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ORIGINAL ARTICLE

Effect of Poly Herbal Formulation "*Karisalai chooranam*" against Detrimental Effects of Psychological Stress

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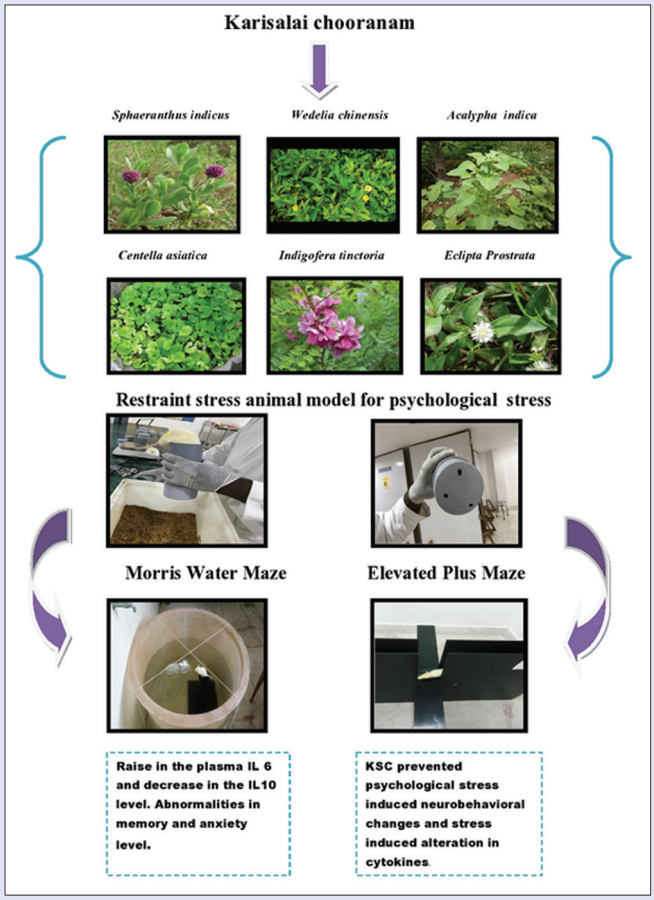
ABSTRACT

Background: The association of psychiatric illness with altered cytokine profile and changes in neurobehavior are well known. "*Karisalai chooranam*" (KSC) is a polyherbal preparation, contains equal proportion of six ingredients namely *Eclipta prostrata* Linn., *Acalypha indica* Linn., *Sphaeranthus indicus* Linn., *Indigofera tinctoria* Linn., *Centella asiatica* Linn., and *Wedelia chinensis*. Restraint stress is reported to be a model for psychological stress. It was hypothesized that KSC might protect against restraint stress-induced detrimental effects. **Objectives:** The objective is to evaluate the efficacy of KSC against detrimental effects of psychological stress. **Materials and Methods:** Male Wistar albino rats were subjected to restraint stress (6 h/day, 21 days). At the end of stress, rats were subjected to elevated plus maze (EPM) and Moris Water Maze studies. Blood was collected and examined for interleukin-6 (IL-6), IL-10 by Enzyme-Linked Immuno Sorbent Assay. **Results:** Restraint stress produced raise in the plasma IL-6 and decrease in the IL-10 level. Abnormalities in memory and anxiety level were seen. The effects of KSC in preventing detrimental effects on spatial recognition skill and reference memory were observed in Morris water maze analysis. In EPM trail, the anxiety developed due to restraint stress was prevented by the KSC. **Conclusion:** KSC was found to have action against psychological stress-induced neurobehavioral changes and stress-induced alteration in cytokines which play important roles in the development of psychological stress.

Key words: Cognition, *Karisalai chooranam*, Psychological stress, Restraint stress, Siddha Medicine

SUMMARY

- *Karisalai chooranam* (KSC), is a Siddha Medicine polyherbal formulation, consisting of six medicinal plants. KSC exhibited its protective action against psychological stress induced abnormality in cognitive skills. Besides, KSC showed its efficacy in preventing development of dysregulation in IL6 and IL10 which are closely associated with psychological stress.



Abbreviations used: EPM: Elevated plus maze; ELISA: Enzyme-Linked Immunosorbent Assay; EDTA: Ethylenediaminetetraacetic acid; HPA: Hypothalamic pituitary adrenal axis; KSC: *Karisalai chooranam*; ROS: Reactive oxygen species.

