Asian Journal of Pharmaceutical Education and Research
Vol -7, Issue-1, January-March 2018
ISSN : 2278 7496

RESEARCH ARTICLE Impact Factor: 4.101

BIOACTIVE GUIDED FRACTIONATION OF *PTEROSPERMUM ACERIFOLIU*M FOR THE SCREENING OF NEUROCHEMICALS AND NEUROENDOCRINE EFFECTS

***Ankur Choubey, Ritu Gilhotra, Santosh Kumar Singh, Gopal Garg**

Department of Pharmacognosy, School of Pharmacy, Suresh Gyan Vihar University, Jaipur

*Corresponding Author's E mail: chaubey.ankur03@gmail.com

Received 18 Sept. 2017; Revised 15 Oct. 2017; Accepted 25 Oct. 2017, Available online 20 Jan. 2018

ABSTRACT

Pterospermum acerifolium is common plant in India is considered carminative, stimulant and emmenagogue. In the present study, ethanol extract of bark of *Pterospermum acerifolium* have been evaluated for Neurochemical study. Treatment with ethanolic extract of *Pterospermum acerifolium* was found to significantly decrease the serum levels of adrenocorticotropic hormone (ACTH), corticosterone (CORT) and -endorphin (-EP) as well as the brain and serum level of norepinephrine (NE). Furthermore, ethanolic extract of *Pterospermum acerifolium* was able to significantly reverse the chronic stress by decreasing the brain and serum levels of the monoamine neurotransmitters dopamine (DA), 5-hydroxytryptamine (5-HT). The results obtained from this study suggested that the memory-enhancing effect of ethanolic extract of *Pterospermum acerifolium* was mediated through regulations of neurochemical and neuroendocrine systems. From the present study it was concluded that the herbal drugs can be potentially used to control the state of CNS disorders. Further investigations are, however, necessary to explore mechanism(s) of action involved in these pharmacological activities.

Keywords: Adrenocorticotropic, Corticosterone (CORT), Dopamine (DA), 5Hydroxytryptamine (5-HT)

INTRODUCTION

Advances in nanoparticulate frameworks for enhanced medication conveyance show an incredible potential for the organization of critical dynamic particles. Strong Lipid Nanoparticles (SLNs) have risen as a contrasting option to other novel conveyance approaches because of different favourable circumstances, for example, plausibility of joining of lipophilic and hydrophilic medications, enhanced physical strength, minimal effort contrasted with liposomes and simplicity of scale-up and producing. In addition, the capability of SLNs in epidermal focusing on, follicular conveyance, controlled medication conveyance, expanded skin hydration because of more noteworthy occlusivity and photostability change of dynamic pharmaceutical fixings has been exceptionally settled. Solid lipid nanoparticles are colloidal transporter frameworks made out of high dissolving point lipids as a strong center covered by surfactants. The term lipid in a more extensive sense incorporates triglycerides, halfway glycerides, unsaturated fats, hard fats and waxes. A reasonable point of preference of SLNs is the way that the lipid lattice is produced using physiological lipids which diminish the threat of intense and ceaseless danger. Exceedingly refined normal strong lipids, for example, stearine portions of organic product bit are minimal effort contrasting

option to the business lipids utilized for SLN generation. This claim to fame material is gotten from indigenous source, accessible in wealth and supplemented with crucial bio-actives. Plants have been used in the conventional human services framework from time immemorial, especially among tribal groups. In India, around 2000 medications utilized are of plant origin.[1] Plant assets are draining all-inclusive at a disturbing rate and various monetarily and therapeutically foremost plant species will anon be wiped out. Therapeutic plants are currently under awesome weight because of their excessive collection or exploitation.[2]

