



Anti-stress activity of different extracts of *Caralluma fimbriata* in wistar rats

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Abstract

Objective: The current investigation was planned to assess adaptogenic activity of aqueous and ethanol extracts of *Caralluma fimbriata*

Methods: Anti-stress of aqueous and ethanol extracts of *Caralluma fimbriata* was appraised by inducing stress in rats through the forceful swimming. Biochemical parameters such as 6- β -OH-cortisol, urinary vanillymandelic acid (VMA), homovanillic acid (HVA), ascorbic acid and 5-hydroxyindoleacetic acid (5HIAA) were estimated to analyze the anti-stress activity. Aqueous and ethanol extracts of *Caralluma fimbriata* were administered orally at dose of 200 and 400 mg/kg body weight one h before the induction of stress to prevent stress-induced urinary biochemical changes in a dose dependent manner.

Results: Nevertheless, in all groups which were treated with aqueous and ethanol extracts of *Caralluma fimbriata*, there was no statistically difference between level of 6- β -OH-cortisol, urinary vanillymandelic acid (VMA), homovanillic acid (HVA), ascorbic acid and 5-hydroxyindoleacetic acid (5HIAA) as compared to normal animals.

Conclusion: The present investigation indicated that aqueous and ethanol extract of *Caralluma fimbriata* have potential anti-stress activity.

Keywords: Adaptogenic activity, *Caralluma fimbriata*, Biochemical parameters

Introduction

Stress is described as an individualized response of an organism to external and internal challenges that can be governed with intricacies [1]. Stress can be aroused by environment, social and pathological conditions arising during the life which induces changes in the immune, endocrine and nervous systems [2]. Hence, stress has been assumed to be involved in the epiopathogenesis of a various ailments such as psychotic disorder (Depression/ Anxiety), endocrine disorder (Diabetes mellitus), male sexual dysfunction, cognitive dysfunction, peptic ulcer, ulcerative colitis, immunosuppression and hypertension [3]. The evidences obtained from previous literatures revealed that main researches were undertaken on alternation of biochemical, neurochemical and molecular effect engendered by stress [4, 5]. The neurotransmitter (epinephrine, norepinephrine, dopamine, and serotonin) and glucocorticoids (cortisol, corticosterone) has a crucial role during stress [3]. Herbal remedies have been investigated for treatment of anti-stress activity [6] by reason of having adaptogenic properties due to their active constituents [7]. *Caralluma fimbriata* belongs to family Asclepiadaceae commonly called as a famine food, appetite suppressant and thirst quencher among tribal populations [8]. This plant has been used traditionally as analgesic, anti-inflammatory and antihyperglycaemic [9]. The

present research was taken up to predict potential antistress activity of aqueous and ethanol extracts of *Caralluma fimbriata*.

Materials and methods

Plant material and preparation of extracts

The leaves of *Caralluma fimbriata* were collected from Chennai, Tamil Nadu, India and authenticated by Green Chem of India, Doddinduvadi District, Bangalore, Karnataka, India; a voucher specimen (CFO-GR-612) was conserved for future references. The fresh leaves of *Caralluma fimbriata* were washed with tap water and air dried for one h. Then it was cut into small pieces, dried in room temperature for two weeks, grounded into powder with the help of hand mill and stored in room temperature. The leaves powder was macerated in the solvents including ethanol 95% (v/v) and water at room temperature, undergoing mechanical shaking for 4 h followed by filtration. The extracts obtained were concentrated in a rotary evaporator at 40 °C and the residue was extracted twice again analogously, there by obtaining the crude solvent extracts.

Chemicals

Homovanillic acid (CAS 306-08-1), vanillymandelic acid (VMA), 6- β -OH-cortisol, 5-hydroxyindoleacetic acid (5HIAA) and ascorbic acid was purchased from Sigma, ST Louis, MO, USA. Acetonitrile and methanol HPLC grade were supplied

from Qualigens, Fischer Scientific, Mumbai. All other chemicals were analytical grade and obtained from local store of Visveswarapura Institute of Pharmaceutical Sciences.

