

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 5th day and 10th 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, Rotarod, 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, Ahiphenam 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 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 serpentina-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 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 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 intraperitoneal 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 40W 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 rotarod 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 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

Despair Swim test

The depression related behavior was tested with the Porsolt 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 15cm 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 Porsolt 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 X5cm) and two closed arm (16X5X12) 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].

$$\text{Percentage reduction in locomotion} = \frac{\text{no of counts /5 min, 60 min after drug administration}}{\text{no of counts /5 min, before drug administration}} - 100$$

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 were 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).

RESULTS

Despair swim test

The results obtained from DST are presented in table 1, indicate that Jessica at a dose of 100mg/kg and 200mg/kg showed increase in immobility time similar to diazepam(1mg/kg, 2mg/kg) and streswin(100mg/kg,200mg/kg) and the results were significant (P<0.01) on 5th and 10th day P values were insignificant on 1st day. The effect of 200mg/kg of Jessica is nearly equal to the effect of diazepam 1mg/kg and streswin 100mg/kg.

Elevated plus maze

The results obtained were presented in table 2 and 3 it indicates that Jessica at doses of 100mg/kg and 200mg /kg has shown a significant increase in number of entries into open arm and time spent in open arm. Whereas, it showed a significant decrease in number of entries and time spent in closed arm when compared to control group. The significant values for Jessica was P<0.05 on 1st day (100mg and

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) 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 200mg/kg) were compare 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

Treatment	Duration of immobility (in Sec) [Mean±SEM]		
	Day 1	Day 5	Day 10
Control 2%gumacacia	139.3 ±3.426	138.8±5.964	155.4±2.794
Diazepam 1mg/kg	162.0±2.711**	174.7±4.230**	198.3±4.76**
Diazepam 2mg/kg	181.9±3.284**	208.6±3.194**	237.5±4.90**
Streswin 100mg/kg	143.9±3.51	160.1±3.576**	185.8±3.733**
Streswin 200mg/kg	156.4±2.531**	189±3.256**	207±3.733**
Jessica 100mg/kg	141.8±3.083	158.6±2.813**	177.6±3.163**
Jessica 200mg/kg	149.2±3.574	169.9±4.22**	192.3±3.733**

** indicates P<0.01

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

Treatment	Number of entries into open arm [Mean±SEM]			Time spent in open arm (sec/5min) [mean±SEM]		
	Day1	Day 5	Day 10	Day 1	Day 5	Day 10
Control 2%gumacacia	1.3±0.2108	2.1±0.3073	2.0±0.2582	8.5±1.478	14.1±1.887	14.3±1.764
Diazepam 1mg/kg	2.6 ±0.2108**	4.8±0.4014**	7.5±0.5**	25±2.017**	55.5±2.952**	111.1±4.339**
Diazepam 2mg/kg	3.3±0.2108**	6.8±0.7032**	11.0±1.033**	36.1±3.24**	75.8±6.67**	137.1±4.956**
Streswin 100mg/kg	2.30±0.2108*	4.5±0.2236**	6.0±0.3651**	18.5±2.837*	44±3.317**	104.5±3.713**
Streswin 200mg/kg	3.0±0.2582**	6.3±0.4215**	8.3±0.7149**	24.8±1.869**	64.3±3.211**	128±4.747**
Jessica 100mg/kg	2.16±0.1667*	3.8±0.3073*	5.1±0.4773**	14.6±2.108*	41.6±1.706**	96.8±4.423**
Jessica 200mg/kg	2.3±0.2108*	6.3±0.4216**	8.1±0.7491**	22±2.352**	60±2.828**	117.3±3.921**

** 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

Treatment	Number of entries into closed arm [Mean±SEM]			Time spent in closed arm (sec/5min) [Mean±SEM]		
	Day 1	Day 5	Day 10	Day1	Day 5	Day 10
Control 2%gumacacia	7.5±0.4282	10±0.8165	15±0.7746	291.5±1.478	285.8±1.887	285.6±1.764
Diazepam 1mg/kg	4.3±0.335**	6.8±0.6009**	5.8±0.3073**	275.0±2.017**	244.5±2.952**	188.8±4.339**
Diazepam 2mg/kg	3.3±0.4216**	4.1±0.3073**	4.3±0.2108**	263.8±3.240**	224.1±6.67**	162.8±4.956**
Streswin100mg/kg	5.6±0.333*	7.1±0.5426**	5.6±0.2108**	281.5±2.837*	256.0±3.317**	195.5±3.713**
Streswin200mg/kg	4.83±0.3073**	4.6±0.2108**	5±0.3651**	275.5±1.928**	237.3±1.994**	172±4.747**
Jessica 100mg/kg	5.83±0.5426*	7.5±0.6908*	7.1±0.5426**	282.3±2.108*	258.3±1.706**	203.1±4.423**
Jessica 200mg/kg	4.83±0.3073**	4.8±0.3073**	6.0±1.095**	278.0±2.352**	240±2.828**	182.6±3.921**

