ANXIOLYTIC ACTIVITY OF AQUEOUS EXTRACT OF NERIUM OLEANDER FLOWER ON EXPERIMENTAL ANIMALS

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ABSTRACT

Plant Nerium oleander (Apocynaceae) is used in Indian folk medicine as an antidote to snake venom, aphrodisiac, tonic for chronic pain in joints and abdomen and to treat skin ulcers. In this study, Psychopharmacological potential of aqueous extract of Nerium oleander flower (AENOF) for its anxiolytic activity was evaluated.

Oral doses (100 mg/kg 200 mg/kg and 400 mg/kg) of AENOF was assayed by using elevated plus maze, hole-board test, light and dark model and social interaction test in mice and rats. The AENOF in elevated plus maze, showed marked increase in the time spent and the entry in open arm where as in hole-board test there was significant increase in head dip count and decrease latency and number of crossing. Likewise, in light and dark model the AENOF showed dose dependent effect with an increase in both latency to enter in dark and time spent in light with decrease in the number of entries. These effects were much more significant at 200mg and 400mg/kg as compared to the known anxiolytic drug diazepam (2mg/kg; p.o). However, in social interaction model, the AENOF at 400mg/kg has significantly increased the interaction time and decreased the locomotors activity. The present results suggest that the AENOF possess some sedative active principles and that were responsible for its anxiolytic activity.
KEYWORDS: Anxiolytic property; *Nerium oleander* flower; Elevated plus maze; Hole-board; Light/dark; Social interaction.

INTRODUCTION

Anxiety disorders are marked by excessive fear (and avoidance), often in response specific objects or situation and in the absence too danger, and they are extremely common in the general population. According to a recent epidemiological study, the life time prevalence any anxiety disorder is 28.8%.\(^1\) Anxiety disorders are associated with impaired work place performance and hefty economic costs.\(^2\) Major drug classes for the treatment of anxiety disorders are Benzodiazepines (BZDs), Selective serotonin-reuptake inhibitors (SSRIs), Tricyclic antidepressant, β-blockers, and Azapirones.\(^3\) All this drug classes currently used are associated with side effects that very occurrence and severity. Like BZDs produce undesirable effects such as drowsiness, ataxia, and sedation, muscle relaxation, insomnia, hepatotoxicity and in addition. They adversely interact with other CNS depressants, particularly alcohol.\(^4,5\) Hence the clinical applications of benzodiazepines as anxiolytic are limited by their side effects. Research has also focused on the development of drugs with fewer side effects, such as sedation, muscle relaxation, and drug dependence. Hence there is needed to look for more efficacious anxiolytic agents with lesser side effects. Many herbal plants available to be best herbs for antianxiety effect. It has been estimated that 43% of anxiety sufferers use some form of complementary therapy. The most popular treatments include herbal medicine.\(^6\) The isolated active constituent of medicinal herbs and after testing some found to be therapeutically active.\(^7\) There are several plants very effective in treating stress / anxiety, such plants include Matricaria bchamomilla (chamomile), Hypericum perforatum (St.Jons wort), Piper methysticum (Kava kava)\(^8\), Pssiflora incarnate (Passion flower).\(^9\) Dolichandrone falcate, contain chrysin flavones is subgroup of flavonoid present in bark\(^10\) and leaves also contain chrysin and Chrys in 7-rutinoside.\(^11\) The active constituent mention above reported to have many biological activities, and one among them is for anxiolytic activity.\(^12\)