Animals

Albino wistar rats (150-200 gm) of either sex obtained from the NIMHANS animal house, Bengaluru and were housed at room temperature in a well-ventilated animal house. The animals had natural day and night cycle with temperature between 25 ± 3 °C. The animals were maintained under standard conditions. The rats had free access to standard rat chow and water *ad libitum* for at least one week prior to use. The study protocol was approved by Institutional Animal Ethics Committee (IAEC), Visveswarapura Institute of Pharmaceutical Sciences, Bangalore (Registration No: 152/1999, renewed in 2012).

Evaluation of anti-stress activity

Calibration curve was obtained for determination of standard value of HVA, VMA, 6- β -OH cortisol and 5-HIAA in urine by using HPLC method [10]. Calibration curve for ascorbic acid was attained to estimate standard value of this biomarker by using spectrophotometer [11]. Rats of either sex weighing between 180–220 gm were divided into five groups (I, II, III, IV & V) each containing six animals. The 24 h urine samples from each group were collected into two different beakers, one containing 5 ml of 10% oxalic acid for the spectrophotometric determination of ascorbic acid at 550 nm and the other containing 0.5 ml of 6N hydrochloric acid for the determination of stress metabolites. The experimental protocol was divided into four phases: In the first phase of the experiment, 24 h urine samples were collected in all the groups and subjected to analysis for all biomarkers to gain the normal value for 4 consecutive d. In the second phase, after a recovery period of one week, the animals were subjected to swimming stress individually. The 24 h urine samples were collected in all the groups and subjected to analysis for finding changes in biomarkers value during stress for 4 consecutive days. In the third phase of the experiment, after a recovery period of one week, the experimental animals were treated as follows for 4 consecutive d. Group 1 rats served as normal control and received 2 ml/kg distilled water, group 2, 3 rats were administrated orally with aqueous extracts of *Caralluma fimbriata* at dose of 200 mg/kg and 400 mg/kg respectively. Group 4, 5 rats received ethanol extracts *Caralluma fimbriata* orally at dose of 200 mg/kg and 400 mg/kg respectively. In the final phase of the experiment, after a recovery period of one week, the administration of *Caralluma fimbriata* extract were done as mentioned in the third phase, one h prior to the daily induction of stress for 4 consecutive d while group I serving as control. The 24 h urine samples were collected in all the groups and subjected to analysis for all biomarkers and the data were noted for 4 consecutive d to estimate the effect of the aqueous and

ethanol extracts of *Caralluma fimbriata* on the stress induced biochemical changes.

Statistical analysis

The data were displayed as mean \pm S.E.M. Statistical analysis was done by using student's paired t-test, where the difference was considered significant if $p < 0.05$.

Results and discussion

Stress is linked to many diseases such as heart disease, stroke, cancer, gastrointestinal problems, necrosis, etc [3]. In view of the increasing investigation towards plants have non-specific resistance (adaptogens) activity towards stress, current research was considered to estimate the effect of aqueous and ethanol extracts of *Caralluma fimbriata* on non-invasive biomarkers. Locus coeruleus in the centre and sympathetic nerves in the periphery release noradrenaline as the mediator [12]. Raphe nuclei in the centre and enterochromaffin cells in the periphery secrete serotonin as the mediator. While neurons of mesolimbic system, nigrostriatal system and tubero infundibular system in the centre and the renal system in the periphery secrete dopamine as the mediator. These amines are metabolized by MAO and COMT to various metabolites for instance; HVA is a metabolite of dopamine, VMA is a metabolite of NA, 5-HIAA is a metabolite of serotonin, 6- β -OH cortisol is a metabolite of cortisol and ascorbic acid is a metabolite of glucose that all of them were appraised as non-invasive biomarkers to divulge the increase in peripheral sympathetic activity during stress to evaluate the anti-stress activity of aqueous and ethanol extracts of *Caralluma fimbriata* [13]. The standard value of HVA, VMA, 6- β -OH cortisol, 5-HIAA and ascorbic acid were obtained by calibration and exhibited respectively in Table 1-5. Figures 1-5 displayed the effect of aqueous and ethanol extracts of *Caralluma fimbriata* on urinary levels of HVA, VMA, 6- β -OH cortisol, 5-HIAA and ascorbic acid biomarkers respectively in normal and stress condition. There was variation in each biomarkers from group to group in normal state. The amount of VMA was found to be low in group I ($0.353 \mu\text{g} \pm 0.019$) and high in group V ($0.429 \mu\text{g} \pm 0.014$). The amount of 5-HIAA was perceived to be low in group II ($0.375 \mu\text{g} \pm 0.014$) and high in group V ($0.518 \mu\text{g} \pm 0.015$). The amount of 6- β -OH cortisol was observed to be low in group V ($0.435 \mu\text{g} \pm 0.016$) and high in group I ($0.518 \mu\text{g} \pm 0.013$). The amount of HVA was noted to be low in group I ($0.109 \mu\text{g} \pm 0.005$) and high in group III ($0.159 \mu\text{g} \pm 0.014$). The amount of ascorbic acid was found to low in group IV ($44.46 \mu\text{g} \pm 1.53$) and high in group I ($54.01 \mu\text{g} \pm 4.49$). The data revealed the significant increase ($P < 0.05$) in urinary level of HVA, VMA, 6- β -OH cortisol, 5-HIAA and significant reduction ($P < 0.05$) in urinary level of ascorbic acid in stress condition. The changes in levels of the urinary metabolites in animals treated with aqueous and ethanol extracts of *Caralluma fimbriata* as compared to normal basal levels were

