Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Ameliorative Effect of Quercetin and Rutin via Modulation of Hypothalamic–Pituitary–Adrenal Axis and Regulation of Fasting Glucose in Chronic Stress-Induced Prediabetes

Mustajab Quraishi, Santosh N. Mokale, Nikhil S. Sakle

Department of Pharmacology, Y. B. Chavan College of Pharmacy, Aurangabad, Maharashtra, India

Submitted: 20-07-2017 Revised: 29-08-2017 Published: 28-06-2018

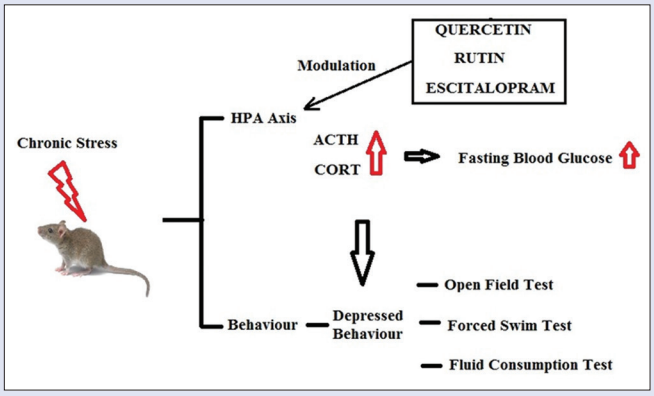
ABSTRACT

Background: Quercetin (QUE) and Rutin (RUT) have nutritive and medicinal values. On the other hand, there are no reports of scientific assessment of its hypothalamo–pituitary–adrenal (HPA) axis modulation in the treatment of prediabetes (DM). **Aim:** The current study was designed to investigate the modulatory effects of QUE, RUT, and escitalopram (ESC) as antidepressants on HPA axis in chronic stress-induced pre-DM in rats. **Materials and Methods:** The experimental protocol was of 5 weeks. Chronic unpredictable mild stress (CUMS) was used as a model of depression to induce pre-DM in rats. The treatment was started at the end of 4th week. After 5th week, the plasma adrenocorticotropic hormone (ACTH), serum corticosterone (CORT), fasting blood glucose (FBG), and behavioral parameters were evaluated. **Results:** Oral administration of QUE (50 mg/kg), RUT (50 mg/kg), and ESC (2.5 mg/kg) to stressed control alleviated HPA axis-associated parameters (ACTH and CORT) and significantly decreased the FBG. Besides this, the depressive effects induced by CUMS were significantly improved as evident from results indicating a promising antidepressant activity. Moreover, submaximal dose of QUE (25 mg/kg) and RUT (25 mg/kg) enhanced the antidepressant activity of ESC (1 mg/kg, p.o.), which suggests that they may act through the HPA axis. **Conclusion:** Current results suggest that chronic stress in rats causes dysregulation of the HPA axis which leads to diabetic-like condition, i.e., "pre-DM." It is possible that QUE, RUT, and ESC may be able to suppress the HPA axis response which could be beneficial for the treatment of stressed diabetic patients.

Key words: Antidepressants, chronic unpredictable mild stress, depression, diabetes, hypothalamo–pituitary–adrenal axis

SUMMARY

- The present study aims to reveal and establish the modulatory effects of quercetin, rutin, and escitalopram as antidepressants on hypothalamo–pituitary–adrenal axis in chronic stress-induced prediabetes in rats.



Abbreviations used: QUE: Quercetin; RUT: Rutin; HPA: Hypothalamic–Pituitary–Adrenal Axis; CUMS: Chronic unpredictable mild stress; ACTH: Adrenocorticotropic hormone; CORT: Corticosterone; FBG: Fasting Blood Glucose; ESC: Escitalopram; FST: Fasting Blood Glucose.

Correspondence:

Prof. Nikhil S. Sakle,
Y. B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Aurangabad - 431 001, Maharashtra, India. E-mail: nikhilsakle@gmail.com

DOI: 10.4103/pm.pm_323_17

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Stress is a term used in behavioral research that indicates "a physical, chemical, or emotional factor to which an individual fails to make a satisfactory adaptation" and it varies from person to person.[1] When the hypothalamus senses stimuli, produces general adaptation syndrome and stimuli are called stressors.[2] It has been well established that stress gives rise to the pathogenesis of a variety of diseases, including psychiatric disorders, endocrine disorders, immune suppression, sexual and cognitive dysfunctions, peptic ulcer, hypertension and heart diseases, ulcerative colitis, anxiety, and depression.[3,4]

A number of clinical studies have given evidence that depression is associated with hyperactivity of the hypothalamo–pituitary–adrenal (HPA) axis.[5] Antidepressant drug treatment normalizes the hyperactivity of the HPA axis with clinical recovery on or after depression.

Chronic stress in animals results in elevated adrenocorticotropic hormone (ACTH) and glucocorticoid levels in plasma and elevated production of corticotropin-releasing hormone (CRH) in the hypothalamic paraventricular nucleus.[6] The increased CRH secretion due to impaired negative feedback to the increment of cortisol is associated with depression.[7] A clear relationship of stress has been established for depression and DM. It may lead to deterioration of glycemic control through the neuroendocrine system in which the

Cite this article as: Quraishi M, Mokale SN, Sakle NS. Ameliorative effect of quercetin and rutin via modulation of hypothalamic-Pituitary-Adrenal axis and regulation of fasting glucose in chronic stress induced prediabetes. Phcog Mag 2018;14:S65-71.

stressors lead to the activation of the HPA axis, leading to various endocrine abnormalities, such as higher corticosterone (CORT) levels, which antagonize the actions of insulin. At the same time, an increase in visceral adiposity is observed, which plays an important role in DM by means of contributing to insulin resistance.[8]

Diabetes is a chronic disease and for the most part a still hidden disease. All diabetic patients go through a process called "pre-DM" or "impaired fasting glucose." It is a condition in which the blood glucose is lower than the DM, but higher than normal fasting blood glucose (FBG) between 100 and 125 mg/dl. It is estimated that up to 70% of people with pre-DM may develop type 2 DM during their lifetime.[9,10]

MATERIALS AND METHODS

Chemicals

Quercetin (QUE) and rutin (RUT) were purchased from Sigma-Aldrich Chemicals Private Ltd (MO, USA). Escitalopram oxalate (ESC) was purchased from Abbott Healthcare Pvt. Ltd., India. All the chemicals were extra pure and of analytical grade.

