Int J Pharma Res Health Sci. 2018; 6 (2): 2364-68
DOI:10.21276/ijprhs.2018. 02.05
Shivalinge G K P *et al.*
CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Study of Anti-anxiety Effect of Nu'Cell - A Polyherbal Formulation in Swiss Albino Mice

Ume Nishar Banu S [1], Shivalinge Gowda K P [1,*], Joyeeta Bhattacharya [1], Nataraj Loganayaki [2], Khader Shareef K S [2], Venkateswarlu K [2]

[1]Dept of Pharmacology, PES College of Pharmacy, 50 ft Road, Hanumanthanagar, Bengaluru-50 , India.
[2]Suguna Foods Pvt. Ltd., Herbal division, Suguna Lifeherbs, 169/1(P), Kottamangalam village, Tirupur Main Road, Madathukulum Taluk, Tirupur District, Tamil Nadu, India.

ARTICLE INFO

Received: 20 Feb 2018
Accepted: 07 Mar 2018

ABSTRACT

The present study is aimed to investigate the anti-anxiety activity of Nu'Cell- a polyherbal formulation using the Elevated Plus Maze (EPM) method and the Light Dark method on Swiss albino mice. The animals were randomly divided into three groups with six animals in each group. The control group, standard drug group and the test group received normal saline 0.1ml, diazepam 2 mg/kg intraperitonially and Nu'Cell 200 mg/kg orally respectively for 10 days. The antianxiety effect was assessed on 10th day. The parameters studied were (a) time spent in each arm of elevated plus maze, (b) number of entries in each arm of elevated plus maze, (c) time spent by mice in each light and dark chambers. The test group showed similar activities as the standard drug treated group by showcasing a significant increase in the time spent in open arms, increase in the open arm entries and also increase in the time spent in light chamber, when compared with the control group, suggesting decrease in fear and increased exploratory activities in mice. The study thus observed anxiolytic-like properties of Nu'Cell similar to that of diazepam. However, further studies are required to explain the exact mechanism of action of our proposed drug.

Keywords: Anxiety, Nu'Cell, Diazepam, elevated plus maze.

1. INTRODUCTION

Anxiety is defined as a subjective sense of unease, dread, or foreboding, and it is an indication to a primary psychiatric condition or can be a component of, or reaction to, a primary medical disease. The primary anxiety disorders are classified according to their duration and course and the existence and nature of precipitants. It affects one eighth of the total population worldwide. [1, 2] There is a high prevalence of

Corresponding author *
Prof Dr. Shivalinge Gowda KP
Dept of Pharmacology, PES College of Pharmacy, 50 ft Road, Hanumanthanagar, Bengaluru-50 , India.
Email: shivalinge65@gmail.com

mental and neurological disorders worldwide and these accounts for 13% of total disability adjusted life years (DALYs) lost due to all diseases and injuries in the world. Anxiety is wide spread, with lifetime prevalence ranging from 13.6 to 28.8% in western countries. Individuals aged between 10 and 25 years are at highest risk for developing an anxiety. [3] The defining features of anxiety disorders according to the "Diagnostic and Statistical Manual of Mental Disorders III edition" (DSM III-R) are symptoms of anxiety and avoidance behaviour. [4] Hyper arousal is also a frequent symptom of which is often seen in the patients with these syndromes. The patients with anxiety disorders generally exhibit two types of syndromes:

(a) Panic disorder and generalized anxiety disorder, in which anxiety is usually the primary symptom.

(b) Phobic disorders, in which anxiety is experienced as if one is confronted by the feared stimulus followed by avoidance, which is very common.

