ANXIOLYTIC ACTIVITY OF JESSICA – A POLYHERBAL FORMULATION

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ABSTRACT

Objective: To study the anxiolytic activity of Jessica- a polyherbal formulation, in rats.

Methods and Material: Swiss albino rats of either sex weighing between 180g-210g were used. The standard anxiolytic, diazepam (1mg and 2mg/kg) standard polyherbal drug, Streswin (100 and 200mg/kg) and the test drug, Jessica (100mg and 200mg/kg) were suspended in 2% gum acacia and administered orally. In acute study the vehicle and test drug were given 40 min prior to experiment, while in chronic study they were given daily once for 10 days. The anxiolytic effect of Jessica (100 and 200mg/kg) was evaluated by using Despair swim test (DST), Elevated Plus maze (EPM), Actophotometer and Rota rod apparatus the animals were explored on the above models on 1st, 5th, 10th day of the treatment plan.

Results: It was observed that Jessica (100 and 200mg/kg) has increased the immobility time in DST, increased the total no of entries and time spent in open arm in EPM, increased the percentage reduction in locomotion in Actophotometer and decreased the time spent on revolving rod in Rota rod on 5 th day and 10 th day. All the results were significant when compared to control group and the effect was similar to that of standard drug diazepam (1 and 2mg/kg) and standard polyherbal formulation Streswin (100 and 200mg/Kg).

Conclusion: Jessica has exhibited anxiolytic activity comparable to diazepam and streswin. So, it can be used as a novel therapeutic strategy for anxiety.

Keywords: Anxiolytic, Jessica, Polyherbal formulation, Elevated plus maze, Despair swim test, Rota rod, Actophotometer.

INTRODUCTION

According to American Psychological Association, Anxiety is a Psychological disorder characterized by apprehension, uneasiness, panic, fear or terror, dread, uncertainty, feeling of tension, worried thoughts and physical changes like increased blood pressure, shortness of breath, rapid heart rate, decreased sex drive, Muscle tension, Dizziness [1]. Anxiety and anxiety spectrum disorders are becoming increasingly prevalent in modern society, requiring new therapeutic approaches and treatments [2-3]. Panic disorder affects about 6 million adults and is twice as common in women as men and often being in late adolescence or early adulthood [4]. Currently the most preferred treatment for anxiety disorder is by pharmacological agents such as benzodiazepines (BZD'S), Beta-blockers and selective serotonin reuptake inhibitors, but all of these exhibits some major side effects such as insomnia, anticholinergic effects, withdrawal and tolerance, weight gain and loss of memory [5]. These side effects have limited their use in patients and gave a scope for alternative medicine which can exhibit its pharmacological effect with minimal or no side effects. Ayurveda is the 5,000 year old holistic Indian art of healing and rejuvenation that is recently gaining more popularity and widely available [6]. 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]. 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 [8]. The numerous herbs like Withania somnifera, Ocimum sanctum, Nardostachys jatamansi, Alpinenam Papaver somniferum etc [6] 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 Practitioners are Kava Calm, Calm ez, 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 Rauvolfia serpentine-15.4mg. The results obtained by Jessica were compared with that of Diazepam, a benzodiazepine- anxiolytic drug and streswin, a standard polyherbal formulation.

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.

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 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 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 intraperitonal route [9].

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 assess 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.

In the present study the anxiolytic activity of ‘Jessica’ was evaluated
using:
1. Despair Swim test (DST)
2. Elevated Plus Maze (EPZ)
3. Actophotometer
4. Rota Rod Apparatus.

Experimental Protocol
All the experiments were carried out between 9.00-14.00hrs in a
dimly illuminated room with 40 W fluorescent bulb at a temperature
of 25±2°C. The animals were subjected to test 60 mins after the
drug administration. Treatment was given for 10 days and the behavioral
parameters were estimated on 1st, 5th and 10th day of treatment.
Animals were sequentially exposed to experimental models such as DST,
EPZ, actophotometer and rota rod apparatus to evaluate
depressant, anxiolytic, locomotor and muscle relaxant action of
the test drug respectively. All the apparatus are cleaned with ethanol
after use in order to mask the odor by the animal.