Animals

Male Wistar rats (body weight 180–220 g) were obtained from Wockhardt Research Centre Pvt., Ltd, Aurangabad, India, and housed in standard conditions (12 h light/dark cycle, 20 ± 2°C; 65% ± 15% relative humidity) with free access to water *ad libitum*. Behavioral experiments were performed between 8.00 and 10.00 am. All the experimental procedures were carried out according to the Institutional Animal Ethics Committee and the efforts were made to minimize animal suffering and to reduce the number of animals used.

Experimental design

After acclimatization to laboratory conditions for a period of 1 week, the rats were randomly divided into seven groups, each containing six animals and maintained as follows:

Rats subjected to the chronic unpredictable mild stress (CUMS) procedure were called as stress rats. Unstressed rats were exposed to behavioral tests and not subjected to the CUMS. Group I: Unstressed control rats receive normal saline orally. Group II: Stress control rats receive normal saline orally. Group III: Stress rats receive QUE (50 mg/kg, p.o.). Group IV: Stress rats receive RUT (50 mg/kg, p.o.). Group V: Stress rats receive ESC (2.5 mg/kg, p.o.). Group VI: Stress rats receive QUE (25 mg/kg, p.o.) and ESC (1 mg/kg, p.o.). Group VII: Stress rats receive RUT (25 mg/kg, p.o.) and ESC (1 mg/kg, p.o.).

Chronic unpredictable mild stress procedure

This model intends to use chronic stress in rats, which develops depressive state.[11] Rats were subjected to different kinds of stressors such as cage tilt of 45°, changes in temperature, tail squeezing, change in the light/dark cycle, wet bedding, and others are summarized in Table 1.[12] In general, stressors were applied at different times each day following a semi-random 2-week schedule. The stress procedure lasted for 4 weeks prior to the behavioral testing. Furthermore, bedding was changed every 3 days and animals were exposed to fresh environment. At the beginning of week 5, the stressed rats received the treatment with QUE, RUT, and ESC. Stressors continued to be applied during the testing phase, except on testing days to avoid the effects of acute stress. At least 12 h of rest was provided between a stressor and a test.[13]

Table 1: Stressors during chronic unpredictable mild stress model

Stressor	Days
Cage tilting (8 or 12 h)	1, 8, 14, 21, 28, 31
Deprivation of water and food for 24 h	6, 18, 26
Cage shaking (3 or 5 h)	3, 11, 25
Tail squeezing (2 min)	5, 16, 22
Damp sawdust (12 or 15 h)	2, 7, 14, 25
Overnight illumination	4, 10, 19, 23, 29
Switching cages	4, 9, 13, 19, 24, 26
Swimming in cold water (4°C)	5, 8, 17, 21, 27
Swimming in hot water (45°C)	7, 12, 18, 23, 29
Empty water bottle	4, 10, 14, 23, 27
Placement in empty cage with water at bottom	5, 9, 15, 17, 24, 30
Inversion of light/dark cycle	6, 11, 17, 22, 28
Noise	1, 8, 12, 19, 24, 26
Placement in empty cage	3, 6, 12, 18, 29
Hot stress in oven at 42°C	6, 12, 24
Cold stress	2, 7, 13, 19, 24
Soiled bedding	13
Soiled bed with cage tilt	15
Testing dark phase and reversed light-dark cycle	20
24 h social isolation	12, 19, 24, 31
Grouped housing	26

Adrenocorticotropic hormone and corticosterone measurement

A volume of 2 ml of blood sample was collected (8:00–10:00 am) by retro-orbital puncture with all aseptic precautions in a plain and EDTA vacutainer. It was allowed to clot for 30 min at room temperature for serum. Serum and plasma were separated by centrifugation at 2500 rpm for 5 min and subsequently stored at −20°C for ACTH and CORT determination. ACTH was determined by the chemiluminescent method using an IMMULITE automatic analyzer (DPC, Los Angeles, CA).[14] The CORT was estimated by automated chemiluminescence immunoassay system (Alpha Prime LS, France) by using Cortisol ELISA kit (DRG, USA).[15]

Fasting blood glucose

FBG was measured after 12 h fasting period with a glucometer (Accu-check Go; Roche Diagnostics, Germany). For the determination, blood samples were collected from the tail vein.[16]

Behavioral tests

Open field test

The arena was used to assess the exploratory activity of the animal over a 5-min period after drug administration. Apparatus was made up of Plexiglass (80 cm × 80 cm × 50 cm) with transparent walls and a black floor, divided into 16 squares of equal area. The following parameters were analyzed using the ANYMAZETM software (Stoelting Co., USA): the number of lines crossed, total distance traveled, the number of entries and time in the center zone, and the number of entries and time in the peripheral zone.[17]

Forced swim test

On the 1st day of the forced swim test (FST) (pretest), the rats were placed individually in a cylinder (50 cm × 23 cm) filled with a 30-cm depth with water (25°C ± 1°C) for 15 min and then were removed from the water, dried with towels, placed in a warmer enclosure for 15 min, and then returned to their home cages. The cylinders were emptied and cleaned between rats. Twenty-four hours after the first exposure to FST, the rats were retested for 5 min under identical conditions. Retest sessions were evaluated by ANYMAZE software (Stoelting Co, USA), which measured the average speed, maximum speed, total distance traveled, total mobility and immobility time, and total immobile and mobile episodes. A rat was rated to be immobile if it was making only movements necessary to keep its head above water.[18]

