Research Article

Effect of Jessica (A Polyherbal Formulation) on the Levels of Neurotransmitters in the Brain of Rats

Renuka Tejasvi. Pusa[*1], D. Satyavati[2], Rajaneekar Dasari[3]
[1]Teegala Krishna Reddy college of Pharmacy, Meerpet, Hyderabad, Andhra Pradesh, India.
[2]Sree Dattha Institute of Pharmacy, Sheriguda, Ibrahimpatnam, Hyderabad, Andhra Pradesh, India.
[3]Malla Reddy Institute of Pharmaceutical Sciences, Maisammaguda, Doolapally, Andhra Pradesh, India.
***Corresponding author's E-mail:** teju.pusa@gmail.com

Accepted on: 06-11-2013; **Finalized on:** 31-01-2014.

ABSTRACT

The aim of the present study was to evaluate the mechanism of action of polyherbal formulation-Jessica for its anxiolytic activity. Anxiety is caused due to imbalance between excitatory and inhibitory neurotransmitters. The present study is to investigate the effect of Jessica on biogenic amines such as Nor adrenaline (NA), dopamine (DA), Serotonin (5-HT) and Gamma amino butyric acid (GABA), In the brain of swiss albino rats after 11 days oral treatment. In the Ayurveda the use of *Embilica officinalis, Nardostachys jatamansi* and *Rauwolfia serpentine* has been reported of have effect on CNS, Polyherbal formulation-Jessica has been made consisting of these herbs to have an potent synergistic anxiolytic .Results showed that Jessica (100 mg/kg and 200mg/kg) has significantly (P<0.01) decreased the levels of NA, DA and Jessica100 and 200mg/kg has significantly (P<0.05) and(P<0.01) decreased the levels of 5-HT respectively in the subcortical region (including the striatum). Jessica has also significantly (P<0.01) increased the level of GABA in the frontal cortex of the rat brain after 11 days treatment, When compared to control group. The results were similar to Standard drug Diazepam (1mg and 2 mg/kg) and polyherbal standard Streswin (100 and 200 mg/kg). Hence it is revealed that Jessica had a potent anxiolytic activity.

Keywords: Anxiety, Dopamine, GABA, Nor adrenaline, Polyherbal Formulation, Serotonin.

INTRODUCTION

Anxiety has been conceptualized as a frequent and serious disorder affecting the world's population, independently of ethnicity and is considered a cardinal symptom of many psychiatric disorders. It affects one- eighth of total population of the world and became a very important area of research interest in psychopharmacology during this decade.[1] Anxiety disorders lead to huge suffering and disability, where there is an associated avoidance behavior and agrophobia can male markedly disrupt family life as well as the life of sufferer. They are common with life time prevalence and often start at childhood and/or adolescence and can have negative effect on all aspects of later life.[2,3]

Anxiety disorders including generalized anxiety disorder (GAD), Specific and social Phobias, Post traumatic stress disorder (PSTD), Obsessive compulsive and panic disorder are typically treated with medications that target Gamma amino butyric acid (GABA) or Serotonergic system and modulate the overall effect.[4] Benzodiazepines and selective Serotonin reuptake inhibitors and β- blockers are most widely prescribed treatment for these disorders. Some forms of anxiety are relatively resistant to treatment with these agents.[5] There are many side effects such as sedation, memory impairment, potential for substance abuse and withdrawal syndrome, sexual dysfunction and weight gain.[6] Non compliance with these pharmacological agents remains a problem leading to increased risk of relapse. Patients with anxiety disorder also show sign of abnormal contextual conditioning. Clinical levels of anxiety may show stronger relationships with innately threatening stimuli. GABA, 5HT and NA have been implicated in putative pathophysiology of anxiety and patients with generalized anxiety disorder demonstrate dysregulation of these neurotransmitters Therefore it has become increasingly apparent that alternative treatment strategies are needed.