Plant *Nerium oleander* is an important medicinal plant of family Apocynaceae commonly known as Kaner in Hindi, is large glabrous evergreen shrub with milky juice. This plant grows in Mediterranean region up to Africa, china, Iran, Iran, Pakistan and India. Leaves are three, shortly stalked, coriaceous, 10-15 cm long, linear lanceolate with dark green colour. Flowers are salver-shaped pink or white scentless without any fragrance.\(^13,14,15,16\) A wide
spectrum of biological activities has been reported with various constituents isolated from different parts of the Nerium oleander. Root, bark, seeds, leaves and flower contains several glycosides\textsuperscript{17,18}, one of which is cardiac glycosides that have a paralysing action on the spinal cord\textsuperscript{19}, pregnanes\textsuperscript{20}, and triterpenes.\textsuperscript{21,22} The plant also contains traces of vitamins A, K, and C\textsuperscript{23}. Rosagenin has been extracted from the bark and has a strychnine-like action. Several flavones (0.5\%) and volatile oils, as well as rubber, fats, sugars and hydrocyanic acid, have been isolated from its leaves (Schvartsman, 1979; Shaw & Pearn, 1979; Pearn, 1987).\textsuperscript{23,24,25} The active constituent mention above has reported many biological activities, such as cardiotonic\textsuperscript{26}, antileprotic and in skin disease\textsuperscript{27}, analgesic, anti-inflammatory, also used to treat eye diseases\textsuperscript{21}, sedative and CNS depressant.\textsuperscript{28} The leaves of Nerium oleander showed CNS depressant activity due to presence of triterpenes\textsuperscript{21,22} and bioactive cardenolides.\textsuperscript{19} The partially purified fractions (B-1 and B-3) of Nerium oleander leaves produced an alteration in general behaviour pattern and reduction in spontaneous locomotors activity.\textsuperscript{28} It is noteworthy that fractions B-1 and B-3 caused sedation at low doses and showed hypnosis at higher doses.\textsuperscript{28} In contrast to barbiturates, there was no excitation prior to induction of sedation which suggests that the behavioural properties of these fractions are similar to benzodiazepines.\textsuperscript{29} Also possess antidiabetic\textsuperscript{30}, diuretic\textsuperscript{27}, anticancer\textsuperscript{17,20,21,22}, antiepileptic\textsuperscript{17}, cathartic and emetic\textsuperscript{17}, anticonvulsant\textsuperscript{31}, Rodenticides and antimalarial\textsuperscript{24,32} and insecticidal\textsuperscript{17,24,32}. These are some more recently evaluated activity on leaves and flowers Antinociceptive, Antifungal, Antibacterial, Antileukemic, immunomodulating activity.\textsuperscript{33} The root is bitter; aphrodisiac, very poisonous, but an antidote to snake venom. It can also be used as good tonic for chronic pain either in abdomen or in joints. For chaneres and ulcers on the penis, it is recommended to apply with the root beaten into a paste with water. The fluid of young leaves is poured into eyes in ophthalmia with copious lachrymation. Moreover, Vydiens consider that the root bark and the leaves of this shrub, if applied externally\textsuperscript{34} are very powerful repellents. The plant can also be used for curing indigestion, fever, ringworm, venereal disease.\textsuperscript{35} The clinical applications of well-known benzodiazepines as anxiolytic agents are limited because of their side effects. Therefore, the purpose of the investigation was to test anti-anxiety property of \textit{Nerium oleander} flower’s extract as new pharmacological agents.
MATERIALS AND METHODS

Plant Material
The *N. oleander* flowers were collected from surrounding area of Gulbarga, Karnataka in the month of March-May, 2011. The voucher specimens (Voucher No Wazid 1) were identified and authenticated by scientist J. Jayanti, Botanical Survey of India, Ministry of Environment and Forest, Govt. of India, Pune, Maharashtra, India, (Annexure A). The flowers of *N. Oleander* were dried in shade, pulverized by a mechanical grinder and passed through mesh sieve to get the coarse powder.

Preparation of extract
Coarsely powdered material was macerated in sufficient quantity of distilled water with small quantity of chloroform to prevent fungal growth and kept for 7 days. During maceration it was shaken twice daily. On seventh day it was filtered & the filtrate was concentrated on water bath (50°C) to remove the solvent and to get sticky dark brown coloured extract i.e. aqueous extract of *N.oleander*. The obtained yield was 19.16%.\(^{36,37}\)

Preliminary phytochemicals screening
The preliminary phytochemical group test of the aqueous extract of dried flowers of *N.oleander* extract was performed for the presence of various active principles (alkaloids, tannins, cardiac glycoside, steroids, flavonoids, reducing sugar, saponins, vitamins) using standard procedures.\(^{36,37,38}\)

Animal
Swiss albino mice of either sex weighing 20-30 gm and albino Wistar rats either sex weighing 200-250 gm were procured from registered breeders (149/1999/CPCSEA, Mahavir Enterprises, Hyderabad.) andb used for studying acute toxicity and anxiolytic activity respectively. The animals were housed under standard conditions of temperature (25±2°C) and relative humidity (30%–70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet (VRK Nutrition, Pune) and water *ad libitum*. Approval at the Institutional Animal Ethics Committee (IAEC; no. 346/CPCSEA, Annexure B) of Luqman College of Pharmacy, Gulbarga was taken for conducting anxiolytic activity on animals.
Drugs and Chemicals
The Diazepam (DPZ) as free sample was gifted by Reliance Formulation Private Limited, Chaekosons Chemic, Ahmadabad; Gujarat, were used as the standard anxiolytic drugs. Distilled water was used as vehicle.