Fluid consumption test

Rats had access to water and 1% sucrose solution both in their home cages for 48 h prior to start of the experiment. Over the next 23 h, the animals were food and water deprived for 23 h. Fluid consumption was recorded by reweighing preweighed bottles of test solution. Bottles were counterbalanced across the left and right sides of the cages throughout the experiment. The percentage preference for sucrose was calculated by determining the percentage of total fluid consumption accounted for by ingestion of the 1% sucrose solution. Sucrose preference was calculated using the following formula: sucrose preference (%) = sucrose intake/(sucrose intake + water intake) × 100.[19,20]

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test and results were expressed as mean ± standard error of the mean (n = 6), $^{a}P < 0.001$, $^{b}P < 0.01$, and $^{c}P < 0.05$, ^{d}nonsignificant.

RESULTS

Effect of treatment on adrenocorticotropic hormone and corticosterone measurement

Hyperactivity of the HPA axis by stressor increases ACTH (69.80%) and CORT (76.31%) level in the stressed rats compared to normal rats as shown in Figure 1a and b. Reversal effects of treatment with QUE (50 mg/kg), RUT (50 mg/kg), and ESC (2.5 mg/kg) have been checked in the present study. In stressed rats, ACTH and CORT levels were significantly decreased by 36.87% and 45.71% ($P < 0.001$) in QUE (50 mg/kg), 49.80% and 57.87% ($P < 0.001$) in ESC (2.5 mg/kg), and 43.96% and 52.11% ($P < 0.001$) in QUE (25 mg/kg) + ESC (1 mg/kg) co-treatment, respectively. On the other hand, RUT (50 mg/kg) 18.55% and 27.27% ($P < 0.001$) and RUT (25 mg/kg) +ESC (1 mg/kg) co-treatment 8.96% and 14.85% ($P < 0.001$) decreased ACTH and CORT levels, respectively. The co-treatment of submaximal doses of QUE (25 mg/kg) with ESC (1 mg/kg) demonstrated a synergistic inhibitory effect on the HPA axis. These results indicated that the administration of QUE, RUT, and ESC co-treatment for 7 days effectively prevented CUMS-induced changes in plasma ACTH and serum CORT levels.

Effect of treatment on fasting blood glucose

We further investigated the role of treatment with QUE, RUT, and ESC on FBG level. Figure 1c indicates that QUE, ESC, and QUE + ESC co-treatment decreases FBG significantly. FBG levels were significantly decreased by 17.81% ($P < 0.001$) in QUE (50 mg/kg), 17.39% ($P < 0.01$) in ESC (2.5 mg/kg), and 15.28% ($P < 0.01$) in QUE (25 mg/kg) +ESC (1 mg/kg) co-treatment compared to RUT (50 mg/kg) 13.59% ($P < 0.05$) and co-treatment with RUT (25 mg/kg) + ESC (1 mg/kg) 15.42% ($P < 0.05$). This significant decrease in FBG is through normalization of dysregulated ACTH and CORT levels.

Effect of antidepressant treatment on open field test

Compared to unstressed control rats, administration of QUE (50 mg/kg), RUT (50 mg/kg), ESC (2.5 mg/kg) as well as co-treatment with QUE (25 mg/kg) + ESC (1 mg/kg) and RUT (25 mg/kg) + ESC (1 mg/kg) to stress rats augmented the number of line crossings (F [6, 35] = 8.526, P = 0.0030), total distance traveled (F [6, 35] = 8.408, P = 0.0031), the number of entries in center zone (F [6, 35] = 6.583, P = 0.8465), and time in the center zone (F [6, 35] = 68.962, $P < 0.0001$) of the apparatus as demonstrated in Figure 2a-d. In addition, at the same doses, it decreased the number of entries in the peripheral zone (F [6, 35] = 40.517, P = 0.3661) and time in the peripheral zone (F [6, 35] = 275.73, P = 0.1178) significantly shown in Figure 2e and f. Group mean heat maps of the rats' center position for all tests are represented in Figure 3. Heat map indicates cumulative time spent in the center and peripheral zones of the apparatus.

Effect of treatment on immobility in forced swim test

The effects of QUE (50 mg/kg), RUT (50 mg/kg), and ESC (2.5 mg/kg) alone and co-treatment, on the average speed, maximum speed, total