The concept of "Reverse Pharmacognosy" is widely used in Ayurveda to identify the drug candidates from a large scale and to validate its clinical efficiency.[7,8] Till now natural products evolved from medicinal plants have provided numerous clinically useful drugs. Four billion people or about 80% of the world population use herbal medicines as an alternative medicine.[9] The numerous herbs like *Withiana somnifera, Ocimum sanctum, Nardostachys jatamansi, Ahiphenam Papaver somniferum* etc.[7] have been used as a component of herbal anxiolytics. Many Ayurvedic Practitioners prefer polyherbal formulations rather than monoherbal formulation due to their synergistic action and good therapeutic efficacy. The various polyherbal drugs prescribed by the practioners are Kava Calm, Calm ezz, Relax and Sleep, Amazing and Natur all calm have active ingredient as St John's wort, Piper methysticum, Withania somnifera, Passiflora etc. The aim of the present study is to evaluate the Polyherbal formulation 'Jessica' for its anxiolytic activity as so far there is no literature available for it. 'Jessica' was developed by- IMIS Pharmaceuticals Pvt Ltd, Vijayawada and each 100 mg of Jessica consists of *Embilica officinalis*-7.6 mg, *Nardostachys jatamansi*-77mg, and *Rauwolfia serpentine*-15.4mg. In our previous study Jessica was evaluated for its anxiolytic effect on rats

by using various behavioral models such as Despair swim test (DST), Elevated plus maze (EPZ), actophotometer and Rota rod apparatus. The results obtained in the study showed that Jessica has significantly decreased the immobility time in DST increased the time spent and number of entries in open arm in EPZ, Decreased the locomotion in actophotometer and decreased the time spent on the revolving rod in the rota rod apparatus, when compared to control. But, the effect was similar to that of Diazepam, a benzodiazepine- anxiolytic drug and streswin, a standard polyherbal formulation[10]. In the present study the levels of brain biogenic amines such NA, DA, 5HT and GABA levels are determined to correlate them with anxiolytic effect of the drugs.

MATERIALS AND METHODS

Drugs and Chemicals

Jessica (IMIS Pharmaceuticals Pvt Ltd, Vijayawada). Diazepam (Ranbaxy Laboratories Ltd, New Delhi, India). Streswin (Siddhayu Ayurvedic Research Foundation Pvt.Ltd, India). All the drugs were suspended in 2% gumacacia (S.d fine-chem limited, India) and were given orally. GABA standard- Lobal chemie, India. Dopamine standard- systacare remedies, India. Norepinephrine – Neon laboratories limited, India. Sertonin-Srigen life science Pvt Ltd, India.

Test animals

Female swiss albino mice weighing from (18-21g) are used for acute toxicity studies. Swiss albino rats of either sex weighing from (180-210g) are used for behavioral studies. The animals were procured from Albino Research and Training Center, Hyderabad. They were housed in groups of four animals per cage and were maintained on a 12:12 hour light/dark cycle at an ambient temperature of 25±2°C. The study protocol was approved by Institutional animal ethics committee, Teegala Krishna Reddy College of Pharmacy, Hyderabad. The animals were acclimatized for one week and were fed on standard laboratory animal fed and water *ad libitum.* Care of animals was taken as per the guidelines of CPCSEA, Department of animal welfare and government of India.

Acute toxicity studies

The procedure for acute toxicity was followed according to OECD guidelines[11] (Organization of economic co-operation and development)423 (Acute toxic class method) animals were observed for 3 hours at 30 min time interval for signs of behavioral, neurological, toxicity and mortality for 24 hrs. For acute toxicity studies mice were divided into five groups, each group consisting of three animals. Each group was administered with vehicle and test drug suspended in 2% gum acacia at a dose of 200mg/kg, 400mg/kg, 800mg/kg, and 1000mg/kg respectively by intraperitonial route.[12]

Female swiss albino mice weighting about 18-22 g were selected and the test drug is administered to mice in the doses as mentioned above. The animals were observed for 3-4 hrs after administration of the drug and up to 14 days to asses toxicity. The mice were observed for behavioral, neurological and autonomic activities before and after drug administration. The onset and signs of toxicity, the overnight mortality were recorded as it indicates toxicity. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on acute toxicity of the test substance. Results allow a substance to be ranked and classified according to the Globally Harmonized system (GHS). It is observed that there is no mortality up to 1000mg/kg body weight and there are no signs of toxicity. Hence, 1/10th and 1/5th (100mg/kg and 200 mg/kg) doses of Jessica were selected to carryout anxiolytic activity.[10]

Experimental Protocol

All the experiments were carried out between 9.00-14.00hrs in a dimly illuminated room with 40W fluorescent bulb at a temperature of 25±2°C. Treatment was given for 11 days on the last day of the treatment the animals were sacrificed by cervical dislocation 60 minutes after drug administration and NA,DA,5HT,GABA levels were estimated by using spectroflourimeter.