It is entrenched that the disturbance of the hypothalamus-pituitary-adrenal (HPA) pivot, a focal pathway to the whole endocrine framework, is frequently key to most wellbeing issues, disorders, infections, and notwithstanding maturing itself. Hyperactive status of the HPA hub can bring about expanding levels of corticotropin-discharging hormone (CRH), adrenocorticotropic hormone (ACTH), and glucocorticoids in the hypothalamus, pituitary, and adrenal cortex, separately. From one perspective, anxiety can likewise adjust the physiological homeostasis which can bring about different neuronal, endocrine, and instinctive dysfunctions. Moreover, stretch is likewise known not psychological capacities, for example, memory, and it has been connected to the pathophysiology of inclination and tension issue. [2-8] A focal component of the anxiety reaction is the actuation of the HPA pivot which can bring about an expansion in plasma levels of glucocorticoids. As a result of their significant consequences for neurons, glucocorticoids can impact conduct, state of mind, and memory process. Neurotransmitter frameworks are additionally required in learning and memory forms, and a considerable piece of learning and memory debilitations is because of changes in neurotransmission. 9-18 It is entrenched that neurotransmitters can meddle with learning securing and memory. In this setting, the memory brokenness depicted in irregular Savda disorder could include an unnecessary creation of corticotropin-discharging hormone (CRH), adrenocorticotropic hormone (ACTH), and glucocorticoids in the hypothalamus, pituitary, and adrenal cortex, separately, under the anxiety condition.

The present venture is done to investigate the capability of natural medications for the treatment of CNS issue with a perspective to perform phytochemical examination and survey Neurochemical screening.

Experimental Design

Plant material

P. acerifolium bark were collected from the local market of Bhopal, (M.P.) during the month of May –July, 2012. The specimens were identified and authenticated by Dr. Zia ul Hassan, Assistant professor, Department of Botany, Saifia College of Science & Education, Bhopal and their herbarium was deposited. The authentication number is

safia/75. These collected specimens were chosen for the extraction process and assessment of Neurochemical activity.

Extraction

Ethanolic Extraction

The plant materials so collected were cleaned properly and washed with distilled water to remove dust particles and dried in shade. The dried drugs were coarsely powdered and then exhaustively extracted with 50% ethanol in Soxhlet apparatus for 72 h. The ethanolic extracts so obtained were freed of solvent under vacuum. (Yield: 9.33 %)

Bioactivity Guided Fractionation of Extracts The solvent free ethanolic extracts were acidified by 2M H_2SO_4, dissolved and extracted with chloroform. Two layers i.e. chloroform and aqueous acid layers were obtained. The chloroform layers were separated and allowed to evaporate. The aqueous acid layers were further basified by ammonium hydroxide and extracted with methanol. Chloroform fractions and aqueous basic fractions were thus obtained (Harborne, 1998).

The different fractions were subjected to qualitative phytochemical investigation to detect different phytoconstituents, TLC and Pharmacological studies.

***In Vivo* Experimental Design**

Animals for experiment

Swiss albino rats were obtained from animal house VNS institute of Pharmacy with due permission from Institutional animal ethical committee (Registration Number. 778/03/c/CPCSEA). Acute toxicity studies were conducted by using albino mice of either sex weighing between 20 and 25 gms and healthy adult male albino rats weighing between150 and 200 gms were selected for the Neurochemical screening. The animals were acclimatized to standard laboratory conditions (temperature: 25±20C) and maintained on 12-h light: 12-dark cycle. They were provided with regular rat chow (Lipton India Ltd., Mumbai, India) and drinking water ad libitum.

Acute Toxicity

In an acute toxicity studies, *pterospermum acerifolium* extract were given single doses of drug. The swiss albino rats were divided into groups. All animals fed with standard rat pelleted diet(Lipton India Ltd. pellets) and had free access to tap water *ad libitum.* Acute toxicity studies were performed according to the OECD guidelines. The doses selected for the study were 50 mg/kg, 100 mg/ kg, 200 mg/ kg, 300 mg/kg, 400 mg/kg for one day. Three animals were taken for each dose. It was observed that the extract don't produce any significant effect on the behaviour of rats. The animals were observed for 3 hours after dose administration and also after 24 and 48 hours.