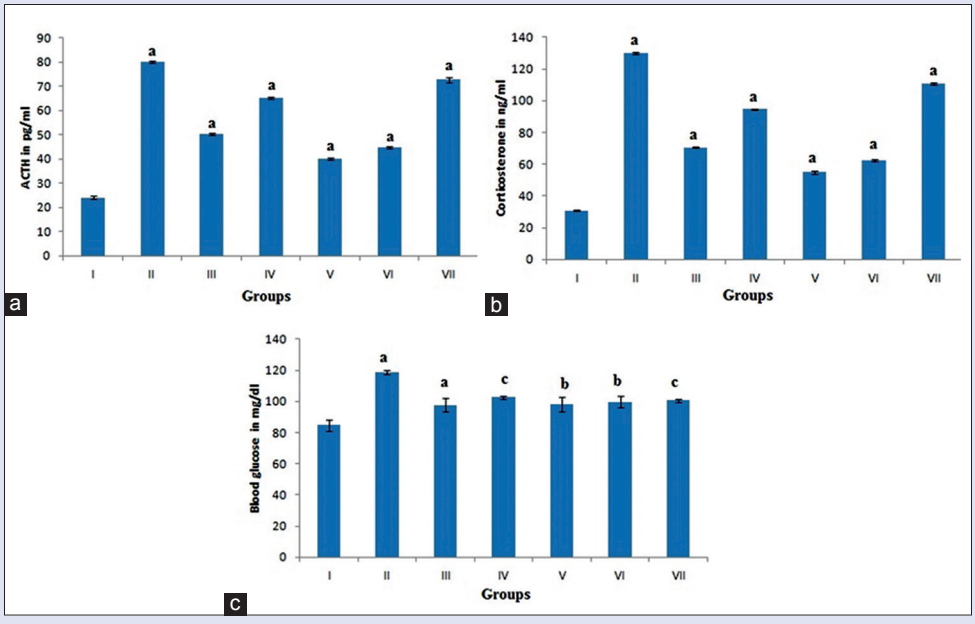


Figure 1: Effect of treatment on (a) Plasma adrenocorticotropic hormone levels (pg/ml), (b) Serum corticosterone levels (ng/ml), and (c) Fasting blood glucose level (mg/dl) in normal and stress rats

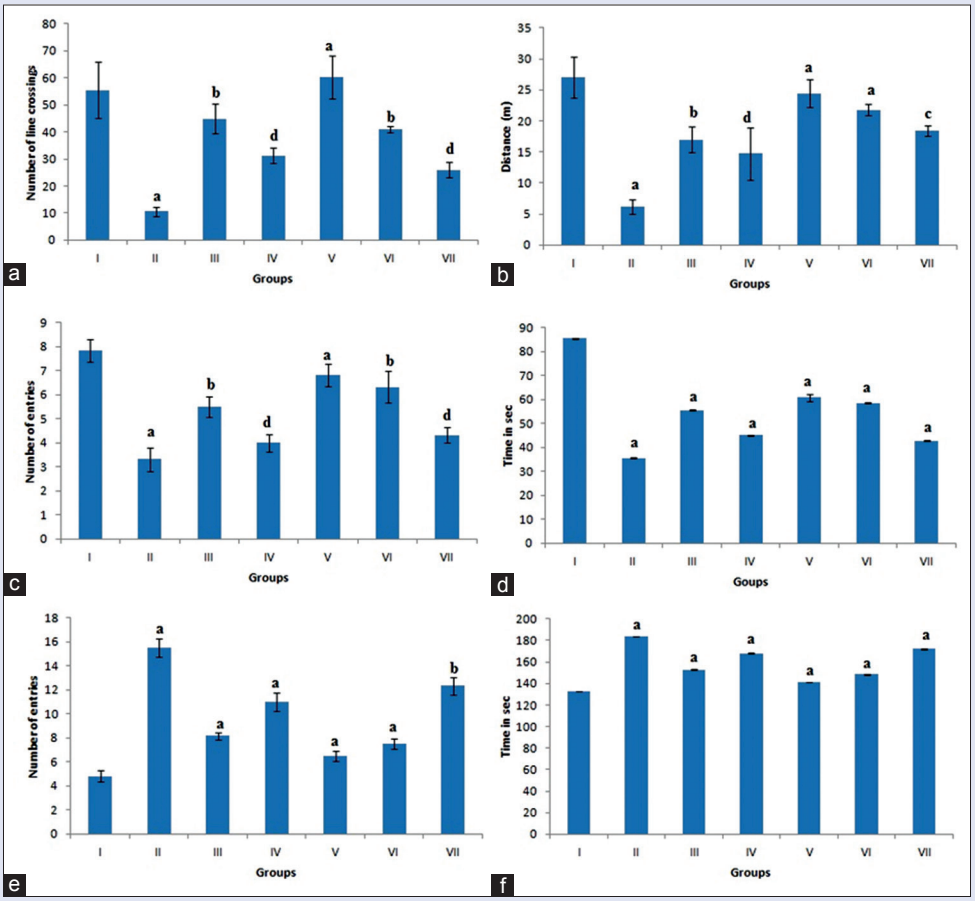


Figure 2: Behavioral data obtained with open field test in various groups. (a) Number of line crossings (b) Total distance traveled (m) (c) Number of entries in center zone (d) Time in center zone (s) (e) Number of entries in corner zone (f) Time in corner zone (s)

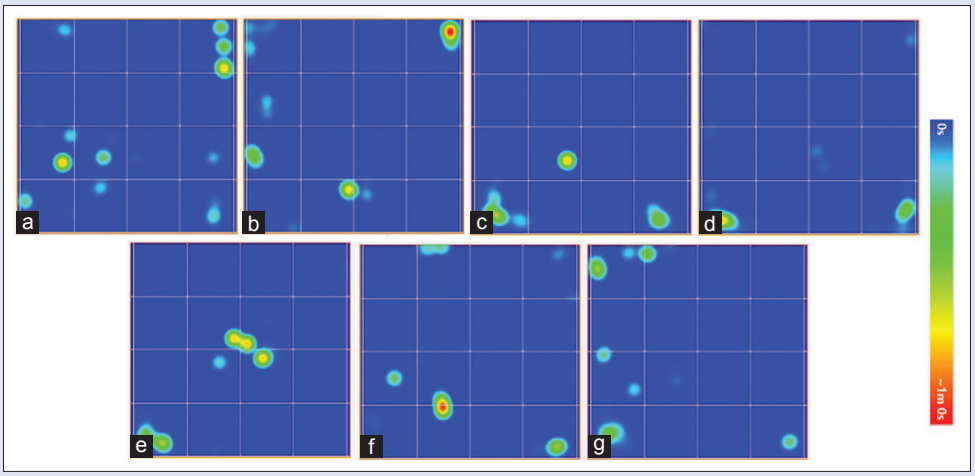


Figure 3: Group mean heat map of the animals' center position for all tests. (a) Unstressed control rats receive normal saline orally (b) stress control rats receive normal saline orally (c) Stress rats receive quercetin (50 mg/kg, p.o.) (d) Stress rats receive rutin (50 mg/kg, p.o.) (e) Stress rats receive escitalopram (2.5 mg/kg, p.o.) (f) Stress rats receive quercetin (25 mg/kg, p.o.) and escitalopram (1 mg/kg, p.o.) (g) Stress rats receive rutin (25 mg/kg, p.o.) and escitalopram (1 mg/kg, p.o.)

