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RESEARCH ARTICLE

Amelioration of Altered Blood Glucose Level by Improving Acetate and Serotonin Level of Brain in Immobilized Stressed Rat

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ABSTRACT

Objective: The present study was designed to determine the effect of acetate and selective serotonin reuptake inhibitors (SSRI) on stress and blood glucose levels in stressed rats. **Materials and Methods:** Animals were divided into five groups (n = 6), treated with 20 mg/kg *p.o.* of fluoxetine (SSRI) and glyceryl triacetate (GTA) at 6 g/kg *p.o.* dose (acetate supplementation) alone and in combination. For 28 days to assess its effect in immobilized stressed rats. Open field and hole board tests were used for the determination of stress in animals, followed by blood glucose levels. **Results:** Animals treated with 20 mg/kg *p.o.* fluoxetine (SSRI), 6 g/kg *p.o* GTA, and SSRI (fluoxetine) + GTA showed significant ($P < 0.01$) stress-resistant activity as compared to negative control. Results also demonstrated that there was a significant ($P < 0.01$) decrease in blood glucose levels in animals treated with 20 mg/kg *p.o.* fluoxetine (SSRI), 6 g/kg *p.o.* GTA, and SSRI (fluoxetine) + GTA. **Conclusion:** The present study concludes that acetate and SSRI supplementation possess stress-resistant and hypoglycemic potential in immobilized stressed rats by enhancing the acetylation of histone.

Keywords: 5-HT1A receptor, acetate supplementation, hyperglycemia, hypoglycemia, selective serotonin reuptake inhibitors, stress

INTRODUCTION

Stress is a major health problem in the population which is involved in the etiopathogenesis of mental disorders, including psychiatric disorders such as anxiety and depression, cognitive dysfunctions, and endocrine disorders including diabetes mellitus, immunosuppression, male sexual dysfunction, peptic ulcer, hypertension, and ulcerative colitis.[1] Chronic stress leads to altered functions of monoamine neurotransmitters especially noradrenalin, dopamine, and 5-hydroxytryptamine,[2] which play a key role in stressful conditions leading to behavioral changes and a cascade of hormonal release from the hypothalamus-pituitary-adrenal (HPA) axis results in various disorders such as anxiety, depression, eating, and sleeping disorders, decreased immune response, and hyperglycemia.[3,4] Significant evidence has been reported in the past few decades focusing on altered neurochemical, biochemical, and molecular mechanisms caused by stress.[5-9]

Chronic stress leads to alteration in the levels of serotonin (5HT), nor-adrenaline, and dopamine in the brain[2] serotonin (5HT) is one of the most potent neurotransmitters, having numerous effects and associated with many behavioral disorders, including anxiety, depression, schizophrenia, etc.[10,11] The serotonin 1A receptor (5-HT1A) is a G-protein-coupled receptor that works as a main

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mediator for serotonergic signaling in the central nervous system[12,13]. The 5HT1A receptor is involved in the development of stress resistance through the epigenetic mechanism of histone acetylation.[14]

Blood glucose level is influenced by food, physical activity, body weight, medications, and genetic factors. It is also reported that mental status affects blood glucose levels. It is postulated that chronic stress increases blood glucose levels by stimulation of the HPA axis in stress, leading to increased secretion of glucocorticoids.[15-18]

Acetate supplementation, using glyceryl triacetate (GTA), increases brain acetyl-coenzyme A (CoA) levels two-fold and attenuates both neuroglial activation and cholinergic cell loss.[19] Dietary acetate supplementation is suggested as a potential therapy for Canavan's disease,[20] is effective in alleviating tremors in a rat model of Canavan's disease,[21] and is neuroprotective in a rat model of traumatic brain injury.[22]

Selective serotonin reuptake inhibitors (SSRIs) inhibit the reuptake of serotonin. As a result, the serotonin stays in the synaptic gap longer than it normally would and may repeatedly stimulate the receptors of the recipient cell. In the short run, this leads to an increase in signaling across synapses in which serotonin serves as the primary neurotransmitter.[23]

As stress-related disease and death mount, it becomes increasingly important to characterize it. Forced immobilization produces both physical and emotional stress.[24] Hence, the present study was undertaken to investigate the effect of immobilized stress on blood glucose levels and the effect of acetate and SSRI supplementation on glucose levels in stress

MATERIALS AND METHODS

Materials

EPM, open field apparatus, wire mesh restrainers, biochemistry analyzer ambica diagnostics (AD)-100, cooling centrifuge (Remi Elektrotechnik Ltd., Vasai, India), micropipette (10–100 µL), anesthetic chamber, weighing machine, etc.

Liquistable glucose kit (AD, Parbhani, Maharashtra, India), Fluoxetine (Macleods Pharma Ltd., Andheri (E), Mumbai, India), glyceryl triacetate (Swastik Acids and Chemicals, Nagpur), and other chemicals of analytical grade, etc.