** 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

Treatment	No of count/5min[Mean±SEM]				Percentage reduction in locomotion [Mean±SEM]		
	Before treatment	After 60 min of treatment			Day 1	Day 5	Day 10
		Day 1	Day 5	Day 10			
Control 2% gum acacia	363.1±5.618	363.1±5.618	351±5.618	359.5±4.365	NA	NA	NA
Diazepam 1mg/kg	236.5±5.69	282.6±3.98**	261.1±5.36**	196.3±2.917**	29.3±0.68**	34.6±0.63**	50.81±0.27**
Diazepam 2mg/kg	233.8±4.24	270±5.59**	217±2.86**	139.6±1.022**	38.1±1.01**	49.9±0.46**	67.7±0.44**
Streswin 100mg/kg	238±6.27	323.6±6.73**	297.8±5.98**	262.6±4.72**	18.6±0.35**	27.09±0.25**	36.9±0.53**
Streswin 200mg/kg	240.5±5.79	276.3±4.01**	221±5.56**	196.3±2.88**	24.3±0.50**	39.58±0.62**	46.3±0.37**
Jessica 100mg/kg	240±3.72	349.6±9.15	315±9.87**	264±6.89**	11.2±0.28**	19.73±0.47**	32.75±0.34**
Jessica 200mg/kg	231±6.83	329±6.99**	270.3±3.35**	220±3.18**	16±0.73**	30.91±0.21**	43.6±0.87**

**indicates P<0.01

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

Treatment	Time spent on revolving rod (sec)		
	Day1	Day 5	Day 10
Control 2%gumacacia	239±4.442	244.5±4.357	246.5±6.386
Diazepam 1mg/kg	184.6±5.031**	179.8±3.772**	129±3.055**
Diazepam 2mg/kg	180.6±4.738**	132.1±3.936**	74.16±3.628**
Streswin 100mg/kg	210.8±5.218**	199.3±5.667**	175.8±4.813**
Streswin 200mg/kg	190±4.42**	155±9.879**	112.8±4.143**
Jessica 100mg/kg	228.1±4.053	212.3±3.73**	195.5±3.757**
Jessica 200mg/kg	231.3±6.83	175.8±5.805**	151.3±5.123**

** indicates P<0.01

DISCUSSION

In Ayurveda it is reported that anxiety and depression are caused due to imbalance of kapha dosha and pitta dosha at somatic level this may be caused due to imbalance between excitatory and inhibitory neurotransmitter [17]. An anxiolytic drug exerts its action by increasing the levels of inhibitory neurotransmitters like GABAergic and Serotonergic neurotransmitters in brain and by decreasing the levels of dopaminergic and adrenergic neurotransmitters in brain. Despair swim test is widely used to assess the antidepressant effect caused mainly due to Mono-amine oxidase inhibitors, tricyclic antidepressants which cause a decrease in immobility time. By taking the same parameter in an opposite way (i.e. increase in immobility time) the drugs causing depressant effect can be evaluated. Immobility is thought to reflect failure to escape from an inescapable condition and development of a passive behavior anxiolytic drugs and CNS depressant drugs increase the immobility time in forced swimming. In the present study Jessica (100mg and 200mg/kg) caused a significant increase in immobility time in rats by reducing the struggling time this effect may be due to calming effect of drug and was comparable with standard anxiolytic diazepam and polyherbal standard streswin. This increase in immobility time may be due to reduction in 5-HT levels or MAO levels.

Anxiolytic effect of any drug can be evaluated by using elevated plus maze, the height and open spaces of the maze induces fear in animals. Rodents generally, have a tendency to prefer closed and dark places. When an animal is kept on the elevated plus maze facing the open arm it showed reduced number of movements by spending most of the time in closed arm. When treated with the anxiolytic drug the number of entries and time spent in open arm was significantly increased and the number of entries and time spent in closed arm was decreased and the results were comparable with the standard drug diazepam (1mg and 2mg/kg) and streswin (100mg and 200mg/kg). The open arm-closed arm approach for anxiolytic effect has worked well in identifying the anxiolytic drugs having potential effect on benzodiazepine/GABA_A receptor. Hence the results suggest that the test drug may possess a positive modulation effect on GABA_A-chloride channels and there by may cause a rise in GABA levels in brain.

Test drug Jessica (100mg and 200mg/kg) has shown a significant percentage reduction in locomotion and the results were comparable with standard drug diazepam (1mg and 2mg/kg) and streswin (100mg and 200mg/kg). This action was possibly due to sedative effect of the drug by acting on benzodiazepine/GABA receptor complex [18].

Jessica has also shown a significant reduction in time spent on revolving rod indicating the presence of myo-relaxant effect and the results were comparable with the standard drug diazepam (1mg and 2mg/kg) and streswin (100mg and 200mg/kg).

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 benzodiazepines/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.

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