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Research Article

QUANTITATIVE ESTIMATION OF 5-HT (SEROTONIN) IN STRESSED RAT'S BRAIN REGIONS UNDER INFLUENCE OF *T. ORIENTALIS* AND *H.RHAMNOIDES* PLANT EXTRACTS

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ABSTRACT
The pattern of serotonin was evaluated in brain of immobilized stressed rat group and in stress plus ethanolic extract of *Trema orientalis* and *Hippophae rhamnoides* along with a group of normal control.

Keywords: *Histamine, Hypothalamus, Thalamus, Cortex, Serotonin, Immobilization Stress, Extract*

INTRODUCTION
The most important mode of stress management with no adverse effect reported with herbal medicine system, which is very common in India. It is believed that our predecessors inherited the estimate; about 70% of Indian population still relies on herbal medicines for various ailments. They are traditionally claimed as restorative and health promoting agents with their overall effect like brain tonic. Medicinal plants have been used as a major source of therapeutic agents by human beings for thousands of years. Ancient people obtained more than 90% of his medicaments form higher plants.
According to Farnsworth(1991) even in a highly developed country like the United States of America, more than 25% of the prescriptions were found to contain one or more plant products in 1980. The importance of plants as a major source of clinical agents is much more in developing countries of Asia, Africa, South America where traditional medicine is practised widely. There has been considerable revival in the use of higher plants for health care purposes during last 3 decades throughout the world. More and more people worldwide globally are switching over to plant drugs, herbal health foods and herbal tea in Europe and the United States for treatment of common ailments.
It was reported earlier that various medicinal plants and their active constituents were used to regularize the levels of various neurotransmiters. Hersaponin isolated from *Bacopa monniera* was found to decrease the brain nor-epinehrine and 5-HT contents of rat (Bhakuni *et al.*, 1969). In my study, it was found that the levels of nor-epinephrine were significantly decreased in different parts of the brain in mandukparni (*C. asiatica*) treated stressed group in comparison to stressed group. Various other workers also reported the anti-*stress* property of some other medicinal plants. Total root extract of Ashwagandha (*W. somnifera*) showed a significant anti-*Stress* activity in widely different *Stress* situation (Bhattacharya et al., 1987). Similarly, *O. sanctum* has also been reported to be an anti-*Stress* agent against various kind of stressor (Redge *et al.*, 1999).
It has been earlier reported that alcoholic extract of *Withania somnifera* increased, the 5-HT and *histamine* levels in normal rats were increased (Singh *et al.*, 1979). Similarly, a gradual reduction in the level of brain 5-HT was found reduced; when jatamansone isolated from *Nardostachys jatamansi* was introduced to the rabbit (Arora *et al.*, 1962a).
Stress leads to a positive increase in brain serotonin levels, increases in dopamine levels and increases in SDH (succinate dehydrogenase) levels, while Holy Basil *O. sanctum* may help people maintain normal levels of these brain chemicals in times of stress (Devi, 2001; Singh, 1999; Agrawal, 1996). In another study animals that received the *O. sanctum* extract showed significant normalization of epinephrine, norepinephrine, serotonin, MAO, and SDH. Epinephrine and norepinephrine are used in coping with stress (Devi, 2001; Singh, 1997; Agrawal, 1996).
The ethanolic extracts of flowers of *Hibiscus rosa sinesis* exhibited raised brain contents of gamma-aminobutyric acid (GABA) and serotonin. The extracts protected animals from maximum electro shock,