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INTRODUCTION

Mental illness and pain are the two most common grounds cited by people who perceive their health condition to be poor.[1] Stress can be defined as external events or conditions that affect an organism.[2] Stress is one of the root causes for pathogenesis for numerous diseases such as hypertension, peptic ulcer, immune suppression, reproductive dysfunction, and behavioral ailments.[3]

Stress causes activation of autonomic sympathetic nervous system, adrenal gland, and hypothalamus.[4] During chronic stress process, the hypothalamic–pituitary–adrenal axis causes consistent elevation of glucocorticoids which leads to functional disorders of the nervous system, endocrine system, and immune systems.[5]

In addition to hypothalamus, hippocampus brain area associated with learning, memory, cognition, and emotion. It has the highest number of glucocorticoid receptors. During stress, high level of glucocorticoids, causes nerve cell atrophy in hippocampus, leading to structural and functional damages.[5]

Besides central nervous system and endocrine pathways, stress also affects the immune system.[6] Prolonged stress increases interleukin-6 (IL-6) and decreases IL-10 level in serum of animal models. In depression, increased level of IL-6 and decreased IL-10 was seen in serum of human samples.

Using plant products for treating human ailments adopted as a natural approach to health care since human civilization evolved. "*Karisalai chooranam*" (KSC) is one of the kayakarpa medicines taken from the Siddha Medicine literature called "Bogamunivar Vaidya Kaviyam 1000."[7] It is made up of six ingredients of plant origin.

MATERIALS AND METHODS

Preparation and standardization of *Karisalai chooranam*

KSC consists of equal parts namely *Eclipta prostrata* Linn. (Vellai Karisalai), *Acalypha indica* Linn. (Kuppaimani), *Sphaeranthus indicus* Linn. (Kottai karanthai), *Indigofera tinctoria* Linn. (Avuri), *Centella asiatica* Linn. (Vallarai), and *Wedelia chinensis* (Portalaikaiyan). The whole plants were taken and cleaned (six ingredients as mentioned above) thoroughly. Equal amount of dried plants were taken and powdered.[7]

In our previous report,[8] standardization of KSC by high-performance thin-layer chromatography chemical profiling using wedelolactone, quercetin, asiaticoside, and rutin as markers was reported. Besides, residue analysis and pesticide analysis were also reported. Microbial load was found to be within limits.[8]

Experimental design

In the efficacy study of KSC, animals were divided into six groups ($n = 6$). Group I was kept as vehicle control (honey). Group II was given only stress, the Group III was administered with diazepam (2 mg/kg) along with stress as a positive control. Group IV, V, and VI were subjected to restraint stress for 3 weeks and also administered with KSC 100, 200, 400 mg/kg dosage along with vehicle.[9] Grouping and treatment details of efficacy study of KSC are shown in Table 1.

Restraint stress tube was prepared according to Chen *et al*. in 2010.[9] It was about 8.5 inches in length and 2.5 inches in breadth cylindrical plastic tube. Its end has few small air holes for ventilation. This tube fits closely to the body size of rat and inhibited motions. Rats are subjected to restraint stress for 6 h per day for 3 weeks. It is one of the animal models for inducing psychological stress. It is illustrated in Figure 1a and b.

Table 1: Grouping and treatment details of efficacy study of *Karisalai chooranam*

Dose	Number of species
Group I - Vehicle control	6 male
Group II - Stress control	6 male
Group III - Diazepam 2 mg/kg body weight + stress	6 male
Group IV - Stress + 100 mg KSC/kg body weight	6 male
Group V - Stress + 200 mg KSC/kg body weight	6 male
Group VI - Stress + 400 mg KSC/kg body weight	6 male

KSC: *Karisalai chooranam*

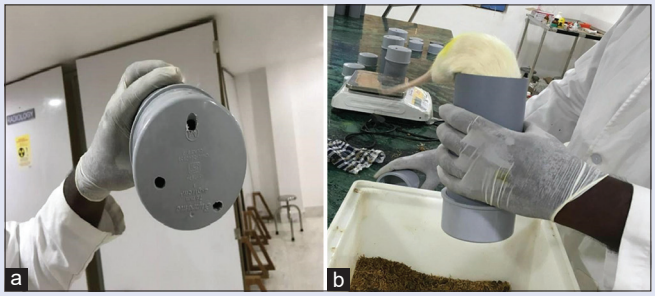

Figure 1: (a) Restraint tube. It was about 8.5 inches length and 2.5 inches breadth cylindrical plastic tube. Its end has few small air holes for ventilation. (b) Placing Wistar albino rat in restraint tube. Wistar albino rat were placed in restraint tube for 6 h per day for 3 weeks

