INTRODUCTION

Mental disorders lead to a significant load of morbidity and disability and few of them may lead to increase in mortality also. Anxiety is the main feature of many psychiatric disorders and also associated with many medical and surgical conditions. Anxiety disorders are among one of the most prevalent mental, emotional and behavioural problems in the world, estimated to affect 3.6% of the global population as of 2015. Present therapy of anxiety gives only temporary relief with various side effects. Aims: The aim of this study is to evaluate the role of Shankhpushpi and Omega-3 fatty acids alone and in combination in anxiety.

Methods: A total number of 36 Wistar rats (n=6) weighing 160-200 gm were used. Herbal drug Shankhpushpi whole plant powder in doses of 100mg/kg b.w. and 400mg/kg b.w. was used. Omega-3 Fatty Acids (500mg/kg b.w.) also administered orally along with higher dose of Shankhpushpi.

Diazepam was given as standard drug for comparison. Elevated Plus Maze and Open Field Test were used for behavioural analysis. Statistical Analysis Used: Data-analysis was carried out by one way ANOVA followed by Dunn’s multiple comparison test using Graph Pad Prism 7.0 software.

Results: Shankhpushpi-100mg did not show significant anxiolytic activity. But treatment with its higher dose, 400mg showed significant (P < 0.001) anxiolytic activity in dose dependent manner. Shankhpushpi 400mg and Omega-3 fatty acids also showed statistically significant (P < 0.05) anxiolytic effect which is comparable to diazepam.

Conclusion: The effect of combination Shankhpushpi (400 mg/kgb.w.) and Omega –3 fatty acids (500mg/kgb.w.) on anxiety was significant and greater than when both drugs were given alone. The encouraging results of the study may reveal the importance of the herbal drugs and nutrients in anxiety.

There are many drugs available to treat anxiety disorders, but they produce various systemic side effects such as, addiction, suicidal tendency, headaches, loss of motor coordination, hallucinations, insomnia and can disrupt everyday activities or exhibit tolerance upon chronic use.

More than 80% of people in developing nations can hardly afford the most basic therapeutic interventions, drugs, and vaccines. Now in the developing countries, a surprisingly large proportion of population choose Complementary and Alternative Medicine (CAM) or traditional medicine (TM) practices. According to World Health Organization (WHO) about 2,860 hospitals provide CAM and TM to patients in India (WHO, 2002). The World Health Organization (WHO) estimates that 80% of the world’s population presently uses these medications for primary health care ( Jarvik ME , 1972). Mechanisms of action for these medicines primarily involve modulation of neuronal transmission, via specific plant

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INTRODUCTION

Mental disorders lead to a significant load of morbidity and disability and few of them may lead to increase in mortality also. Anxiety is the main feature of many psychiatric disorders and also associated with many medical and surgical conditions. Anxiety disorders are among one of the most prevalent mental, emotional and behavioural problems in the world, estimated to affect 3.6% of the global population as of 2015, or about 264 million people in an analysis (Madhar M ., 2001) of 10 Indian studies on psychiatric morbidity, concluded that prevalence rates for anxiety neurosis and hysteria were 18.5 and 4.1 per 1000 population respectively. Anxiety is a feeling of discomfort, such as fear that can be mild or severe. Anxiety is a normal emotional behaviour, but when it is severe or chronic, it becomes pathological and can lead to various pathological and psychiatric disorders. Serotonin, dopamine, and norepinephrine – are the neurotransmitters responsible for anxiety disorder.

There are many drugs available to treat anxiety disorders, but they produce various systemic side effects such as, addiction, suicidal tendency, headaches, loss of motor coordination, hallucinations, insomnia and can disrupt everyday activities or exhibit tolerance upon chronic use.

