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Research Article

EVALUATION OF THE EFFECT OF *COSTUS IGNEUS* ON LEARNING AND MEMORY IN NORMAL AND DIABETIC RATS USING PASSIVE AVOIDANCE TASK

SHASHIKANTH CHETTY[1], SHALINI ADIGA[2*], SHIVKUMAR REDDY[1]

[1, 2]**Department of Pharmacology, Kasturba Medical College, Manipal University, Manipal 576104, Karnataka, India. Email:drshaliadiga@gmail.com**

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ABSTRACT

Objective: To evaluate the effect of *Costusigneus* on learning and memory in normal and diabetic rats using passive avoidance test.

Methods: Wistar rats were divided into 6 groups namely control, diabetic control *Costusigneus*(CI) alcoholic extract treated normal, and diabetic groups(250&500mg/kg). For induction of diabetes, a single dose of streptozotocin was injected (35 mg/kg i.p). After 30 days of oral administration of respective drug to different groups, blood glucose and body weight were measured and rats were assessed for learning and memory using passive avoidance test. Entrance latency to the dark compartment and time spent in the dark compartment was analysed using ANOVA, followed by Tukey's post hoc test.

Results: Diabetic rats showed impairment in acquisition trial of passive avoidance test. Diabetic rats treated with *C.igneus* showed significant reduction in the entrance latency and increased the time spent in the dark compartment during acquisition trial retaining their normal behaviour. During the post-shock retention testing at 24 and 48 hours, treatment with *C.igneus* showed significant increase in the entrance latency and decrease in the time spent in the dark room. The blood glucose of these rats was reduced to normal levels. The treated normal rats showed similar behaviour as the saline control.

Conclusion: *C.igneus* prevents learning and memory deficit which is otherwise impaired in diabetic rats. Hypoglycaemic activity of *C.igneus* is largely accountable for this protective effect on cognition.

Keywords: *Costusigneus*, diabetes, hyperglycaemia, learning and memory.

INTRODUCTION

Diabetes mellitus is a serious metabolic disorder characterised by a state of chronic hyperglycaemia. As per International Diabetes Federation (IDF), 366 million people were suffering from diabetes mellitus worldwide in 2011and it is estimated that by 2035 this will rise to 592 million[1].Diabetes results from either insufficient quantity of insulin or insensitivity to the available insulin. Hyperglycaemia may lead to both acute and chronic complications and the latter being major source of morbidity. Chronic complications include cardiovascular diseases, retinopathy, chronic renal failure, nerve damage both central as well as peripheral, and deficiency in the antioxidant defence system and lipid profile disorders [2-4]. Diabetes causes both electrophysiological and structural alterations of the brain which leads to impairment of cognitive function[5].Various literature reports have revealed that diabetic patients have moderate deficit in learning and memory [6-8].

Streptozotocin induced diabetes provides an experimental model for insulenopenic type 1 diabetes mellitus. Experimental diabetes has shown both structural and physiological changes in the central as well as the peripheral nerve fibres [9,10]. Hyperglycaemia plays a vital role in the development of cognitive deficit observed in diabetic patients. However, the exact mechanism through which hyperglycaemia mediates this effect is not clear. Hyperglycaemia alters the cognitive function through variety of mechanisms including activation of polyol pathway[11],excessive production of advanced end glycation products (AGEs)[12],and generation of reactive oxygen species. This results in the production of free radicals leading to membrane lipid peroxidation and destruction of the brain cells[13]. *Costusigneus* also known as Feirycostusor Spiral flag belongs to family *Costaeceae*[14]. *Costusigneus* is also appropriately called as insulin plant because of its hypoglycaemic activity[15].In India, diabetic patients consume *C.igneus* leaves to maintain their blood glucose level within normal limits[16].*C.igneus*is known to possess antioxidant activity [16, 17].In view of the above it is justifiable to unearth the potential of *C.igneus*as an alternate mode of treatment for cognitive deficit observed in diabetes. Hence, we planned to evaluate the effect of ethanolic extract of *Costusigneus* on learning and memory in both normal and diabetic rats.

MATERIALS AND METHODS

The study was carried out after obtaining the Institutional Animal Ethics Committee clearance (IAEC/KMC/100/2011-2012).

Chemicals

Streptozotocin (STZ) was obtained from sigma chemicals, St Louis, MO, USA. Buffers and other reagents used were of analytical grade.

