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RESEARCH ARTICLE

EFFECT OF OJASVITA CHOCOLATE (OC) ON ANXIETY, STRESS AND MEMORY MANAGEMENT IN ALBINO WISTAR RATS

Arvind Varchaswi, Dr. Ravi Kumar Reddy, M. and *Ramakanth Reddy, K.

Source Natural Foods and Herbal Supplements Limited, Hyderabad-90

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ABSTRACT

Now a day's many dietary supplements are available in the present market. Source Natural Foods and Herbal Supplements limited, Hyderabad also came up with a dietary supplementOjasvita Chocolate which is charged with the addition of seven herbs extracts namely With aniasomnifera, Bacopamonniera, Ecliptaalba, Curculigoorchioides, Celastruspaniculatus, Asparagus racemosus and Convolvulus pluricaulis. The present study evaluates brain function activity of the dietary supplement since the herbs used have rich neuropharmacological significance. Effect of our supplement on Neuro pharmacological conditions like Stress, Anxiety and Memory are evaluated by conducting locomotor and open field testfor antianxiety and in case of antistress swimming test was performed and finally memory enhancer activity was evaluated by using Light & dark explorations, Object recognition test and Elevated plus maze test. Ojasvita Chocolate (OC) at 0.54 gm/kg has enhanced antianxiety activity, moderate anti-stress activity and exhibited moderate memory enhancement in light & dark, elevated plus test. Where, object recognition test shows significant activity when compared with standard on experimental animals.

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INTRODUCTION

Anxietyis a state of excessive fear, characterized by motor tension, sympathetic hyperactivity, apprehension and vigilance syndromes. Anxiety may interfere with intelligence, psychomotor function and memory. Anxiety disorders include generalized anxiety disorder, social anxiety disorder, specific phobia, panic disorder with and without agoraphobia, obsessive- compulsive disorder, post-traumatic stress disorder, anxiety secondary to medical condition, acute stress disorder, and substance-induced anxiety disorder (Poonam Mahendra, 2011). The anxiety and worry are associated with the following six symptoms Restlessness, Being easily fatigued, Difficulty concentrating, Irritability, Muscle tension and Sleep disturbance. Stress can be described as the sum of total reactions of the body, which disturb the normal physiological condition and result in a state of threatened homeostasis. Stress is anphenomenon internationally recognized by the advancement of industrialization in a demanding civilization. Thus, every individual is likely to face stressful situations in day-to-day life. Memory is the major ability to encode, store, retain and subsequently recall information and past experiences in the human brain. It can be thought of in general terms as the use of past experience to affect or influence current behavior (Kaur, 2010).

***Corresponding author:* Ramakanth Reddy, K.,**
Source Natural Foods and Herbal Supplements Limited, Hyderabad-90.

Memory is the natural counter part of learning. Poor memory, low retention and slow recall are common problems in today's stressful and competitive world. Age, stress, emotions are conditions that leads to cognitive disorders (Desai, 2009). Cognitive deficits have long been recognized as severe and consistent neurological disorders associated with numerous psychiatric and neuro-degenerative states such as senile dementia, multi-infract dementia, Parkinson's disease, Huntington's chorea (Reddy, 1997) and Alzheimer's disease, amnesia, delirium, depression and schizophrenia are the results of impairments in learning and memory (Wangkhem, 2011). The present study is to investigate the brain functions activity of the Ojasvita Chocolate(OC) in experimental animals.

Experimental Animals

Species: Albinorats/mice

Strain: Wistar

Sex: EitherSex

Source: Acharya B.M Reddy College OfPharmacy.

Bodyweight: 140 - 200g/25-30g

Identification: By cage card and corresponding color body markings. Numberofanimals per dose group: 3 Male and 3 female

Acclimatization: One week in experimentalroom.

Selectionofanimals: After acclimatizationthe animals weresubjected toa grossobservation to ensure thatthe selected rats arein goodstate ofhealth. Rats were randomly selected for final allotment to the study.

Environmentalcondition:Airconditionedroomswithoptimalair changesperhour,relativehumidity, temperature and elimination cycle set to 12 hour light and 12 hour indark.

Accommodation:Group housed in polypropylene cages with stainless steel grilltop.Facilities for food and water bottle and bedding of clean paddy husk.

Diet:'Amrut' brand pellet feed was provided *adlibitum*.

Water: Purified filtered water was provided *ad libitum* in polypropylene bottles with stainless steel sippertubes.

