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Evaluation of the Protective Effect of Andrographolide in Chronic Immobilization Stress Induced Memory Deficits and other Physiological Changes in Rats

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Abstract

Aim Present study was designed to evaluate the protective effect of andrographolide on chronic stress (CS) mediated biochemical alterations and memory deficits in rats. **Methods** Two hour immobilization for ten days was used to induce chronic stress. Physiological and biochemical parameter related to stress such as body weight, adrenal gland weight, ulcer index, plasma glucose, plasma cholesterol were measured in normal, control and drug treated groups. Lipid peroxidation using serum Thiobarbituric Acid reactive Substances (TBARS) and free radical scavenging using serum reduced glutathione (GSH) were measured to assess effect of CS and andrographolide treatment on oxidative stress and inherent antioxidant mechanisms. Effect of CS on memory was evaluated using Elevated Plus Maze (EPM). Memory assessment was done on day 2 and Day 10 of stress induction protocol. Transfer Latency (TL) time was noted as an index of memory retrieval. **Results** CS produced dysfunctional homeostasis as indicated by stress mediated increase in plasma glucose, ulcer index and adrenal gland weight and stress induced decrease in plasma cholesterol and body weight. Furthermore, lipid peroxidation was evident by increased serum TBARS and decreased serum GSH contents. CS resulted in memory deficits as evident from increase in TL time on day 2 and day 10 of retrieval trials. Administration of Andrographolide (50 mg/kg *p.o.*, 100 mg/kg *p.o.* and 150mg/Kg *p.o.*) resulted in a dose dependant decrease in the lterations of physiological, biochemical parameters and stress induced memory deficits. **Conclusion** Andrographolide may possess adaptogenic properties and could be further explored for use as anti-stress agent.

Keywords
Andrographolide, Antioxidant, Chronic Stress, Memory Deficits.

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INTRODUCTION

Stress is a common phenomenon associated with life style nowadays. Due to a marked increase in the office going executives which spend a substantial part of their daily routine on computers and other gadgets, sedentary lifestyle has been flourishing. Moreover to competitive office environment, people are being repeatedly and consistently subjected to stressful stimuli which negatively affect their physiological processes. Physiologically, stress activates the Hypothalamo-Pituitary-Adrenal (HPA) axis and central monoaminergic systems [1]. HPA activation increases the release of Corticotrophin Releasing Hormone (CRH) from the hypothalamic paraventricular nucleus (PVN), causing the secretion of Adrenocorticotropin (ACTH) from anterior pituitary, which itself releases glucocorticoids from the adrenal cortex [2, 3].

Excessive and persistent release of glucocorticoids is documented to affect the homeostatic functions of the body and is also involved in the pathogenesis of a wide array of pathological conditions like coronary heart disease [4], hypertension, gastric ulcers [5], diabetes [6], immuno-suppression [7], mental depression [8], and memory loss. There could be variation in the type and intensity of these resultant pathological changes which depends on type, intensity, and the duration of an involved stressor and the strain\sex differentiation of the subjects [9]. Although, stress represents as an unavoidable phenomenon, yet, no specific pharmacological intervention is currently known to medical sciences which could be ascribed as curative for stress and associated memory loss. There has been a long quest for finding an effective way to enhance body's resistance against stress and associated pathological changes.

Many plants have been investigated in the ethnomedical research for their action as adaptogens or anti-stress agents [10]. Some successful results were reported from *Panax ginseng* [11], *Bacopa monniera* [12], *Withania somnifera* [13], *Emblica officinalis* [10], *Ocimum sanctum* [14, 15] and *Evolvulus alsinoides* [16] on different types of stressors and stress induction protocols.

Andrographis paniculata (Burm. F) Nees, commonly known as the "King of Bitters" throughout tropical and subtropical Asia, Southeast Asia, and India. In India, A. paniculata is known as "Kalmegh" in China it is known as "Chuan-Xin-Lian"; in Thailand it is known as "Fah Tha Lai"; in Malaysia it is known as "Hempedu bumi"; in Japan it is known as "Senshinren"; and in Scandinavian countries it is known as "green chiretta" [17]. Extracts of this plant and andrographolide have shown to be immunostimulatory [17, 18] antiviral [19] and antibacterial [20]. As major active constituent, andrographolide exhibits a broad range of biological activities, such as anti-inflammatory, antibacterial, antitumor, antidiabetic, antimalarial, and hepatoprotective [21]. However, to the best of our knowledge, effect of andrographolide on chronic stress induced memory deficits has not been documented so far. Therefore, the present study was designed to determine the adaptogenic and anti-amnestic effect of andrographolide in chronic stress.