Major pharmacotherapeutic agents used for the treatment of anxiety disorders includes Selective Serotonin Reuptake Inhibitors (SSRIs) like paroxetine, Serotonine Norepinephrine Reuptake Inhibitors (SNRIs) like venlafaxine, Tricyclic antidepressants (TCAs) like imipramine and Nortriptyline, Benzodiazepine anxiolytics like diazepam and clonazepam, Non benzodiazepine anxiolytic buspirone and Anticonvulsant anxiolytics like gabapentin and tigabine. [5] Although effective treatments like anxiolytic drug therapy or cognitive behavioural therapy exist, many patients remain untreated or experience adverse effects of these synthetic drugs or they do not get benefited from full symptom control. It has been estimated that 43% of complementary therapy users are effected from anxiety hence the most popular treatments include herbal medicines. [6] Pathophysiology of anxiety includes neurobiology abnormalities of noradrenergic, serotonergic, GABAergic and glutamatergic transmission. Involvement of these systems is reflected in the efficacy of benzodiazepines, selective serotonin reuptake inhibitors as well as selective serotonin and noradrenalin reuptake inhibitors in the treatment of anxiety. [7] Since their introduction to the human race, the benzodiazepines have remained the most commonly prescribed treatment for anxiety, despite of many side-effects such as muscle relaxation, sedation, ataxia, amnesia, pharmacological dependence, and memory disturbance. Thus, to eradicate the harmful effects, researchers have been focusing on safer and effective natural anxiolytics from herbal medicine. [8]

In present era, a sudden holocaust of mental disorders, and recognition of severe side effects and addiction liabilities associated with long term administration of widely prescribed synthetic drugs have aroused the attention of researchers towards natural resources. Plants like *Valeriana officinalis,* [9] *Hypericum perforatum,* [10, 11] *Bacopa monniera,* [12] *Coriandrum sativum* [13] have been used extensively in various traditional systems of therapy because of their adaptogenic and psychotropic properties. Some herbal medicines, such as *Rhodiola rosea* and *Crocus sativus*, with mood elevating effects also display anxiolytic activity, which may be due to modulation of neurological pathways (GABAergic, serotonergic, and noradrenergic systems). [7] Medicinal plants are now becoming more widely used by people all over the world. People understand the gentle strength of these natural remedies. However, no systematic study was carried out on Nu'Cell- a polyherbal formulation for anxiolytic activity. Hence the present work is proposed to explore the anti-anxiety activity of Nu'Cell- a polyherbal formulation using the most proposed models for anxiety i.e., the Elevated Plus Maze method and the Light Dark method using Swiss albino mice.

2. METHODS

Experimental animals:

Swiss albino mice weighing 20-30g (1-2 months old) were used. All the animals were procured from, Adita Biosys Pvt Ltd, Tumakur, CPCSEA Registration No: 1868/PO/Bt/S/16/CPCSEA (with health certificate of the animals), and were maintained under controlled condition of temperature (23 ± 2^{o}C), humidity (50±5%) and 12 hour light and dark cycles. The animals were randomized into experimental and control group and housed in sanitized polypropylene cage containing sterile paddy husk as bedding. They also had free access to standard pellet as diet and water. An Ethical clearance was obtained from Institutional Animal Ethics Committee no PESCP/IAEC/34/2016 and conducted according to the Indian National Science Academy guidelines for the use and care of experimental animals.

Acute oral toxicity study [14]

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation for Development (OECD), revised draft guidelines 425. Swiss albino mice weighing between 20g to 30g were used for acute toxicity study to determine LD50 of Nu'Cell – a polyherbal formulation developed by Suguna foods and Pvt Ltd. The median dose was then selected for the complete study. Prior to dosing, animals were fasted overnight before being weighed. Following the period of fasting, the body weight of each animal was determined and the dose was calculated according to the body weight. Single animals were dosed in sequence usually at 48 h intervals. Using the default progression factor, doses were selected from the sequence as 1.75, 5.5, 17.5, 55, 175, 550 and 2000. Since no estimation of the drug's lethality was available, the dosing was initiated at 175 mg/kg till 2000 mg/kg as recommended in OECD Guidelines 425. The LD50 was calculated by the changes in the observations for the main test at 2000 mg/kg body weight for 14 days.