More than 80% of people in developing nations can hardly afford the most basic therapeutic interventions, drugs, and vaccines. Now in the developing countries, a surprisingly large proportion of population choose Complementary and Alternative Medicine (CAM) or traditional medicine (TM) practices. According to World Health Organization (WHO) about 2,860 hospitals provide CAM and TM to patients in India (WHO, 2002). The World Health Organization (WHO) estimates that 80% of the world’s population presently uses these medications for primary health care ( Jarvik ME , 1972). Mechanisms of action for these medicines primarily involve modulation of neuronal transmission, via specific plant

EFFECT OF SHANKHPUSHPI (CONVOLVULUS PLURICAULIS) ALONE AND ITS COMBINATION WITH OMEGA-3 FATTY ACID ON ANXIETY IN AN ANIMAL MODEL OF RATS

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metabolites binding to neurotransmitter or neuromodulator receptors, and via alteration of neurotransmitter synthesis and general function so they are now preferred for treatment of psychiatric disorders. Herbal medicines have wide range of therapeutic actions in psychiatric illness which may include antidepressant, anxiolytic, nootropic (cognitive enhancing), sedative, hypnotic and analgesic effects (Spinella M., 2001). People who consume vegetables and fruits, phytochemicals are believed to reduce the risk of various diseases such as cardiovascular diseases, cancer as well as neurodegenerative disorders.

These are economical and free from side effects Many herbalists believe that isolated ingredients have lesser beneficial clinical effects than whole plant extracts. Shankhpushpi of Ayurvedic Pharmacopoeia of India consists of the whole plant of *Convolvulus pluricaulis* (CP) Choisy (Convolvulaceae) and considered as “Medhya Rasayana” in ayurvedic texts Agarwal P. et al. (2014). Plants other than C. pluricaulis like *Evolvulus alsinoids* (EA) Linn. (Convolvulaceae), *Clitoria ternatea* (CT) Linn. (Papilionaceae) and *Canscora decussata* (CD) Schult. (Gentianaceae) were also used as Shankhpushpi by some practitioners. Plants *Convolvulus pluricaulis* belongs to family – Convolvulaceae, genus – Convolvulus and species – pluricaulis. This plant have anxiolytic, tranquilizing, antidepressant, antistress, antiinmnesic, antioxidant, hypolipidemic, immunomodulatory, analgesic, antifungal, antibacterial, antidiabetic, antinociceptive and beneficial cardiovascular activity. This herb induces a feeling of calm and peaceful state of mind, good sleep and a relief in anxiety, stress and mental fatigue.

The brain contains high proportion of omega-3 PUFAs and their derivatives, which regulate several biological processes such as neurotransmission, cell survival and anti-neuroinflammation, and thereby mood and cognition. In addition, higher cerebrospinal fluid levels of 5-hydroxy-indoleacetic acid (5-HIAA), a metabolite of serotonin and an indicator of brain serotonin turnover, has been shown to be associated with high plasma concentration of omega-3 PUFAs among healthy subjects (Vinot N. et al., 2011). Omega-3 PUFAs are anti-inflammatory and anti-oxidative, and therefore could be beneficial in depression and anxiety (Vancassel S. et al.,2008).

Since the literature shows that there is ample evidence regarding the role of Shankhpushpi and Omega-3 fatty acids in anxiety, but only few scientific studies have been done in this regard and none have been done to see the combined effect of Shankhpushpi and Omega-3 fatty acids on anxiety. Therefore the present study has been planned to evaluate the effect of Shankhpushpi alone and with Omega-3 fatty acid in anxiety.

**METHODS**

The study was conducted in the Department of Pharmacology and Therapeutics of our University. Ethical clearance was obtained from the Institutional Animal Ethics Committee before conducting the study (project no. – 92/IAEC/2018)

**Experimental animals and rearing conditions**

Adult healthy albino Wistar rats of either sex, weighing 160-200 gm were used in the study. Animals were obtained from CPCSEA – certified animal house [Indian Institute of Toxicology Research (IITR), Lucknow]. They were allowed to access normal rat pellet diet and water ad libitum and were kept in Institutional Animal House under temperature controlled environment [25±2ºc] with 12 hours light dark cycle. The animals were housed for one week prior to the experiments to acclimatize to new environment.

**Dosage forms, Doses and Sources of the drugs**

Following drugs were used in this study. Following drugs were used in this study.