The protocol of memory enhancer activity was approved by the Institutional Animal Ethical Committee (IAEC) of Acharya & B.M Reddy College of Pharmacy (Reg.No: 997/C/06/CPCSEA), Soldevanahalli, Bangalore, Karnataka, as per the guidelines of CPCSEA.

MATERIALS AND METHODS

Antianxietyactivity

Locomotoractivity

The locomotor activity was evaluated using by Actophotometer, the rats were divided into three groups (n=6). Group-I received with water, Group-II received with diazepam (0.1 mg/kg, p.o), Group-III treated with **OC**orally administered respectively. Each animal were placed individually in actophotometer and the basal activity score of all the animals were recorded for 10 min at 0 day and after 7th and 14th day of drugtreatment (Alagarswamy, 2006).

Open fieldtest

The test provides simultaneous measures of locomotion, exploration and anxiety was used for this study. The open field is a 400 × 400 × 300 mm arena with thin black stripes painted across the floor, dividing it into 16 quadratic blocks. Group-I received with water, Group-II received with diazepam (0.1 mg/kg, p.o), Group-III treated with **OC** administered orally with oral canula respectively. Mouse was placed in the center of arena and an observer quantified the spontaneous ambulatory locomotion of each mouse for 5 min at 0, 7thand14th day of treatment. During this period, the number of squares crossed and number of rearing weremeasured[7].

Antistress activity

Swimming test

Method: Albino mice (20-25 g) were randomly divided into 3 groups. The Group I serve as control with swimming stress. Group II, III, were received water, Fluoxetine (15 mg/kg, p.o) and **OC**respectively orally. After 1 hour mice will be placed individually in a Plexiglas cylinder (height 40 cm, diameter 10 cm) filled with tap water (27 ± 2°C) to a height of 18 cm. Two swimming sessions was conducted: a 15 min (training) pre-test followed 24 hour later by a 6 min test. After the pre-test, the animals were removed from the water, dried before a room heater and returned back to their home cages. The total duration of immobility behavior was record during the second 6 min test. Mouse was judged immobile, when it remains floating in water, in an upright position making only small movements to keep the head abovewater[8].

Memoryenhancer

Light and darkexplorations

Method: As a paradigm of memory, two-compartment exploratory model of Crawley and Goodwin has been validated pharmacologically,behaviorally and physiologically. This two-chambered method titrates the natural tendency of rat to explore a novel environment, against the aversive properties of brightly lit open field. The time spent in lit area and exploratory behaviour seemed to be the most reliable parameter for assessing memory enhancing activity. The light and dark box consists of two compartments: one light area(27L×27W×27H cm) was painted white, and the other dark area (18L×27W×27H cm) was painted black. The two compartments will be separated by partition with tunnel (7.5×7.5 cm) to allow passage from one compartment to other. All animals of groups either scopolamine, **OC**compound or standard drug (Piracetam 100 mg/kg, p.o.) treated were placed individually in the specially designed light and dark exploration chamber in the center of the dark area facing the wall opposite to the tunnel. Following parameters in a 5 minutes session wasrecorded (Saima Khaliq, 2012).

The total time spent in the illuminated part of cage

Object recognitiontest

Method: A wooden chamber ofdimensions (35cm×35cm×35 cm) was used in low light condition (about 40 lx) during the light phase of the light/dark cycle. The general procedure, as described elsewhere, consisted of three different phases which are, a habituation phase, an acquisition phase, and finally retention phase. On the 1st day (habituation phase), rats were individually subjected to a single familiarization session of 10 min, during which they were introduced in the empty arena, in order to become familiar with the apparatus. On the 7th and 14th day (acquisition phase) animals were subjected to a single 10-min session, during which floor-fixed two objects (A and B) were placed in a symmetric position in the central line of the arena, 10 cm from each and 8 cm from the nearest wall (each object occupies approximately 5 cm space by its size). The two objects are made of the same wooden material with the similar color and odor, with different in shape but identical in size. Rats were allowed to explore the objects in the open field (Tursun Alkam, 2011). The exploration time on each object was shown (as seconds) to indicate the exploring activity of rats. On the 8th and 15th day (retention phase), rats were allowed to explore the open field in the presence of two objects: the familiar object A and a novel object C in different shapes from object A and B but in similar color and size (A and C). A recognition index (for retention session), calculated for each rats, was expressed as the ratio (TC×100)/(TA + TC), where TA and TC are the time spent during retention phase on object A and object C, respectively. The time spent exploring

any object (nose pointing toward the object at a distance≤1 cm, but not mounting on the object or playing with the object) was recorded (using stopwatch) by hand (Tursun Alkam, 2011).