Grouping and dosage

For behavioral studies Swiss albino rats were divided into seven groups each group consisting of six animals. The treatment to different groups was given as follows. All the drugs/ vehicle were given orally.[10]

Group I- control group-vehicle (2% gum acacia)

Group II- diazepam low dose- 1mg/kg

Group III- diazepam high dose -2mg/kg

Group IV- Streswin- 100mg/kg

Group V- Streswin- 200mg/kg

Group VI- Jessica- 100mg/kg

Group VII-jessica-200mg/kg

Biochemical Estimation

Preparation of tissue

The animals from each group were killed by cervical dislocation 60 minutes after drug administration on 11th day of treatment. The animals were decapitated and the whole brains were dissected out, weighed and kept on ice for further processing.[13]

For Nor-adrenaline, Dopamine and Serotonin

Preparation of Reagents

1. HCl-Butanol solution: 0.85 ml of 37% hydrochloric acid dissolved in 1 liter n-butanol.
2. Heptane
3. 0.1M HCl: 0.85 ml conc. HCl to 100ml H_2O

Sample Preparation

From the whole brain the sub cortical region (including striatum) was separated. Weight of wet tissue was taken and was homogenized in 5-6 ml of HCl n-Butanol for about 2 minutes. The homogenate was then subjected to cold centrifuged for 10 min at 2000rpm. A 4 ml of aliquot supernatant phase was taken and was added to 20 ml capacity of centrifugal tube containing 10 ml of heptane and 1.24ml HCl of 0.1M and was vigorously shacked. Then the contents were again centrifuged under same conditions as above in order to separate the organic and aqueous phase. The overlying organic phase was discarded and aqueous phase of 1.3 ml was then taken in each of the two test-tubes for estimation of DA, NA and 5HT. All the steps were carried out at 0^{o}c. (Note: Amount of HCl butanol is taken depending on the weight of the wet tissue i.e for 1.5-5mg of wet tissue 0.1ml of HCl butanol is taken hence in correlation to this about 5-6ml of HCl-butanol is used this is to get adequate amount of supernatant liquid for analysis.[14]

Estimation of Nor-adrenaline and Dopamine [13, 14]

Preparation of Reagents

1. 0.4 M HCl: 3.4ml conc.HCl to 100ml H_2O.
2. Sodium acetate buffer (pH-6.9): 2.88ml of 1M acetic acid (5.7ml of Glacial acetic acid to100 ml distilled water) + 27.33ml of 0.3M sodium acetate (4.08g of sodium acetate to 100ml distilled water) and volume is made up to 100ml with distilled water. pH is adjusted with sodium hydroxide solution
3. 5M NaOH: 20 g of sodium hydroxide pellets dissolved in distilled water and volume is made up to 100 ml with distilled water.
4. 0.1 M Iodine solution (in Ethanol): 4g of pot. Iodide+ 2.6 g of iodine dissolved in ethanol volume is made up to 100ml.
5. Na_2SO_3 solution: 0.5g Na_2SO_3 in 2 ml H_2O + 18 ml 5 M NaOH.
6. 10M Acetic acid: 57 ml of glacial acetic acid dissolve in distilled water up to 100ml.

Procedure

To the 0.8 ml of aqueous phase, 0.2ml 0.4M HCl and 0.4 ml of Sodium acetate buffer (pH 6.9) were added, followed by 0.4 ml iodine solution (0.1M ethanol) for oxidation. The reaction was stopped after 2 min by addition of 0.4 ml Na_2SO_3 solution. 0.4ml acetic acid is added after 15 min. The solution was then heated to 100^{o}C for 6 min when the sample again reached room temperature, excitation and emission spectra were read from the spectroflourimeter. The readings were taken at 330-375 nm for dopamine and 395-485 nm for nor-adrenaline.