**Test Drugs**

**Shankhpushi (Convolvulus pluricaulis)**

Whole plant powder was purchased from market (Organic Shankhpushi - *Convolvulus pluricaulis*, whole plant powder) administered in two doses of 100mg/kg b.w. and 400mg/kg b.w. (Vancassel S. et al., 2008 and Malik J. et al.,2011) orally through feeding cannula using distilled water as a vehicle

**Omega – 3 Fatty Acids**

Capsules of Omega-3 fatty acids (Maxepa) were obtained from Merk, Germany and administered in a dose of 500mg/kg b.w. (Malik J. et al.,2015) orally through feeding cannula using Tween 20 as a vehicle

**Standard Drug**

**Diazepam**

Diazepam was purchased from government authorized medical store and given in a dose of 2mg/kg b.w. as Intraperitoneal injection (Carlezon WA et al., 2005).

**Animal Groups**

A total number of 36 Wistar rats (n=6) were included in the study. They were kept in Institutional Animal House under standard conditions. All the animals received normal rat pellet diet and water ad libitum. All the animals were allowed to get acclimatized to the new environment for period of two weeks. Rats were divided into 6 groups, each group containing 6 rats.

**Flow diagram 1(a) Depicting animal groups and treatment**
Meenu Yadav et al., Effect Of Shankhpushpi (Convolvulus pluricaulis) Alone and its Combination With Omega-3 Fatty Acid On Anxiety in an Animal Model of Rats

Grouping
Albino Wistar rats weighing between 160-200 gm were randomly divided into 6 experimental groups, each group containing 6 rats (Figure: 1a).

Group I: Control - Rats were administered distilled water (1ml p.o.)
Group II: Rats were administered Shankhpushpi (Convolvulus pluricaulis) whole plant powder (100 mg/kg b.w. p.o.) (Vancassel S. et al., 2008)
Group III: Rats were administered Shankhpushpi (Convolvulus pluricaulis) whole plant powder (400 mg/kg b.w. p.o.) (Vancassel S. et al., 2008)
Group IV: Rats were administered Omega 3 fatty acids (500 mg/kg b.w. p.o.) (Malik J. et al., 2015)
Group V: Rats were administered Shankhpushpi (Convolvulus pluricaulis) whole plant powder (400 mg/kg b.w. p.o.) (Vancassel S. et al., 2008) and Omega 3 fatty acids (500 mg/kg b.w.p.o) (Malik J. et al., 2015)
Group VI: Rats were administered standard drug (Diazezapm 2 mg/kg b.w. i.p.) injection (Carlezon WA et al., 2005).

Behavioural Animal Model for Anxiety
Induction of Anxiety: Cold Exposure model was used for induction of anxiety. Cold exposure in a small refrigerator set up at 4 ± 2°C lasting for 30 min was used for inducing stress (Kumar S. et al., 2011). Rats were exposed to this environment and their behaviour was observed throughout the stress experiment along with body temperature monitoring. This sharp fall in temperature leads to a sharp increase in the levels of adrenocorticoids that leads to the development of stress response.

Behavioural animal model of rodents for anxiety was used to assess the effect of Shankhpushpi (Convolvulus pluricaulis) - CP and Omega -3 fatty acids (o-3FAs) in anxiety with the help of Elevated Plus Maze and Open Field Test.

Effect on Anxiety
By Using Elevated plus Maze test
The Elevated Plus Maze test is well established animal model for testing anxiolytic drug (Jaggi A S et al., 2011). The Elevated Plus Maze is based on the two different behaviour: the rodent tendency to explore the new environment and its aversion to light and open space. The apparatus was made of wood consisting of two open arms (40 x 10 cm) and two closed arm (50cm x 10cm x 40cm) and a central platform (10cm x 10cm), arranged in such a way that the two arms of each type were opposite to each other to give the apparatus a plus sign appearance. The entire maze was elevated to a height of 50 cm above the floor. Rats were brought to testing room 1 hour prior to test. Each rat was placed at centre of the elevated plus maze with its head facing the open arms. The dose administered schedule was so adjusted that each rats having its turn on elevated plus-maze apparatus 30 min after diazepam and 60 minutes after the oral administration of the herbal drug doses and vehicle. All the animals were received the respective drugs for 10 days of experimental period. The findings were recorded on the 10th day.