Elevated plus maze task

In the last day of psychological stress elevated plus maze (EPM) trial was performed. All of the rats in experimental groups were subjected to EPM for normal duration of 5 min to assess the anxiety levels in rodents. The EPM consisted of two open arms measuring 16 cm × 5 cm, two closed arms, measuring 16 cm × 5 cm × 12 cm and a central platform 5 cm × 5 cm. The central platform was connected with the two arms. The EPM was raised to a height of 25 cm above the flooring level. Each Wistar albino rat was placed at the center of the EPM with its head facing toward an open arm and the stop watch was started and following parameters were noted for 5 min. (a) Number of entries in open and closed arms (An arm entry defined as the entry of four paws into the arm) (b) Time spend in the open arm and time spend in the closed arm.[10]

The Morris water maze task

In the last week of psychological stress, the water maze task was carried out 1 h after restraint stress, according to the method presented in Ebrahimpour *et al*. in 2017.[11] The task was utilized to determine cognitive skills such as spatial recognition and reference memory. Swimming training in the absence and presence of a platform was given. After the training period, during the test run, time taken to reach the platform was recorded as escape latency on 17th, 18th, 19th, and 20th days. This parameter was considered as a sign of spatial recognition.

On the last day of the trial, each rat was subjected to "probe trial." The platform was eliminated and time spent in the quadrant in which the platform had been placed initially was measured. This particular parameter was considered as a sign of reference memory. At the end of the trial, all animals were weighed and noted accurately. Animals were provided anaesthesia and performed necropsy. Plasma was collected and aliquots were saved at −80°C for analysis of corticosterone, IL-6 and IL-10. Organs such as brain, thymus, adrenal, and spleen were weighed and recorded.

Estimation of interleukin-6

IL-6 was estimated utilizing Ray bio rat-IL-6 Enzyme-Linked Immunosorbent Assay (ELISA) kit. Assay kit consists of a 96-well plate coated with antibody specific for rat IL-6. IL-6 contained in a sample was bound to the wells. After the binding of sample IL-6, biotinylated anti-rat IL-6 antibody was added. Subsequently, Horseradish Peroxidase conjugated Streptavidin (HRP)-conjugated streptavidin was added. After washing, 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate solution was added to the wells and color developed. The stop solution was added before reading at 450 nm. Standards (40–10000 pg/ml) were run from which the concentration of test samples were calculated. Tests were run in duplicate.[12]

Estimation of interleukin-10

IL-10 was estimated by Ray bio rat IL-10 ELISA kit. Kit consists of 96-well plate coated with an antibody specific for rat IL-10. IL-10 contained in a sample was bound to the wells. After the binding of sample IL-10, biotinylated anti-rat IL-10 antibody was added. Subsequently, HRP-conjugated streptavidin was added. After washing, TMB substrate solution was added to the wells and color developed. The stop solution was added before reading at 450 nm. Standards (10–6000 pg/ml) were run from which the concentration of test samples were calculated. Tests were run in duplicate.[13]

Statistical analysis

All the results are presented as means ± standard error of mean. Statistical significance was analyzed using one way analysis of variance with Tukey's test as *post-hoc* analysis by Graph Pad prism (7.04) software (GraphPad, San Diego, California, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

Restraint stress and interleukin-6

IL-6 level in plasma was significantly increased in stress control group when compared to vehicle control group. IL-6 level was decreased in KSC treated groups (100, 200, and 400 mg/kg) compared to stress control. It is summarized in Figure 2.

Restraint stress and interleukin-10

IL-10 level was reduced significantly in stress control when compared to vehicle control. IL-10 level was higher in KSC treated groups (100, 200, and 400 mg/kg bw) compared to stress control group [Figure 3].

Restraint stress and memory

Spatial recognition trial was carried out on the 17th, 18th, 19th, and 20th days. Changes in the escape latency were statistically insignificant on 17th, 18th, and 19th days between vehicle control and stress group. Spatial recognition trial on 20th day showed significant increase in the escape latency in stress group compared to vehicle control, indicating the development of abnormality in spatial recognition memory of the stressed group. In the drug-treated groups (100, 200, 400 mg/kg), escape latency time was reduced in the 100 mg/kg, 200 mg/kg, and in 400 mg/kg groups significantly. This indicates efficacy of KSC in preventing stress-induced changes in spatial recognition memory [Figures 4 and 5].

