Original Article

Amelioration Of Altered Blood Lipid Profile By Improving Acetate And Serotonin Level Of Brain In Immobilized Stressed Rat

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DOI: 10.47750/pnr.2021.12.02.13

Abstract

Aim: Present study was designed to determine the effect of acetate and Selective serotonin reuptake inhibitors (SSRI) on stress and blood lipid profile in stressed rat.

Materials and Methods: Animals were divided in 5 groups (n=6) , treated with 20 mg/kg *p.o.* of Fluoxetine (SSRI) and glyceryl triacetate (GTA) at 6g/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 test were used for determination of stress in animals followed by blood lipid level.

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 significant ($p<0.01$) decrease in blood lipid level in animals treated with 20 mg/kg *p.o* Fluoxetine (SSRI), 6 g/kg *p.o* GTA and SSRI (Fluoxetine) + GTA.

Conclusion: Present study concludes, Acetate and SSRI supplementation posses stress resistant and antihyperlipidemic potential in immobilized stressed rats may be by enhancing the acetylation of histone.

Keywords: Stress, 5-HT1A receptor, Acetate supplementation, SSRI, Antihyperlipidemic activity

INTRODUCTION

Stress is a major health problem in population which is involved in the etiopathogenesis of mental disorders including psychiatric disorders such as anxiety and depression, cognitive dysfunctions, endocrine disorders including diabetes mellitus, immunosuppression, male sexual dysfunction, peptic ulcer; hypertension, ulcerative colitis etc. [1]. Chronic stress leads to altered functions of monoamine neurotransmitters specially noradrenalin, dopamine and 5-hydroxytryptamine [2], which play avital role in stressful conditions leading to behavioral changes and cascade of hormonal release from the hypothalamus–pituitary–adrenal axis (HPA) results in various disorders like anxiety, depression, eating and sleeping disorders, decreased immune response, hyperglycemia, [3,4]. Significant evidence reported in the last few decades focusing on altered neurochemical, biochemical, and molecular mechanism caused by stress [5-9]. Chronic Stress leads to alteration in the levels of Serotonin (5HT), Nor-adrenaline and Dopamine in brain [10] Serotonin (5HT) is one of the most potent neurotransmitters, having numerous effects and associated with many behavioural disorders including anxiety, depression, schizophrenia etc.[11 & 12]. The serotonin 1A receptor (5-HT1A) is a G-protein-coupled receptor which works as a main mediator for serotonergic signalling in the central nervous system[13 & 14]. The 5HT1A receptor is involved in the development of stress resistance through epigenetic mechanism of histone acetylation [15].

Blood lipid profile is influenced by nutrition, physical activity, body weight, medications and genetic factors.[16&17]It is also reported that mental status affects blood lipids.[18]It is postulated that stress increases blood lipids through increasing hepatic lipoprotein lipase activity by a stimulating sympathetic neuronal response.[19] Many studies investigated the influence of mental factors on the blood lipid profile. Dimsdale in his review suggested that the free fatty acids and total cholesterol levels increases after acute and/or chronic stress.[20] Another study by Tabriz, Iran, in 2007 demonstrated persons those who were exposed to stress have increased level of serum triglycerides[21]. Some studies have investigated a probable connection among low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HLD-C) levels and stress.[22&23]

Acetate supplementation, using glyceryl triacetate (GTA), increases brain acetyl-coenzyme A (CoA) levels two-fold and attenuates both neuroglial activation and cholinergic cell loss [24]. Dietary acetate supplementation is suggested as a

potential therapy for Canavan's disease [25], is effective in alleviating tremors in a rat model of Canavan's disease [26] and is neuroprotective in a rat model of traumatic brain injury [27].
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.[28]
As stress related disease and death mount, it becomes increasingly important to characterise it. Forced immobilisation produces both physical and emotional stress [29]. Hence, the present study was undertaken to investigate the effect of immobilised stress on blood lipid profile, effect of acetate and SSRI supplementation on blood lipid profile in stress.

MATERIAL AND METHOD

Animal

Eight weeks old healthy Sprague-dawley rats (weighing 180-250g) were used in the present study. Animals were housed in polypropylene cages with wire mesh top and husk bedding and maintained under controlled conditions of light (10h-light: 14h- 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. Protocol was approved by IAEC of P. wadhwani college of Pharmacy, Yavatmal.

Experimental design:

The animals were divided in 5 groups, 6 animals in each group as fallow:-
Group-I: -Normal control group (animals will be treated with saline solution only)
Group-II: -Negative control group (animals were subjected for stressful condition)
Group-III: - Animals were subjected for stressful condition and treated with 20 mg/kg *p.o.*SSRI (Fluoxetine) alone
Group-IV: - Animals were subjected for stressful condition and treated with 6g/kg *p.o.*GTA alone
Group-V: -Animals were subjected for stressful condition and were treated with 20 mg/kg*p.o.* SSRI (Fluoxetine) + 6g/kg *p.o.* GTA

Induction of stress in rats

Rats were assigned to a daily restraint stress for 6 hour's × 28 days [30] in a wire mesh restrainer.[31] 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 grey 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. [32]

Hole-board test

The apparatus 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-dippings (when both eyes disappeared into the hole) were recorded for 5 min.[33]

Determination of Blood lipid profile

Blood from each animal was collected at 28th day. Serum was prepared by 15-min centrifugation at 3000 rpm by using cooling centrifuge. The lipid level (triglyceride, Cholesterol and HDL) was determined by using the kit according to the manufacturer's instructions (Ambica Diagnostics). LDL was calculated using formula.