Estimation of Neurochemicals

Estimation of Adrenocorticotropic (ACTH), Corticosterone (CORT), and β Endorphin (β-EP)

The blood was collected and centrifuged at 4◦C; the serum was stored at −80◦C before assay. Serum levels of ACTH, CORT, and β-EP were determined using ELISA kit (obtained from R&D Systems). The sensitivity of the assay was 1.0 ng/mL. Intra-assay and interassay coefficients of variation were less than 4.85% and 6.08%, respectively. The test was performed according to the manufacturer's specification.

Adrenocorticotropic (ACTH), corticosterone (CORT), and β-endorphin (β-EP) kits were obtained from R&D Systems, USA. Norepinephrine (NE, purity ≥ 97%), dopamine (DA, purity ≥ 99%), 5-hydroxytryptamine (5-HT, purity ≥ 99%), 3,4–dihydroxyphenylamine (DOPAC, purity ≥ 99%),and 3,4-dihydroxybenzylamine (DHBA, purity ≥ 98%) were obtained from Sigma Co., Ltd., USA. All other reagents were of analytical grade.

Surgicals

Haemostatic sponge: AbGel, Absorbable gelatin sponge USP, Srikrishna Laboratories, and Mumbai, India.

Sterile sutures: Ethicon 4-0, Non-absorbable surgical sutures USP. Mersilk (Braided silk black). Ethicon 4-0, Absorbable surgical sutures USP (Catgut), Johnson and Johnson, India.

Surgical Needle: Curved surgical needles were obtained from Pricon Surgical, New Delhi, India.

Drug Administration.

Estimation of Neurochemicals

Estimation of Adrenocorticotropin (ACTH), Corticosterone (CORT), and β Endorphin (β-EP).

The blood was collected and centrifuged at 4◦C; the serum was stored at −80◦C before assay. Serum levels of ACTH, CORT, and β-EP were determined using ELISA kit (obtained from R&D Systems). The sensitivity of the assay was 1.0 ng/mL. Intra-assay and interassay coefficients of variation were less than 4.85% and 6.08%, respectively. The test was performed according to the manufacturer's specification.

Reagents:

Adrenocorticotropin (ACTH), corticosterone (CORT), and β-endorphin (β-EP) kits were obtained from R&D Systems, USA. Norepinephrine (NE, purity ≥ 97%), dopamine (DA, purity ≥ 99%), 5-hydroxytryptamine (5-HT, purity ≥ 99%), 3,4–dihydroxyphenylamine (DOPAC, purity ≥ 99%),and 3,4-

dihydroxybenzylamine (DHBA, purity ≥ 98%) were obtained from Sigma Co., Ltd., USA. All other reagents were of analytical grade.

Drug Administration.

In the preliminary investigation, the animals were divided into four matched groups namely;

Wistar rats were divided into four groups of six rats each. Group I served as normal group. Animals of Group II serves as model group and III-IV group were administered orally with respective test drugs , between 7:30 am–9:30 am daily during 14 days. These doses were calculated according to the conversion table of equivalent effective dose ratios from human to animals based on the body surface area. Food was withdrawn from the animals 2 h prior to drug administration but water was allowed freely. The PTetoh pretreatment groups received the same electric foot-shock one hour after drug administration (8:30 am–10:30 am).

Group I = Normal group (0.5% Sodium carboxyl methyl cellulose (CMC-Na) solution (20 mL/kg, b.w.)

Group II = Standard Group (1 mg/kg, p.o)

Group III = Aqueous basic fraction of P. acerifolium ethanolic extract at the oral dose of (2.53 g/kg)

Group IV = Chloroform fraction of P. acerifolium ethanolic extract at the oral dose of 2.53 g/kg)

Measurements of Monoamine Neurotransmitters by HPLC-FCD.