Elevated plusmaze

Method: Elevated plus maze served as extroceptive behavioral model to evaluate learning and memory in rats. The elevated plus maze consisted of two open arms and two closed arms (50cmx10cmx40cm) with an open roof arranged so that the two arms are opposite to each other. The maze used was elevated to a height of 50 cm. On the 14th day respectively each rat was placed at end of the open arm, facing away from the central platform. Transfer latency is time taken by the rats to move in to the covered arm with all its four paws, transfer latency was recorded. If the animals did not enter in to one of the covered arms within90s, it was gently pushed in to one of the two covered arms and transfer latency was assigned as 90s. The rats were allowed to explore the maze for 10s and returned to the home cage. The measurement of transfer latency on the 8thday and on 15th day served as parameter for retention of memory. All the animals were treated for 15 days and on 8th and 15th day of treatment period all the bioactive treated animals was subjected to scopolamine (1 mg/kg i.p) 60 minutes after administration of OC compounds and piracetam, except the first group which served as vehiclecontrol (Mani Vasudevan and Milind Parle, 2011).

Statisticalevaluation: Here the data were expressed as mean ± standard error of mean. Statistical comparisons were made by using one-way ANOVA followed by Dunnet multiple comparison test. The results were considered as statistically significant if the value of P is <0.05.

RESULTS AND DISCUSSION

Antianxietyactivity

Table 1. Effect of OC on anxiety (counts) in rats at weekly intervals by actophotometer

Days	Treatment group (Counts)		
	Control (Distilled water, 5ml/kg)	Diazepam (0.1 mg/kg,p.o)	OC (2.7 gm/kg, p.o.)
0 DAY	348.0±6.53	354.0±9.03	336.8±6.47
7th DAY	378.0±10.07	309.0±7.06**	320.4±6.32**
14th DAY	363.0±5.96	281.2±5.34**	306.2±6.03**

Note: All values are expressed in Mean ± SEM; the results were analyzed using Prism, version-5. One way analysis of variance (ANOVA) test followed by Dunnett's test was used to analyze the results, ** p< 0.01 was considered as statistically significant compared to control.

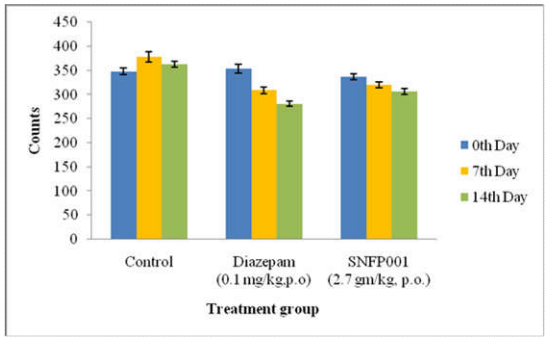

Fig. 1. Effect of OC on anxiety (counts) in rats at weekly intervals by actophotometer

Locomotoractivity

Table 2. Effect of OC on anxiety (No.of line crossing) in rats at weekly intervals by open field

Days	Treatment group (No. of line crossing)		
	Control (Distilled water, 5ml/kg)	Diazepam (0.1 mg/kg,p.o)	OC (2.7 gm/kg, p.o.)
0 DAY	40.6±3.48	39.8±2.67	45.4±3.04
7th DAY	46.8±3.91	33.4±2.94*	36.4±3.23
14th DAY	57.6±1.91	30.0±1.76**	32.4±2.97**

Note: All values are expressed in Mean ± SEM; the results were analyzed using Prism, version-5. One way analysis of variance (ANOVA) test followed by Dunnett's test was used to analyze the results, ** p< 0.01 was considered as statistically significant compared to control.

