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. 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.

Anxiety disorders including generalized anxiety disorder (GAD), Specific and social Phobias, Post traumatic stress disorder (PSTD), Obsessive compulsive and panic disorder (GAD), Specific and social Phobias, Post traumatic stress disorder (PSTD), Obsessive compulsive and panic disorder 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. 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. The numerous herbs like Withania somnifera, Ocimum sanctum, Nardostachys jatamansi, Ahiphenam Papaver somniferum etc. have been used as a component of herbal anxiolytics. Many Ayurvedic Practitioners prefer polyherbal formulations rather than monoherbol formulation due to their synergistic action and good therapeutic efficacy. The various polyherbal drugs prescribed by the practitioners 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 Emblica 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 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. 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.

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

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ABSTRACT

The aim of the present study was to evaluate the mechanism of action of polyherbal formulation-Jessica for its anxiolytic activity. Jessica has been made consisting of these herbs to have a 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.
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. 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% gum acacia (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 (Organization of economic cooperation 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 intraperitoneal route.

Female swiss albino mice weighing 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 assess toxicity. The mice were observed for behavioral, neurological and autonomic activities before and after drug administration. The onset of signs of toxicity, 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.

**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.

- **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.

**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 H2O
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°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 Dopamine13, 14

Preparation of Reagents

1. 0.4 M HCl: 3.4ml conc.HCl to 100ml H2O.
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. Na2SO3 solution: 0.5g Na2SO3 in 2 ml H2O + 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 Na2SO3 solution. 0.4ml acetic acid is added after 15 min. The solution was then heated to 100°C for 6 min when the sample again reached room temperature, excitation and emission spectra were read from the spectrofluorimeter. 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°C for 10 min. When the samples reached equilibrium with the ambient temperature, readings were taken at 360-470nm in the spectrofluorimeter. 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 100 ml water then to prepare 10% solution by adding 9 ml water to 100%(w/v) of trichloroacetic acid.
2. Preparation of 0.5M carbonate-bicarbonate 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°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 0.032% tartaric acid). After 10 min fluorescence at 377/455 nm in a spectrofluorimeter 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).

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 noradrenaline, 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

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Dopamine (ng/g of wet tissue)</th>
<th>Noradrenaline (ng/g of wet tissue)</th>
<th>Serotonin (ng/g of wet tissue)</th>
<th>GABA (ng/g of wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2% gum acacia</td>
<td>391.33 ± 3.22</td>
<td>284.5 ± 3.22</td>
<td>158.3 ± 3.21</td>
<td>547.66 ± 3.127</td>
</tr>
<tr>
<td>Diazepam</td>
<td>1 mg/kg</td>
<td>299.16 ± 2.301**</td>
<td>257 ± 2.490**</td>
<td>83.83 ± 3.554</td>
<td>1098.33 ± 2.906**</td>
</tr>
<tr>
<td>Diazepam</td>
<td>2 mg/kg</td>
<td>204.33 ± 2.092**</td>
<td>213 ± 2.113**</td>
<td>64.83 ± 2.358**</td>
<td>1528.33 ± 2.871**</td>
</tr>
<tr>
<td>Streswin</td>
<td>100 mg/kg</td>
<td>332.83 ± 2.613**</td>
<td>268.16 ± 3.188**</td>
<td>128.66 ± 3.432**</td>
<td>834.8 ± 1.887**</td>
</tr>
<tr>
<td>Streswin</td>
<td>200 mg/kg</td>
<td>317.83 ± 2.358**</td>
<td>229.16 ± 2.272**</td>
<td>103.3 ± 4.731**</td>
<td>966.8 ± 2.182**</td>
</tr>
<tr>
<td>Jessica</td>
<td>100 mg/kg</td>
<td>359.66 ± 2.552**</td>
<td>263 ± 2.155**</td>
<td>145.3 ± 4.761*</td>
<td>653.33 ± 1.926**</td>
</tr>
<tr>
<td>Jessica</td>
<td>200 mg/kg</td>
<td>327.5 ± 2.045**</td>
<td>234.5 ± 3.394**</td>
<td>134.66 ± 7.18**</td>
<td>786.5 ± 3.739**</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM from 6 rats. P<0.01 ** and P<0.05 * as compared to control group.
stem, hypothalamus and hippocampus, due to chronic stress. Anxiolytic activity was suppressed upon local microinjection of nicotine into the dorsal hippocampus, and it gives the evidence that anxiolytic response is due to suppressing the serotonin release in hippocampus. 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 stresswin (100 and 200mg/kg).

It has been earlier reported that, Amygdala plays an important role in acquisition, consolidation and retention or expression of fear. 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. 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 amygdala. In most of the researches it is found that Intra-amygdaloid infusion of D1 agonists and antagonists elicits anxiogenic and anxiolytic effects respectively on conditionized 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. 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 D2 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-HT1A 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 D2 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.

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