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 drug/ 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

Despair Swim test
The depression related behavior was tested with the Perssalt swim
test used to evaluate “behavioral despair”. It is a measure of failure to
seek escape from an aggressive stimulus. The rats were forced to
swim individually in a transparent cylinder containing water at 32°C
to a depth of 30 cm, the depth was adjusted so that the animal must
swim or float without touching its hind limbs or tail to the bottom
and the water level must be 15 cm from top of cylinder such that
animal cannot jump. A Pre-test was conducted 24 hrs before the
administration of the drug and all the animals were allowed to swim
for 15 min, then they are removed dried with a towel and returned
back to the cage. During the second test session i.e. 40 mins after
treatment on 1st, 5th and 10th day rats were allowed to swim for 10
mins As suggested by Perssalt the data (immobility time) was
recorded in the last 4 mins of the total 10 mins test session for each
animal. Rodents (Rats and mice) will generally swim, animals
treated with anxiolytic drugs or depressive drugs will stop
swimming and will float. Floating time was considered as a measure
of depression like behavior as the animal stopped swimming and
made minimal movements to keep its head above water. This
indicates that animal has given up finding no escape route. The
floating time is measured by using a stopwatch. Any rat which did
not swim or float is immediately removed from the water at the end
of the test session animals are dried and returned to their cages. The
results are represented in table 1[10].

Elevated plus maze
The elevated plus maze apparatus consist of two open arm (16
5Xcm) and two closed arm (165X12) having an open roof and is
elevated to a height of 50cm from ground the arms are arranged
around a central square. The animal were gently placed on the
central square facing the open arm and the no of entries and time
spent in open arm and no of entries and the time spent in closed arm
for a duration of 5min was noted on 1st, 5th and 10th day the entry
into a arm is considered only when all the four paws of an animal are
into the arm. The results are represented in table 2 and 3[11, 12].

Locomotor activity
The locomotor activity was measured by using an actophotometer. It
is equipped with 6 photo cells in the outer wall. Interruptions to the
signals of photocell beam due to locomotor activity were recorded
by means of a six digits counter. Each animal was observed for a
period of 5 min. The animal was placed individually and the
actophotometer was turned on. The animal was made familiarized
with the instrument for 2 min then the basal activity score was
noted as counts/5 min for next 5 min. The basal activity score was
noted for all the animals before the administration of the drug on
the first day and 60 min after administration of the drug on 1st, 5th
and 10th day of treatment. Percentage decrease in motor activity was
calculated and is represented in table 4. The results are represented
in table 4[13, 14, 15].

Skeletal Muscle Relaxant Activity
Muscle relaxant activity is measured by using Rota rod apparatus
initially animals were trained on the apparatus. The rotarod was set
at 25rpm and animals which remained on the revolving rod for 3
min or more after low successive trials are included in the study.
After 2 hrs of vehicle/drug administration the fall of time was noted
on 1st, 5th, 10th day and is compared with the control group. The
difference in the fall off time from rotating rod between the control
and treated rat was taken as muscle relaxation index of the drug.
The results are represented in table 5[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, stresswin and control were analyzed by one way analysis
of variance (ANOVA).
200mg/kg) and P<0.01 on 10 day (100mg/kg) on 5 and 10 day (200mg/kg). Diazepam (1mg and 2mg/kg) and streswin (100mg/kg, 200mg/kg) showed P<0.01 on 1,5 and 10 day. The effect of 200mg/kg of Jessica is nearly equal to the effect of diazepam 2mg/kg and streswin 200mg/kg.

**Locomotor activity**

The results obtained were presented in table 4 and it indicates that Jessica has shown a significant reduction in locomotion on 1,5,10 day of the treatment. It also shows significant percentage reduction in locomotion. Results of Jessica (100mg and 200mg/kg) diazepam (1mg and 2mg/kg) and streswin (100mg and 200mg/kg) were compared with control group it showed a significant effect with P<0.01.

**Skeletal Muscle relaxant activity**

The results obtained were presented in table 5 and it indicates that Jessica (100mg and 200mg/kg) has significantly decreased the time spent on the revolving rod 1, 5 and 10 day of treatment. Results of Jessica (100mg and 200mg/kg) diazepam (1mg and 2mg/kg) and streswin 100mg and 20mg/kg were compared to control group it showed significant effect with P<0.01. However Jessica didn’t show any significant effect on first day of the treatment.