Each rat was observed for a total of 5 mins on the apparatus. Entry into an arm was defined as the point when the animal places all four paws onto that arm. The entire experiment, every precaution was taken. Room level lighting was kept consistent during test. The procedure was conducted in a quiet room. The apparatus was cleaned properly between trials to eliminate the possible bias due to the odour left by the previous animals.

Assessment: During the 5 min experiment following behaviour of the rats was recorded
- Number of entries in open arm
- Number of entries in closed arm
- Time spent in open arm
- Time spent in closed arm

The mean ± SEM of each treated group was determined and compared.

By Using Open Field Test
Developed by Calvin S. Hall. Animals such as rats and mice show a natural dislike to brightly lit areas. They also have a tendency to explore a perceived threatening stimulus. The result of these two conflicting drives is anxiety. Decreased anxiety results in increased exploratory behaviour. Increased anxiety will results in less locomotor motion and preference for the edges and corner of the field.

The two factors influence anxiety-like behaviour in the open field. The first is social isolation resulting from the physical separation from cage mates when performing the test. The second is the stress created by the brightly lit, unprotected, novel test environment.

Apparatus: Rats were transported to the test room. Open Field Test consists of wooden box of measurement of 70cm×70cm×45cm. The floor of the area was divided into 16 units by black painted lines. The entire room except the open field was kept dark during experiment. Then each rat was placed in the centre of box and was allowed to freely explore for the duration of 5 minutes of the test session.

Assessment: Behavioural aspects noted were (Pellow S. et al., 1985 and Nahata A. et al., 2009)
1. Ambulation: This was measured in form of the number of squares crossed by the animal.
2. **Rearings**: Number of times the animal stood on its hind limbs.
3. **Self-grooming**: Number of times the animal groomed facial region and licked/washed/scratched various parts of its body.
4. **Activity in center**: Number of central squares crossed by the animal.
5. **Faecal droppings**: Number of faecal droppings excreted during the period. All faecal pellets were removed and all spots of urination were wiped out. The floor and walls of the maze quadrant was sprayed with 95% ethanol and wiped down with a clean paper towel. Ethanol solution was allowed to completely dry prior to testing other rat.

On last day of behavioural study all rats were sacrificed by high dose of pentobarbitone-100mg/kg as a method of euthanasia.

**Statistical analysis**

Data were expressed as mean ± SEM. Statistical analysis was carried out by one way ANOVA followed by Dunn’s multiple comparison test. p<0.05 was considered to be statistically significant.

**RESULTS**

**Behavioural test**

**By using Elevated plus Maze (EPM) test**

In Table 1a and 1b, the effect of CP-100, CP-400, ω-3 FAs, CP-400 + ω-3 FAs and Diazepam have been summarized. CP-100 did not cause significant increase in time spent in open arm. But treatment with CP-400 showed significant increase (p<0.001) in time spent in open arm compared to control. So Shankhpushpi showed anxiolytic activity in dose dependent manner. CP-400 and ω-3 FAs showed significant (p<0.001) increase in time spent in open arm as compared to control. CP-400 + ω-3 FAs showed significant (p<0.05) increase in time spent in open arm as compared to when both of these were given alone. CP-400 + ω-3 FAs showed similar anxiolytic activity as standard treatment - Diazepam. The values have been summarized in Table – 1a, and these are also shown graphically in Figure – 2a. Treatment with Shankhpushpi (**Convolvulus pluricaulis**) also showed increase in number of entries in open arm in dose dependent manner. CP-400 and ω-3 FAs showed significant (p<0.001) increase number of entries in open arm as compared to control Group. CP-400 + ω-3 FAs showed significant increase (p<0.05) in number of entries in open arm as compared to when these drugs were given alone CP-400 & ω-3 FAs. The reference drug Diazepam significantly increased (p<0.05) the number of entries as well as the duration of stay in the open arms as compared to CP-400 & ω-3 FAs (Group - III and Group – IV), indicating anxiolytic activity. Combined treatment with CP-400 + ω-3 FAs showed anxiolytic activity which is comparable to Diazepam. The values have been summarized in Table – 1b, and these are also shown graphically in Figure – 2b.

**Table 1** Effect of Shankhpushpi (**Convolvulus pluricaulis**), omega-3 fatty acids and combination treatment on exploratory activity of the rats in Elevated Plus-Maze apparatus.