Norepinephrine (NE), Dopamine (DA), Serotonin or 5-hydroxytryptamine (5-HT)

Levels of monoamine neurotransmitters (NE,DA, 5-HT, and) in serum and brain were measured by HPLC coupled with a fluorescence detector (FCD). Mice were sacrificed immediately after exposure to the stress. Blood was sampled into EDTA-containing tubes at 10:00 am, and separated in a refrigerated centrifuge at 10,000×g for 10 min at 4◦C. The serum was stored at −80◦C until assayed. After blood collection, the brains were quickly removed, frozen in liquid nitrogen, and stored at −80◦C until assayed. To determine serum monoamine neurotransmitter levels, an equal volume of 0.1M HCl was added to the serum samples containing 200 μg/mL of DHBA as an internal standard. The samples were then shaken and mixed for 1.5min in ice water. One drop of concentrated HCl was then added to the solution and mixed in ice water for another 1.5 min and then centrifuged at 3000 rpm, 4◦C for 10 min. The samples of brain tissue were homogenized in ice water solution of 0.1M HCl. Then, 0.1M HCl solution was added to the samples (1 μL/1mg tissue) containing 200 μg/mL of DHBA as an internal standard and centrifuged at 18000 rpm, 4◦C for 10min. The samples were filtered through 0.45 μm microfilters (MFS Inc., USA).

Aliquots (10 µL) of supernatant were injected into a reverse phase HPLC column (condition: Agilent 110180 high-voltage pump coupled to a fluorescence detector, chromatographic column ZORBAX ODB C18 4.6mm × 150mm × 5 mm, voltage 121V, and wavelength 360 nm). All the brain samples were weighed on an electronic scale prior to HPLC analysis, and the results were expressed as ng of monoamine/mg of wet weight tissue.[19-22]

Statistical analysis

Statistical evaluation of the data was done by Student's t test. (Graph PAD Instat software, Kyplot). A value of $p<0.05$ was considered to be significant.

Results and discussion

Effects of *P.acerifolium* on the Serum Levels of ACTH, CORT, and β-EP in the Chronic Stress Mice.

Table 1-3 and figure 1-3 showed that the serum levels of ACTH, CORT, and β-EP were markedly increased ($P < 0.01$) in the chronic stress mice (Gr. II) when compared to the normal group (Gr. I). Oral administration of *P.acerifolium* at doses of 2.53 g/kg, (Gr. III, IV) for 14 days caused a decrease of the levels of ACTH, CORT, and β-EP in the serum when compared to the model group (Gr. II).

Table 1: Effects of *P.acerifolium* on the serum level of ACTH

GROUP	ACTH (pg/mL)
Gr. I	16.0 ± 3.12
Gr. II	32.10 ± 4.14*
Gr. III	22.17 ± 3.90**
Gr. IV	21.60 ± 4.29**

*Results are given as means ±SEM, when compared to the normal group (Gr. I), $P < 0.01$.