found to be non-significant. Aqueous and ethanol extracts of *Caralluma fimbriata* significantly ($P<0.05$) diminished urinary levels of VMA, in group II - V. Significant reduction ($P<0.05$) in 5-HIAA, 6- β -OH cortisol and HVA was found in group III, IV

and V. Significant increase ($P<0.05$) in urinary ascorbic acid levels was perceived in group III, IV and V compared to their respective stress condition.

Table 1: Standard values of homovanillic acid (HVA)

Concentration (ng/ml)	Peak Area Ratio		
	Trial-I	Trial-II	Average
25	0.041	0.043	0.042
50	0.090	0.089	0.089
100	0.183	0.179	0.181
250	0.470	0.471	0.470
500	0.922	0.928	0.925
1000	2.012	2.003	2.009
2500	5.050	5.285	5.168
5000	10.274	10.207	10.241

Table 2: Standard values of vanillylmandelic acid (VMA)

Concentration (ng/ml)	Peak Area Ratio		
	Trial-I	Trial-II	Average
25	0.056	0.060	0.058
50	0.088	0.102	0.095
100	0.188	0.208	0.198
250	0.546	0.522	0.534
500	1.007	1.142	1.074
1000	2.428	2.646	2.537
2500	6.100	6.349	6.224
5000	12.743	12.881	12.812

Table 3: Standard values of 6- β -hydroxy cortisol (6- β -OH cortisol)

Concentration (ng/ml)	Peak Area Ratio		
	Trial-I	Trial-II	Average
100	0.826	0.828	0.827
250	2.809	2.110	2.459
500	4.196	4.020	4.108
1000	8.244	9.013	8.628
2500	19.860	19.320	19.590
5000	39.350	40.320	39.835

Table 4: Standard values of 5-hydroxy indole acetic acid (5-HIAA)

Concentration (ng/ml)	Peak Area Ratio		
	Trial-I	Trial-II	Average
25	0.026	0.036	0.031
50	0.077	0.079	0.078
100	0.157	0.157	0.157
250	0.333	0.337	0.335
500	0.672	0.677	0.674
1000	1.345	1.354	1.349
2500	3.574	3.481	3.528
5000	6.955	7.024	6.989

Table 5: Standard values of ascorbic acid

Concentration (ng/ml)	Peak Area Ratio		
	Trial-I	Trial-II	Average
5	0.029	0.028	0.028
10	0.074	0.077	0.075
20	0.148	0.158	0.153
30	0.251	0.243	0.247
40	0.321	0.332	0.326
50	0.407	0.409	0.408