MATERIALS AND METHODS

Animals

Sprague Dawley albino rats of either sex weighing 250±5 g were utilized for the present study. They were maintained of standard rat chow (Sheetal Animal Feeds Ltd., Amritsar, India) and tap-water *ad libitum*. Their housing was done in college animal house maintaining natural cycles of light and dark. Care of the animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Ministry of Environment and Forests, Government of India.

Drugs and Chemicals

Andrographolide was purchased from TCL Chemicals Pvt. Ltd., Chennai, India. It was suspended in 0.5% w/v solution of carboxy methyl cellulose. Thiobarbituric acid was purchased from Hi-media chemicals. Reduced Glutathione (GSH), 5, 5- dithiobis (2-nitro benzoic acid) (DTNB), and were obtained from CDH, Mumbai, India. 1, 1, 3, 3-tetramethoxy propane was procured from Sigma-Aldrich, USA. Glucose estimation kit, Cholesterol estimation kits were purchased from ERBA diagnostics, Germany. Other chemicals and reagents used were purchased from Rankem Mumbai, India. All the reagents and chemicals employed in the study were of analytical grade.

Experimental Procedure

Induction of Chronic Stress

Chronic stress was produced as per the method described by Kvetnansky [22]. Rats were immobilized using a plastic rat restrainer. Immobilization was done for 2 hours daily for 10 days.

Elevated Plus Maze Model for Evaluation of Memory

The elevated plus maze was used as an exteroceptive behavioral model for evaluation of learning and memory. The apparatus consisted of two open arms (50 cm × 10 cm) and two covered arms (50 cm × 10 cm × 40 cm). The arms extended from a central

platform (10 cm × 10 cm), and the maze was elevated to a height of 50 cm from the floor. On the first day, each rat was placed on the end of an open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by the rat to move into any one of the covered arms with all its four legs. TL was recorded on the first day. If the rat did not enter into one of the covered arms within 90 s, it was gently pushed into one of the two covered arms and the TL was assigned as 90 s. The rat was allowed to explore the maze for 10 s and then was returned to its home cage. Memory retention was examined 24 h after the first day trial on the second day [23, Parle and Singh, 2004). Long term memory was evaluated on 10th day.

Biochemical Paramaters

Collection of Sample

Blood samples for biochemical estimation were collected after sacrificing the animal. The plasma and serum were collected aseptically and preserved until used for biochemical assays.

Estimation of Plasma Glucose Concentration

Blood plasma concentration was estimated by using glucose estimation kit which employed Draper's method [25] for estimation.

Estimation of Plasma Cholesterol Concentration

Plasma cholesterol concentration was estimated by using cholesterol estimation kit which employed Allain's method [26] for estimation.

Estimation of serum thiobarbituric acid reactive substances (TBARS)

The quantitative estimation of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation in serum was performed according to the method of Satoh [27].

Estimation of ulcer index

Ulcer Index (UI) was scored according to the method of Main & Whittle [28].

Evaluation of adrenal gland weight and Body Weight

At the end of study (on 10th Day), body weight of each animal in each group was measured and average weight of each group was calculated and analyzed. Thereafter, after sacrifice of animals, kidneys were dissected out and adrenal gland was located as a small protuberance over the upper medial portion of the kidney. The adrenal gland was carefully dissected out and weighed.

Experimental Protocol

Five groups were employed in the present study. Each group comprised of 06 Sprague Dawley rats of either sex.

Statistical Analysis

The results were expressed as mean ± standard error of means (S.E.M). The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's test was used in *post hoc* analysis for comparison between different groups. The $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of Andrographolide on chronic stress induced changes in plasma glucose level:

Chronic Stress (CS) produced significant increase in plasma glucose and serum corticosterone levels as compared to normal rats group, as noted on 10th day after stress protocol. Treatment with Andrographolide (50 mg/kg *p.o.*, 100 mg/kg *p.o.* and 150mg/Kg *p.o.*) significantly attenuated stress induced rise in plasma glucose (Figure 1) level in a dose dependent manner.

Effect of Andrographolide on chronic stress induced changes in plasma cholesterol level:

Chronic stress produced significant decrease in plasma cholesterol level as compared to normal rats group. Treatment with Andrographolide (50 mg/kg *p.o.*, 100 mg/kg *p.o.* and 150mg/Kg *p.o.*) significantly attenuated stress induced decrease in plasma cholesterol level (Figure 2) in a dose dependent manner.

Effect of Andrographolide on chronic stress induced changes in oxidative stress markers:

Chronic stress produced significant increase in serum thiobarbituric acid reactive substances (TBARS) and decrease in reduced glutathione levels as compared to normal rats group. Treatment with Andrographolide (50 mg/kg *p.o.*, 100 mg/kg *p.o.* and 150mg/Kg *p.o.*) significantly attenuated stress induced rise in TBARS and decrease in glutathione levels in a dose dependent manner. (Figures 3 and 4).