Animals

Animals were obtained from as well as maintained at the central animal house under standard conditions. Adult albino male rats of Wistar strain, weighing between 200 and 250 grams were selected for the study. They were housed under controlled conditions of temperature (23 ± 2ºC) and humidity (50 ± 5%) on a 12 hour light/dark cycle. Each animal was housed in separate labelled polypropylene cages with sterile husk bedding. Water and food pellets were provided *ad libitum*.

Preparation of ethanolic extract of *Costusigneus*

The leaves of *Costusigneus* were collected locally and authenticated. Leaves were shade dried at room temperature for about a week and were finely powdered. The powder was loaded into Soxhlet extractor in batches of 200 g each and was subjected to extraction for 30-40 hours with 95% ethanol by reflux condensation at 60-80ºC. After extraction, the extract was transferred to a china dish; the solvent was distilled off and concentrated on a water bath at a temperature below 50ºC to syrup consistency. Then it was dried and stored in a desiccator. The yield was about 10%.

Diabetes induction

The protocol used to induce diabetes in rats resembles the type 1 diabetes of human beings [18, 19].Rats were fasted overnight prior to streptozotocin (STZ) induction. STZ was dissolved in a sodium citrate buffer (pH 4.5). STZ solution was injected intraperitoneally, at a dose of 35 mg/kg[19,20] and 10% glucose water was supplied to avoid sudden hypoglycaemia post-injection[18].Blood glucose measurement was performed seven days after STZ injection[18].

Blood was drawn from the tail vein and glucose level was determined using a glucometer. Rats with blood glucose levels more than 250 mg/dL were considered as diabetic.

Treatment

Animals were divided into six groups. Normal control and diabetic control rats received equi volume of normal saline and four *C. igneus* treated groups namely normal/CI 250, diabetic/CI 250 and normal/CI 500, diabetic/CI 500 received 250 mg/kg and 500 mg/kg of ethanolic extract of *C. igneus* respectively. After 30 days of oral administration of respective drug in different groups, blood glucose was measured and rats were assessed for learning and memory using passive avoidance test.

Passive avoidance test

The apparatus consisted of two compartments. An illuminated rectangular larger compartment (50 × 50 × 35 cm) having grid floor which is connected through an opening (6 cm × 6 cm) to a smaller dark compartment (15 cm × 15 cm). This dark compartment is provided with electrifiable grid. The connection between the two compartments can be closed with a sliding door made of transparent Plexiglas.

Procedure

The experiment was performed by the method of Bures J at el[21], Narayanan SN et al[22] with modifications. The experiment was performed in three stages: 1) an exploration test, 2) aversive stimulus, and 3) a retention test. During each trial rat was kept in the centre of larger compartment facing opposite to the dark compartment. Each trial lasted for three minutes during which the rat was allowed to explore both the compartments. Two parameters were noted during the trial 1) time taken by the rat to enter the dark compartment for the first time and 2) the total time spent by the rat in the dark compartment, using a stop-watch. After the three exploration trials, rat was once again kept in the centre of larger compartment. Once the rat entered the dark compartment the sliding door present between the two compartments was closed and three strong electric foot shock (50 Hz, 1.5 mA, and 1 s duration) was given at 5 seconds interval. The retention test was performed after 24 and 48 hours. During the retention, the latency time required for the rat to enter the dark compartment and total time spent in the dark compartment were recorded.

Statistical Analysis

The results were analysed for statistical significance using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, using SPSS computer software (version 15.0). A p-value<0.05 was considered as statistically significant.

RESULTS

Exploration and acquisition test

The entrance latency result of the exploration trial is depicted in figure 1. Time taken by the rats of all the groups to enter the dark compartment decreased from first trial to third trial and the decrease in entrance latency was statistically significant in all groups except for diabetic control group.

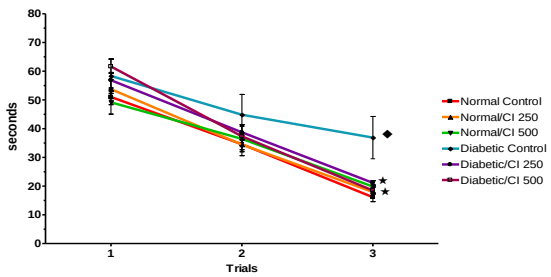

Fig. 1: Effect of *Costusigneus* on entrance latency in normal and diabetic rats during acquisition trial in Passive avoidance model

Values are in Mean ± SEM (n=6)

◆ p<0.05 (vs. Normal Control)

★ p<0.05(vs. Diabetic Control)

One-way ANOVA followed by Tukey's post hoc test

Figure 2 shows total time spent in the dark compartment by the rats. In each successive trial the rats spent more time in dark compartment. Normal rats treated with *C. igneus* spent almost equal time as compared to normal control group suggesting no difference in their behaviour. Diabetic rats treated with *C. igneus* spent more time in the dark compartment in each successive trial when compared to diabetic control group rats and the difference was statistically significant only in the third trial.