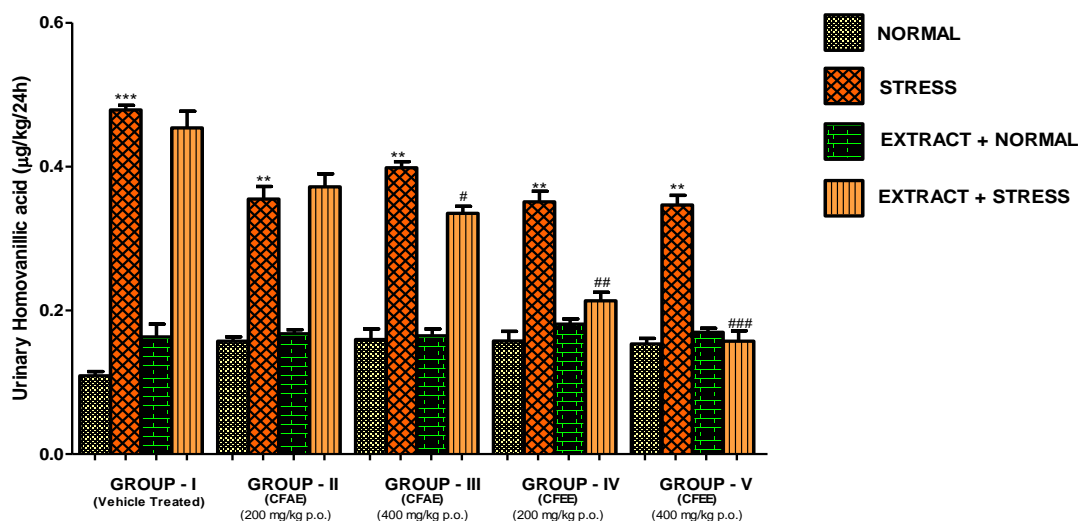


Figure 1: Influence of aqueous and ethanol extracts of *Caralluma fimbriata* on 24 h urinary levels of HVA in normal and stress induced rats. n = 6, Values are mean \pm S.E.M, statistical analysis was done by using student's paired t-test. ***P<0.0001, **P<0.001 significant difference from normal condition of the corresponding groups; ####P <0.0001, ###P <0.001, #P <0.01 significant difference from stress condition of the corresponding groups.

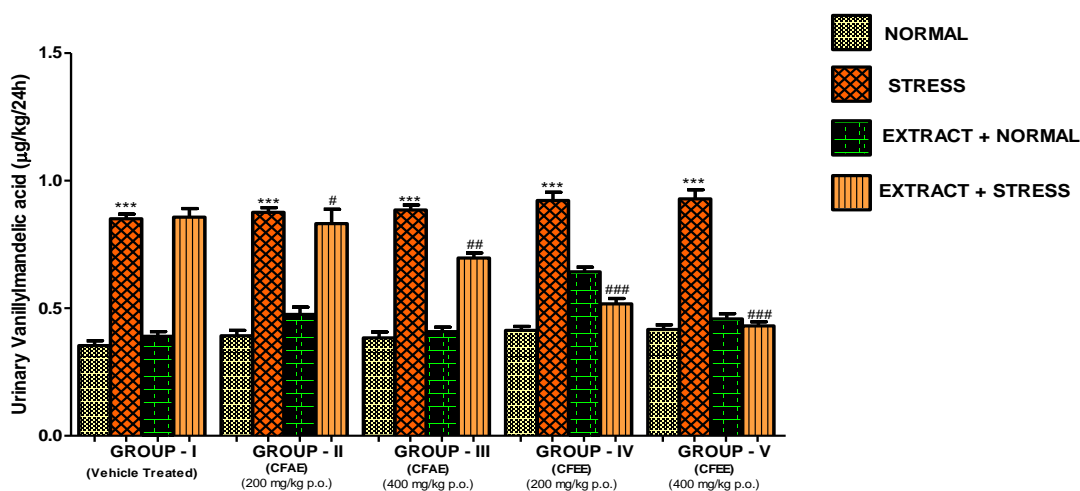


Figure 2: Influence of aqueous and ethanol extracts of *Caralluma fimbriata* on 24 h urinary levels of VMA in normal and stress induced rats. n = 6, Values are mean \pm S.E.M, statistical analysis was done by using student's paired t-test. ***P<0.0001 significant difference from normal condition of the corresponding groups; ####P <0.0001, ###P <0.001, #P <0.01 significant difference from stress condition of the corresponding groups.