**Table 1: Results of despair swim test**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration of immobility (in Sec) [Mean±SEM]</th>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 2% gumacacia</td>
<td></td>
<td>139.3±4.26</td>
<td>138.8±5.964</td>
<td>155.4±2.794</td>
</tr>
<tr>
<td>Diazepam 1mg/kg</td>
<td></td>
<td>162.0±2.711**</td>
<td>174.7±4.230**</td>
<td>198.3±4.76**</td>
</tr>
<tr>
<td>Diazepam 2mg/kg</td>
<td></td>
<td>181.9±3.284**</td>
<td>208.6±3.194**</td>
<td>237.5±4.90**</td>
</tr>
<tr>
<td>Streswin 100mg/kg</td>
<td></td>
<td>149.3±5.51</td>
<td>160.1±3.576</td>
<td>185.8±3.733**</td>
</tr>
<tr>
<td>Streswin 200mg/kg</td>
<td></td>
<td>156.4±2.531**</td>
<td>189.9±2.856</td>
<td>207.5±3.733**</td>
</tr>
<tr>
<td>Jessica 100mg/kg</td>
<td></td>
<td>141.8±3.083</td>
<td>158.6±2.813</td>
<td>177.6±2.163**</td>
</tr>
<tr>
<td>Jessica 200mg/kg</td>
<td></td>
<td>149.2±3.574</td>
<td>169.9±4.22**</td>
<td>192.3±3.733**</td>
</tr>
</tbody>
</table>

** indicates P<0.01

**Table 2: Results of Elevated plus maze showing no of entries and time spent in open arm:**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of entries into open arm [Mean±SEM]</th>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 2% gumacacia</td>
<td></td>
<td>1.3±0.2108</td>
<td>2.1±0.3073</td>
<td>3.0±0.2582</td>
</tr>
<tr>
<td>Diazepam 1mg/kg</td>
<td></td>
<td>2.6±0.2108**</td>
<td>4.6±0.4014**</td>
<td>6.0±0.2804**</td>
</tr>
<tr>
<td>Diazepam 2mg/kg</td>
<td></td>
<td>3.3±0.2108**</td>
<td>6.8±0.7032**</td>
<td>6.0±0.3651**</td>
</tr>
<tr>
<td>Streswin 100mg/kg</td>
<td></td>
<td>2.3±0.2108*</td>
<td>4.5±0.2236**</td>
<td>6.0±0.3651**</td>
</tr>
<tr>
<td>Streswin 200mg/kg</td>
<td></td>
<td>3.0±0.2582</td>
<td>6.3±0.4215**</td>
<td>8.3±0.7149**</td>
</tr>
<tr>
<td>Jessica 100mg/kg</td>
<td></td>
<td>2.1±0.2108</td>
<td>3.8±0.3073*</td>
<td>5.1±0.4773**</td>
</tr>
<tr>
<td>Jessica 200mg/kg</td>
<td></td>
<td>2.3±0.2108**</td>
<td>6.3±0.4216**</td>
<td>8.1±0.7491**</td>
</tr>
</tbody>
</table>

** indicates P<0.01 and * indicates P<0.05

**Table 3: Results of Elevated plus maze showing no of entries and time spent in closed arm**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of entries into closed arm [Mean±SEM]</th>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 2% gumacacia</td>
<td></td>
<td>7.5±0.4282</td>
<td>10.0±0.8165</td>
<td>15.0±0.774</td>
</tr>
<tr>
<td>Diazepam 1mg/kg</td>
<td></td>
<td>4.3±0.335**</td>
<td>6.8±0.6009**</td>
<td>5.8±0.3073**</td>
</tr>
<tr>
<td>Diazepam 2mg/kg</td>
<td></td>
<td>3.3±0.4216**</td>
<td>6.4±0.3703**</td>
<td>4.3±0.2108**</td>
</tr>
<tr>
<td>Streswin100mg/kg</td>
<td></td>
<td>5.6±0.333</td>
<td>7.1±0.5426**</td>
<td>5.6±0.2108**</td>
</tr>
<tr>
<td>Streswin200mg/kg</td>
<td></td>
<td>4.8±0.3073**</td>
<td>4.6±0.2108**</td>
<td>5.6±0.3651**</td>
</tr>
<tr>
<td>Jessica 100mg/kg</td>
<td></td>
<td>5.8±0.5426*</td>
<td>7.5±0.6908**</td>
<td>7.1±0.5426**</td>
</tr>
<tr>
<td>Jessica 200mg/kg</td>
<td></td>
<td>4.8±0.3073**</td>
<td>4.8±0.3073**</td>
<td>6.0±0.1995**</td>
</tr>
</tbody>
</table>