Effect of Andrographolide on chronic stress induced changes in ulcer index, adrenal gland weight and body weight:

Chronic stress produced significant increase in ulcer index and adrenal gland weight and decrease in the body weight as compared to normal rats group. Treatment with Andrographolide (50 mg/kg *p.o.*, 100 mg/kg *p.o.* and 150mg/Kg *p.o.*) significantly attenuated stress induced rise in ulcer index and adrenal gland weight and decrease in the body weight in a dose dependent manner. (Table1.)

Effect of Andrographolide on chronic stress induced memory deficits.

Chronic stress produced significant increase in transfer latency (TL) period as compared to normal

rats group on both day 2 and day 10. Treatment with Andrographolide (50 mg/kg *p.o.*, 100 mg/kg *p.o.* and 150mg/Kg *p.o.*) significantly decreased transfer latency period in a dose dependent manner (Table 2).

DISCUSSION

Chronic stress (CS) resulted in an evident and significant increase in the level of plasma glucose in the present study. Furthermore, CS also resulted in gastric ulcers, reduction in body weight and adrenal gland weight. These stress mediated changes may be attributed to stress induced activation of Hypothalamo-Pituitary-Adrenal (HPA) axis [29, 30]. Chronic HPA stimulation results in functional hypertrophy of adrenal gland [31, 32] resulting in excessive corticosterone release [33]. Stress induced changes in plasma glucose and cholesterol levels may be attributed to corticosterone mediated increased metabolic needs to cater to the enhanced demands of the body organs during stress [34, 35]. Stress mediated increase in the gastric ulcer index is widely attributed to activation of paraventricular nucleus in brain which regulates the gastric secretions [36].

Moreover, in the present study, chronic stress (CS) caused impairment of memory as indicated by decrease in the performance on elevated plus maze test. Chronic stress has been documented to affect specific brain structures like hippocampus and cause cognitive dysfunction like learning and memory deficits [37, 38]. The hippocampus possesses high number of Glucocortocoid Receptors (GRs) in mammalian brain [39]. Chronic stress mediated increase in corticosterone levels has been implicated to induce neuronal atrophy and cell death in the hippocampus, while leaving other brain areas intact, neuronal atrophy might be a result of damage to synaptic terminal structures [40, 41]. Therefore, the noted stress induced memory deficits may be a consequence of neurodegenration due to increase in corticosterone levels.

The present results demonstrate that administration of Andrographolide (50 mg/Kg, 100 mg/Kg and 150 mg/Kg) significantly attenuated chronic stress induced increase in glucose levels, adrenal gland weight and gastric ulcerations. Further, Andrographolide also normalized stress induced reduction in cholesterol level and body weight. These results highlight the potential of andrographolide as an anti-stress agent or an adaptogenic substance in stressful events. Our present results support the traditional as well as experimental claims of adaptogenic potential of Andrographolide.

Pre-treatment with Andrographolide also afforded significant benefit in post-stress memory deficits (both acquisition and retrieval) as is evident from a dose dependent decrease in transfer latency period on day 2 as well as on day 10. To the best of our knowledge, it is the first report demonstrating the beneficial effect of Andrographolide in chronic stress induced memory deficits.

The present results may possibly be linked to andrographolide mediated normalization of the stress activated HPA axis. Andrographolide may have may have produced its effect directly on the adrenal gland in order to decrease stress mediated corticosterone secretion as is evident from decrease in adrenal hypertrophy in andrographolide treated animal groups. However, the present data is a qualitative estimate of effect of andrographolide in chronic stress but it does not delineate the molecular mechanism of Andrographolide mediated decrease in corticosterone secretion.

Further, in the present study, CS resulted in significant elevation of TBARS (a marker of lipid peroxidation) and reduction in the levels of glutathione (an endogenous anti-oxidant). Stress has been well documented to increase the production of free radicals [42], which has also been linked to hyper-activation of HPA axis with consequent increase in corticosterone secretion [43]. Conversely free radicals may also participate in HPA over activation and increase in corticosterone secretion by producing damage to hippocampal neurons, which maintain the homeostasis of HPA axis by negative feedback mechanisms [44].

There have been reports suggesting the crucial role of free radicals in stress induced biochemical imbalance and associated pathological outcomes [45, 46, 47]. Stress induced memory deficits may also be linked to excessive free radical production probably due to their neuronal damaging effects [45]. However, administration of Andrographolide attenuated CS associated increase in oxidative stress in terms of reduction in TBARS and elevation of glutathione levels, suggesting that free radical scavenging property of Andrographolide may also be playing a key role in its adaptogenic and anti-amnesic activity. Andrographolide is a well-known potent antioxidant [48]. This hypothesis is supported by other reports demonstrating the anti-stress effects of antioxidants such as vitamin C [49], Lipoic acid [50], Vitamin E [51] and *Triphala* [52].