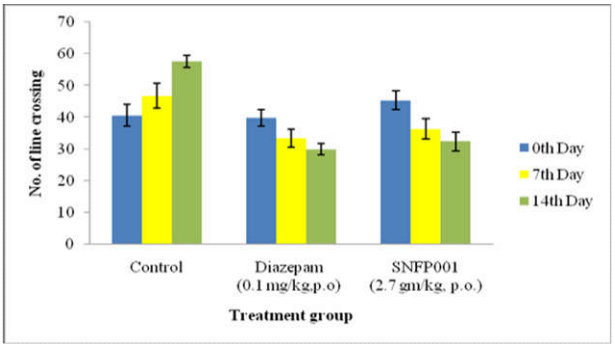

Fig. 2. Effect of OC on anxiety (No.of line crossing) in rats at weekly intervals by open field

Antistress activity

Table 3. Effect of OC on anxiety (No. of rearing) in rats at weekly intervals by open field

Days	Treatment group (No. rearing)		
	Control (Distilled water, 5ml/kg)	Diazepam (0.1 mg/kg,p.o)	OC (2.7 gm/kg, p.o.)
0 DAY	11.6±0.74	13.0±1.09	11.6±1.28
7th DAY	12.8±1.06	7.4±0.81**	10.6±0.5
14th DAY	13.6±1.43	6.6±0.67**	9.6±1.83

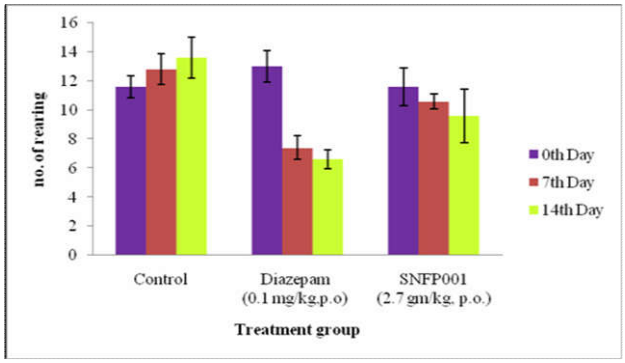

Fig. 3. Effect of OC on anxiety (No. rearing) in rats at weekly intervals by open field

Table 4. Effect of OC on strees (Immobility in sec) in rats at weeklyintervals

Days	Treatment group (Immobility in sec)		
	Control (Distilled water, 5ml/kg)	Fluoxetine (15 mg/kg, p.o)	OC (2.7 gm/kg, p.o.)
0 DAY	95.50±3.93	83.83±4.45	90.33±6.58
7th DAY	124.33±4.4	165.17±5.22**	147.50±6.14*
14th DAY	154.50±4.68	188.00±5.7**	175.17±5.91*

Note: All values are expressed in Mean ± SEM; the results were analyzed using Prism, version-5. One way analysis of variance (ANOVA) test followed by Dunnett's test was used to analyze the results, ** p< 0.01 was considered as statistically significant compared to control.

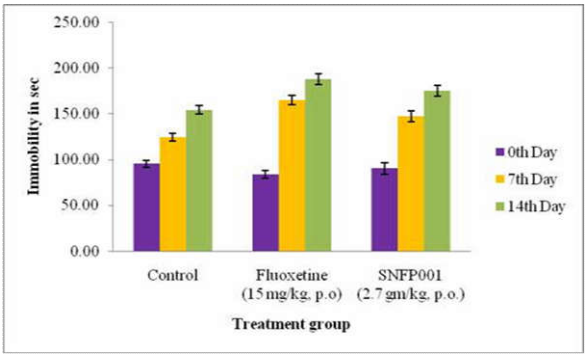

Fig. 4. Effect of OC on stress (Immobility in sec) in rats at weekly intervals

Table 5: Effect of OC on time spent (s) in light and darkmodel

Days	Treatment group (Total time spent in the illuminated part in sec)		
	Control (Distilled water, 5ml/kg)	Piracetam (100 mg/kg, p.o)	OC (2.7 gm/kg, p.o.)
0 DAY	32.8±2.59	37.4±2.85	34.8±2.35
7th DAY	47.8±3.2	67.2±6.03*	52.2±3.48
14th DAY	49.6±3.17	75.4±5.5**	64±2.57*

Note: All values are expressed in Mean ± SEM; the results were analyzed using Prism, version-5. One way analysis of variance (ANOVA) test followed by Dunnett's test was used to analyze the results, * p< 0.05, ** p< 0.01 was considered as statistically significant compared to control.