electrical kindling and pentylenetetrazole-induced convulsions (Kasture, 2000). The Ze 117 extract of Hypericum perforatum in a dose-dependent inhibition was seen on NE and 5-HT uptake into brain slices. The Ze 117 extract was more selective for the uptake of NE than for that of 5-HT (Kientsch *et al.,* 2001).
The search for effective and safe alternatives from natural sources especially plant products should, therefore, continue. Forced immobilization is one of the best explored models of stress in rats and the role of serotonin, norepinephrine (NE), dopamine (DA) is well documented. *Ginkgo biloba* extract (14 mg/kg p.o.) restored restraint stress-induced elevation in whole rat's brain levels of catecholamines (NE, DA), 5-HT and plasma corticosterone to near normal levels (Shah *et al.,* 2003). *Evolvulus alsinoides* at a dose of 200 mg/kg p.o. found effective in acute studies was administered 45 min prior to stress for 7 days. *Evolvulus alsinoides* reduced the stress induced perturbations similar to *Panax quinquefolium* (100 mg/kg p.o.), a well known adaptogen (Kiran, 2005).
Caesalpinia bonduc Roxb. (Caesalpiniaceae) seed extracts were screened for adaptogenic activity using cold stress model and swim endurance model, the seed coat as well as kernel extracts showed significant antistress activity when administered orally at a dose of 300 mg/kg (Kannur *et al.,* 2006). In CUS model (different stressors for 7 days), pretreatment with *Bacopa monniera* (40 and 80 mg/kg) and *Panax quinquefolium* significantly elevated the levels of NA, DA and 5-HT levels in cortex and levels of NA and 5-HT in hippocampus regions (Naila *et al.,* 2007). Peppermint or *Mentha piperita* also known as *M. balsamea* Willd, extract (0.1gm/kg) was given to 2h restraint stressed animals. The results were consistent with antistress effect of Mentha piperita and suggest a role of brain Serotonin and Dopamine in antistress effect (Perveen *et al.,* 2012).

MATERIALS AND METHODS

Animal Selection

Adult Charles Foster albino rat's strains (175-250g) of either sex were obtained from Central Animal House of the Institute and were distributed into three experimental groups. The rats were housed in gp of ten in polypropylene cages at ambient temperature of 250C and 45-55% RH, with a 12:12 h light/dark cycle. Animal were provided with commercial food pellets (Brooke Brand-Lipton, India) and water ad libitum unless stated otherwise ("Principles of laboratory animal care" (NIH publication no. 85-23, revised 1985) guideline were followed.

Experiment: The study has been divided into three groups and in each series 10 rats of Experiment was divided in to three groups:

Gp (I): The rats were selected for present study were kept in normal laboratory condition and served as control gp and received only vehicles.

Gp (II): In this gp Immobilization stress was given to all rats for 15 minutes daily for 15 days and received only DW.

Gp (III): In this gp Immobilization stress was given to all the rats along with mentioned doses of alcoholic extracts of *Trema orientalis* and *H.rhamnoides* daily for 15 days orally.

Selection of the plants: Different medicinally active parts of the plant mentioned below were procured from Department of Pharmacy, IMS.

Table 1: Experimental studied plants

S.N.	Scientific Name	Family	Part used	Extraction	Dose for 15 days
1	*Trema orientalis(Goal)*	*Ulmaceae*	Root and stem	90% ethanol	80 mg/kg/day
2	*Hippophae rhamnoides (Badriphal)*	*Eleagnaceae*	Fruit	do	65 mg/kg/day
3	*Nardostachys jatamansi (Jatamansi)*	*Valerianaceae*	Rhizome	do	15 mg/kg/day

Crude Organic Extraction

Different test drugs were shade dried and course powder of given sample was taken for extraction by hot percolation method in 90% ethyl alcohol. Extraction was done by by soxhlet apparatus continuously for 90 hours.Temp. was maintained at 70ºC. The obtain organic extract was solidified to a pasty mass over thermostatic water bath at 70ºC. The extracts of test drugs were separately suspended in Dw in 5% gum acasia and given to all the rats before introducing stress.

Method for the Quantitative Measurement of Serotonin (5-HT)

At the end of the 15th day the rats of all groups were sacrificed and different parts of the rat brain were dissected out. Different brain regions serotonin was measured in different dissected parts like hypothalamus, thalamus and cerebral cortex. 5-HT was extracted in n-butanol from the tissue homogenate then transferred to a buffer medium. Buffer solution containing 5-HT was then reacted with ninhydrin to yield fluorescence which was measured with the help of spectrophotofluorometer.

Reagents: 0.4 N perchloric acid (PCA), 10% $ZnSO_4$ (Zinc sulphate), 1.0 N NaOH, 10 N NaOH, 0.5 M borate buffer (pH 10.6), 0.1 M borate buffer (pH 10) NaCl, n-butanol, n-heptane, 0.05 M sodium-potassium phosphatase buffer (pH 7.0) and 0.1 M ninhydrin solution.