**Results are given as means ±SEM, when compared to the model group (Gr. II), $P < 0.05$.

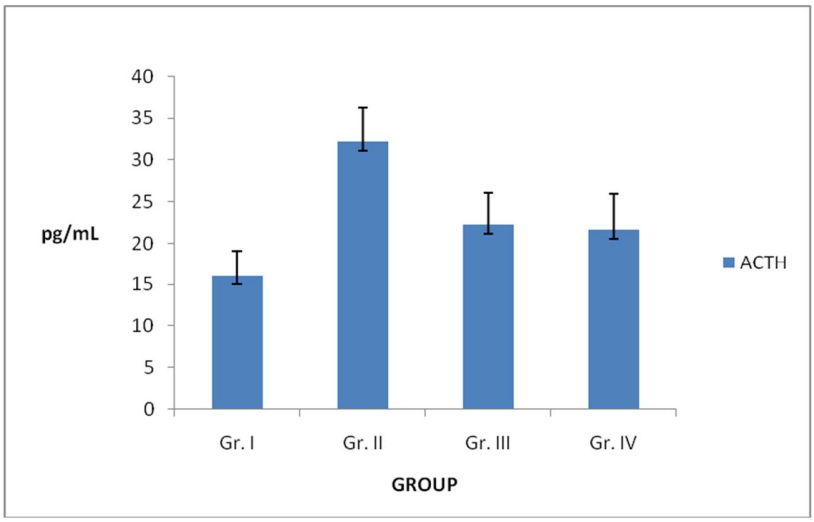

Figure 1: Effects of *P.acerifolium* on the serum level of ACTH

Table 2: Effects of *P.acerifolium* on the serum level of CORT

GROUP	CORT (pg/mL)
Gr. I	12.10 ± 4.9
Gr. II	29.23 ± 4.5*
Gr. III	21.98 ± 5.6**
Gr. IV	23.51 ± 3.34 **

*Results are given as means ±SEM, when compared to the normal group (Gr. I), $P < 0.01$.

**Results are given as means ±SEM, when compared to the model group (Gr. II), $P < 0.05$.

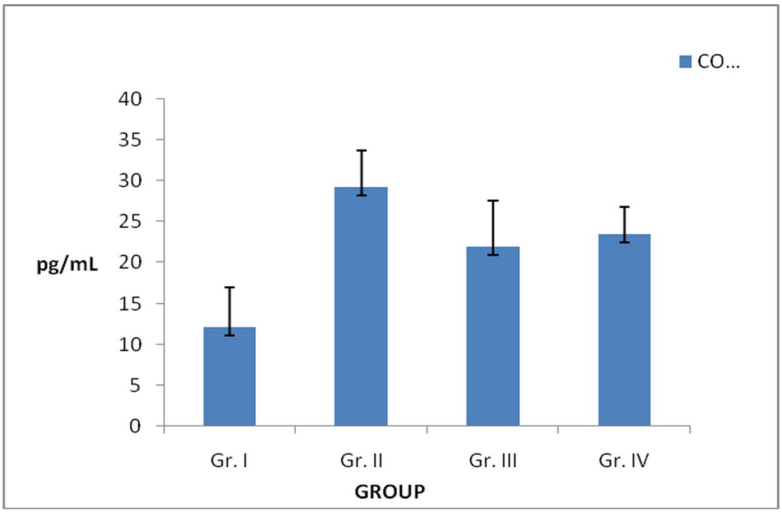

Figure 2: Effects of *P.acerifolium* on the serum level of CORT

Table 3: Effects of *P.acerifolium* on the serum level of β-EP

GROUP	*β*-EP (pg/mL)
Gr. I	154.17 ± 27.19
Gr. II	256.21 ± 23.12*
Gr. III	205.30 ± 34.25**
Gr. IV	198.09 ± 19.65**

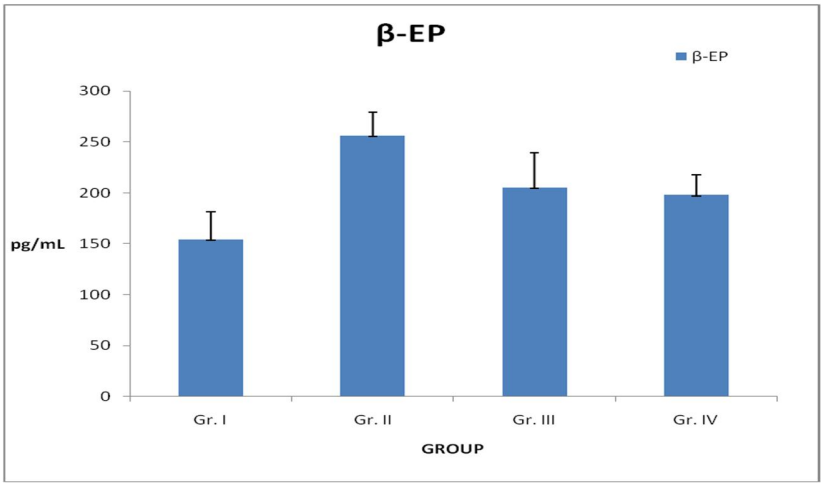

Figure 3: Effects of *P.acerifolium* on the serum level of β-EP

Effects of *P.acerifolium* on the Contents of Monoamine Neurotransmitters of Brain and Serum in the Chronic Stress Mice.

