level of 5-hydroxytrptamine brought back to the normal like fluoxetine type of effect (Figure 7). On serum glucose levels at day 14 (Figure 8), all three doses of hydroethanol and chloroform extracts-treated groups showed a statistically significant (p<0.001) decrease in

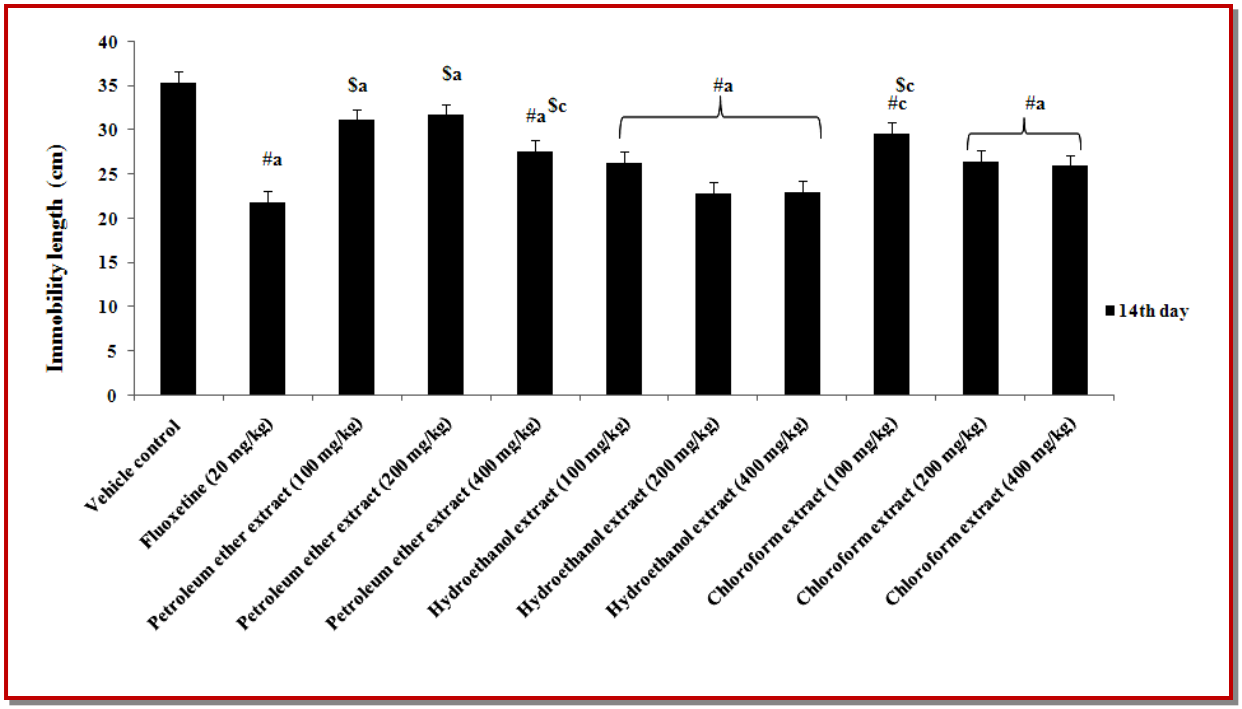

Figure 5: Effect of *Nyctanthes arbortristis* extracts on immobility length on kymograph paper using tail suspension model (TST) at 14th day

Statistical analysis of data was carried by one-way ANOVA followed by Tukey-Kramer multiple comparisons test. The values are Mean ± SEM for each group (n=6), P value less than 0.05 was considered as significant. # Vehicle treated control vs all groups, $ Fluoxetine vs all groups. [a]p<0.001, [b]p<0.01, [c]p<0.05