distance traveled, total mobility and immobility time, and total immobile and mobile episodes in the FST are shown in Figure 4a-g. One-way ANOVA revealed that the average speed ($F[6, 35] = 8.565$, $P = 0.0140$), maximum speed ($F[6,35] = 10.242$, $P = 0.0266$), total distance traveled ($F[6,35] = 9.447$, $P = 0.1232$), and total mobility time ($F[6, 35] = 17.763$, $P = 0.0458$) increased significantly. Treatment to CUMS-exposed rats significantly reduced

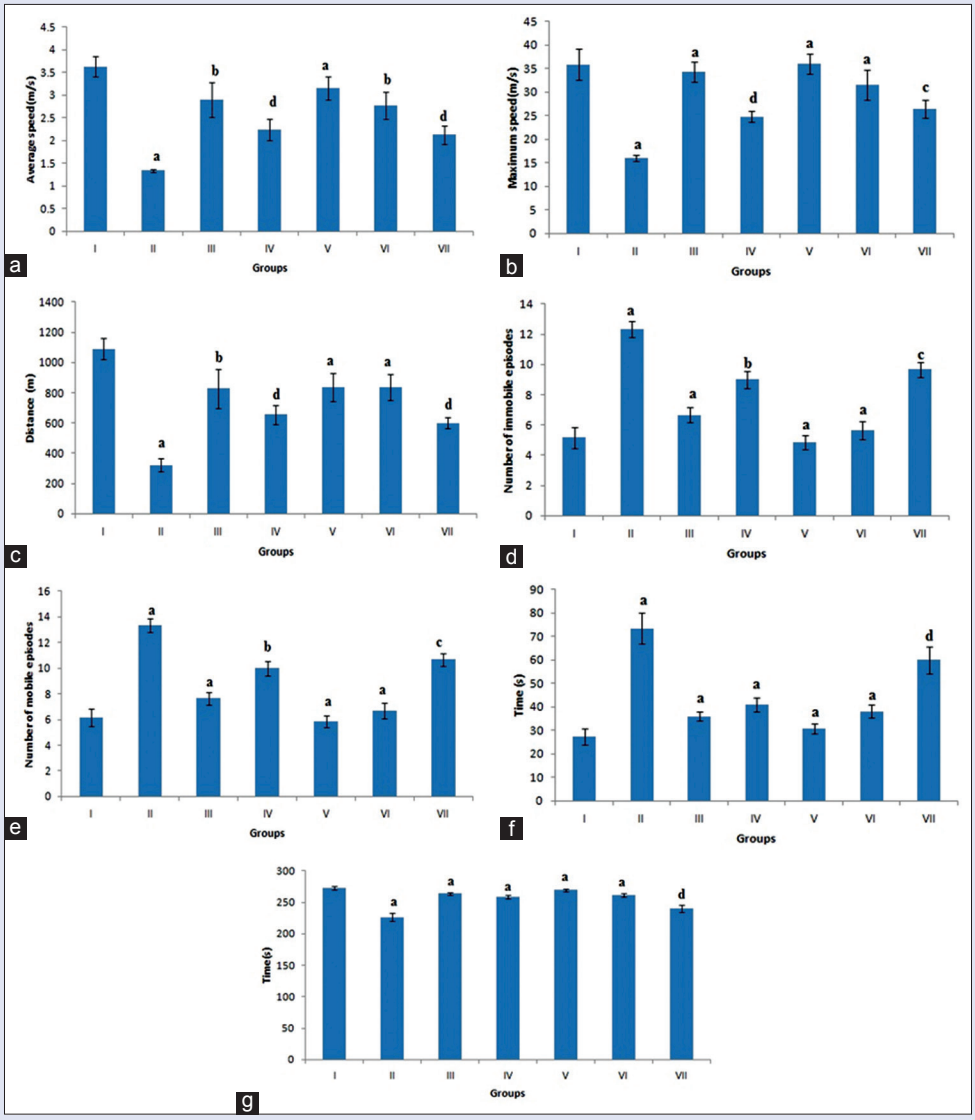


Figure 4: Parameters observed in forced swim test (a) average speed (m/s) (b) maximum speed (m/s) (c) total distance traveled (m) (d) total immobile episodes (e) total mobile episodes (f) total time immobile (s) (g) total time mobile (s)

total immobile episodes (F [6, 35] = 24.457, P = 0.9800) and mobile episodes (F [6, 35] = 24.457, P = 0.9800) with total immobility time (F [6, 35] = 17.763, P = 0.0458). Combined submaximal dose of QUE (25 mg/kg) with ESC (1 mg/kg) potentiated antidepressant-like activity significantly by increasing mobility and decreasing immobility time, whereas RUT (25 mg/kg) with ESC (1 mg/kg) showed nonsignificant effect.

Effect of antidepressant treatment on fluid consumption tests

No differences were observed between unstressed control and stressed rats in total fluid consumption test (water and 1% sucrose solution) over the period of 5 weeks of the study (F [6, 35] = 2.965, P = 0.4935) [Figure 5a]. In contrast, the stressed rats consumed significantly less sucrose solution by the end of the 5th week (F [6, 35] = 252.02, P = 0.1355) [Figure 5b]. Drinking of the sucrose solution accounted for a total fluid consumption in the stressed and in the control rats (F [6, 35] = 69.834, P = 0.4526) [Figure 5c].