Animal

Eight-week-old healthy Sprague-Dawley rats (weighing 180–250 g) were used in the present study. Animals were housed in polypropylene cages with a wire mesh top and husk bedding and maintained under controlled conditions of light (10 h- light: 14 h- dark), temperature (22 ± 3°C), and humidity (approximately 50 ± 10%) and fed with standard pellet diet and water were used for the entire animal study. The protocol for all the animal studies was approved by the P. Wadhwani College of Pharmacy Institutional Animal Ethics Committee with approval no. 650/PO/Re/S/2002/CPCSEA/2016/03, date- March 12, 2016.

Experimental Design

The animals were divided into 5 groups, 6 animals in each group as follow:

- Group-I: Normal control group (positive control)
- Group-II: Negative control group (animals were subjected to stressful conditions)
- Group-III: Animals were subjected to stressful conditions and treated with 20 mg/kg *p.o.* SSRI (fluoxetine) alone
- Group-IV: Animals were subjected to stressful conditions and treated with 6 g/kg *p.o.* GTA alone
- Group-V: Animals were subjected to stressful conditions and were treated with 20 mg/kg *p.o.* SSRI (fluoxetine) + 6 g/kg *p.o.* GTA

Induction of Stress in Rats

Rats were assigned to a daily restraint stress for 6 h's × 28 days[25] in a wire mesh restrainer.[26] This kind of restrainer will only restrict the movements of the animal without causing any pain, discomfort, or suffocation.

Determination of Stress in Rats

Open-field test

A large plywood box (75 × 75 × 29 cm) painted gray with a black grid (15 × 15 cm squares) on the floor was used for investigational testing. The number of rears (animal on hind limbs), number of grid boxes entered (front 2 paws over a line), time in center 9 squares, and latency to leave the corner box initially were measured for 10 min.[27]

Hole-board test

The apparatus is composed of a gray wooden box (50 cm × 50 cm × 50 cm) having four equidistant holes 3 cm in diameter in the floor. Total locomotor activity (numbers of squares crossed) and the number and duration of head dipping (when both eyes disappeared into the hole) were recorded for 5 min.[28]

Determination of Blood Glucose

Blood from each animal was collected on the 28th day. Serum was prepared by 15 min centrifugation at 3000 rpm using a cooling centrifuge. The glucose level was determined using the liquistable glucose (glucose oxidase/peroxidase method) kit according to the manufacturer's instructions (AD).

Measurement of Adrenal Gland Weight

On the 28th day, all rats were euthanized and their adrenal glands were removed and weighed immediately. The adrenal weight was reported as the ratio of 100 mg of the animal's total body weight.[29]

Statistical Analysis

The experimental results were represented as mean ± standard deviation. Statistical analysis was performed by one-way analysis of variance for stress resistance activity of ethanolic extract of *Cassia occidentalis Linn*. The biochemical parameters in blood were statistically analyzed and compared using the Dunnett's test in the InStat software.

RESULTS

Table 1 shows the effect of acetate supplementation and SSRI (fluoxetine) on stressed rats using hole-board test. Group II shows the significant decrease ($P < 0.01$) in the number of box crossing and nose-poking behavior as compared to Group I, but Group III, Group IV, and Group V show significant ($P < 0.01$) increase in the number of box crossing and nose poking behavior as compared to Group II.
Table 2 shows the effect of acetate supplementation and SSRI (fluoxetine) on stressed rats using open field test. Group II shows a significant decrease ($P < 0.01$) in the number of boxes entered or latency to inside portion or duration of time and a significant increase ($P < 0.01$) in the number of rears as compared to Group I, but Group III, Group IV, and Group V show significant increase ($P < 0.01$) in the number of box entered or latency to inside portion or duration of time and significant decrease ($P < 0.01$) in the number of rears as compared to Group II.
Table 3 shows the effect of acetate supplementation and SSRI (fluoxetine) on the glucose level of stressed rats. Group II shows a significant increase ($P < 0.01$) in the level of glucose as compared to Group I, but Group III, Group IV, and Group V show a significant ($P < 0.01$) decrease in the level of glucose as compared to Group II.
Table 4 shows the effect of acetate supplementation and SSRI (fluoxetine) on the weight of the adrenal gland of stressed rats. Group II shows a significant increase ($P < 0.01$) in the weight of the adrenal gland as compared to Group I. Group III, Group IV, and Group V show significant ($P < 0.01$) decrease in the weight of the adrenal gland as compared to Group II.