Estimation of serotonin

Preparation of reagents

1. O-phthaldialdehyde (OPT) reagent: (20 mg in 100 ml conc. HCl)

Procedure

To 1.2 ml aqueous extract 1.5 ml of OPT reagent was added. The fluorophore was developed by heating to 100^{o}C for 10 min. When the samples reached equilibrium with the ambient temperature, readings were taken at 360-470nm in the spectroflourimeter. Tissue blanks for dopamine and nor-adrenaline were prepared by adding the reagents of oxidation step in reverse order (sodium sulphite before iodine). For serotonin tissue blank 1.5 ml conc. HCl without OPT was added.

Internal standard: (500µg/ml each of noradrenaline, dopamine and serotonin are prepared in distilled water:HCl-butanol in 1:2 ratio.

Estimation of brain GABA content [15]

Preparation of reagents

1. *Preparation of 10% (w/v) Trichloroacetic acid (TCA)* Prepare 100% (w/v) solution by dissolving 2.2g of TCA in 1 ml water then to prepare 10% solution by adding 9 ml water to 100%(w/v) of trichloroacetic acid.
2. *Preparation of 0.5M corbonate-bicorbonate 1 buffer (pH 9.95)*

Step 1: Preparation of Solution A (0.5M sodium carbonate) Dissolve 5.3 g of sodium carbonate in 100 ml water.

Step 2: Preparation of Solution B (0.5M sodium bicarbonate) Dissolve 4.2 g of sodium bicarbonate in 100 ml water.

Step 3: Add 30 ml of solution A to 70 ml of solution B

3. *Preparation of copper tartarate reagent*

Step 1: Preparation of solution A (copper II sulphate), 7 grams cupric sulfate pentahydrate in 100 ml distill water containing just two drops of dilute sulfuric acid**.**

Step 2: Preparation of solution B aqueous (Potassium sodium tartarate) also known as Rochelle salt and a strong alkali (commonly sodium hydroxide) 35g of potassium tartarate and 12g of Na-OH in 100ml if distilled water.

Step 3: Equal volumes of the two mixtures are mixed to get the final solution, which is a deep blue color.

Procedure

The gamma amino butyric acid content in brain was estimated according to Lowe et al., (1958). The separated forebrain region was blotted, weighed and placed in 5ml ice- cold trichloroacetic acid (10% w/v), then homogenized and centrifuged at 10,000 rpm for 10 min at 0^{o}C. A sample (0.1 ml) of tissue extract was placed in 0.2

ml of 0.14M ninhydrin solution in 0.5M carbonate-bicarbonate 1 buffer (pH 9.95), kept in a water bath at 60°C for 30 min, then cooled and treated with 5 ml of copper tartarate reagent (0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid). After 10 min fluorescence at 377/455 nm in a spectroflourimeter was recorded for the resulting compound formed due to reaction of GABA with ninhydrin (alkaline medium) in the presence of glutamate.

(Internal standard: For different concentration 20,40,60,80 and 100µg of GABA is mixed with 1.5µM glutamic acid were dissolved in 0.1ml 10% trichloroacetic acid (w/v).[16]

Statistical analysis

All the data obtained where subjected to statistical analysis by using Instat graph pad version 3.05 and are expressed as Mean ± SEM .The statistical comparison were made by Dunnett's test P values less than 0.05 were considered as significant. All the activities of Jessica, Diazepam, streswin and control were analyzed by one way analysis of variance (ANOVA).

RESULTS

Pre treatment with Jessica (100 and 200mg/kg) for 10 days had significantly decreased the levels of nor adrenaline, dopamine and serotonin in sub cortical region (including striatum) and significantly increased the GABA levels in the forebrain when compared to control group. Similar effect was observed with diazepam (1and 2 mg/kg) and streswin (100 and 200mg/kg). However, the effect of 100 and 200 mg/kg of Jessica was less than (1and 2 mg/kg) of diazepam and (100 and 200 mg/kg) of streswin. All the values exhibit significance of p<0.01 in case of serotonin Jessica at a dose of 100mg/kg showed a significance p<0.05. The values are given in table 1 and figure 1.