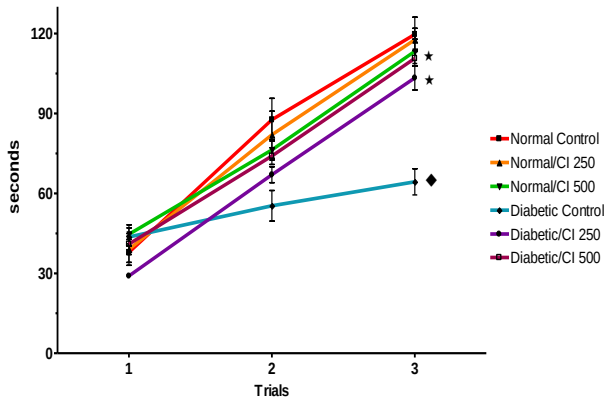

Fig. 2: Effect of *Costusigneus* on the time spent in the dark compartment by the normal and diabetic rats during acquisition trial in Passive avoidance model

Values are in Mean ± SEM (n=6)

◆ p<0.001 (vs. Normal Control)

★ p<0.05 (vs. Diabetic Control)

One-way ANOVA followed by Tukey's post hoc test

Retention test

During the memory retention test, difference was noted in the mean latency to enter the dark compartment between the control group and the normal rats treated with *C. igneus* after an aversive stimulus at 24 hours and 48 hours. This was found to be nonsignificant.

The entrance latency to the dark compartment for diabetic control group was less when compared to rest of the groups. The latency was approximately three times less and significant both at 24 hours and 48 hours of aversive stimulus as compared to diabetic treated groups (figure 3).

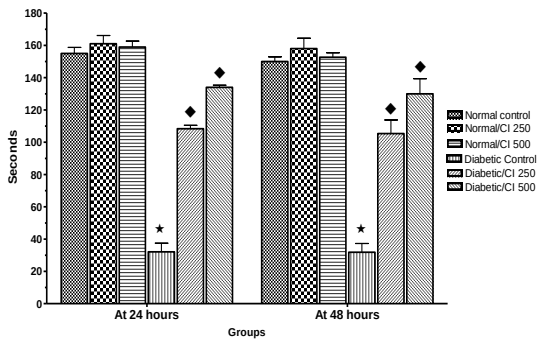

Fig. 3: Effect of *Costusigneus* on entrance latency in normal and diabetic rats during retention test in Passive avoidance model

Values are in Mean ± SEM (n=6)

★p<0.001 (vs. Normal Control)

◆p<0.001 (vs. Diabetic Control)

One-way ANOVA followed by Tukey's post hoc test

Figure 4 shows the total time spent in the dark compartment after the aversive stimulus. There was minimal variation in time spent between the rats belonging to normal control group and the normal rats treated with *C. igneus*. Diabetic control group rats spent significantly more time in the dark compartment after the aversive stimulus when compared to diabetic rats treated with *C. igneus*as observed at 24 hours and 48 hours respectively.

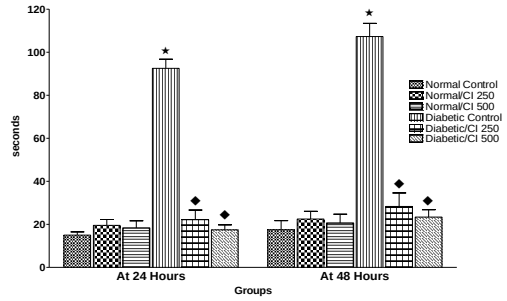

Fig. 4: Effect of *Costusigneus* on the time spent in the dark compartment by the normal and diabetic rats during retention test in Passive avoidance model

Values are in Mean ± SEM (n=6)

★p<0.001 (vs. Normal Control)

◆p<0.001(vs. Diabetic Control)

One-way ANOVA followed by Tukey's post hoc test

Blood glucose level

The blood glucose levels determined at the onset and at the end of the treatment are presented in Table 1. The blood glucose level in the diabetic control group was significantly more when compared to the normal control group at the end of the experiment.