In probe trial, time spent in the target quadrant was measured which is higher in KSC-treated (100, 200, and 400 mg/kg) groups when compared to stress control group.

Restraint stress and anxiety

EPM trial was performed on 21st day of restraint stress. All the rats were exposed to EPM for 5 min to assess the anxiety levels in rodents.

Entries in open arm were lower in the stress group, in relation to the vehicle control group. The number of entries in open arm was higher in the KSC 100, 200 and 400 mg/kg groups in relation to the stress control group.

Open arm time was lower in the stress control group, in relation to vehicle control group. Open arm time was higher in the KSC treated 100, 200 and 400 mg/kg groups in relation to the stress control group.

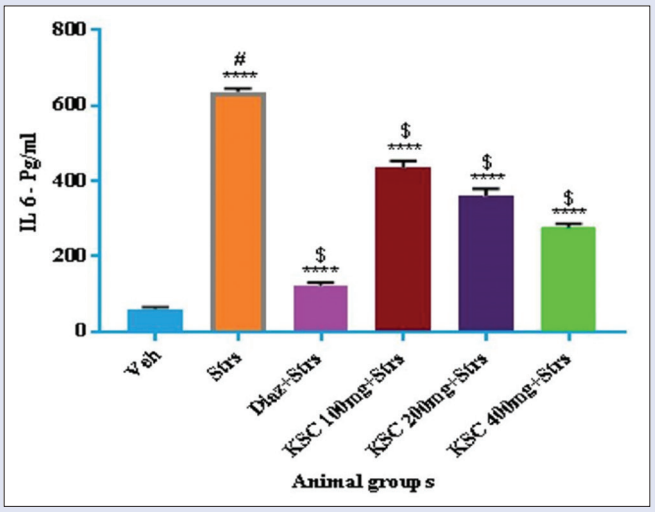

Figure 2: Effect of *Karisalai chooranam* on interleukin-6 after restraint stress. Data are expressed as mean ± standard error of mean ($n = 6$). The significance is fixed at $P < 0.05$, #Stress group compared with vehicle group. ($P < 0.0001$), $Drug-treated groups (Diazepam, *Karisalai chooranam* 100, 200, 400 mg/kg) compared with stress group. ($P < 0.0001$, <0.0001, <0.0001, <0.0001)

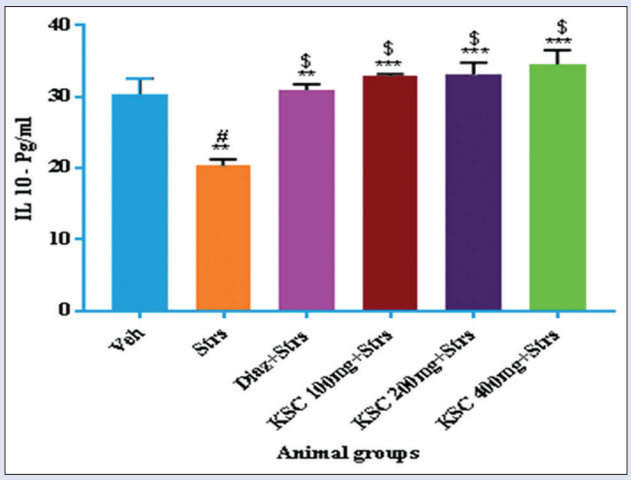

Figure 3: Effect of *Karisalai chooranam* on interleukin-10 after restraint stress. Data are expressed as mean ± standard error of mean ($n = 6$). The significance is fixed at $P < 0.05$, #Stress group compared with vehicle group. ($P < 0.0053$), $Drug-treated groups (Diazepam, *Karisalai chooranam* 100, 200, 400 mg/kg) compared with stress group, ($P < 0.0036$, <0.0008, <0.0007, <0.0003)