Table 1: Effect of acetate supplementation and SSRI (fluoxetine) on stressed rats using hole board test

Groups	Number of box crossing	Number of nose poking
Group I	36.33±2.36	43.00±1.78
Group II	6.33±1.36**	5.00±2.36**
Group III	26.66±2.08@@	30.66±2.25@@
Group IV	32.66±1.52@@	37.66±2.36@@
Group V	37.33±1.36@@	38±0.89@@

Values are expressed in mean±SD, (n=6). **P<0.01, when compared to Group I; @@P<0.01, compared to Group II. SSRI: Selective serotonin reuptake inhibitors

Table 2: Effect of acetate supplementation and SSRI (fluoxetine) on stressed rats using open-field test

Groups	No. of box entered	No. of rears	Latency to an inside portion of the field (s)	Duration of time in the inside portion of the field (s)
Group I	190.19±5.85	40.1±3.49	113.90±4.71	44.20±2.56
Group II	123.96±6.53**	58.31±5.99**	92.34±3.79**	15.80±2.61**
Group III	200.10±3.45@@	45.13±3.01@@	105.15±0.32@@	33.22±2.62@@
Group IV	184.75±4.88@@	39.81±1.76@@	111.45±4.04@@	35.37±2.05@@
Group V	227.29±8.73	57.99±2.77	118.90±1.84@@	56.91±1.46@@

Values are expressed in Mean±standard deviation, (n=6). **P<0.01, when compared to Group I; @@P<0.01, compared to Group II. SSRI: Selective serotonin reuptake inhibitors

Table 3: Effect of acetate supplementation and SSRI (fluoxetine) on blood glucose of stressed rats

Groups	Blood glucose (mg/dL)
Group I	121.08±3.18
Group II	178.72±2.13**
Group III	162.89±2.1@@
Group IV	155.43±2.12@@
Group V	132.56±2.4@@

Values are expressed in mean±standard deviation, (n=6). **P<0.01, when compared to Group I; @@P<0.01, when compared to Group II. SSRI: Selective serotonin reuptake inhibitors

Table 4: Effect of acetate supplementation and SSRI (fluoxetine) on weight of adrenal gland/body weight

Groups	Weight of adrenal gland/body weight
Group I	13.65±0.17
Group II	15.58±0.13**
Group III	14.96±0.12@@
Group IV	14.5±0.18@@
Group V	14.22±0.17@@

Values are expressed in mean±standard deviation, (n=6). **P<0.01, when compared to Group I; @@P<0.01, when compared to Group II. SSRI: Selective serotonin reuptake inhibitors

DISCUSSION

The present study evaluates the hypoglycemic effect of acetate and SSRI supplementation by virtue of its stress-resistant potential.

In this study, stress was produced in animals by immobilization with a physical restrainer for 28 days.[25] Among the different stress models, immobilization is mostly used and accepted for studying stress-induced alterations[30] as immobilization-induced stress is useful for the assessment of central as well as peripheral mechanisms of stress-induced deficits, and for determining the effect of drugs on these deficits.[31] Open field test and hole board test are some of the most used models for the determination of stress in rodents.[32-34]

Result showed significant increase in the blood glucose level in stressed animals. This increase in the level of blood glucose is due to the stimulation of the HPA axis in stress, leading to increased secretion of glucocorticoids, which promotes gluconeogenesis and inhibits utilization of glucose.[15-18] This was supported by an adrenal gland study, which showed an increase in the weight of the adrenal gland in stressed animal.

Histone acetylation in the brain is responsible for stress-resistant activity. The histone acetylation state is actively maintained by the opposing activities of two enzyme families: Histone acetyltransferases (HATs) and histone deacetylases (HDACs). Acetylated histones serve as epigenetic markers or 'tags', which recruit HATs and other bromodomain-containing proteins. HDACs remove the acetyl group from lysine/arginine residues in the amino-terminal tails of core histones and other proteins, thus reversing the effects of the HATs.[35] Acetate supplementation, using GTA, reduces stress by increasing brain acetyl-CoA levels two-fold and attenuates both neuroglial activation and cholinergic cell loss.[19] In this study, a single oral dose of GTA shows stress-resistant activity because it increases the proportion of acetylated brain H3K9, H4 acetylated at lysine 8 (H4K8) and H4K16, with no changes in the acetylation state of histone H3 acetylated at lysine 14(H3K14), histone H4 acetylated at lysine 5 (H4K5), or histone H4 acetylated at lysine 12 (H4K12). It also decreases brain HDAC activity and HDAC2 expression with no changes in brain HAT activity.[36]

In our finding SSRI (fluoxetine) reduces stress in rats, this is because it inhibits reuptake of serotonin as a result, the serotonin stays in the synaptic gap longer than it normally would, and may repeatedly stimulate the receptors of the recipient cell. In the short run, this leads to an increase in signaling

across synapses in which serotonin serves as the primary neurotransmitter.[23] The elevated level of serotonin by SSRI (fluoxetine) activates the 5HT1A receptor, resulting in the acetylation of histone, and as mentioned earlier, histone acetylation is responsible for the reduction of stress.[14]

CONCLUSION

Physical restraint for 28 days (6 h/day) significantly increases blood glucose levels in rats. Acetate supplementation and SSRI at the dose of 6 g/kg and 20 mg/kg, respectively, shows significant stress resistance activity and have a pronounced effect on blood glucose level in stressed rats.

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