DISCUSSION

The HPA axis is an important system activated in response to stress. Chronic stress causes a series of physiological changes in body and increases sympathetic and HPA tone and the sympathetic and HPA systems greatly influence energy metabolism, suggesting that they are possible mediators of the metabolic changes that occur following chronic stress. Hyperactivation of HPA axis in chronic stress impairs negative feedback mechanism which leads to the elevated production of ACTH and glucocorticoids (cortisol in humans and CORT in rodents) from the pituitary gland and adrenal cortex, respectively.[21,22] Previous studies have clearly shown that 3–4 weeks' exposure to stressors causes increased level of corticosteroids.[23] The occurrence of type 2 DM and pre-DM increased sharply in the recent years.[24]

Several clinical studies linked that the hyperactivation of the HPA axis associated with depression leads to various endocrine abnormalities.[25] Depression is the most common form of mental illness and is supposed to contribute in the development or progression of DM.[26] The link between depression and DM might be bidirectional and there might exist a few

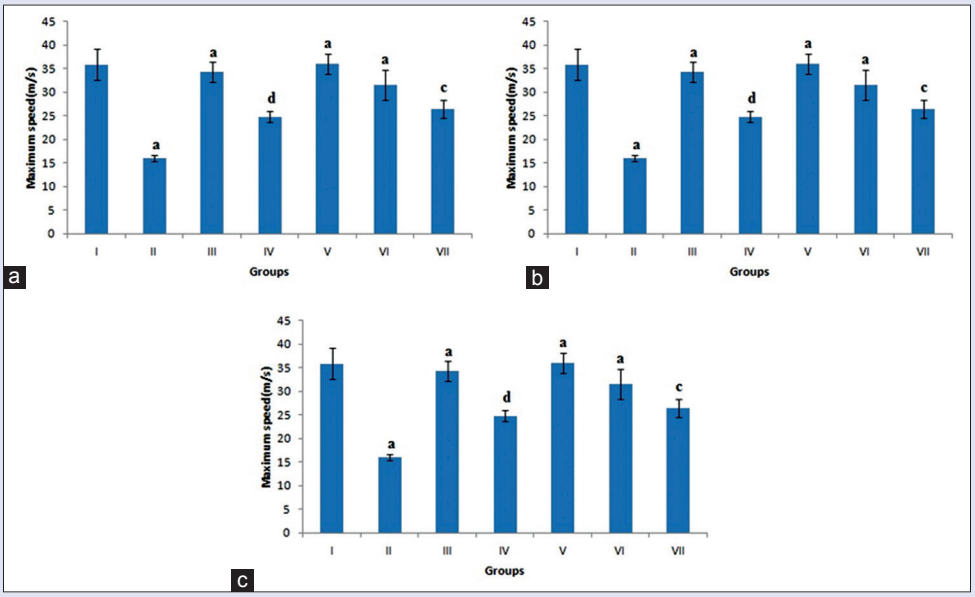


Figure 5: Effect of treatment on fluid consumption test (a) total fluid consumption (g) (b) Sucrose consumption (g) (c) percentage sucrose preference in normal and stressed rats

common physiological and etiological factors.[27] Activation of the HPA axis with elevated cortisol was noticed in both DM and depression.[28,29]

It is hypothesized that chronically high cortisol level (as reported in about 50% of patients with depression) might result in obesity, insulin resistance, and type 2 DM.[30] If depression is an underlying cause for the development of type 2 DM, people with depression would be at an increased risk of Pre-DM.[31]

The HPA axis directs the body's neuroendocrine response to stressors through influence and feedback between the hypothalamus, the anterior pituitary, and the cortex of the adrenal glands.[32] The selective serotonin re-uptake inhibitors (SSRIs) are the most commonly prescribed antidepressants because of their safety profile and efficacy.[33,34] They have been suggested in the treatment of depressed patients with DM, as they may cause hypoglycemia and weight loss in addition to their antidepressant properties.[35]

SSRIs such as citalopram and ESC are considered as drugs of choice in depression. Initial hyperactivation of HPA system is downregulated to normal levels by citalopram and ESC in depressed patients.[36,37] This normalization is linked with improved clinical outcome.[38] Population uses some phytochemical preparations, alternative to synthetic antidepressant drugs, with relative success.[39]

Based on the hypothesis, in our present study, the CUMS caused hyperactivity of the HPA axis in rats, as indicated by the increased level of ACTH and CORT. This neuroendocrine response leads to increase in FBG, resulting in prediabetic-like condition in rats. Treatment with antidepressant drugs, such as QUE, RUT, and ESC, attenuated the stress-induced increased level of ACTH, CORT, and FBG. Moreover, co-treatment of QUE (25 mg/kg) and RUT (25 mg/kg) with ESC (1 mg/kg) showed synergistic effect in reducing ACTH, CORT, and FBG levels. This indicates that the antidiabetic effects of QUE, RUT, ESC, and their combination are due to suppression of the HPA axis.

Open field test (OFT), FST, and fluid consumption tests are used to access the depressive behavior of rats.[40] Reduced exploration in OFT is suggested as an assessment indicator of depression-like behaviors in the stressed rats, which might be due to lethargy, apathy, and bodily neglect. Our results indicated that treatment with QUE, RUT, and ESC produced significant antidepressant-like effects, when assessed in OFT. What is noteworthy is that, the combination of QUE (25 mg/kg) and RUT (25 mg/kg) with ESC (1 mg/kg) produces synergistic effect and is supposed to be mediated via hyperactivated HPA axis to normal levels in CUMS depressed rats. The red area shown in heat maps denotes maximum occupancy and is the maximum value calculated across all tests in the experiment.[41]

Four weeks of CUMS exposure led to increased depression-like symptoms in stress rats when compared to unstressed control rats as evident from increased immobility in FST.[42] This model is apt for a broad spectrum of antidepressants, which significantly decrease the immobility time.[43] The results of the present study showed that treatment with QUE, RUT, and ESC evoked antidepressant-like effects in the FST by increasing mobility and decreasing immobility time.