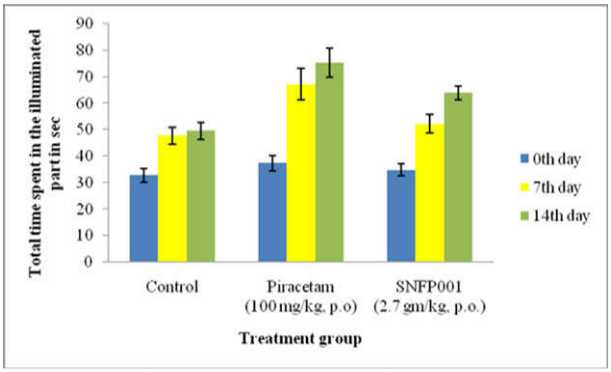

Fig. 5. Effect of OC on time spent (s) in light and dark model

Table 6. Effect of OC on recognition index for retentionphase

Days	Treatment group (Recognition index for retention phase)		
	Control (Distilled water, 5ml/kg)	Piracetam (100 mg/kg, p.o)	OC (2.7 gm/kg, p.o.)
14th DAY	16.0±5.20	53.58±4.02**	59.56±2.71**

Note: All values are expressed in Mean ± SEM; the results were analyzed using Prism, version-5. One way analysis of variance (ANOVA) test followed by Dunnett's test was used to analyze the results, ** p< 0.01 was considered as statistically significant compared to control.

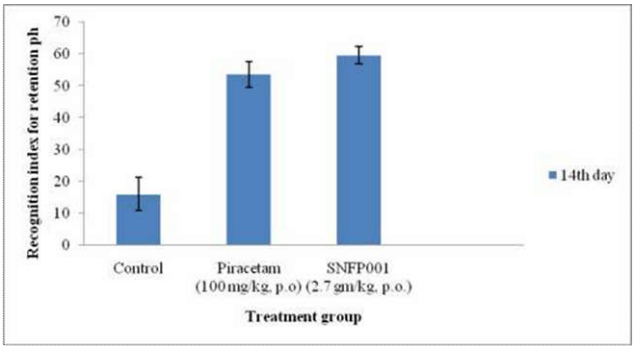

Fig. 6. Effect of OC on recognition index for retention phase

Table 7. Effect of OC on transfer latency (s) in elevated plusmaze

Days	Treatment group (Transfer latency in sec)		
	Control (Distilled water, 5ml/kg)	Piracetam (100 mg/kg, p.o)	OC (2.7 gm/kg, p.o.)
0 DAY	81.8±3.77	80.2±3.47	88±2.00
7th DAY	81.6±5.15	71.6±3.65	81.4±3.73
14th DAY	86.2±2.78	65.4±3.40**	72.8±2.63*

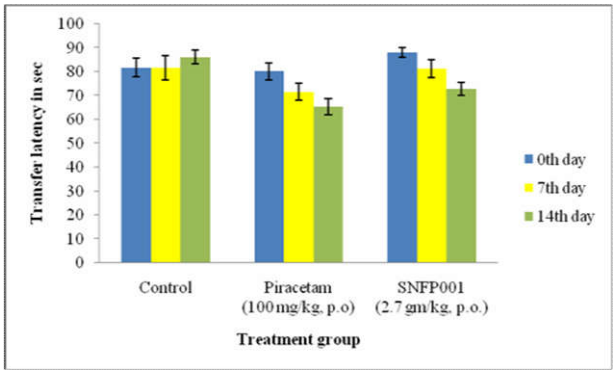

Fig. 7. Effect of OC on transfer latency (s) in elevated plus maze

Swimmingtest

The results are revealed that OC (2.7 gm/kg, p.o.) shows significant (p<0.01) decrease counts in locomotor activity compared to control at 7th and 14thday (Table 1 and Fig1). In open filed study, OC shows significant (p<0.01) decrease number of line crossing on 14thday only whereas, number of rearing does not changes significantly (Table 2, 3 and Fig 2, 3). In swimming test, OC shows significant (p<0.05) increase in immobility time compared tocontrol at 7th and 14thday (Table 4 and Fig 4). In light & dark test, OC shows significant (p<0.05) total time spent in illuminated area compared to control on 14th day only (Table 5 and Fig 5). In object recognition test OC shows significant (p<0.01) recognition index for retention phase on 14th day (Table 6 and Fig 6). Whereas elevated plus test, OC shows significant (p<0.05) transfer latency compared to control (Table 7 and Fig 7).

Conclusion

In present study, The OC (2.7 gm/kg, p.o.) exhibits enhanced antianxiety activity and moderate activity in antistrees on experimental animals. OC samples exhibits moderate activity of memory enhancer in light & dark test and elevated plus test whereas, object recognition test shows enhanced activity compared to control.

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