Preparation of Drugs
The extracts were suspended in the vehicle in such concentrations as to administer 100, 200 and 400 mg/kg doses to mice and rats through per oral route. All drugs were freshly prepared before each experiment. DPZ (2mg/kg; p.o) used as standard.

Acute Toxicity Study
The acute toxicity of aqueous extract of N.oleander flowers was determined in female albino mice. Animal were fasted overnight prior to the experiment. Fixed dose (Annexure-2) method of CPCSEA, OECD guideline No. 425; was adopted for the study. One fifth and one tenth of LD50 cut off (2000 mg/kg) values taken as screening dose.\textsuperscript{[39]}

Elevated plus Maze Model (EPM):
After 30 minutes DPZ and 45 minutes extract/vehicle of oral administration the elevated plus maze test was performed in five groups of six albino mice, in 2% of gum acacia (vehicle control); 2mg/kg DPZ (drug control); 100, 200 and 300mg/kg of AENOF to the group I-V was done respectively. Elevated plus-maze test consists of a plus shaped maze, elevated 25 cm above ground level. It has two open (16 X 5 cm) arms and two closed (16 x 5 x 12 cm) arms having an open roof. The test mice was placed in the central square area (5X5 cm) of the plus maze and time spends by the animals in open arm during a 5 min observation period was noted. The plus maze was carefully cleaned with a wet towel after each animal test. The following parameters were calculated for each animal; a) open arm time, b) close arm time, c) open arm entry, d) closed arm entry.\textsuperscript{[40,41,42,43]}

Test for exploratory activity in mice (Hole-Board Test; H/B)
Exploratory behaviour was assessed using the board-hole test. The apparatus consists of wooden chamber (40x40x25 cm) with 16 holes (diameter 3 cm) on the floor, elevated to the height of 50cm, from the ground so that the rats could peep through the holes, in a dimly illuminated room. The rats were divided into five groups (six animals/group). DPZ (2mg/kg, p.o) was used as the positive control and AENOF at doses of 100, 200 and 400 mg/kg., p.o., in the three remaining groups. Rats were placed individually in the centre of the apparatus,
and the latency to the first head dips, number of head dips in the holes, total time spend with
the head dips, number of rearings, total locomotors activity (numbers of squares crossed) was
immediately counted during two or three consecutive periods of 5minutes each after
30minutes of DPZ and 45minutes of extract/vehicle administration.\[44]\n
**Light-dark model transition test in mice (L/D)**

The light-dark apparatus is a rectangle box of 40 x 60 x 20 cm (l x b x h), which is divided in
to two-compartment chamber, 40 x 20 cm is for the dark compartment and 40x40 cm served
as light compartment, separated by a wall with a round hole (7 cm diameter). Extract/vehicle
or standard drug is administered through per oral route. Forty five minutes after oral
administration of extract/vehicle and 30minutes after DPZ, the mouse are placed individually
on the light compartment and observe. Time spent in light and dark zones and number
crossings in light and dark zone are observed during this observation period for a period of
5min.\[45,46,47,48]\n
**Social interaction test(S I)**

Pairs of male rats were placed in a test box for 10 min and the time they spent in active social
interaction was scored. Maximum active interaction was found when the rats were tested
under low light with familiar condition. When the light level increased or in unfamiliar test
condition active social interaction decreased.

A total of 30 male albino rats 220-250 gm divided into five groups of six animals (three
pairs) each. Standard drug (Diazepam) was administered 30 min prior to testing and extracts
were administered p.o. 45 min prior to testing. They were housed singly for 5 days before the
experimental test, and were allowed food and water ad libitum. During this period they were
weighed and handled daily and the position of the cages in the rack was changed so that all
rats received equal experience of the different levels of illumination. The rats were randomly
assigned to ‘low light and unfamiliar’ test conditions. The test box was 65 x 65 cm with wall
47 cm high. Pairs of rats were placed in this box for 10 min and their behaviour observed on a
television monitor in an adjacent room. The following behaviours were scored: sniffing,
nipping, grooming, following, mounting, kicking, boxing, wrestling, and jumping on,
crawling and under or over the partner.\[49,50\]
Statistical analysis
The values were expressed as (n=6) mean ± SEM. The results were subjected to statistical analysis by using ANOVA followed by Dennett’s- t - test to calculate the significance difference if any among the groups. P<0.05 was considered as significant.