The percentage preference for sucrose consumption was thus significantly reduced in the stressed rats. Anhedonia in CUMS-exposed rats decreased self-stimulation of reward pathways in the brain and in decreased preference for a dilute sucrose solution as confirmed in the study.[44,45] QUE, RUT, and ESC treatment alleviated the depression-like behavior caused by CUMS, as indicated by an increased sucrose preference in rats.

In summary, the results obtained here suggest that the involvement of chronic stress in rats is through dysregulation of the HPA axis which leads to diabetic-like condition. It is possible that change in FBG level by normalization of HPA axis and associated parameters (ACTH and CORT) is through QUE, RUT, and ESC treatment. It is possible that QUE, RUT, and ESC may be able to suppress the HPA axis response which could be beneficial for the treatment of stress diabetic patients.

CONCLUSION

The present study showed that CUMS induces prediabetes in rat through the downregulation of HPA Axis. Thus, QUE, RUT and ESC are able to suppress HPA mediated response and effective in stress diabetic condition.

Acknowledgements

The authors are thankful to Mrs. Fatima Rafiq Zakaria, Chairman, Maulana Azad Educational Trust, Dr. Rafiq Zakaria Campus, Aurangabad 431001 (MS), India, for providing the laboratory facility.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Jaggi AS, Bhatia N, Kumar N, Singh N, Anand P, Dhawan R, *et al*. A review on animal models for screening potential anti-stress agents. Neurol Sci 2011;32:993-1005.
2. Rao NV, Pujar B, Nimbal SK, Shantakumar SM, Satyanarayana S. Nootropic activity of tuber extract of *Pueraria tuberosa* (Roxb). Indian J Exp Biol 2008;46:591-8.
3. Piato AL, Detanico BC, Linck VM, Herrmann AP, Nunes DS, Elisabetsky E, *et al*. Anti-stress effects of the "tonic" *Ptychopetalum olacoides* (Marapuama) in mice. Phytomedicine 2010;17:248-53.
4. Chandola T, Britton A, Brunner E, Hemingway H, Malik M, Kumari M, *et al*. Work stress and coronary heart disease: What are the mechanisms? Eur Heart J 2008;29:640-8.
5. Duman RS, Aghajanian GK, Sanacora G, Krystal JH. Synaptic plasticity and depression: New insights from stress and rapid-acting antidepressants. Nat Med 2016;22:238-49.
6. Butterweck V, Winterhoff H, Herkenham M. St John's Wort, hypericin, and imipramine: A comparative analysis of mRNA levels in brain areas involved in HPA axis control following short-term and long-term administration in normal and stressed rats. Mol Psychiatry 2001;6:547-64.
7. Demir EA, Gergerlioglu HS, Oz M. Antidepressant-like effects of quercetin in diabetic rats are independent of hypothalamic-pituitary-adrenal axis. Acta Neuropsychiatr 2016;28:23-30.
8. Dhavale HS, Panikkar V, Jadhav BS, Ghulghule M, Agari AD. Depression and diabetes: Impact of antidepressant medications on glycaemic control. J Assoc Physicians India 2013;61:896-9.
9. Butacnum A, Chongsuwat R, Bumrungpert A. Black tea consumption improves postprandial glycemic control in normal and pre-diabetic subjects: A randomized, double-blind, placebo-controlled crossover study. Asia Pac J Clin Nutr 2017;26:59-64.
10. Li X, Lian FM, Guo D, Fan L, Tang J, Peng JB, *et al*. The rs1142345 in TPMT affects the therapeutic effect of traditional hypoglycemic herbs in prediabetes. Evid Based Complement Alternat Med 2013;2013:327629.
11. Willner P, Muscat R, Papp M. Chronic mild stress-induced anhedonia: A realistic animal model of depression. Neurosci Biobehav Rev 1992;16:525-34.
12. Farooq RK, Isingrini E, Tanti A, Le Guisquet AM, Arlicot N, Minier F, *et al*. Is unpredictable chronic mild stress (UCMS) a reliable model to study depression-induced neuroinflammation? Behav Brain Res 2012;231:130-7.
13. Mineur YS, Belzung C, Crusio WE. Effects of unpredictable chronic mild stress on anxiety and depression-like behavior in mice. Behav Brain Res 2006;175:43-50.
14. Djordjević J, Cvijić G, Davidović V. Different activation of ACTH and corticosterone release in response to various stressors in rats. Physiol Res 2003;52:67-72.
15. Jameel MK, Joshi AR, Dawane J, Padwal M, Joshi A, Pandit VA, *et al*. Effect of various physical stress models on serum cortisol level in wistar rats. J Clin Diagn Res 2014;8:181-3.
16. Amin A, Tuenter E, Exarchou V, Upadhyay A, Cos P, Maes L, *et al*. Phytochemical and pharmacological investigations on *Nymphoides indica* leaf extracts. Phytother Res 2016;30:1624-33.
17. Santos EC, Bicca MA, Blum-Silva CH, Costa AP, Dos Santos AA, Schenkel EP, *et al*. Anxiolytic-like, stimulant and neuroprotective effects of *Ilex paraguariensis* extracts in mice. Neuroscience 2015;292:13-21.
18. Kaminska K, Rogoz Z. The antidepressant- and anxiolytic-like effects following co-treatment with escitalopram and risperidone in rats. J Physiol Pharmacol 2016;67:471-80.
19. Lin YH, Liu AH, Xu Y, Tie L, Yu HM, Li XJ, *et al*. Effect of chronic unpredictable mild stress on brain-pancreas relative protein in rat brain and pancreas. Behav Brain Res 2005;165:63-71.