Elevated plus-maze (EPM) model of anxiety

The plus-maze apparatus consists of two open arms (16×5 cm) and two closed arms (16×5×12cm.) having an open

roof, with the plus-maze elevated (25 cm) from the floor, was used to observe anxiolytic behaviour in mouse. The animals were fasted 18 h prior to the experiment. Test drugs were administered orally using tuberculin syringe fitted with oral cannula. The dose administration schedule was so adjusted that each mouse was having its turn on the elevated plus-maze apparatus 45 min after the administration of the dose. Each mouse was placed at the centre of the elevated plus-maze with its head facing the open arms. During this 5 min experiment, the behaviour of the mouse was recorded as (a) preference of the animal for its first entry into the open or closed arms, (b) the number of entries into the open or closed arms, (c) average time spent by the animal in each of the arms (average time = total duration in the arms/number of entries). During the entire experiment, the animals were allowed to socialize. Every precaution was taken to ensure that no external stimuli can invoke anxiety in the animals. Similar observations were recorded for the standard group (Diazepam 2 mg/kg intraperitoneally (i.p)) as well as the control group (vehicle, 0.1 ml per os (p.o)). The number of entries into and the time spent in each of the two types of arms were counted during a 5-minute test period. The open-arm entries and open-arm time was used as an indicator of anxiety, and the number of entries into the closed arms as an indicator of the reduction of spontaneous locomotion in mice. A mouse was considered to have entered an arm when all its four paws will be on that arm. [15-17]

Eighteen mice weighing between 20-30g were divided into three groups (n=6) comprising six mice in each group.

Group I animals were treated with normal saline 0.1 ml orally for 10 days.

Group II animals were treated with standard drug diazepam at a dose of 2mg/kg i.p. for 10 days.

Group III animals were given Nu'cell at a dose of 200mg/kg p.o. for 10 days.

On 10th day, the animals were taken for the screening of anxiolytic activity.

Light Dark Test

The apparatus consists of two 20cm×10 cm×14 cm plastic boxes: one is dark and the other one is transparent. The mice were allowed to move from one box to the other through an open door between the two boxes. A 100W bulb placed 30 cm above the floor of the transparent box was the only light source in the room. A mouse was put into the light box facing the hole. The transitions between the light and the dark box and time spent in the light box were recorded for 5 minutes immediately after the mouse stepped into the dark box. The number of crossings through the partition between the light and the dark chamber were compared with total activity counts during the 5 min. [18] Eighteen mice weighing between 20-30g will be divided into three groups (n=6) comprising six mice in each group.

Group I: animals were treated with normal saline 0.1 ml orally for 10 days.

Group II: animals were treated with standard drug diazepam at a dose of 2mg/kg i.p. for 10 days.

Group III animals were given Nu'cell at a dose of 200mg/kg p.o. for 10 days.

On 10th day, after half an hour of administration of the test compounds, the animals were taken for the screening of anxiolytic activity.

3. RESULTS

The mice treated with Nu'cell have shown significant increase in time spent in the open arm (2.353±0.6416 sec) when compared to the normal control group (0.7933±0.176sec). Nu'Cell treated animals have also shown significant increase in open arm entries (9.833±1.014) when compared to the normal control (4.667±1.054). Similarly Diazepam treated groups have shown significant increase in the time spent and No. of entries in the open arm (2.443±0.457sec and 10.33±1.520 respectively) when compared to the normal control groups. Whereas, Nu'Cell and Diazepam treated groups have shown significant decrease in the time spent in the closed arm (1.098±0.3206sec and 1.533±0.2155sec respectively) when compared to the normal control group (3.013±0.4393sec). Similarly, Nu'Cell and Diazepam treated groups have shown significant decrease in closed arm entries (3.000±1.414 and 4.500±1.384) when compared to the control group (8.333±0.9189). These results support the anti-anxiety effect of Nu'cell.