Therefore, it may be proposed that Andrographolide mediated antioxidant actions and normalization of hyper-activated HPA axis with subsequent decrease in corticosterone secretion (may also be due to antioxidant action) is responsible for its preventive effects in chronic stress and chronic unpredictable stress induced alterations in functional homeostasis

and memory deficits. Nevertheless, further studies are needed to explore the adaptogenic potential of Andrographolide in other models of stress and to delineate the mechanism of Andrographolide mediated normalization of stress induced hyper-activation of HPA axis.

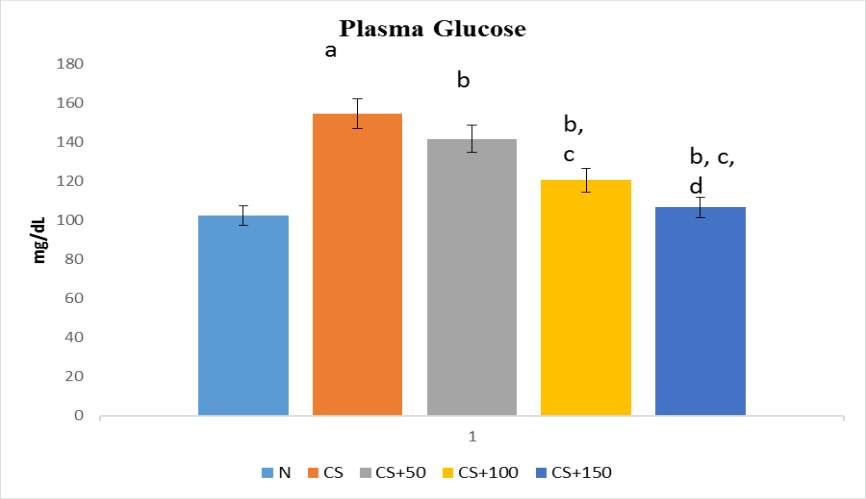

Figure1. Changes in plasma glucose under chronic stress (CS) of the normal, stress and Andrographolide treated groups. Results are represented as the mean ± S.E.M. with n = 6 in each group. [a] *p*<0.05, as compared to the N group; [b] *p*<0.05, as compared to CS group; [c] *p*<0.05, as compared to chronic stress + 50 mg/Kg Andrographolide (CS+50) group; [d] *p*<0.05, as compared to chronic stress + 100 mg/Kg Andrographolide (CUS+100) group; [e] *p*<0.05, as compared to chronic stress + 150 mg/Kg Andrographolide (CUS+150) group.

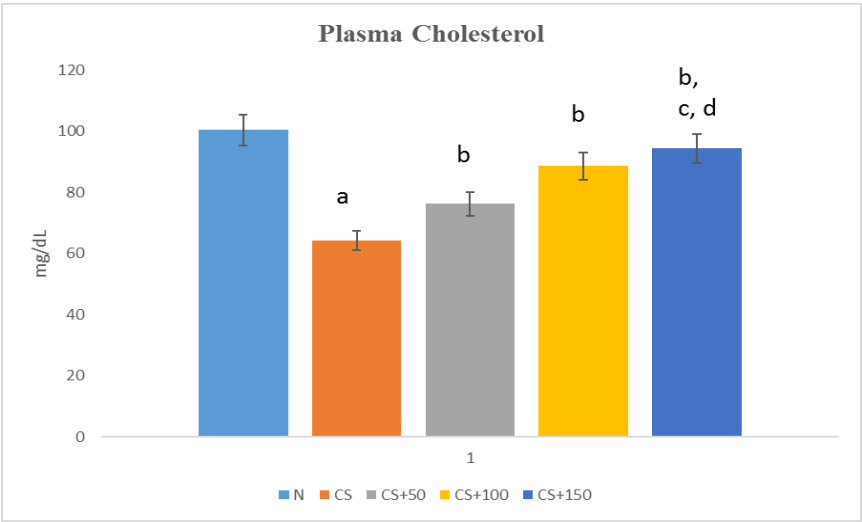

Figure 2. Changes in plasma cholesterol under chronic stress (CS) of the normal, stress and Andrographolide treated groups. Results are represented as the mean ± S.E.M. with n = 6 in each group. [a] *p*<0.05, as compared to the N group; [b] *p*<0.05, as compared to CS group; [c] *p*<0.05, as compared to chronic stress + 50 mg/Kg Andrographolide (CS+50) group; [d] *p*<0.05, as compared to chronic stress + 100 mg/Kg Andrographolide (CUS+100) group; [e] *p*<0.05, as compared to chronic stress + 150 mg/Kg Andrographolide (CUS+150) group.