RESULTS
Preliminary phytochemical screening: Result obtained in this exercise, AENOF revealed the presence of carbohydrates (reducing sugar), cardiac glycoside, flavonoids, alkaloids, tannins, vitamin C& D. The results are presented in Table 1.

Assessment of anxiolytic activity
Elevated plus Maze Model: The behavioural effects of AENOF at doses as 100, 200, 400 mg/kg and diazepam, on the behaviour of mice in the elevated plus maze test were summarized in Table 2 and Figure 1 to 4.

Diazepam has increased the percentage of time spent in open arms significantly (P < 0.001, Figure. 1) and of arm entries in open arms significantly (P < 0.01, Figure. 3), whereas the percentage of time spent in closed arms has decreased significantly (P < 0.001, Figure. 2) and of entries in closed arms has decreased significantly (P < 0.01, Figure. 4) as compare to control group. It was seen that the AE 100 has no significant result but AE 200 has increased percentage of time spent and of entries in open arm significantly (P < 0.05, Figure. 1 and 3) whereas the percentage time spent in closed arm has decreased significantly (P < 0.05, Figure. 2) but the percentage of entries in to closed arm has no significantly decreased as compare to control. The studies with that of AE 400 shows significant results as compare to control group. AE 400 has significantly increased the percentage time spent and arms entries in open arms (P < 0.01, Figure. 1 and 3) whereas in closed arm it has decreased (P < 0.01, Figure. 2 and 4) as compare to control group.

Table 1. Preliminary Phytochemical group tests for the Aqueous extract of Nerium Oleander Flower:

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Aqueous extract of flowers of Nerium oleander Linn</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>2. Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>3. Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>4. Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>5. Tannins</td>
<td>+</td>
</tr>
<tr>
<td>6. Saponins</td>
<td>+</td>
</tr>
</tbody>
</table>

- Absence, + Presence.
Table 2. Effect of AENOF on EPM test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>% open arm time (s) ± SEM</th>
<th>% close arm time (s) ± SEM</th>
<th>% open arm entries ± SEM</th>
<th>% close arm entries ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle</td>
<td>29.37±2.98</td>
<td>70.63±2.98</td>
<td>39.12±4.63</td>
<td>60.87±4.63</td>
</tr>
<tr>
<td>II</td>
<td>Diazepam 2mg/kg</td>
<td>73.70±4.47***</td>
<td>26.30±4.47**</td>
<td>63.17±3.44**</td>
<td>38.503±4.51**</td>
</tr>
<tr>
<td>III</td>
<td>AE 100 mg/kg</td>
<td>39.17±7.45</td>
<td>60.83±7.45</td>
<td>45.65±3.25</td>
<td>54.35±3.25</td>
</tr>
<tr>
<td>IV</td>
<td>AE 200 mg/kg</td>
<td>56.70±9.77*</td>
<td>44.97±9.44</td>
<td>56.25±6.16*</td>
<td>43.75±6.16</td>
</tr>
<tr>
<td>V</td>
<td>AE 400 mg/kg</td>
<td>63.43±5.97**</td>
<td>36.57±5.97**</td>
<td>62.33±4.84**</td>
<td>37.68±24.83**</td>
</tr>
</tbody>
</table>

All values are mean ± SEM, N=6, *p < 0.05, **p < 0.01, ***p < 0.001 (one way ANOVA followed by Dennett’s’ test)

Fig.1. Effect of various doses of AENOF (100, 200, 400 mg/kg) on % open arm time in 5-min EPM

Fig.2. Effect of various doses of AENOF (100, 200, 400 mg/kg) on % close arm time in 5-min EPM
Fig. 3. Effect of various doses of AENOF (100, 200, 400 mg/kg) on % open arm entries in 5-min EPM

Fig. 4. Effect of various doses of AENOF (100, 200, 400 mg/kg) on % close arm entries in 5-min EPM

**Hole-Board Model:** The effects of AE 100, AE 200, AE 400 mg/kg and diazepam were summarized in Table 3 and Figure 5 to 7. Diazepam revealed that, significantly decrease head dip latency (P < 0.001, Figure 5) and increase head dip count (P < 0.001, Figure 6) and locomotion was decreased (P < 0.001, Figure 7) as compare to control group.