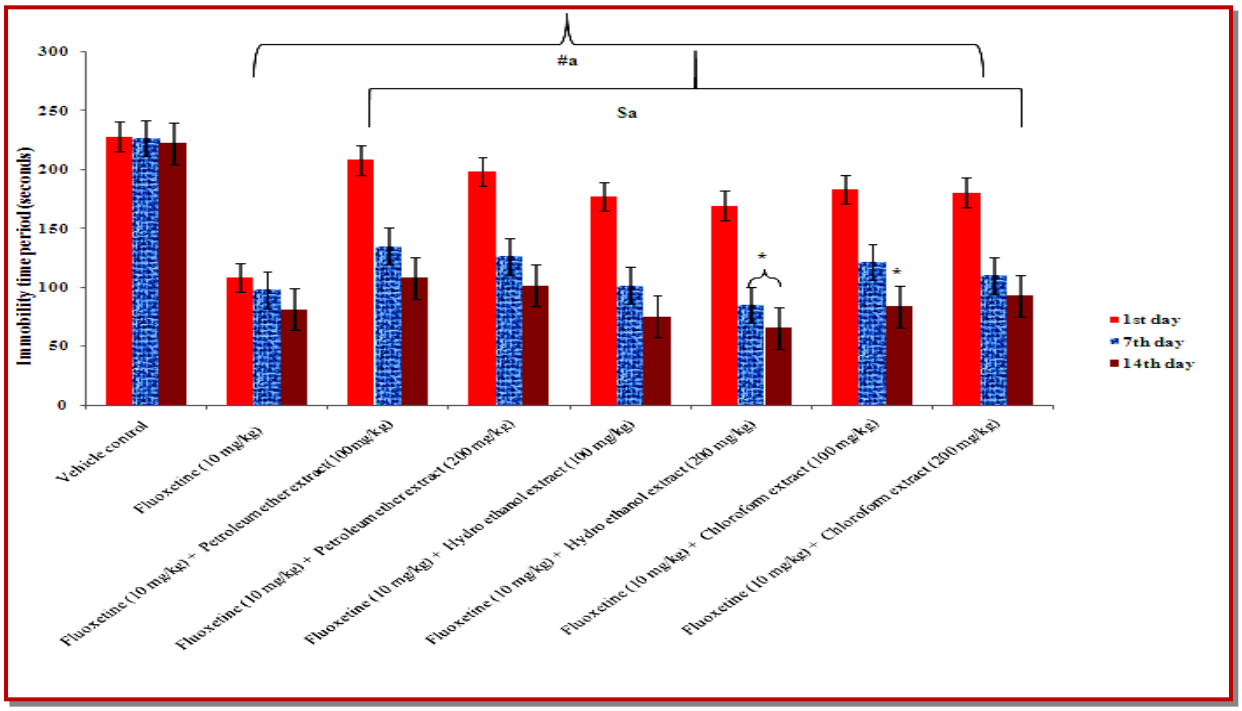

Figure 6: Combination effect of different extracts of *Nyctanthes arbortristis* with Fluoxetine on immobility time in tail suspension test

Statistical analysis of data was carried by one-way ANOVA followed by Tukey-Kramer multiple comparisons test. The values are Mean ± SEM for each group (n=6), P value less than 0.05 was considered as significant. # Vehicle treated control vs all groups, $ Fluoxetine vs all groups. [a]p<0.001, [b]p<0.01, [c]p<0.05. * Non-significant (Fluoxetine vs all extracts)