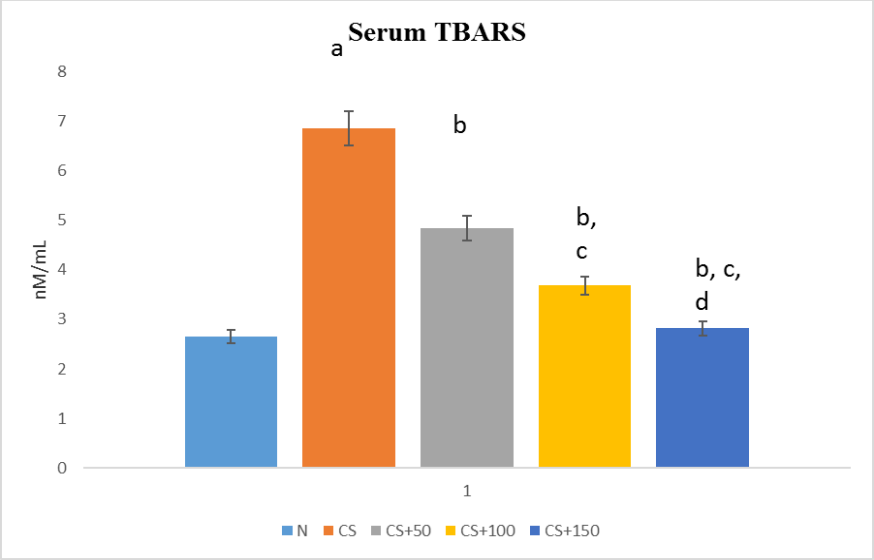

Figure 3. Changes in serum TBARS under chronic stress (CS) of the normal, stress and Andrographolide treated groups. Results are represented as the mean ± S.E.M. with n = 6 in each group. [a] *p*<0.05, as compared to the N group; [b] *p*<0.05, as compared to CS group; [c] *p*<0.05, as compared to chronic stress + 50 mg/Kg Andrographolide (CS+50) group; [d] *p*<0.05, as compared to chronic stress + 100 mg/Kg Andrographolide (CUS+100) group; [e] *p*<0.05, as compared to chronic stress + 150 mg/Kg Andrographolide (CUS+150) group.

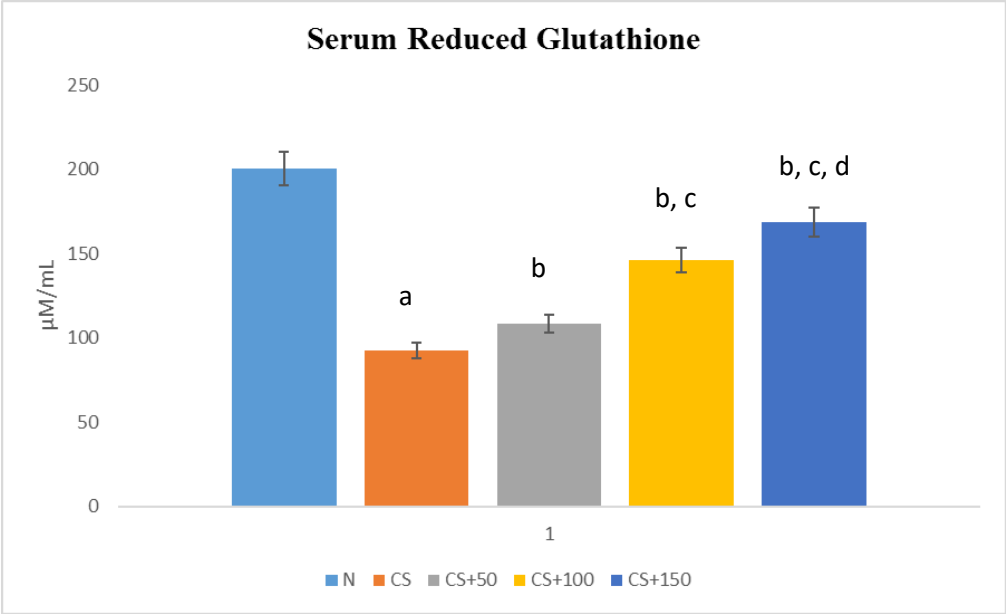

Figure 4. Changes in serum reduced glutathione under chronic stress (CS) of the normal, stress and Andrographolide treated groups. Results are represented as the mean ± S.E.M. with n = 6 in each group. [a] *p*<0.05, as compared to the N group; [b] *p*<0.05, as compared to CS group; [c] *p*<0.05, as compared to chronic stress + 50 mg/Kg Andrographolide (CS+50) group; [d] *p*<0.05, as compared to chronic stress + 100 mg/Kg Andrographolide (CUS+100) group; [e] *p*<0.05, as compared to chronic stress + 150 mg/Kg Andrographolide (CUS+150) group.