(a) Time spent in open and closed arms (b) Entries in open and closed arms

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Open arm</th>
<th>Closed arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>1.17 ± 0.17</td>
<td>3.89 ± 0.17</td>
</tr>
<tr>
<td>II</td>
<td>CP + 100mg/kg</td>
<td>1.83 ± 0.31</td>
<td>3.83 ± 0.31</td>
</tr>
<tr>
<td>III</td>
<td>CP - 400mg/kg</td>
<td>8.17 ± 0.31</td>
<td>3.34 ± 0.21</td>
</tr>
<tr>
<td>IV</td>
<td>ω-3 Fatty acid</td>
<td>7.43 ± 0.31</td>
<td>4.67 ± 0.31</td>
</tr>
<tr>
<td>V</td>
<td>CP + 400mg/kg + ω-3 FAs</td>
<td>10.78 ± 0.36</td>
<td>3.13 ± 0.27</td>
</tr>
<tr>
<td>VI</td>
<td>Diazepam</td>
<td>11.00 ± 0.32</td>
<td>3.06 ± 0.31</td>
</tr>
</tbody>
</table>

* All values are Mean ± S.E.M. (n=6)
* a: p< 0.001 as compared to control
* b: p< 0.05 as compared to group III and group IV
* One way ANOVA followed by Dunn’s multiple comparison test.
**Open field Test**

In Table-2 the effect of CP-100, CP-400, ω-3 FAs, CP-400 + ω-3 FAs and Diazepam have been summarized. CP-400 and ω-3 FAs alone showed much significant anxiolytic (p<0.001) behaviour when compared to control group. CP-100 did not show any significant effect when compared to control. CP-100 and CP-400 showed dose dependent action. CP-400 + ω-3 FA significantly increased the ambulatory activity, rearings, self-groomings and activity in center and decreased in faecal droppings as compared (p<0.05) to CP-400 & ω-3 FA when given alone. Combination of CP-400 + ω-3 FA showed approximately similar comparable effect with the Diazepam. The values have been summarized in Table – 2, and these are also shown graphically in Figure – 3a and 3b.

**Table 2** Effect of Shankhpushpi (Convolvulus pluricaulis), Omega-3 fatty acids and combination treatment on open field exploratory behaviour in rats:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Ambulation (s)</th>
<th>Rearings</th>
<th>Self-grooming</th>
<th>Activity in center</th>
<th>Faecal droppings</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>26.17 ± 1.22</td>
<td>2.17 ± 0.1</td>
<td>3.15 ± 0.03</td>
<td>2.5 ± 0.22</td>
<td>4.3 ± 0.42</td>
</tr>
<tr>
<td>II</td>
<td>CP (100 mg/kg p.o.)</td>
<td>28.5 ± 1.31</td>
<td>3.17 ± 0.03</td>
<td>4.33 ± 0.27</td>
<td>3.67 ± 0.25</td>
<td>2.5 ± 0.22</td>
</tr>
<tr>
<td>III</td>
<td>CP (400 mg/kg p.o.)</td>
<td>51.5 ± 2.45</td>
<td>6.17 ± 0.17</td>
<td>8.67 ± 0.31</td>
<td>5.5 ± 0.35</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>IV</td>
<td>ω-3 FA (500 mg/kg p.o.)</td>
<td>46.5 ± 1.54</td>
<td>5.6 ± 0.10</td>
<td>7.87 ± 0.36</td>
<td>5.1 ± 0.34</td>
<td>1.8 ± 0.26</td>
</tr>
<tr>
<td>V</td>
<td>CP (400 mg/kg p.o.) + ω-3 FA</td>
<td>68.56 ± 1.65</td>
<td>8.17 ± 0.31</td>
<td>12.05 ± 0.31</td>
<td>7.67 ± 0.31</td>
<td>0.33 ± 0.22</td>
</tr>
<tr>
<td>VI</td>
<td>Diazepam</td>
<td>69 ± 1.40</td>
<td>8.43 ± 0.21</td>
<td>12.83 ± 0.31</td>
<td>7.97 ± 0.31</td>
<td>0.34 ± 0.22</td>
</tr>
</tbody>
</table>

- All values are Mean ± S.E.M. (n=6), a: p< 0.001 as compared to control, b: p< 0.05 as compared to group III and group IV
- *: p< 0.05 as compared to group IV
- One way ANOVA followed by Dunn’s multiple comparison test

**DISCUSSION**

**Anxiety** is a feeling of discomfort, such as uneasiness or fear that can be mild or severe. Serotonin,dopamine, and norepinephrine—are disturbed in anxiety states.