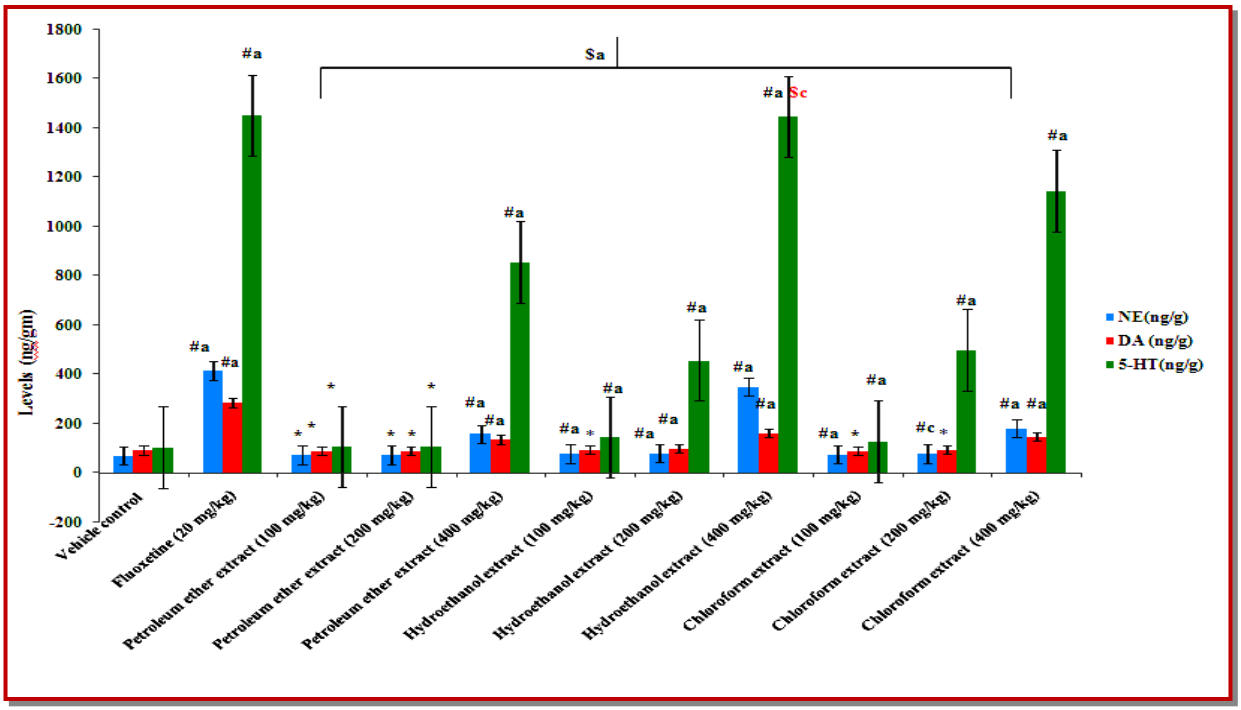

Figure 7: Effect of *Nyctanthes arbor-tristis* extracts on norepinephrine, dopamine, 5-HT levels at 14th day in mice using FST model

Statistical analysis of data was carried by one-way ANOVA followed by Tukey-Kramer multiple comparisons test. The values are Mean ± SEM for each group (n=6), P value less than 0.05 was considered as significant. # Vehicle treated control vs all groups, $ Fluoxetine vs all groups. [a]p<0.001, [b]p<0.01, [c]p<0.05. * Non-significant (vehicle treated control vs all groups)