Table 1: Results showing the level of neurotransmitters in the brain

Group	Dose	Dopamine (ng/g of wet issue)	Noradrenaline (ng/g of wet tissue)	Serotonin (ng/g of wet tissue)	GABA (ng/g of wet tissue)
Control	2% gum acacia	391.33 ± 3.22	284.5 ± 3.22	158 ± 3.215	547.66 ± 3.127
Diazepam	1 mg/kg	299.16 ± 2.301**	257 ± 2.490**	83.83 ± 3.554	1098.33 ±2.906**
Diazepam	2 mg/kg	204.33 ± 2.092**	213 ± 2.113**	64.83 ± 2.358**	1528.33 ±2.871**
Streswin	100 mg/kg	332.83 ± 2.613**	268.16 ± 3.188**	128.66 ± 3.432**	834.8 ± 1.887**
Streswin	200 mg/kg	317.83 ± 2.358**	229.16 ± 2.272**	103.3 ± 4.731**	966.8 ± 2.182**
Jessica	100 mg/kg	359.66 ± 2.552**	263 ± 2.155**	145.3 ± 4.761*	653.33 ± 1.926**
Jessica	200 mg/kg	327.5 ± 2.045**	234.5 ± 3.394**	134.66 ± 7.18**	786.5 ± 3.739**

Values are expressed as mean±SEM from 6 rats. P<0.01 ** and P<0.05 * as compared to control group

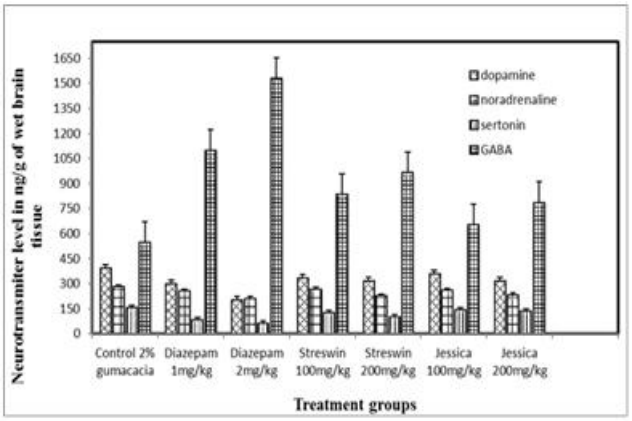

Figure 1: Results showing the level of neurotransmitters in the brain

DISCUSSION

From the previous data obtained from the animal and human experimentation, which defined the key role in the transmitters in the pathology of anxiety.

GABA appears to play an important role in the pathogenesis of several neuropsychiatric disorders. GABA is the major inhibitory neurotransmitter which counterbalances the action of the excitatory neurotransmitters.[17] Most of the traditional agents used to treat psychiatric disorders show their effect, by enhancing GABA activity, while some of the newer agents may exert their therapeutic effects exclusively via GABAergic system associated with benzodiazepine receptor and modulate GABA levels. In our present study after 11 days treatment with 100 and 200 mg/kg of Jessica showed significant enhancement of GABA levels in frontal cortex region of rat's brain,[18] when compared to control group. The effect was similar to Diazepam (1 and 2 mg/kg) and streswin (100 and 200mg/kg).

Another major neurotransmitter involved in the pathophysiology of anxiety is serotonin (5HT). Evidence which implicate the role of serotonergic system with anxiety suggests that increased brain 5-HT activity causes anxiety.[19] Whereas, 5-HT1A receptor agonists induced anxiolytic effect. The major clinically used anxiolytic drug (benzodiazepines) act on dorsal raphe nuclei and reduces their rate of firing there by selectively decreases the 5-HT levels. Evidence proposed the involvement of increased serotonin release in frontal cortex and amygdala in generalized anxiety disorder. Stress associated anxiety and altered cognitive performance is due to increase in the levels of 5-HT concentrations in frontal cortex, brain

stem, hypothalamus and hippocampus, due to chronic stress. Anxiolytic activity was suppressed upon local microinjection of nicotine into the dorsal hippocampus, it gives the evidence that anxiolytic response is due to suppressing the serotonin release in hippocampus.[20] In our present study after 11 days treatment with 100 and 200 mg/kg of Jessica has showed significant reduction of serotonin levels in sub cortical region (including striatum) of rat's brain, when compared to control group. The effect was similar to Diazepam (1 and 2 mg/kg) and streswin (100 and 200mg/kg).