Various anxiolytic drugs available for treating anxiety states are Benzodiazepines, Monoamine oxidase inhibitors, Tricyclic antidepressants (TCAs), Selective serotonin uptake inhibitors (SSRIs) and tranquilizers especially buspirone. In addition to being expensive, these medications can have side effects such as, addiction, suicidal tendency, hallucinations, insomnia, headaches, loss of motor coordination, and can impair day to day routine activities.

Now a days there is increasing trend across the globe of using herbal drugs as Complementary Alternative Medicine (CAM) especially for neurodegenerative and mental disorders and also for physical well-being. Conventional standard treatment has its own serious adverse drug reaction and potential harm in long term treatment as mentioned above. Herbal drugs, traditional medicines and nutritional therapy are safer and of better compliance. Herbal medicines cause prevention of mental illnesses, rejuvenate body system, harmonise and extend the lifespan and also safer, more affordable, easily assessable than the conventional treatment. In 1992, the WHO develop criteria and general principles to guide research work on evaluating herbal medicines (WHO, 2006).

Shankhpushpi (Convolvulus pluricaulis) proved its scientific potential in CNS depression, anxiolytic, tranquilizing, antidepressant, antistress, neurodegenerative, antiinflammatory, hypolipidemic, immunomodulatory, analgesic, antifungal, antibacterial, antidiabetic, antiulcer, anticatatonic, and cardiovascular activity (Ferri CP et al., 2005). It contains several types of alkaloids, flavanoids, and coumarins as active chemicals that bring about its biological effects (Ferri CP et al., 2005). ‘Charak Samhita’ has described Shankhpushpi as one of the best Medhya rasayanay or brain tonic ( Vas Cj et al., 2001). Omega -3 fatty acids have regulatory effects on immunomodulation, anti-inflammation, signal transduction, neurotransmission and neuroprotection which support its preventive benefits on mood and anxiety disorder.

Therefore in our study we tried to combine herbal drug CP and nutrient ω-3 FAs for evaluating their antidepressant activity.

For assessment of antianxiety, Cold Stress ( Jaggi AS et al., 2011) was given to all groups to produce stress induced anxiety model and Elevated Plus Maze and Open Field test were used to assess anxiety levels.

**Elevated Plus Maze (EPM)**

In EPM, CP-400 increased the time spent in open arms more than the control and CP-100. Similarly CP-400 also increased open arms entries more than the control and CP-100 which shows its dose dependent action. ω-3 FAs alone also increased the time spent in open arms and increase in open arms entries more than the control groups. Results of anti-anxiety effect of these drugs have shown similar beneficial responses as was found in other previous studies ( Sethiya NK et al., 2010 and Perez MA et al., 2013). Combination of CP - 400 and ω-3 FAs produced greater significant (p<0.05) increase in time spent in open arms and open arms entries than both of these when given
alone and results are comparable to Diazepam. (Table – 1a and 2b, Figure – 2a and 2b)

Open Field Test (OFT)

Anxiolytics have been shown to increase the open field ambulation, rearings, self-groomings and activity in the center and a decrease in the number of faecal droppings as a result of their administration. In OFT, CP-400 group have shown significant (p<0.001) anxiolytic effect, which was resulted in, increased in ambulation, rearings, self-grooming, activity in center, and decreased faecal droppings as compared to control. CP-100 has shown non-significant anxiolytic effect as compared to CP-400 group which represented its dose dependent action. ω-3 FAs have also shown significant (p<0.001) anxiolytic effect as compared to control in form of increase in ambulation, rearings, self-grooming, activity in center, and decreased faecal droppings as compared to control. These results showed similar beneficial response as shown in previous studies (Sethiya NK et al., 2010 and Perez MA et al., 2013). But combination of these two CP-400 and ω-3 FAs produced significant (p<0.05) increase in ambulation, rearings, self-grooming, activity in center, and decrease in faecal droppings as compared to when both of these were given alone and which was comparable to Diazepam (Table – 2, Figure – 3a and 3b)