Further analysis showed that AE 100 was insignificant in above paradigms, whereas AE 200 shown decreased head dip latency significantly (P < 0.01, Figure 5) and increase head dip count (P < 0.01, Figure 6) but the locomotion shown insignificant result as compare to control group. The AE 400 has decreased head dip latency significantly (P < 0.001, Figure 5).
and increase head dip count (P < 0.001, Figure 6) whereas the locomotion shown decreased significantly (P < 0.01, Figure 7) as compare to control group.

Table 3. Effect of AENOF on Mice in Hole-Board (H/B) test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Head dip</th>
<th>Latency</th>
<th>Head dip count</th>
<th>No. of crossing</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>CONTROL</td>
<td>51.67±10.53</td>
<td>23.33±1.25</td>
<td>149.8±4.56</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>DIAZEPAM 2mg/kg</td>
<td>11.33±1.97***</td>
<td>39.17±2.30***</td>
<td>110±5.09***</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>AE 100 mg/kg</td>
<td>37.50±7.70</td>
<td>25.50±0.56</td>
<td>142.8±7.38</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>AE 200 mg/kg</td>
<td>21.17±2.35**</td>
<td>32.83±2.13**</td>
<td>133±5.68</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>AE 400 mg/kg</td>
<td>14.33±2.10***</td>
<td>34.50±2.07***</td>
<td>120.8±3.51**</td>
<td></td>
</tr>
</tbody>
</table>

All values are mean ± SEM, n=6, *p < 0.05, **p < 0.01, ***p < 0.001 (one way ANOVA followed by Dunnett’s ‘t’ test)

Fig. 5. Effect of various doses of AENOF (100, 200, 400 mg/kg) on % on Latency to head dip in 5-min Hole-Board test

Fig. 6. Effect of various doses of AENOF (100, 200, 400 mg/kg) on % on Head dip count dip in 5-min Hole-Board test
Fig.7. Effect of various doses of AENOF (100, 200, 400 mg/kg) on Locomotion in 5-min Hole-Board test

**Light and Dark test:** Results of the light/dark test shows in table 4 and figure 8 to 10. Diazepam treatment group shown increased latency to enter in dark compartment significantly (P < 0.001, Figure 8) and significantly increased time in light area (P < 0.01, Figure 9), and number of tunnel crossing was decreased significantly (P < 0.01, Figure 10) as compare to control group. The AE 100 has not significant in above paradigms as compare to control group. But the AE 200 shown the latency to enter in dark compartment increased significantly (P < 0.05, Figure 8) and significantly increased time in light area (P < 0.05, Figure 9), and number of tunnel crossing was significantly decreased (P < 0.05, Figure 10). The AE 400 has shown increased latency to enter in dark significantly (P < 0.01, Figure 8) and significantly increased time in light area (P < 0.01, Figure 9), and number of tunnel crossing was significantly decreased (P < 0.01, Figure 10) as compare to control group.

Table 4. Effect of AENOF on Behaviour of Mice in Light and Dark (L/D) test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Latency to enter in dark</th>
<th>Time Spent in light area</th>
<th>Tunnel crossing</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>CONTROL</td>
<td>16±2.44</td>
<td>100.5±4.91</td>
<td>22.17±1.93</td>
</tr>
<tr>
<td>II</td>
<td>DIAZEPAM 2mg/kg</td>
<td>35±1.88***</td>
<td>172.2±13.13**</td>
<td>12.83±1.35**</td>
</tr>
<tr>
<td>III</td>
<td>AE 100 mg/kg</td>
<td>18.50±2.38</td>
<td>139±20.57</td>
<td>17.33±1.92</td>
</tr>
<tr>
<td>IV</td>
<td>AE 200 mg/kg</td>
<td>29.67±4.11*</td>
<td>153±14.25*</td>
<td>14.67±1.47*</td>
</tr>
<tr>
<td>V</td>
<td>AE 400 mg/kg</td>
<td>33.83±3.85**</td>
<td>170.5±12.94**</td>
<td>12.33±1.43**</td>
</tr>
</tbody>
</table>

All values are mean ± SEM, N=6, *p < 0.05, **p < 0.01, ***p < 0.001 (one way ANOVA followed by Dunnett’s ‘t’ test)
Fig. 8. Effect of various doses of AENOF (100, 200, 400 mg/kg) on Latency to enter in dark (sec) in 5-min Light and dark test.