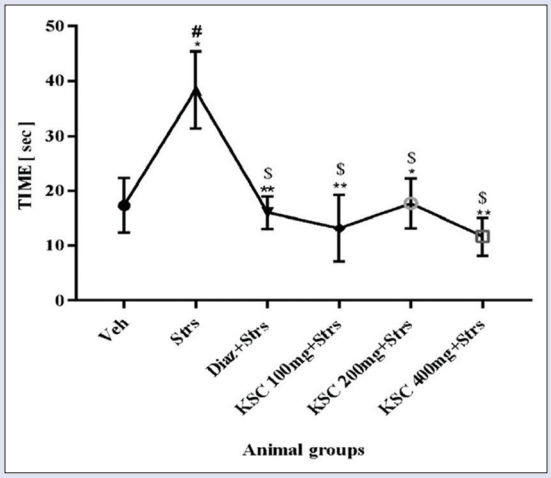

Figure 4: Effects of *Karisalai chooranam* on spatial recognition-Morris Water Maze trial: Escape latency on 20th day. Data are expressed as mean ± standard error of mean ($n = 6$). The significance is fixed at $P < 0.05$, #Stress group compared with vehicle group. ($P < 0.0388$), $Drug-treated group (Diazepam, *Karisalai chooranam* 100, 200, 400 mg/kg) compared to stress group, ($P < 0.0055$, <0.0020, <0.0101, <0.0011)

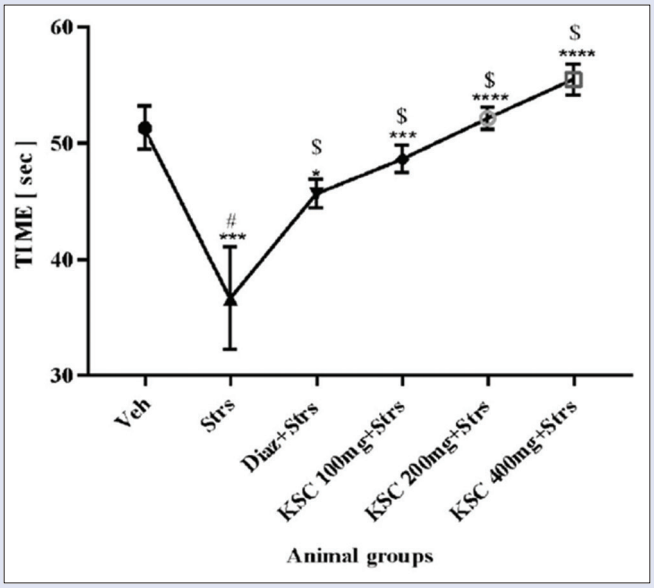

Figure 5: Effects of *Karisalai chooranam* on reference memory-Morris Water Maze: Probe trial, Time spent on target quadrant on 21st day. Data are expressed as mean ± standard error of mean ($n = 6$). The significance is fixed at $P < 0.05$, #Stress group compared with vehicle group. ($P < 0.0005$), $Drug-treated group (Diazepam, *Karisalai chooranam* 100, 200, 400 mg/kg) compared to stress group, ($P < 0.0170$, <0.0010, <0.0001, <0.0001)

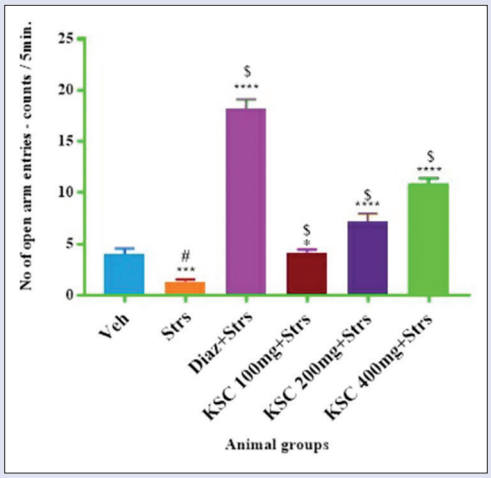

Figure 6: Effects of *Karisalai chooranam* on anxiety using the elevated plus maze trial, Number of entries in open arm. Data are expressed as mean ± standard error of mean ($n = 6$). The significance is fixed at $P < 0.05$, #Stress group compared with vehicle group. ($P < 0.0009$), $Drug-treated group (Dia, *Karisalai chooranam* 100, 200, 400 mg/kg) compared to stress group, ($P < 0.0001$, <0.0351, <0.0001, <0.0001)