However, the treatment with *C. igneus* significantly reduced the blood glucose level in a dose dependent manner in diabetic treated groups when compared to the diabetic control group. But no significant differences in glucose levels were observed when *C.igneus* was administered to nondiabetic rats and it was comparable to the normal control values.

Body weight

The effect of *C. igneus* extract on body weight changes of normal and diabetic rats are shown in the Table 1. During the 30 days of observation of the treated diabetic rats there was weight gain relative to day 0 i.e., before the start of the treatment.

The untreated diabetic rats lost 17% of body weight. No significant changes in body weight were observed with normal rats in both the control and treated groups.

Table 1: Effect of *Costusigneus* on blood glucose level and body weight in normal and diabetic rats

Groups	Blood glucose level (mg/dl) Mean ± SEM		Body weight (g) Mean ± SEM	
	Before treatment	After treatment	Before treatment	After treatment
Normal Control	105.50 ± 2.125	104.16 ± 2.574	228.16 ± 2.151	235.16 ± 1.681
Normal/CI 250	102.33 ± 1.475	109.00 ± 1.316	232.66 ± 2.231	239.33 ± 1.891
Normal/CI 500	103.00 ± 1.460	103.33 ± 1.646	234.25 ± 2.485	241.33 ± 1.646
DiabeticControl	315.00 ± 3.974	419.16 ± 5.403★	199.33 ± 1.686	169.83 ± 4.760★
Diabetic/CI 250	295.66 ± 2.962	121.66 ± 2.603◆	198.66 ± 1.837	214.16 ± 1.400◆
Diabetic/CI 500	309.83 ± 6.920	101.83 ± 1.329◆	202.33 ± 1.855	232.00 ± 1.460◆

Values are in Mean ± SEM (n=6)

★p<0.05 (vs. Normal Control)

◆ p<0.05(vs. Diabetic Control)

One-way ANOVA followed by Tukey's post hoc test

DISCUSSION

The results of our study showed that administration of ethanolic extract of *Costusigneus* prevents the development of learning and memory deficit which is otherwise impaired in diabetic rats. However, the extract treatment could do no better to the normal rats.

In the current study, diabetic control group showed no significant decrease in the time taken to enter the dark compartment over the three acquisition trials as compared to normal control and treated groups. This suggests that diabetic rats had alteration in their innate behaviour and also failed to learn over the time (decline in cognitive function). We also noted shorter latency to enter into the dark compartment in the diabetic control group during the memory retention trial (24 hours and 48 hours after the aversive stimulus) in the passive avoidance test indicating memory impairment in these animals. These results were in accordance with other studies that have also documented cognitive impairment in streptozotocin-induced diabetes mellitus [23,24]. However when diabetic rats were treated with *C. igneus* there was decrease in the time taken to enter the dark compartment over the three acquisition trials suggesting diabetic treated rats maintained their innate behaviour and also showed improvement in learning tendency. Diabetic treated rats also showed an increase in entrance latency into the dark compartment after the aversive stimulus suggesting memory retention in them. Cognition of diabetic rats treated with *C.igneus* was comparable to that of normal rats. However, normal rats administered with *C. Igneus* did not show significant difference in learning and memory capabilities when matched with normal control group.

In the current study, streptozotocin administration in rats depicted hyperglycaemia which was consistent with previous study reports. *C.igneus*, also called as insulin plant, is known for its hypoglycaemic activity [15].We found a dose dependent effect of *C.igneus* on fasting blood glucose levels in diabetic rats. The beneficial effect of *C.igneus* on blood glucose was reported in animal models [15,17],as well as in human beings[16].However, our study showed that the extract treatment did not alter the blood glucose in non-diabetic rats. This implies that the mechanism by which *C. igneus* reduces blood glucose may be attributed to its insulin-sensitizing effect rather than the insulin releasing action. However the exact mechanism remains uncertain.

Although hyperglycaemia is said to have a predominant role in the development of learning and memory deficit in diabetes, the exact mechanism remains elusive. Various suggested mechanisms include activation of polyol pathway leading to accumulation of sorbitol in the cranial nerves [11,25],excessive formation of advanced glycationend products [12,26],activation of protein kinase C[27] and shunting of glucose towards the

hexosamine pathway[28]. Hence maintaining the blood glucose level within normal limits in diabetic patients is of utmost important in preventing occurrence of cognitive impairment. In conclusion, the protective effect of *C.igneus* extract on learning and memory may be attributed mainly to its hypoglycaemic effect and partlyto its antioxidant property. Further studies are required for the confirmation of the active principles involved in its learning and memory enhancement.

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