Table 4-6 and Figure 4-6 showed an increase ($P < 0.05$) of NE level in the serum of the chronic stress mice (Gr. II) and a decrease of the serum levels of DA, when compared to the normal group (Gr. I). Oral administration of *P.acerifolium* during 14 days at doses of aqueous and chloroform extract of doses 2.53 g/kg (Gr. III, Gr. IV) was able to decrease the NE levels ($P < 0.01$), while the levels of DA, Figures 5 and 6 showed similar results with an increase of the NE level ($P < 0.01$) but a decrease in the levels of DA ($P < 0.01$) in the brain of the chronic stress mice (Gr.II), when compared to the normal group (Gr. I). All doses of *P.acerifolium* (2.53 g/kg) were found to reduce the concentration of NE in the brain ($P < 0.01$) when compared to the stress mice (Gr.II).

Table 4: Effects of *P.acerifolium* on the serum level of NE (ng/mL)

GROUP	NE (ng/mL)
Gr. I	422.22 ± 20.19
Gr. II	695.55 ± 23.10*
Gr. III	629.30 ± 30.29**
Gr. IV	602.19 ± 18.60**

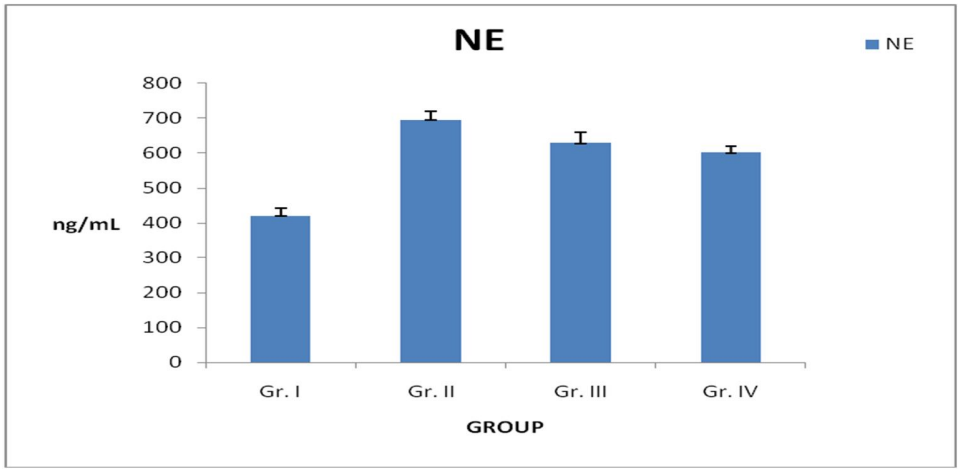

Figure 4: Effects of *P.acerifolium* on the serum level of NE (ng/mL)

Table 5: Effects of *P.acerifolium* on the serum level of 5-HT (ng/mL)

GROUP	5-HT (ng/mL)
Gr. I	100.5 ± 14.19
Gr. II	40.50 ± 21.10*
Gr. III	104.5 ± 21.25**
Gr. IV	103.11 ± 15.65**