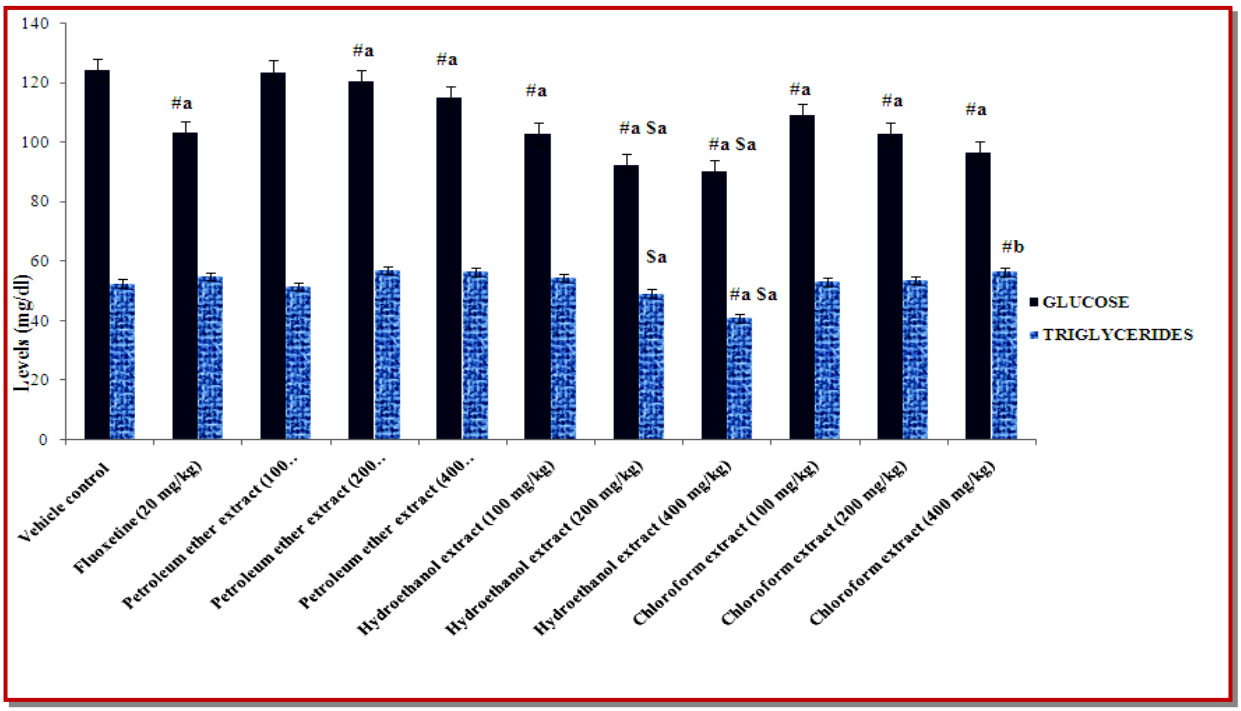

Figure 8: Effect of *Nyctanthes arbor-tristis* extracts on serum glucose and triglycerides levels at day 14 in mice using FST model

Statistical analysis of data was carried by one-way ANOVA followed by Tukey-Kramer multiple comparisons test. The values are Mean ± SEM for each group (n=6), P value less than 0.05 was considered as significant. # Vehicle treated control vs all groups, $ Fluoxetine vs all groups. [a]p<0.001, [b]p<0.01, [c]p<0.05

glucose levels when compared with vehicle-treated control group. In triglyceride levels, hydroethanol extract and chloroform extracts at 400 mg/kg showed a higher statistical significant ($p<0.001$) difference when compared with vehicle-treated control group. In both the parameters, levels were decreased with hydro-ethanol extract (200 mg/kg and 400 mg/kg) showed a statistical significant ($p<0.001$) difference when compared with fluoxetine-treated group.

GCMS analysis

On the basis of pharmacological results in our study, two extracts (hydroethanol and chloroform extracts) were analyzed by GCMS (Table I). Chloroform extracts showed the presence of 8 compounds, out of which thioctic acid which was also known as α-lipoic acid (m/z 73) showed a highest abundance (95-100%). The other compounds were also detected like benzoic acid derivative (m/z 356), stearic acid (m/z 341), gallic acid (m/z 283), 1-ecosine (M-H ecosane derivative m/z 282), nondecane (m/z 267), kaempferol (m/z 191 and 207) and D-mannose (M/Z 147). Similarly, hydroethanol extract confirmed the presence of compounds which include narigenin (m/z 91 and 291) showed a high abundance peak (95-100%), kaempferol (m/z 189), spermidine (m/z 175), behenic acid (m/z 135), lignoceric acid (m/z 117), L-arginine (m/z 115) and oleic acid (m/z 77). Both the extracts contained one common compound which was kaempferol showed at different m/z i.e 189 for hydroethanol extract and m/z 191 and 207 for chloroform extract

Table I

GC-MS analysis of hydroethanol and chloroform extracts of *Nyctanthes arbor-tristis*

SL. No.	Compound	m/z ratio
	Chloroform extract	
1	Thioctic acid (α-lipoic acid)	73
2	D-Mannose	147
3	Kaempeferol	191, 207
4	Nonadecane	267
5	1-eicoscene	282
6	Gallic acid	283
7	Stearic acid	341
8	Benzoic acid	356
	Hydroethanol extract	
9	Naringenin	91, 209
10	Lignoceric acid	117
11	Behenic acid	132
12	Spermidine	175
13	L-arginine	115
14	Kaempeferol	189

Discussion

The present study shows that hydroethanol extract contains number of active compounds like flavonoids, triterpenoids, phenols, tannins, fatty acids and alkaloids whereas chloroform and petroleum ether extracts failed to show the response for these active constituents except triterpenoids and flavonoids. The TLC (thin layer chromatography) of this plant extracts showed R_f values in the range of 0.1 to 0.92, which was confirmed by the presence of triterpenoids and flavonoids.