Groups	Avg. body weight (gm) day 10	Adrenal gland weight (mg)	Ulcer index
Normal	250.3 ± 2.5	4.7 ± 0.4	0
CS	167.8 ± 3.6[a]	7.8 ± 0.4[a]	2.2 ± 0.1[a]
CS + 50 mg/kg Andrographolide	200.1 ± 3.3[b]	6.6 ± 0.3[b]	1.40 ± 0.05[b]
CS + 100 mg/kg Andrographolide	2 26.5 ± 4.4[b,c]	5.4 ± 0.3[b,c]	0.23 ± 0.04[b,c]
CS + 150 mg/kg Andrographolide	242.4 ± 3.4[b,c,d]	4.9 ± 0.3[b,c,d]	0.16 ± 0.02[d,e]

Table 1: Changes in average body weight, adrenal gland weight and ulcer index under chronic stress of the normal, stress and Andrographolide-treated groups.

(N= Normal; CS= Chronic Stress)

Values are represented as the mean ± S.E.M, n = 6 in each group.

[a] denotes $p<0.05$, Vs N group

[b] denotes $p<0.05$, Vs CS group

[c] denotes $p<0.05$, Vs CS+50 mg/kg Andrographolide group

[d] denotes $p<0.05$, Vs CS+100 mg/kg Andrographolide group

Groups	Average Transfer Latency time on day 2	Average Transfer Latency time on day 10
Normal	16.5 ± 0.5	6.8 ± 0.3
CS	38.2 ± 0.7 [a]	36.0 ± 0.7 [a]
CS + 50 mg/kg Andrographolide	27.8 ± 0.8 [b]	21.5 ± 0.8 [b]
CS + 100mg/kg Andrographolide	18.8 ± 0.3 [b,c]	14.3 ± 0.4 [b,c]
CUS + 150mg/kg Andrographlide	25.8 ± 0.3 [b,c,d]	17.5 ± 0.6 [b,c,d]

Table 2: Changes in average transfer latency time on day 2 and day 10 under chronic stress of the normal, stress and Andrographolide-treated groups.

(N= Normal; CS= Chronic Stress)

Values are represented as the mean ± S.E.M, n = 6 in each group.

[a] denotes $p<0.05$, Vs N group

[b] denotes $p<0.05$, Vs CS group

[c] denotes $p<0.05$, Vs CS+50 mg/kg Andrographolide group

[d] denotes $p<0.05$, Vs CS+100 mg/kg Andrographolide group

REFERENCES

1. Kvetnansky, R., Pacak, K., Fukuhara, K., Viskupic, E., Hiremagalur, B., Nankova, B., Goldstein, D.S., Sabban, E.L. and Kopin, I.J., Sympathoadrenal system in stress. Interaction with the hypothalamic-pituitary-adrenocortical system. *Ann NY Acad Sci.,* 771, 131-158, (1995).
2. Pacak, K., Kvetnansky, R., Palkovits, M., Fukuhara, K., Yadid, G., Kopin I.J. and Goldstein, D.S, Adrenalectomy augments in vivo release of norepinephrine in the paraventricular nucleus during immobilization stress. Endocrinology. Sep;133(3):1404-10, (1993).
3. Venihaki, M., Gravanis, A. and Margioris, A.N., Comparative study between normal rat chromaffin and PC12 rat pheochromocytoma cells: Production and effects of Corticotropin Releasing Hormone (CRH). *Endocrinology*, 138, 698-704, (1997).
4. Roy, M.P., Kirschbaum, C. and Steptoe, A., Psychological cardiovascular and metabolic correlates of individual differences in cortisol stress recovery in young men. *Psychoneuroendocrinology,* 26, 375-391, (2001).
5. Yadin, E. and Thomas, E., Stimulation of the lateral septum attenuates immobilization-induced stress ulcers. *Physiol. Behav.,* 59, 883-886, (1996).
6. Fitzpatrick, F., Christedd, N., Durant, S., Dardenne, M., Nunez, E.A. and HomoDelarche, F., Glucocorticoids in non-obese diabetic (NOD) mouse Basal serum levels, effect of endocrine manipulation and immobilization stress. *Life Sci.,* 50, 1063-1069, (1992).
7. Purret, S.B., Quantitative aspects of stress-induced immunomodulation. *Int. Immunol. Pharmacol.,* 1, 507-520, (2001).
8. Gareri, P., Falconi, U., Fazio, P. and De Sarro, G., Conventional and new antidepressant drugs in the elderly. *Prog. Neurobiol.,* 61, 353-396, (2000).
9. Kioukia-Fougia, N., Antoniou, K., Bekris, S., Liapi, C., Christofidis I. and Papadopoulou-Diafoti, Z., The effect of stress exposure on hypothalamic-pituitary-adrenal axis, thymus, Thyroid hormones and glucose levels. *Prog. Neuropharmacol. Biol. Psychiatry.,* 26, 823-830, (2002).
10. Rege, N.N., Thatte, U.M., Dhanukar, S.A., Adaptogenic properteis of six rasayana herbs used in ayurvedic medicine. *Phytotherapy Res.,* 13, 275-291, (1999).
11. Rai, D., Bhatia, G., Sen, T. and Palit, G., Anti-stress effects of *Ginkgo biloba* and *Panax ginseng* a comparative study. *J. Pharamcol. Sci.,* 93, 458-464, (2003a).
12. Rai, D., Bhatia, G., Palit, G., Pal, R., Singh, S and Singh, H.K., Adaptogenic effects of *Bacopa monniera*