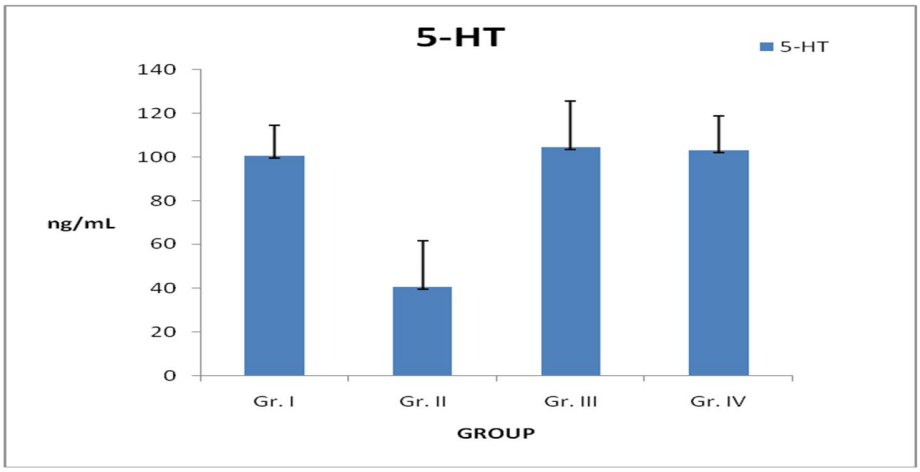

Figure 5: Effects of *P.acerifolium* on the serum level of 5-HT (ng/mL)

Table 6: Effects of *P.acerifolium* on the serum level of DA (ng/mg)

GROUP	DA (ng/mg)
Gr. I	2900.25 ± 21.19
Gr. II	2212.50 ± 18.10*
Gr. III	4475.5 ± 20.25**
Gr. IV	5225.11 ± 29.65**

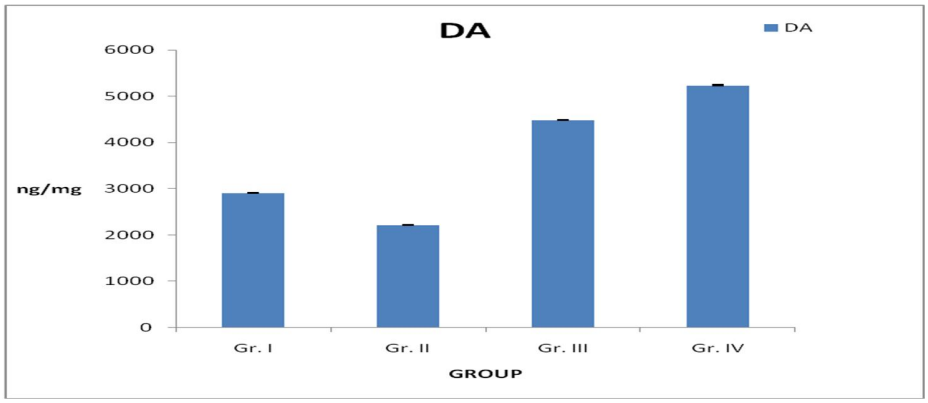

Figure 6.27: Effects of *P.acerifolium* on the serum level of DA (ng/mg)

Conclusion:

Treatment with ethanolic extract of *Pterospermum acerifolium* was found to significantly decrease the serum levels of adrenocorticotropic hormone (ACTH), corticosterone (CORT) and -endorphin (-EP) as well as the brain and serum level of norepinephrine (NE). Furthermore, ethanolic extract of *Pterospermum acerifolium* was able to significantly reverse the chronic stress by decreasing the brain and serum levels of the monoamine neurotransmitters dopamine (DA), 5-hydroxytryptamine (5-HT). The results obtained from this study suggested that the memory-enhancing effect of ethanolic extract of *Pterospermum acerifolium* was mediated through regulations of neurochemical and neuroendocrine systems.