Our results clearly indicated that, the plant extract exhibited significant antidepressant activity after exposure of stress in mice using both models (FST and TST). In FST model, the results clearly showed that the hydroethanol extracts of *N. arbor-tristi*s possess best protective effect from day 7 to day 14 in stress induced depression model. The percentage mobility was also very high. In climbing parameter, the petroleum ether extract (400 mg/kg) showed an excellent response which was similar to fluoxetine treated group. In swimming behavior, all extracts at higher dose showed statistical significant improvement when compared to vehicle treated group which was same type of effect noted by fluoxetine treated group in modified. In TST model, similar type of results was found with all extracts. In modified TST model no statistical significant results were found in the behavior when compared between fluoxetine treated group and all extracts. The immobility length was also decreased constantly in chloroform as well as hydro ethanol extract as the doses increased. In combination of hydroethanol extract (100 mg/kg) with fluoxetine (10 mg/kg) and chloroform (100 mg/kg) with fluoxetine (10 mg/kg) showed a synergistic and additive type of effect at last both the intervals.

Our findings also suggesting that the antidepressant like effect of our plant extracts was shown by the improvement in swimming behavior which was further confirmed by 5-hydroxytrptamine levels. In our study, only hydroethanol and chloroform extract (400 mg/kg) showed a statistical significant ($p<0.001$) reduction in glucose and triglycerides levels indicated that, the plant extract may have potential to maintain blood glucose and lipid levels via indirectly inhibition during pathogenesis of depression.

The presence of triterpenoids and flavonoids was also reported in previous study (Shah et al., 2012). The characteristic of swimming and the climbing behaviors

analyzed by the prediction of the possible neurotransmitters involved as an antidepressant like action of *N. arbor-tristis* extracts. Earlier reports suggesting that, decrease level of monoamines is due to stretched stressful conditions and has been associated with wide range of peripheral and central disorders like depression and anxiety (Jayanthi et al., 2005). One of the scientists (Reneric and Lucki, 1998) reported in his experimental model (FST) that, when the animal is treated with a drug, it may increase neurotransmitter levels like serotonin, norepinephrine and dopamine in the nerve terminals which improve swimming and climbing behavior. An increase in all the three neurotransmitters and improvement in the behavior could be due to inhibition of monoamine oxidase (MAO) activity in the brain.

Improvement in swimming behavior by HT levels was found in our study and a lot of evidences were established which provide a proof of concept showing a decreases in immobility period and increases in swimming behavior with combination of fluoxetine treatment through 5-hydroxytrptamine reuptake mechanism (Page Michelle et al., 1999). Our results also showed a same type of effect and may add in a proof of concept evidences demonstrated synergistic and additive action. The combined treatment of fluoxetine with *N. arbotristis* extracts has been evaluated for the first time in our study but the effect of fluoxetine with other plant extracts has been evaluated previously on *Annona cherimolia* and *Agele marmelos* (Vazquea et al., 2012).

The hydroethanol and chloroform extracts both showed a common compound called kaempferol at different peaks (m/z 189 in hydroethanol extract and m/z 191 & 207 in chloroform extract). This peak was also reported and compared with pure kaempferol spectra data (Tsujimoto et al., 2013). Further, the presence of this compound in our plant was also reported by other researcher (Talakal et al., 2000; Paul et al., 1997; Saxena et al., 2002). The hydroethanol extract showed repeated abundance of narigenin (m/z 91 & 291) as in our spectra and compound was confirmed by comparing the spectra (Tsujimoto et al., 2013). Similar type of constituent was present in *N. arbor-tristis* reported by different researchers (Sah et al., 2012; Tripathi et al., 2010). Similarly, chloroform extract also showed thioactic acid (α-lipoic acid; m/z 73) having repeated abundance as shown in spectra and compared with the spectra of pure compound as reported by Kusano and Fukushima, (2011).