It has been earlier reported that, Amygdala plays an important role in acquisition, consolidation and retention or expression of fear.[22] It is observed that there is a sustain and significant increase in nor-epinephrine release in frontal cortex and in several other regions of brain, such as hypothalamus, amygdala and locus coeruleus, due to some of the naturalistic stimuli such as exposure to novel cage, cause and variety of stressful events, including emotional stress.[21] This gives the evidence that the negative stimuli such as anxiety or fear are caused due to an increase in the levels of nor adrenaline, Most of the drugs such as β-blockers which attenuate the increase of nor adrenaline are clinically used as anxiolytic. Dopamine may exert either inhibitory or excitatory activity in the extra pyramidal centers. Evidence obtained in different species suggest that dopamine predominantly exhibits inhibitory activity on single neurons of the brain. Dopamine plays a crucial role in the pathogenesis of anxiety. As, it has an effect on trafficking of impulses between the basolateral (BLA) and central nuclei (CeA) of amygdala by modulating a cortical brake which the medial prefrontal cortex exerts on the anxiogenic output of the amygdale.[22] In most of the researches it is found that Intra-amygdaloid infusion of D1 agonists and antagonists elicits anxiogenic and anxiolytic effects respectively on conditioned and non-conditioned models of fear/anxiety suggesting an anxiogenic role for D1 receptors in amygdala. Anxiolytic drug buspirone exerts its effect by blocking dopamine receptor located in the nerve terminals. The effects of D2 agonists and antagonists depending on the nature of the threat the animal experiences in anxiety models.[23] D1 receptors control the trafficking of impulse from cortical and BLA regions to BLA and CeA nuclei respectively and help in danger recognition facilitating conditioned–unconditioned associations by the retrieval of the affective properties of the unconditioned stimuli, whereas D_2 receptors help to cope with aversive environmental stimulus. In our present study after 11 days treatment with 100 and 200 mg/kg of Jessica had shown significant reduction of Nor-epinephrine and dopamine levels in sub cortical region (including striatum) of rat's brain, when compared to control group.

Hence, the possible mechanism involved in the anxiolytic effect of Jessica is by reducing the neuronal firing of the serotonergic system by acting on presynaptic 5-HT_{1A} and by increasing the GABAergic activity by acting on GABA-A. Therefore reducing the neuronal firing of the adrenergic and dopaminergic system by acting on the β and D_1 receptors of the brain respectively.

CONCLUSION

The results suggest the influence of Jessica on the levels of GABA, NA, DA and 5HT there by suggesting Jessica as a potent anxiolytic drug having benzodiazepine agonist like action. However In present study the role of other neurotransmitters, such as histamine, acetylcholine, and peptides, is not studied Therefore further study can be carried out to know the effect of the drug on these neurotransmitters and on the memory.

Acknowledgement: We would like to thank like to thank Management and staff of Teegala Krishna Reddy College of Pharmacy, Meerpet and Malla Reddy Institute of Pharmaceutical Sciences, Maisammaguda, Doolapally for their support and encouragement throughout the research work and IMIS Pharmaceuticals Pvt Ltd, Vijayawada for providing the free sample of Jessica.