** indicates P<0.01 and * indicates P<0.05

**Table 4: Results of locomotor activity in actophotometer showing no of counts/5min and percentage reduction in locomotion**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No of count/5min[Mean±SEM]</th>
<th>Percentage reduction in locomotion [Mean±SEM]</th>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 2% gumacacia</td>
<td>363.1±5.618</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Diazepam 1mg/kg</td>
<td>236.5±5.69</td>
<td>282.6±3.98**</td>
<td>261.1±5.36**</td>
<td>196.3±2.917**</td>
<td>29.3±0.68**</td>
</tr>
<tr>
<td>Diazepam 2mg/kg</td>
<td>233.8±4.24</td>
<td>270.5±5.99**</td>
<td>217.2±2.86**</td>
<td>139.6±1.022**</td>
<td>38.1±0.48**</td>
</tr>
<tr>
<td>Streswin 100mg/kg</td>
<td>238±6.27</td>
<td>323.6±6.73**</td>
<td>297.8±5.98**</td>
<td>262.6±4.72**</td>
<td>18.6±0.35**</td>
</tr>
<tr>
<td>Streswin 200mg/kg</td>
<td>240±5.79</td>
<td>276.3±4.01**</td>
<td>221.5±5.56**</td>
<td>196.3±2.88**</td>
<td>24.3±0.50**</td>
</tr>
<tr>
<td>Jessica 100mg/kg</td>
<td>240±3.72</td>
<td>349.6±9.15</td>
<td>315±9.87**</td>
<td>264±6.89**</td>
<td>11.2±0.28**</td>
</tr>
<tr>
<td>Jessica 200mg/kg</td>
<td>231±6.83</td>
<td>329±6.99**</td>
<td>270±3.35**</td>
<td>220±3.18**</td>
<td>16.0±0.73**</td>
</tr>
</tbody>
</table>

** indicates P<0.01
The present study evaluated the muscle relaxant activity of aqueous extracts of sapindus trifoliate (pericarp) in Swiss albino mice. In the test, muscle relaxant activity was measured by the duration of muscle relaxation on a revolving rod. The results showed that the test drug Jessica (100mg and 200mg/kg) significantly increased the time spent on the revolving rod, indicating a significant sedative effect of the drug by acting on benzodiazepine/GABA receptor complex [18].

**CONCLUSION**

Results obtained in the present study suggest that the polyherbal formulation Jessica has a dose-dependent effect. These results also suggest a probable involvement of benzodiazepine/GABA receptor along with 5-HT receptor and provide the evidence that Jessica can be used as a potent anxiolytic drug. However, further studies are required at molecular level to evaluate its exact mechanism of action.

**ACKNOWLEDGEMENT**

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**REFERENCES**


### Table 5: Results for skeletal muscle relaxant activity on Rotarod showing time spent on revolving rod (sec)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time spent on revolving rod (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 2% gumacacia</td>
<td>Day 1: 239±4.42, Day 5: 244.5±4.357, Day 10: 246.5±5.386</td>
</tr>
<tr>
<td>Diazepam 1mg/kg</td>
<td>Day 1: 184.6±5.031**, Day 5: 179.8±3.772**, Day 10: 193±5.055**</td>
</tr>
<tr>
<td>Stresswin 100mg/kg</td>
<td>Day 1: 210.8±5.218**, Day 5: 199.8±5.667**, Day 10: 175.8±4.815**</td>
</tr>
<tr>
<td>Jessica 100mg/kg</td>
<td>Day 1: 220.1±4.053, Day 5: 212.3±3.73, Day 10: 195.5±5.757**</td>
</tr>
<tr>
<td>Jessica 200mg/kg</td>
<td>Day 1: 231.3±6.83, Day 5: 175.8±5.805**, Day 10: 15.1±5.123**</td>
</tr>
</tbody>
</table>

** indicates P<0.01