The antidepressant like effect in this plant may be due to the presence of narigenin (hydroethanol), thioactic acid (chloroform extract) and kampferol (both extracts) which was also confirmed by other reported studies having antidepressant effect of these constituents (Yi et al., 2014; Yi et al., 2012; Silva et al., 2013). Many studies reported to possess antidepressant like effect after administration of these individual active constituents in mice model like oleic acid (Zhang et al., 2009; Jain and Panchagnula, 2003), kaempeferol (Ghasmzadeh and Ghasemzadeh, 2011; Zhang et al., 2014), thioctic acid (Silva et al., 2013; Salzar, 2010), gallic acid (Dhingra and Chhillar, 2012) and naringenin (Zhang et al., 2014). They were basically found to have synergistic and additive effect with these plants, the possible mechanism may be due to the generalized increased in the monoaminergic turnover. Fluoxetine is selective serotonin reuptake inhibitor facilitates serotonergic neurotransmission. No doubt, catecholamine and 5-hydroxytryptamine are implicated in etiology of anxiety and depression, the positive effect of these drugs in TST seems to be due to increased availability of these neurotransmitters at the postsynaptic receptor sites. The combined treatment may increases the monoamine levels at postsynaptic sites.

A growing body of research evidences indicate that besides the depletion of serotonin and catecholamine neurotransmitters, depression could result from various other pathophysiological mechanisms as well like depression may inhibit neurogenesis *via* modulation of hypothalamic-pituitary-adrenal (HPA) axis and the brain derived neurotrophic factor (BDNF) in the hippocampus. There may be up-regulation of 5-HT_{1A} receptors in the brain (Sapolsky, 2000). Another mechanism in the pathophysiology of depression is the activation of innate immunity *via* mitogen activated protein kinases and nuclear factor-κB (NF-κB) which may leads to increased release of interferon-α and other cytokines affecting metabolism of monoamine neurotransmitters (Xu et al., 2007).

The animal experiments show that physical and psychological stress-induced depressions are accompanied by lowered antioxidant levels and increased oxidative stress in the brain resulting damage of fatty acids and proteins (Cho et al., 2009; Kubera et al., 2011). Lipid peroxidation is also one of the factors which decreases the membrane integrity and inhibition of lipid repair enzymes such as lysophosphatidyl choline acyltransferase and fatty acyl-CoA synthetase (Schaller and Graf, 2004). Though antioxidant activity of extracts was not determined in the present study, earlier reports indicate that this plant possesses good antioxidant activity (Megashri and Gopal, 2012; Michael et al., 2013; Hussain and Ramteke, 2012). The protective effect of hydroethanol extract may not be related to excitatory or inhibitory affects in locomotors function but it may also associated with the modulation of dopaminergic and serotonergic neurotransmission. An antioxidant effect of this extract against oxidative stress may be somewhat

responsible for its observed neuroprotective effects. Recent study reported that the depression may be positively associated with the development of type 2 diabetes mellitus through various inflammatory pathways such as IL-1β, TNF-α. IL-6 may contribute to systemic inflammation, as these cytokines are able to cross the blood-brain barrier and enter into the circulation. These mediators have the potential to interact with insulin receptor and induce pancreatic β-cell dysfunction, which leads to the development of diabetes mellitus (Staurt and Baune, 2012).

Conclusion

The leaves of *N. arbor-tristis* showed good antidepressant activity in mice. Of the three extracts tested, hydroethanol extracts showed a very significant and promising antidepressant like activity. The extracts restored the brain level of 5-hydroxytrptamine that are implicated in depression.

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Ethical Issue

Mice were used with the prior approval of the Institutional Animal Ethics Committee (MMCP/IEC/12/02).

Conflict of Interest

Authors declare no conflict of interest

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