(Brahmi). *Pharmacol. Biochem. Behav.,* 75, 823-830, (2003b).
13. Muruganandam, A.V. and Bhattacharya, S.K., Adaptogenic activity of *Withania somnifera:* an experimental study using a rat model of chronic stress. *Pharmacol Biochem Behav.,* 75, 547-555, (2003).
14. Bhargava, K.P. and Singh, N. Anti-stress activity of *Ocimum sanctum* Linn. *Indian J. Med. Res.,* 73, 443-451, (1980).
15. Ravindran, R., Rathinaswamy, S.D. Samson, J. and Senthilvelan, M., Noise-Stress-Induced Brain Neurotransmitter Changes and the Effect of *Ocimum sanctum* (Linn) Treatment in albino Rats. *J. Pharm. Sci.,* 98, 354-360, (2005).
16. Siripurapu, K.B., Gupta, P., Bhatia, G., Maurya, R., Nath, C. and Palit, G., Adaptogenic and anti-amnesic properties of *Evolvulus alsinoides* in rodents. *Pharmacol. Biochem. Behav.,* 81, 424-432, (2005).
17. Kumar Ashok BS, Lakshman K, Jayaveera KN, Nandeesh R, Manoj B, Ranganayakulu D. Comperative in vitro anthelmintic activity of three plants of Amaranthaceae family. *Archives of Biological Sciences*, 62 (1): 185-189, (2010)
18. Rajagopal S., Kumar, R. A. Deevi, D. S. Satyanarayana, C. and Rajagopalan, R., Andrographolide, a potential cancer therapeutic agent isolated from Andrographis paniculata," *Journal of Experimental Therapeutics and Oncology*, vol. 3, no. 3, pp. 147–158, (2003).
19. Calabrese, C. Berman, S. H. Babish J. G. et al., A phase I trial of andrographolide in HIV positive patients and normal volunteers, *Phytotheraphy Research*, vol. 14, no. 5, pp. 333–338, (2000).
20. Singha RR, Roy S, Dey S., Antimicrobial activity of *Andrographis paniculata*. *Fitoteripia*, 74: 692-694(2003).
21. K. Jarukamjorn and N. Nemoto, Pharmacological aspects of Andrographis paniculata on health and its major diterpenoid constituent andrographolide. *Journal of Health Science*, vol. 54, no. 4, pp. 370–381, (2008).
22. Kvetnansky, R. and Mikulai, L., Adrenal and urinary catecholamines in rats during adaptation to repeated immobilization stress. *Endocrinology,* 81, 738-743, (1970).
23. Itoh, J., Nabeshima, T. and Kameyama, T., Utility of an elevated plus maze for the evaluation of nootropics, scopolamine and electro convulsive shock. *Psychopharmacology,* 101, 27-33, (1990).
24. Parle, M. and Singh, N., Animal models for testing Memory. *Asia Pacific. J. Pharmacol,*16, 101-120, (2004).
25. Draper HH and Hadley M., Malondialdehyde determination as index of lipid peroxidation. *Methods in Enzymology*, 186:421–431, (1990).
26. Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W. and Paul, C.F., Enzymatic determination of total plasma cholesterol. *Clin. Chem.,* 20(470), 470-474, (1974).
27. Satoh, K., Serum lipid peroxide in cerebrospinal disorder determined by a new colorimetric method. *Clin. Chem. Acta.,* 90, 37-43, (1978).
28. Main, I.H.M. and Whittle, B.J.R., Investigation of the vasodilator and antisecretory role of prostaglandins in the rat gastric mucosa by use of non-steroidal anti-inflammatory drugs. *Br. J. Pharmacol.,* 53, 217-224, (1975).
29. Akil, H.A. and Morano, M.I. Stress. In: Bloom, FE and Kupfer, D.J. (Eds). *Psychopharmacology: The fourth generation in progress*. New York: Raven Press, pp. 773-785, (1995).
30. Levine, S., Development determinants of sensitivity and resistance to stress. *Psychoneuroendocrinology,* 30, 939-946, (2005).
31. Selye, H. The physiology and pathology of exposure to stress. *Montreal Acta.,* 950, 4-13, (1950).
32. Walker, C., Perrin, M., Vale, W. and Rivier, C., Ontogeny of stress response in the rats: role of pituitary and the hypothalamus. *Endocrinology,* 118, 1445-1451, (1986).
33. Rivier, C. and Plotsky, P.M., Mediation by corticotropin releasing factor of adrenohypophysial hormone secretion. *Annu. Rev. Physiol.,* 48, 475-489, (1986).
34. McEwen, B.S., The neurobiology of stress: from serendipity to clinical relevance. *Brain Res.,* 886, 172-189, (2000).
35. Makara, G.B. and Haller, J., Non-genomic effects of glucocorticoids in the neural system evidence, mechanisms and implications. *Prog. Neurobiol.* 65, 367-390, (2001).
36. Zhang, J. and Zheng, F., The role of paraventricular nucleus of the hypothalamus in stress-ulcer formation in rats. *Brain Res.,* 61, 203-209, (1997).
37. Kim, J.J. and Yoon, K.S., Stress: metaplastic effects in the hippocampus. *Trends Neurosci.,* 21, 505-509, (1998).
38. Kim, J.J. and Diamond, D.M., The stressed hippocampus, synaptic plasticity and lost memories. *Nature Rev. Neurosci.* 3, 453-462, (2002).
39. Eichenbaum, H. A cortical-hippocampal system for declarative memory. Nat. Rev. 1, 41-50, (2000).
40. Magarinos, A.M., Verdugo, J.M.G. and Mc Evans, B.S., Chronic stress alters synaptic terminal structures in hippocampus. *Proc. Natl. Acad. Sci* USA*.,* 94, 14002-14008, (1997).
41. Herbert, J., Goodyer, I.M., Grossman, A.B., Hastings, M.H., De Kloet, E.R., Lightman, S.L., Lupien, S.J., Roozendaal, B. and Seckl, J.R., Do corticosteroids damage the brain? *J. Neuroendocrin.,* 18, 311–393, (2006).
42. Liu, J., Wang, X. Mori, A., Immobilization stress-induced antioxidant defense changes in rat plasma: effect of treatment with reduced glutathione. *Int. J. Biochem.,* 26, 511-517, (1994).
43. Liu, J., Wang, X. Shigenaga, M.K, Yeo, H.C., Mori A. and Ames B.N., Immobilization stress causes oxidative damage to lipid, protein, and DNA in the brain of rats. *FASEB. J.,* 10, 1532–1538, (1996).