Table 1: Table showing anti-anxiety effect of Nu'Cell a polyherbal formulation by EPM test

Gp	Groups	Time spent in each arm in seconds		No. of entries in each arm	
		Open	Close	Open	Close
I	(Normal control)	0.7933±0.176	3.013±0.4393	4.667±1.054	8.333±0.9189
II	(Diazepam)	2.443±0.457**	1.533±0.2155**	10.33±1.520**	4.500±1.384**
III	(Nu'Cell)	2.353±0.6416**	1.098±0.3206**	9.833±1.014**	3.000±1.414**

Each bar represents the Mean ± SEM (n = 6) done by one-way ANOVA followed by Bonferroni method of Statistics. **P < 0.05 when compared to normal control group.

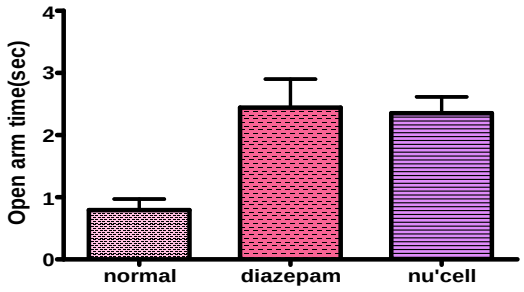

Fig 1: Effect of Nu'Cell on time spent in open arm

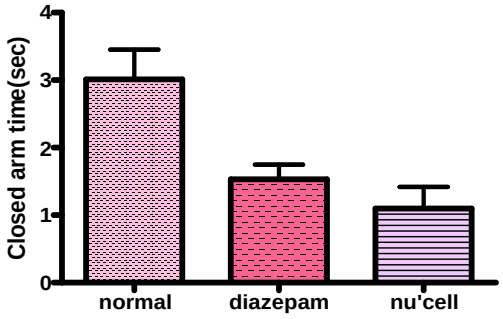

Fig 2: Effect of Nu'Cell on time spent in closed arm

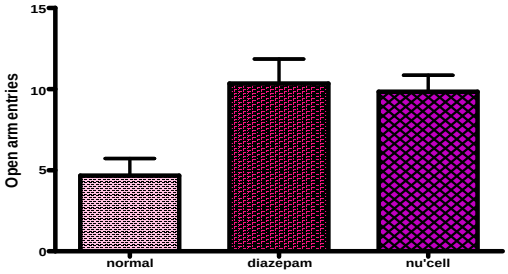

Fig 3: Effect of Nu'Cell on open arm

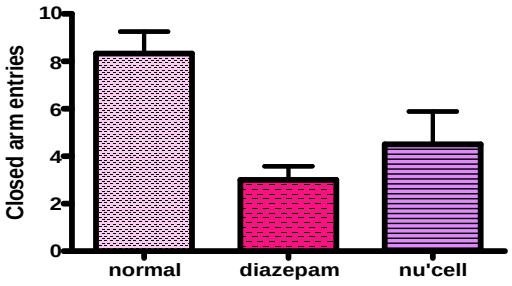

Fig 4: Effect of Nu'Cell on closed arm

The Nu'Cell and Diazepam treated groups have shown significant increase in the time spent in the light box (2.448±0.3208 and 2.560±0.5196 respectively) when compared to the normal control group (0.9100±0.1884). Whereas, the Diazepam treated group shows significant decrease in the time spent in dark box (1.868 ± 0.634) and Nu'Cell treated group were found non-significant (2.662 ± 0.310) when compared to the control group (3.620 ± 0.340). These results showed the anti-anxiety effect of Nu'Cell.

Table 2: Table showing anti-anxiety effect of Nu'Cell by Light Dark model

Gp	Group Name	Time spent in each box in seconds	
		Light	Dark
I	Normal control	0.9100±0.1884	3.620 ± 0.340
II	Diazepam	2.560±0.5196*	1.868 ± 0.634*
III	Nu'Cell	2.448±0.3208*	2.662 ± 0.310^{ns}

Each bar represents the Mean ± SEM (n = 6) done by one-way ANOVA followed by Bonferroni method of Statistics. *P < 0.05 when compared to normal control group. Ns: Not Significant

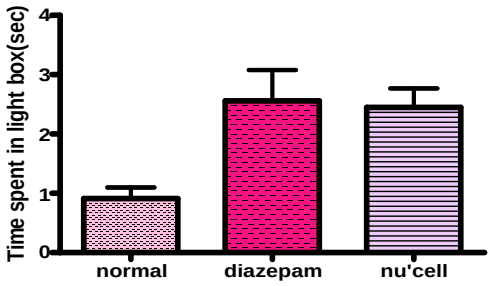

Fig 5: Effect of Nu'Cell on time spent in light box.