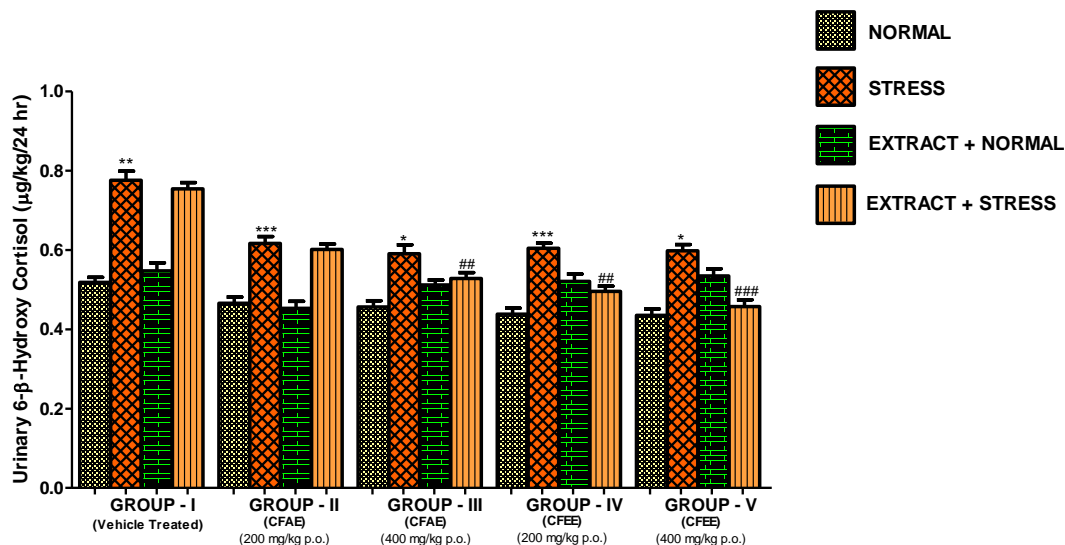


Figure 3: Influence of aqueous and ethanol extracts of *Caralluma fimbriata* on 24 h urinary levels of 6-β-OH cortisol in normal and stress induced rats. n = 6, Values are mean ±S.E.M, statistical analysis was done by using student's paired t-test. ***P<0.0001, **P<0.001, *P<0.01 significant difference from normal condition of the corresponding groups; ###P <0.0001, ##P <0.001 significant difference from stress condition of the corresponding groups.

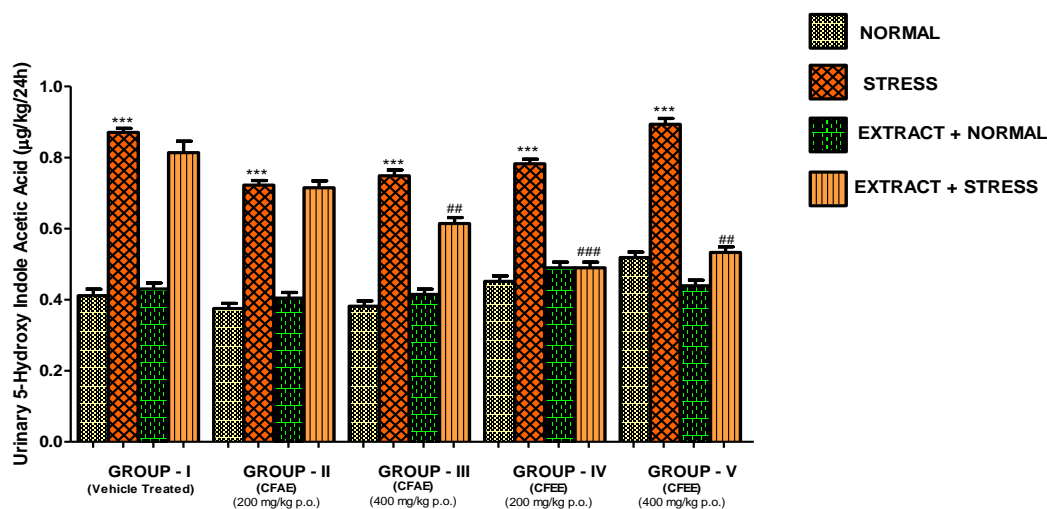


Figure 4: Influence of aqueous and ethanol extracts of *Caralluma fimbriata* on 24 h urinary levels of 5-HIAA in normal and stress induced rats. n = 6, Values are mean ±S.E.M, statistical analysis was done by using student's paired t-test. ***P<0.0001 significant difference from normal condition of the corresponding groups; ###P <0.0001 and ##P <0.001 significant difference from stress condition of the corresponding groups.