44. Sapolsky, R.M., Krey, L.C. and McEwen, B.S., The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocr. Rev.,* 7, 284-301, (1986).
45. Liu, J. and Mori, A., Stress, aging and brain oxidative damage. *Neurochem. Res.* 24, 1479-1497, (1999).
46. Olivenza, R, Moro, M.A, Lizasoain, I, Lorenzo, P., Fernandez, A.P., Rodrigo, J., Bosca, L. Leza, J.C., Chronic stress induces the expression of inducible nitric oxide synthase in rat brain cortex. *J. Neurochem.,* 74, 785-791, (2000).
47. Madrigal J.L., Hurtado O., Moro M.A., Lizasoain, I., Lorenzo, P., Castrillo, A, Bosca, L. and Leza, J., The increase in TNF-alpha levels is implicated in NF-kappaB activation and inducible nitric oxide synthase expression in brain cortex after immobilization stress. *Neuropsychopharmacology,* 26,155-163, (2002).
48. Chen W, Su H, Feng L, Zheng X., Andrographolide suppresses preadipocytes proliferation through glutathione antioxidant systems abrogation. *Life Sci*. 2016 Jul 1; 156:21-29, (2016).
49. Kelly, G.S., Nutritional and botanical interventions to assist with the adaptation to stress. *Altern Med. Rev.,* 4(4), 249-265, (1999).
50. Akpinar, D., Yargicoglu, P., Derin, N., Aliciguzel, Y. and Agar, A., The effect of lipoic acid on antioxidant status and lipid peroxidation in rats exposed to chronic restraint stress. *Physiol. Res.,* 56, 163-171, (2007).
51. Al-Ayadhi, L.Y., Korish, A. A and Al-Tuwaijri, A.S., The effect of vitamin E, L-arginine, N-nitro L-arginine methyl ester and forskolin on endocrine and metabolic changes of rats exposed to acute cold stress. *Saudi Med. J.* 27(1), 17-22, (2006).
52. Dhanalakshmi, S., Devi, R.S., Srikumar, R., Manikandan, S. and Thangaraj, R., Protective effect of Triphala on cold stress-induced behavioral and biochemical abnormalities in rats. *Yakugaku Zasshi,* 127(11), 1863-1867, (2007).