STATISTICAL ANALYSIS

The experimental results were represented as Mean ± SD. Statistical analysis was performed by one way ANOVA for antistress activity of SSRI (Fluoxetine) and Glyceriltriacetate alone in combination. The Biochemical parameters in blood were statistically analyzed and compared by using Dunnette test in the Instat software.

Result

Table 1 shows the effect of acetate supplementation & 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 behaviour as compared to Group I, but Group III , Group IV and Group V shows significant ($p<0.01$) increase in the number of box crossing and nose poking behaviour as compared to Group II.

Table 1. Effect of acetate supplementation & 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, SD= Standard Deviation

Table 2 shows the effect of acetate supplementation & SSRI (Fluoxetine)on stressed rats using Open field test. Group II shows the significant decrease (p<0.01) in the number of box entered or latency to inside portion or duration of time and significant increase (p<0.01) in the number of rears as compared to Group I, but Group III, Group IV and Group V shows 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 2. Effect of acetate supplementation & SSRI (Fluoxetine) on stressed rats using Open field test

GROUPS	No. Of box entered	No. Of rears	Latency to inside portion of the field (sec.)	Duration of time in the inside portion of the field (sec.)
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±SD, (n=6)
** P<0.01, when compared to Group I; @@P<0.01, compared to Group II, SD= Standard Deviation

Table 3 shows the effect acetate supplementation & SSRI (Fluoxetine)on Cholesterol, triglyceride, LDL and HDL level in stressed rats. Group II shows the significant increase (p<0.01) in the level of Cholesterol, triglyceride, LDL as compared to Group I, but Group III , Group IV and V shows significant (p<0.01) decrease in the level of Cholesterol, triglyceride, LDL as compared to Group II. Whereas HDL level significantly(p<0.01) decreased in Group II as compared to Group I, and significantly (p<0.01) increased in Group III, Group IV and V as compared to Group II.

Table 3. Effect of acetate supplementation & SSRI (Fluoxetine) on blood lipid profile of stressed rats

Groups	Cholesterol	Triglycerides	HDL	LDL
Group I	62.39±1.69	191.04±5.84	32.72±0.64	27.27±0.28
Group II	93.87±1.62**	235.49±2.75**	24.28±0.6**	63.95±0.34**
Group III	73.99±1.63@@	215.74±1.78@@	27.75±1.36@@	54.21±0.82@@
Group IV	69.19±0.97@@	212.08±2.67@@	29.74±1.24@@	39.08±0.64@@
Group V	64.55±1.31@@	195.98±2.57@@	32.96±1.3@@	37.65±0.18@@

Values are expressed in Mean±SD, (n=6)
** P<0.01, when compared to Group I; @@P<0.01, when compared to Group II, SD= Standard Deviation

DISCUSSION

Present study evaluates the antihyperlipidemic activity of acetate and SSRI supplementation by the virtue of its stress resistant potential.

In this study stress was produced in animal by immobilization with physical restrainer for 28 days.[30]Among the different stress models, immobilization is mostly used and accepted for studying stress-induced alterations [34] 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.[35] Open field test and Hole board test are some of the mostly used models for determination of stress in rodents.[36,37&38]

Numerous experimental animal studies have provided empirical support for a definite relationship between stress and lipid concentrations. In present study Cholesterol level was significantly raised in stress group which might be due to disturbed rate of synthesis and excretion [39] or due to the effect of epinephrine on lipoprotein lipase, hormone sensitive lipase and hepatic lipase [40]. In current study, elevated level of triglyceride was observed in rats exposed to physical restraint stress. The observed increased level of triglycerides in this study may be due to the stress induced catecholamine flow which activate lipolysis in adipose tissue and increase the free fatty acid flow to the liver where increased triglyceride synthesis and secretion occurs. The LDL and VLDL are well recognized as a risk factor and HDL as a protective factor against arteriosclerosis [41]. In the present study, observed the elevated level of LDL, VLDL and diminished level of HDL in the rats exposed to physical restraint stress may be due to activation of sympathetic system in stress. [42]

Histone acetylation in 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. Histone deactylases (HDACs) remove acetyl group from lysine/arginine residues in the amino-terminal tails of core histones and other proteins, thus reversing the effects of the HATs [43]. Acetate supplementation, using glyceryl triacetate (GTA), reduces stress by increase in brain acetyl-coenzyme A (CoA) levels

two-fold and attenuates both neuroglial activation and cholinergic cell loss [24]. In this study a single oral dose of GTA shows stress resistant activity may be because it increases the proportion of acetylated brain H3K9, It may also decreases brain HDAC activity [44].

In our finding SSRI (fluoxetine) reduces stress in rats, this is because it inhibit 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. [28] The elevated level of serotonin by SSRI (fluxetine) may activates 5HT1A receptor, resulting in the acetylation of histone and as mentioned earlier histone acetylation is responsible for the reduction of stress. [15]

CONCLUSION

Physical restraint for 28 days (6 hrs/day) significantly increases the level of the TC, TG, LDL, decreases the level of HDL in rats. Acetate supplementation and SSRI at the dose of 6gm/kg and 20mg/kg respectively shows significant stress resistance activity & have pronounced effect on lipid profile in stressed rats.

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