References

1. Davis S, Long-lasting effects of acute stress on the neurochemistry. Psychopharmacology. 1995; 118:267-72.
2. Abramson LY, Modeling psychopathology in the laboratory. Psychopathology Experimental Models. Maser JD, Seligman MEP (eds.), San Francisco, Freeman;1978. p. 1-26.
3. Adamec R, Walling S, Burton P. Long-lasting, selective, anxiogenic effects of feline predator stress in mice. Physiol Behav. 2004;83:401-10.
4. Akiskal HS, Interaction of biologic and psychologic factors. Acta Psychiatr Scandium 1985; 71:131-9.
5. Davidson JR, Meoni P, Haudiquet V, Cantillon M, Hackett D.Achieving remission with venlafaxine and fluoxetine in major depression: Its relationship to anxiety symptoms. Depress Anxiety. 2002; 16:4-13.
6. Deakin.JFW, Roles of serotoninergic systems in escape. Psychopharmacology, Academic Press: London. 1983**;** 12: 149-93.
7. Deakin.JFW, Distinct roles of 5HT subsystems in panic, anxiety and depression. Biol Psychiatry 1991; 1: 305-307.
8. Deakin JF. 5 -HT, antidepressant drugs and the psychosocial origins of depression. J Psychopharmacol. 1996; 10:31-8.
9. Deakin JFW. Social stress, steroid levels, 5-HT and depression. J Psychopharmacol. 1997;11:A93-7.
10. Deakin JF. Depression and antisocial personality disorder: Two contrasting disorders of 5HT function. J Neural Transm Suppl. 2003; 64:79-93.

11. Deb S, Lyons I, Koutzoukis C, Ali I, McCarthy G. Rate of psychiatric illness 1 year after traumatic brain injury. Am J Psychiatry. 1999;156:374-8.
12. Denenberg VH. Open-field bheavior in the rat: What does it mean?. Ann N Y Acad Sci. 1969; 159:852-9.
13. Edwards B, Clarke V. The psychological impact of a cancer diagnosis on families: The influence of family functioning and patients' illness characteristics on depression and anxiety. Psychooncology. 2004;13:562-76.
14. Edwards DA, Griffis KT, Tardivel C. Olfactory bulb removal: Effects on sexual behavior and partner-preference in male rats. Physiol Behav. 1990;48:447-50.
15. Evans DL, Charney DS, Lewis L, Golden RN, Gorman JM, Krishnan KR, *et al.* Mood disorders in the medically ill: Scientific review and recommendations. Biol Psychiatry. 2005;58:175-89.
16. Fann JR, Uomoto JM, Katon WJ. Sertraline in the treatment of major depression following mild traumatic brain injury. J Neuropsychiatry Clin Neurosci. 2000;12:226-32.
17. Fedoroff JP, Starkstein SE, Forrester AW, Geisler FH, Jorge RE, Arndt SV, *et al.* Depression in patients with acute traumatic brain injury. Am J Psychiatry. 1992;149:918-23.
18. Feinstein AR. The pre-therapeutic classification of co-morbidity in chronic disease. J Chronic Dis. 1970; 23:455-68.
19. Ferré P, Fernández Teruel A, Escorihuela RM, García E, Zapata A, Tobeña A, *et al.* Struggling and flumazenil effects in the swimming test are related to the level of anxiety in mice. Neuropsychobiology. 1994; 29:23-7.
20. Fehr C, Schleicher A, Szegedi A, Anghelescu I, Klawe C, Hiemke C, *et al.* Serotonergic polymorphisms in patients suffering from alcoholism, anxiety disorders and narcolepsy. Prog Neuropsychopharmacol Biol Psychiatry. 2001; 25:965-82.
21. Fernandez-Ruiz J, Díaz R, Hall-Haro C, Vergara P, Fiorentini A, Nuñez L, *et al.* Olfactory dysfunction in hereditary ataxia and basal ganglia disorders. Neuroreport. 2003; 14:1339-41.
22. Feske U, Frank E, Kupfer DJ, Shear MK, Weaver E. Anxiety as a predictor of response to interpersonal psychotherapy for recurrent major depression: An exploratory investigation.Depress Anxiety. 1998;8:135-41.