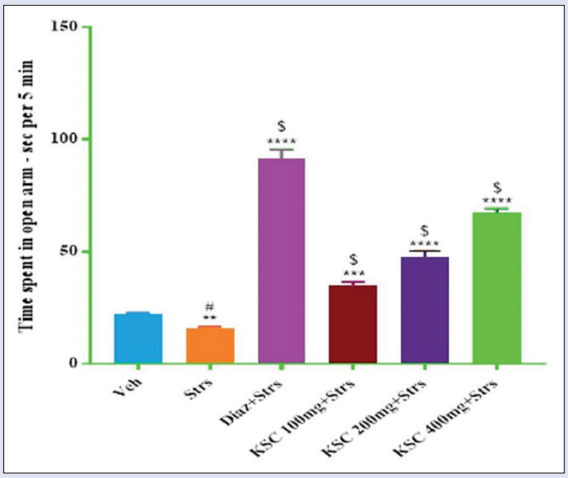

Figure 7: Effects of *Karisalai chooranam* on anxiety using the elevated plus maze trial, Time spent in open arm. Data are expressed as mean ± standard error of mean ($n = 6$). The significance is fixed at $P < 0.05$, #Stress group compared with vehicle group. ($P < 0.0017$), $Drug-treated group (Diazepam, *Karisalai chooranam* 100, 200, 400 mg/kg) compared to stress group, ($P < 0.0001$, <0.0002, <0.0001, <0.0001)

Decrease in the open arm entries and open arm time indicate the development of anxiety due to restraint stress in the stress control group. Increase in open arm entries and open arm time in the KSC-treated groups indicate efficacy of trial drug in preventing stress-induced anxiety [Figures 6 and 7].

DISCUSSION

The association of altered cytokine profile and neurobehavioral changes with psychiatric illness is well known. Psychological stressors can stimulate transient increases in pro inflammatory cytokines. The generation of IL-6 and other pro-inflammatory cytokines can be directly induced by depression and stressful experiences.[14] Low serum level of IL-10 in adult depression patients have already been reported.[15] Besides, Voorhees *et al*. in 2013, reported that prolonged restraint stress in animals, can cause increased IL-6 and decreased IL-10 in the serum.[16] These findings strongly correlate to the present study.

In this study, it was found that 3 weeks of restraint stress, produced a rise in the plasma IL-6 and decrease in the IL-10 level. Moreover, decline in cognitive skills of stressed animals and enhanced anxiety level of stressed animals were also observed. These abnormalities were prevented by the KSC.

In our previous report,[17] elevated corticosterone level was also observed in the animals which were subjected to stress. Furthermore, in the stressed group, high-lipid peroxidation and depletion of superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity in the hypothalamus and hippocampus brain regions were also observed. The decline in antioxidant enzymes and elevation in lipid peroxidation were prevented when KSC was administered.[17]

There are already many reports pertaining to restraint stress-induced oxidative damage to nervous system. Oxidative damage to the hippocampus after exogenous administration of corticosterone has already been reported by Sato *et al*. in 2010.[18] Excessive reactive oxygen species (ROS) can cause oxidative damage to brain especially hypothalamus and hippocampus. Damage to hippocampus cells ultimately can cause deficiency in cognitive functions including memory and neurobehavioral changes. In addition to that, the possible role of elevated IL-6 in mood disorders and depression, has already been reported.[19,20] These studies correlate well with our findings. During Morris water maze trial, decline in the cognitive skills especially spatial recognition skill and reference memory functions of rats were observed in the stress group. Collective impact of abnormally elevated corticosterone, excessive IL-6 and excessive ROS on brain areas namely hypothalamus and hippocampus might have affected the memory function of stressed rats.

Besides disturbance in memory functions, abnormal anxiety was also observed in the present study during elevated plus maize trial. Increased ROS can cause neurobehavioral changes as reported by Salim, in 2017.[21] The influence of oxidative stress mechanisms in psychiatric illnesses has already been reported.[22-24] These findings explain the neurobehavioral changes especially abnormal anxiety observed in the present study.

Disturbances in memory functions and abnormal anxiety developed due to restraint stress were prevented by the poly herbal formulation, KSC. Protective action of KSC against restraint stress-induced neurobehavioral changes could possibly due of its mechanism of action in preventing development of oxidative stress induced disruptions in hypothalamus and hippocampus functions and also because of its efficacy in preventing development of dysregulation in IL-6 and IL-10 cytokines.

CONCLUSIONS

Psychological stress can affect cognition especially memory functions and anxiety levels. Besides, it can alter cytokine homeostasis. KSC was found to have action against psychological stress induced Neuro behavioral changes and also against stress induced alteration in cytokines, IL-6 and IL-10 which are closely related to psychological stress. KSC preparation will be a promising alternative treatment in stress management and it requires further clinical studies in human subjects.

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Conflicts of interest

There are no conflicts of interest.

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