Fig. 9. Effect of various doses of AENOF (100, 200, 400 mg/kg) on Time spent in the light chamber (sec) in 5-min Light and dark test.

Fig. 10. Effect of various doses of AENOF (100, 200, 400 mg/kg) on Tunnel crossing in 5-min Light and dark test.
Social interaction test of AENOF in rats: The effects of aqueous extract of NOF at doses as AE 100, AE 200, AE 400 (mg/kg) and diazepam on social interaction test were shown in Table 5 and Figure 11 and 12. Diazepam has increase in social interaction time (sec) significantly (P < 0.001, Figure 1) and decrease locomotor activity (P< 0.01, Figure 12) as compare to control group. The AE 100 has not shown significant result of S.I time and locomotor activity as compare to control group. The AE 200 has shown increased social interaction time significantly (P < 0.01, Figure 11) and has insignificant in locomotor activity as compare to control group. The AE 400 has shown increased social interaction time significantly (P < 0.001, Figure 11) whereas decreased locomotion significantly (P < 0.01, Figure 12) as compare to control group.

Table 5. Effect of AENOF on Social Interaction (S I) test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Social interaction time</th>
<th>Locomotion</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>CONTROL</td>
<td>84.83±5.16</td>
<td>147±7.54</td>
</tr>
<tr>
<td>II</td>
<td>DIAZEPAM</td>
<td>151.7±8.80***</td>
<td>117.2±5.31**</td>
</tr>
<tr>
<td>III</td>
<td>AE 100 mg/kg</td>
<td>79.17±6.39</td>
<td>139±3.20</td>
</tr>
<tr>
<td>IV</td>
<td>AE 200 mg/kg</td>
<td>112.5±2.91**</td>
<td>121.5±7.10</td>
</tr>
<tr>
<td>V</td>
<td>AE 400 mg/kg</td>
<td>144.2±5.79***</td>
<td>118.7±3.95**</td>
</tr>
</tbody>
</table>

All values are mean ± SEM, N=3, *p < 0.05, **p < 0.01, ***p < 0.001 (one way ANOVA followed by Dunnett’s t’ test)

Fig.11. Effect of various doses of AENOF (100, 200, 400 mg/kg) on Social interaction time (sec) in 10-min Social Integration test
In light and dark model, It indicate that the diazepam (2mg/kg) treated mice significantly increased the latency to enter in dark and time spent in light chamber, and shown
significant reduction in the total entry. AENOF (200 & 400mg/kg) treated rats shown increased the latency to enter in dark and time light chamber, and shown significant reduction in the tunnel crossing.

The Social interaction time was increased and no. of crossing was decreased significantly in case of diazepam treated animals as compared to the control animals. The AENOF at 200, 400 mg/kg doses treated rats shown an increased in social interaction time and the no of line crossing was decreased significantly as compared to the control animals. Thus reinforcing the hypothesis that it has anxiolytic activity.

Earlier reports on the chemical constituents of the plants and their pharmacology suggest that plants containing flavonoids, saponins and tannins possess activity against many CNS disorders.\(^{[25]}\)

Phytochemical tests of AENOF revealed the presence of flavonoids. It may possible that the mechanism of anxiolytic action of AENOF could be due to the binding of any of these phytochemicals to the GABAA-BZD complex. In support of this, it has been found that flavones bind with high affinity BZD site of the GABAA receptor.\(^{[26]}\) The plant *Nerium oleander flower* also contains flavones which may be responsible for its anxiolytic activity. So the anxiolytic activity of AENOF might involve an action on GABAergic transmission or effects on serotonergic transmission or due to its mixed aminergic potentiating effect.

**CONCLUSION**

From the above observations we can conclude that aqueous extract of *Nerium oleander flower* shown anxiolytic activity at both the dose (200 and 400 mg/kg) level which is comparable with the standards. However, it requires further studies to elucidate the possible mechanism involved and its use in human beings.

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