REFERENCES

1. Douglas H, Lamb. Speech anxiety: Towards a theoretical conceptualization and preliminary scale development. Communication monographs, 39(1), 1972, 62-67.
2. Murphy JM, Trends in depression and anxiety; men and women, Acta psychiattrscand, 73(2), 1986, 113-127.
3. Twenge JM, The age of anxiety? Birth Cohort change in anxiety and neuroticism, 1952-1993, Journal of Personality and social Psychology, 79(6), 2000, 1007-1021.
4. Robins LN Regier DAeds, Psychiatric disorder in America. The epidermologic catchment area study, New York The free press, 1991.
5. Sandhya S, Vinod KR, Sravan Kumar, Herbs used for brain disorders Hygeia, Journal for drugs and medicine, 2 (1), 2010, 38-45.
6. KD Tripathi, Jaypee Brothers Medical Publishers (P) Ltd, Essentials of medical Pharmacology, New Delhi, 6th ed, 2008.
7. Jonathan Klemens, B.s Bro, RPh, FAAIM, Herbs used for psychotropic or behavior modifying activity. The online journal of the American Association of integrative medicine, 2006. www.aaimedicine.com/jaaim/sep06/Herbs.pdf
8. Quoc-Tuan DO, Phillippee Bernard, Reverse Pharmacognosy: a new concept for accelerating natural drug discovery, Advances in Phytomedicine, 2, 2006, 1-20.
9. HN Gopala Krishna, RB Sangha, N Misra, MRSM Pai, Anti anxiety activity of NR-ANX-C, a polyherbal preparation in rats, 38(5), 2006, 330-335.
10. P. Renuka Tejasvi, D. Satyavati, Sk. Arifa Begum, Anxiolytic activity of Jessica- A Polyherbal Formulation. International Journal of Pharmacy and Pharmaceutical Science, 5(4), 2013, 384-388.
11. OECD guidelines for testing of chemicals, Acute oral toxicity- Acute toxic class method, 423.

12. Roll R, Hofer-Bosse T, Kayser D, New Perspectives in Acute Toxicity Testing of Chemicals, Toxicol. Lett. Suppl., 31, 1986, 86.

13. Dilip Kumar Pal, Determination of Brain Biogenic Amines in Cynodon Dactylon Pers. and Cyperus Rotundus L. Treated Mice, International Journal Of Pharmacy And Pharmaceutical Sciences, 1(1), 2009, 190-197.

14. Schlumpf M, Lictensteiger W, Langemann H, Waser PG, Hefti F, A Fluorimetric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine in milligram amounts of brain tissue, Biochemical Pharmacology, 2(34), 1974, 2337-2346.

15. Lowe IP, Robins E, Eyerman GS, The fluorimetric measurement of glutamic decarboxylase measurement and its distribution in the brain, Journal of neurochemistry, 5(4), 1958, 8-18.

16. GS Taiwe, E Ngo Bum, T Dimo, E Talla, N Weiss, A Dawe, FCO Moto, N Sidiki. PD Dzeufiet, M De Waard, Antidepressant, Myorelaxant and antianxiety like Effects of Nauclea Latifolia Smith (Rubiaceae) Roots Extracts in Murine Models, International Journal of Pharmacology, 6(4), 2010, 364-371.

17. Li K, Xu E, The role and the mechanism of gamma-aminobutyric acid during central nervous system development, Neurosci Bull, 24(3), 2008, 195–200.

18. D A McCormick, GABA as an inhibitory neurotransmitter in human cerebral cortex, AJP - JN Physiol, 62, 1989, 51018-1027.

19. Baldwin D, Rudge S, The role of serotonin in depression and anxiety, International Clinical Psychopharmacology, 9 (4), 1995, 41-45.

20. Kerry J. Ressler, Charles B. Nemeroff MD, Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders, Depression and Anxiety, 12(2), 2000, 2-19.

21. M Davis, The Role of the Amygdala in Fear and Anxiety, Annual Review of Neuroscience, 15, 1992, 353-375.

22. Miguel Pérez de la Moraa, Andrea Gallegos-Caria, Yexel Arizmendi-Garcíaa, Daniel Marcellinob, Kjell Fuxeb, Role of dopamine receptor mechanisms in the amygdaloid modulation of fear and anxiety: Structural and functional analysis, 90(2), 2010, 198–216.

23. Oleh Hornykiewicz, Dopamine (3-Hydroxytyramine) and Brain Function, Pharmacological Reviews, 18(2), 1966, 925-964.

Source of Support: Nil, **Conflict of Interest:** None.