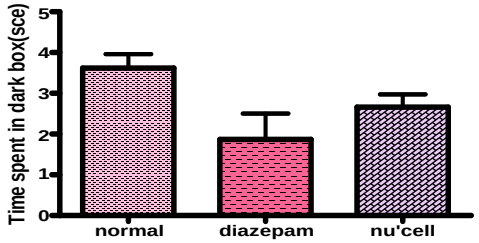

Fig 6: Effect of Nu'Cell on time spent in dark box

4. DISCUSSION [19, 20]

The elevated plus maze is currently one of the most widely used models of animal anxiety and has been validated for use with both rats and mice. In the elevated plus maze, the open arms are more fear provoking than the closed arms. The number of entries and time spent in open arms to closed arms reflects the safety of closed arms with relative fearfulness of open arms. The reduction in entry and time spent in open arm are the indications of high level of fear or anxiety. Anxiolytic drugs increase the proportion of entries and time spent in open arm. In the light and dark box model, the brightly lit environment is a noxious environment stressor that inhibits the exploratory behaviour of animals. Reduction in the time spent in the light chamber was regarded as the marker of anxiety. In the present study, animals that received diazepam showed a significant increase in the time spent in open arms and the number of open arm entries. They showed a decrease in time spent and number of entries in closed arms of elevated plus maze. The animals also showed an increase in the time spent in light chamber in the light and dark arena paradigm. All these suggest decreased fear, an increased exploratory behaviour and the behavioural dis-inhibitory effect of the standard anxiolytic. The test compound, Nu'Cell a polyherbal formulation increased the time spent and in open arms and also increased the open arm entries in the elevated plus maze model. The anti-anxiety effects of Nu'Cell in the elevated plus maze were comparable with those following the administration of diazepam and compared with normal saline treated. In light and dark model, the test drug significantly increased the time spent in light chamber, when compared with normal saline treated suggesting decreased

fear, and increased exploratory behaviour of the animal. As the test drug possesses anxiolytic–like effect similar to that of diazepam and like diazepam, Nu'Cell may also act through the GABA receptors.

5. CONCLUSION

The present study demonstrates the anxiolytic activity of Nu'Cell at a dose of 200 mg/kg as comparable to diazepam. The mice treated with Nu'Cell in EPM model showed reduction in the time spent and the entries in the closed arm. It was also observed that the mice have explored for a greater period of time in the open arm, which indicated the anti- anxiety potential of the Nu'Cell. Similar results were also obtained from the Light Dark model, where the animals treated with Nu'Cell spent maximum amount of time in the light box compared to the dark box. Further studies are required to elucidate the exact mechanism of action of Nu'Cell in treatment of anxiety. This research work was aimed at finding the anxiolytic potential of Nu'Cell cell developed by Suguna Foods Pvt Ltd, Coimbatore and has provided us with a deep insight for its use in treating anxiety disorders. However, further studies confirming its potential is certainly warranted.