The results obtained from present research exhibited that HVA, VMA, 5-HIAA, 6-β-OH cortisol and ascorbic acid were expelled in urine daily at certain levels (basal values). The stress modified the neurotransmitter levels and ascended level of HVA, VMA, 5-HIAA, 6-β-OH cortisol and reduced ascorbic acid excretion. Administration of aqueous and ethanol extracts of *Caralluma fimbriata* to normal animals did not modify HVA, VMA, 5-HIAA, 6-β-OH cortisol and ascorbic acid in comparison with basal values but preliminary administration of aqueous and ethanol extracts of *Caralluma*

fimbriata to stress induced rats revealed the decrease in urinary HVA, VMA, 5-HIAA, 6-β-OH cortisol and increased the ascorbic acid levels in dose dependent manner. The previous phytochemical analysis of *Caralluma fimbriata* displayed the presence of pregnane glycosides, flavonoids and saponins which could be assumed to be responsible for anti-stress activity [14]. Literature survey evinced that these bioactive compounds can attach to the GABAA-BZDS complex, eventually, increase GABA level and reduces dopamine and decline plasma corticosterone level that cause

to diminish level of VMA as it is a metabolite of norepinephrine which is synthesized by dopamine and 6- β -OH cortisol respectively [15]. These phytoconstituents manifested D2 receptor blockers activities which cause to reduce serotonin level that leads to diminish level of HVA [16]. They also can avert tryptophan hydroxylase enzyme which is responsible for biosynthesis of 5-HT (serotonin)

therefore it can reduce level of 5-HIAA eventually as metabolite of serotonin [17]. Ascorbic acid acts a co-factor in the biosynthesis of NE from DA that it causes to decrease concentration of ascorbic acid in urine. Consequently, the effects of these bioactive phytoconstituent on reduction of synthesis of dopamine can impact on the increase of urinary level of ascorbic acid.

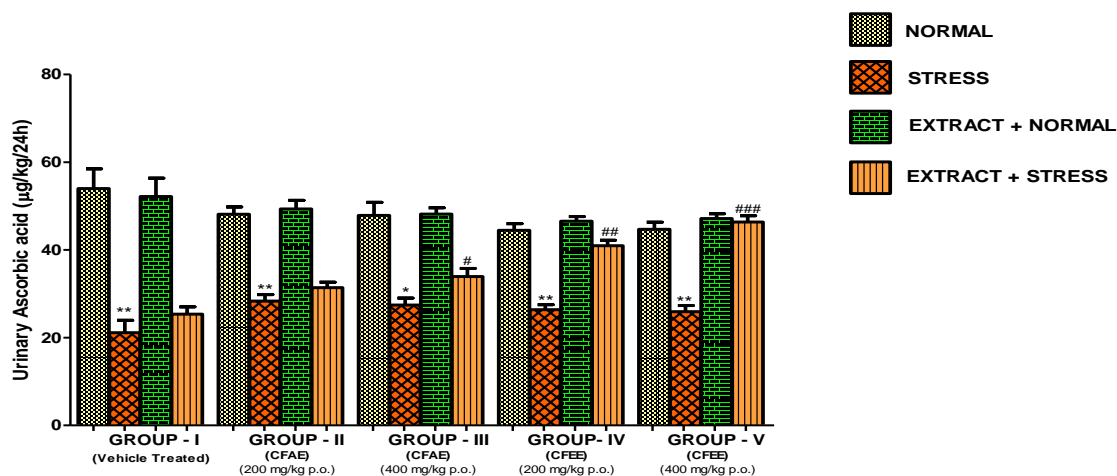


Figure 5: Influence of aqueous and ethanol extracts of *Caralluma fimbriata* on 24 h urinary levels of ascorbic acid in normal and stress induced rats. n=6, Values are mean \pm S.E.M, statistical analysis was done by using student's paired t-test. ***P<0.0001, **P<0.001 significant difference from normal condition of the corresponding groups; ###P <0.0001, ##P <0.001, #P <0.01 significant difference from stress condition of the corresponding groups.

Conclusion

The current appraisal exhibited that aqueous and ethanol extracts of *Caralluma fimbriata* have anti-stress potential due to impede the stress induced increase of adrenergic, serotonergic, cortisol and dopaminergic mechanism. Future evaluations can assess on the levels of neurotransmitters in different regions of brain, as there is limited data that examines the correlation of CNS and urinary neurotransmitters in animals.

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Conflict of Interest: None declared

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