20. Luo DD, An SC, Zhang X. Involvement of hippocampal serotonin and neuropeptide Y in depression induced by chronic unpredicted mild stress. Brain Res Bull 2008;77:8-12.
21. Porter RJ, Gallagher P. Abnormalities of the HPA axis in affective disorders: Clinical subtypes and potential treatments. Acta Neuropsychiatr 2006;18:193-209.
22. Ulrich-Lai YM, Herman JP. Neural regulation of endocrine and autonomic stress responses. Nat Rev Neurosci 2009;10:397-409.
23. Pothion S, Bizot JC, Trovero F, Belzung C. Strain differences in sucrose preference and in the consequences of unpredictable chronic mild stress. Behav Brain Res 2004;155:135-46.
24. Chen S, Zhang Q, Dai G, Hu J, Zhu C, Su L, *et al*. Association of depression with pre-diabetes, undiagnosed diabetes, and previously diagnosed diabetes: A meta-analysis. Endocrine 2016;53:35-46.
25. Varghese FP, Brown ES. The hypothalamic-pituitary-adrenal axis in major depressive disorder: A Brief primer for primary care physicians. Prim Care Companion J Clin Psychiatry 2001;3:151-5.
26. Lustman PJ, Clouse RE. Depression in diabetic patients: The relationship between mood and glycemic control. J Diabetes Complications 2005;19:113-22.
27. Mezuk B, Eaton WW, Albrecht S, Golden SH. Depression and type 2 diabetes over the lifespan: A meta-analysis. Diabetes Care 2008;31:2383-90.
28. Moulton CD, Pickup JC, Ismail K. The link between depression and diabetes: The search for shared mechanisms. Lancet Diabetes Endocrinol 2015;3:461-71.
29. Giordano R, Guaraldi F, Berardelli R, Karamouzis I, D'Angelo V, Marinazzo E, *et al*. Glucose metabolism in patients with subclinical Cushing's syndrome. Endocrine 2012;41:415-23.
30. Knol MJ, Twisk JW, Beekman AT, Heine RJ, Snoek FJ, Pouwer F, *et al*. Depression as a risk factor for the onset of type 2 diabetes mellitus. A meta-analysis. Diabetologia 2006;49:837-45.
31. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2008;34:S62-9.
32. Jones T, Moller MD. Implications of hypothalamic-pituitary-adrenal axis functioning in posttraumatic stress disorder. J Am Psychiatr Nurses Assoc 2011;17:393-403.
33. MacGillivray S, Arroll B, Hatcher S, Ogston S, Reid I, Sullivan F, *et al*. Efficacy and tolerability of selective serotonin reuptake inhibitors compared with tricyclic antidepressants in depression treated in primary care: Systematic review and meta-analysis. BMJ 2003;326:1014.
34. Sclar DA, Robinson LM, Skaer TL, Galin RS. Trends in the prescribing of antidepressant pharmacotherapy: Office-based visits, 1990-1995. Clin Ther 1998;20:871-84.
35. Goodnick PJ, Henry JH, Buki VM. Treatment of depression in patients with diabetes mellitus. J Clin Psychiatry 1995;56:128-36.
36. Nikisch G, Eap CB, Baumann P. Citalopram enantiomers in plasma and cerebrospinal fluid of ABCB1 genotyped depressive patients and clinical response: A pilot study. Pharmacol Res 2008;58:344-7.
37. Nothdurfter C, Schmotz C, Sarubin N, Baghai TC, Laenger A, Lieb M, *et al*. Effects of escitalopram/quetiapine combination therapy versus escitalopram monotherapy on hypothalamic-pituitary-adrenal-axis activity in relation to antidepressant effectiveness. J Psychiatr Res 2014;52:15-20.
38. Flandreau EI, Bourke CH, Ressler KJ, Vale WW, Nemeroff CB, Owens MJ, *et al*. Escitalopram alters gene expression and HPA axis reactivity in rats following chronic overexpression of corticotropin-releasing factor from the central amygdala. Psychoneuroendocrinology 2013;38:1349-61.
39. de Freitas CM, Busanello A, Schaffer LF, Peroza LR, Krum BN, Leal CQ, *et al*. Behavioral and neurochemical effects induced by reserpine in mice. Psychopharmacology (Berl) 2016;233:457-67.
40. Ge JF, Xu YY, Qin G, Peng YN, Zhang CF, Liu XR, *et al*. Depression-like behavior induced by nesfatin-1 in rats: Involvement of increased immune activation and imbalance of synaptic vesicle proteins. Front Neurosci 2015;9:429.
41. Patel TP, Gullotti DM, Hernandez P, O'Brien WT, Capehart BP, Morrison B 3rd, *et al*. An open-source toolbox for automated phenotyping of mice in behavioral tasks. Front Behav Neurosci 2014;8:349.
42. Bhatt S, Radhakrishnan M, Jindal A, Devadoss T, Dhar AK. Neuropharmacological evaluation of a novel 5-HT3 receptor antagonist (6g) on chronic unpredictable mild stress-induced changes in behavioural and brain oxidative stress parameters in mice. Indian J Pharmacol 2014;46:191-6.
43. Pao LH, Lu SW, Sun GG, Chiou SH, Ma KH. Three Chinese herbal medicines promote neuroproliferation *in vitro*, and reverse the effects of chronic mild stress on behavior, the HPA axis, and proliferation of hippocampal precursor cell *in vivo*. J Ethnopharmacol 2012;144:261-9.
44. Willner P, Towell A, Sampson D, Sophokleous S, Muscat R. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology (Berl) 1987;93:358-64.
45. Pardon M, Pérez-Diaz F, Joubert C, Cohen-Salmon C. Age-dependent effects of a chronic ultramild stress procedure on open-field behaviour in B6D2F1 female mice. Physiol Behav 2000;70:7-13.