6. REFERENCES

1. Dipiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM. Pharmacotherapy A Pathophysiologic Approach. VII ed: The McGraw-Hill Companies; 2008.
2. Hwa Y, Jeong P, Jin H, Hwan S, Sukjil S, Bang H. Anxiolytic - like effects of Gensinosides on the Elevated Plus-Maze Model. Prog Neuro Psychopharmacology Biol Psychiatry. 2005; 28:1621-25.
3. Maribel R, Rube R, Alejaedro Z, Jaime T. Flavonoids from *Tilia americana* with anxiolytic activity in plus maze test. J Ethnopharmacol. 2008; 118:312-7.
4. American Psychiatric Association. Diagnosis and Statistical manual of mental disorders. 4th ed. Washington. American Psychiatric Association. 2000; P. 429-84.
5. Grundmanna O, Nakajimab J-I, Kamatab K, Seob S, Butterwecka V. Kaempferol from the leaves of *Apocynum venetum* anxiolytic activities in the Elevated plus maze test in mice. Phytomedicine. 2009; 16: 295-302.
6. Emst E. Herbal remedies for anxiety a systemic review of controlled clinical trials. Phytomedicine. 2006; 13: 205-8.
7. Kourosh Saki, Mahmoud Bahmani, Mahmoud Rafieian-Kopaei. The effect of most important medicinal plants on two important psychiatric disorders (anxiety and depression)-a review. Asian Pac J Trop Med. 2014; 7(1): 34-42.
8. Ying Cui, Chunlei Rong, Junming Wang, Can Cui, Li Wang, Zhiyi Feng, *et al*. Mechanism-based anti-anxiety effects of polysaccharides extracted from Shudihuang (*Radix Rehmanniae* Preparata) by two-dimensional electrophoresis analysis in rat hippocampus proteins. J Tradit Chin Med. 2013; 33(4): 524-30.
9. Leathwood PD and Chauffard F. Aqueous extract of *Valerian* reduces latency to fall asleep in man. Planta Medica. 1995; 51: 144-8.
10. Sanchez-Mateo CC, Bonkanka CX, Prado B, Rabanal RM. Antidepressant activity of some *Hypericum reflexum* L. fil. Extracts in the forced swimming test in mice. J Ethanopharmacol. 2007; 112: 115-21.
11. Caccia S. Antidepressant like components of *Hypericum perforatum* extracts. An overview of their pharmacokinetics and metabolism. Curr Drug Metab. 2005; 6: 531-43.
12. Sairam K, Dorababu M, Goel RK, Bhattacharaya SK. Antidepressant activity of standardized extract of *Bacopa monniera* in experimental models of depression in rats. Phytomedicine. 2002; 9: 207-11.
13. Emamghoreishi M, Khasaki M, Aazam MF. *Corainder sativam*. Evaluation of its anxiolytic effect in the elevated plus maze. J Ethano Pharmacol. 2005; 96: 365-70.
14. Swapnil S. Khadke, Deshbandhu R. Pachauri, Swapnil D. Mahajan. An acute oral toxicity study of *Gnidia glauca* (Fresen.) Gilg. in albino rats as per OECD Guideline 425. IJPRIF. 2011; 2(3):787-91.
15. Kamaldeep Dhawan, Suresh Kumar, Anupam Sharma. Anti-anxiety studies on extracts of *Passiflora incarnata* Linneaus. J of Ethnopharmacol. 2001; 78: 165–70.
16. Shyamjith Manikkoth, Chandrashekar R, Rao SN. Anti-anxiety effect of ethanolic extract of leaves of *Tylophora indica*. IJRAP. 2013; 4(1):127-9.
17. Alireza Komaki, Faeghe Hoseini, Siamak Shahidi, Negar Baharlouei. Study of the effect of extract of *Thymus vulgaris* on anxiety in male rats. JTCM. 2015; 1-5.
18. Mrunal. S, Clement Atlee. W, S.R.S Ashok_Bharathi and Mohamed Farook. Antianxiety effect of methanolic extract of *Bauhinia racemose* (Lamk) stem bark in mice. IJPBS. 2011; 2(2): 217-24.
19. Van der poel AM. A note on "stretched att ention", A behavioral element indicative of an approach-avoidance conflict in rats. Animal Behav. 1979; 27:446-50.
20. Bhagwat V, Mukta N, Shoeb A, Maskeri R, Venkatesh V, Rai A. Evaluation of anxiolytic activity of vanillin in wistar albino rats. IJNPND. 2013; 3(2):96-101.

Conflict of Interest: None

Source of Funding: Nil