Possible explanations and mechanisms for antianxiety effect of Convolvulus pluricaulis which has been reported in various previous studies are as follows

- It regulates the production of the stress hormones, adrenaline, and cortisol (Sethiya NK et al., 2010).
- Potential antioxidant activity can leads to beneficial effect of antianxiety. (Sethiya NK et al., 2010).
- It shows anti-stress effect by normalizing hyperglycemia, plasma corticosterone, creatinine kinase and adrenal hypertrophy that are modulated in various stress conditions (Sethiya NK et al., 2010).
- These above effects are mainly due to the presence of phenolic contents and flavonoids in Shankpushpi.

Possible mechanisms for antianxiety effect of ω-3 FAs as reported in various studies done so far are as follows

- Stress decreases docosahexaenoic acid (DHA) content in the phospholipid membranes of glutamatergic neurons at the amygdaloid complex, whereas it increases arachidonic acid (AA) content. AA is released from the phospholipid membranes to the cytoplasm by cytoplasmic phospholipase A2 (cPLA2) activity and is transformed into endocannabinoid (eCb), which in turn inhibits GABA release from presynaptic neurons (Rapoport SI, 2008 and Häring M et al. 2012). ω-3 FAs supplementation increases DHA content in the phospholipid membranes of excitatory neurons; which in turn decrease AA levels in the cytoplasm of neurons. Thus, inhibitory transmission could be reduced in the amygdala; decreasing anxiety and plasma corticosterone levels in the stressed rats.
- The anxiolytic effect of ω-3 FAs supplementation is due to increased serotonin level which has a key role in the regulation of anxiety-like behaviours.

In our present study it may be due to the combined effect of proposed mechanisms of the two i.e. Convolvulus pluricaulis and ω-3 FAs.

Combination of Convolvulus pluricaulis – whole plant powder, dose- 400mg/kg b.w. p.o.) along with Omega-3 fatty acid (500mg/kg b.w.) has not been studied previously; therefore present study with above combination is the first study of its kind to evaluate its effect in anxiety.

We analysed screening test and biochemical parameter and got result that Convolvulus pluricaulis in dose of 400 mg /kg , Omega -3 fatty acids in dose of 500 mg /kg and their combination can provide better and earlier benefits with very less side effects in learning & memory enhancement and relieving anxiety. Combination treatment is much better and which was comparable to standard drugs treatment.

CONCLUSION

The present study has been designed to evaluate the antianxiety effect of Shankpushpi (Convolvulus pluricaulis), Omega-3 fatty acids separately and of their combination. These effects were compared with the standard drug - Diazepam. Convolvulus pluricaulis has antianxiety effect in dose dependent manner. Omega-3 fatty acids (500mg/kg b.w. p.o.) also has antianxiety effect which was comparable to the effect of Convolvulus pluricaulis at dose of 400mg/kg b.w. The effect of combination Convolvulus pluricaulis (400 mg/kgbw) and Omega –3 fatty acids (-500mg/kgbw) on anxiety was significant and greater than when both drugs were given alone and comparable to standard drug – Diazepam. This anxiolytic activity of Convolvulus pluricaulis and Omega-3 fatty acids was achieved without any impairment in motor co-ordination in contrast to Diazepam which has skeletal muscle relaxant activity in antianxiety dose. The results of present study are encouraging and may reveal the importance of the herbal drugs and nutrients in anxiety states. These drugs and their combination may also be used for longer duration, in any age group of patient without causing any serious side effects.

Future Scope: Short time period and inadequate funding support are the limitation of study. The results are encouraging and reveal the importance of the herbal drugs and nutrients in anxiety states. According to the results, Convolvulus pluricaulis in dose of 400 mg /kg, Omega-3 fatty acids in dose of 500 mg/kg and their combination might have the potential usefulness in the management of anxiety states as alternative/add-on, preventive as well as therapeutic treatment. It may also be used for longer duration, in any age group of patient without causing any serious side effects.

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References