Tissue preparation: Tissue were weighted and hemogenized in 6 ml of 0.4 N PCA. After centrifugation the supernatent was collected and residue again centrifuged with 2 ml 0.4 N PCA and collected the supernatent again in the same tube.

Procedure: Supernatent, obtained from tissue or plasma was adjusted to pH 10.0 (approximately) by 10 N NaOH and added 0.5 ml of 0.5 M borate buffer (pH 10.0). The content was saturated with NaCl (1.5 gm approximately) and 10 ml of n-butanol was added. The tubes were shaken for exactly 5 minutes on a vortex mixer and the aqueous layer was removed by aspirator. The organic phase was shaken for 2 minutes with 2 ml of 0.1 M borate buffer, (pH 10.6, previously saturated with NaCl). Then 8 ml of butanol layer was placed in a separate tube containing 4 ml of 0.05 M phosphate buffer (pH 7.0) and 10ml n-heptane. After shaking for 2 minutes the organic phase was carefully removed.

3.6 ml of buffer layer was taken in a tube containing 0.3 ml of 0.1 M ninhydrin solution. The tubes were incubated at 75^0C for exactly 30 minutes then cooled at room temperature for 30 minutes. The fluorescence was measured in a spectrophotofluorometer at 385/490 mu excitation and emission wavelength respectively. The values were calculated by using the standard curve of 5-HT, processed in the same manner as for tissue serotonin (5-HT) estimation.

Animal model for immobilization stress: Following method of Roger *et al.,* (1978).

RESULTS AND DISCUSSION

Table 2: Effect of drug *Trema orientalis* on *5-HT* levels in different parts of rat brain following Immobilisation stress

Groups	Average 5-HT in µg/gm of wet tissue (Mean±S.D.)		
	Hypothalamus	Thalamus	Cortex
Control group (N=10)	1.570±0.2128	1.040±0.1262	0.786±0.1980
Immobilization Stress group (N=10)	2.638±0.3786	1.298±0.2726	1.290±0.2714
Immobilization Stress + test drug group (N=10)	1.820±0.3812	0.920±0.1746	0.788±0.1896

Serotonin also showed varying pattern in different parts of rat brain in normal control series. Average *5HT* levels in various parts of the brain were found different. The average *Serotonin* of this group was found 1.570±0.2128 µg/gm of wet tissue in hypothalamus and 1.040±0.1262 µg/gm in thalamus and 0.786±0.1980 µg/gm in cortex. But after 15 days of immobilization stress Immobilization stress produce significant changes in brain biogenic amines in different part of brain like hypothalamus, thalamus and

cortex. After 15 days of immobilization Stress, significant elevation of 5-HT was found in each part of the brain hypothalamus ($p<0.001$), thalamus($p < 0.02$) and cortex($p < 0.001$). When drug *Trema orientalis* was given along with immobilization Stress after 15 days, 5-HT level was found significantly decreased in hypothalamus($p < 0.001$), thalamus($p < 0.01$) and cortex($p < 0.001$) (Table 2, Figure 1).

Table 3: Effect of drug *Hippophae rhamnoides* on 5-HT levels in different parts of rat brain following Immobilization Stress

Groups	Average *5-HT* in µg/gm of wet tissue (mean ±S.D.)		
	Hypothalamus	Thalamus	Cortex
Control group (N=10)	1.562±o.2102	1.036±0.1262	0.763±0.1962
Immobilization Stress group (N=10)	2.682±0.3680	1.288±0.2622	1.284±0.2764
Immobilization Stress + test drug group (N=10)	1.812±0.3612	0.986±0.1632	0.786±0.1920

Serotonin was measured in control group different parts of brain and average Serotonin was found 1.562±o.2102 µg/gm of tissue in hypothalamus, 1.036±0.1262 µg/gm wet tissue in thalamus and 0.763±0.1962 µg/gm of tissue in cortex. After 15 days, in Immobilization stress group, 5-HT was found significantly elevated in different part of brain like hypothalamus ($p<0.001$), thalamus ($p<0.02$) and cortex ($p<0.001$). After 15 days, 5-HT was found significantly decreased in thalamus ($p<0.001$) and cortex ($p<0.001$) where as in hypothalamus the difference was found significant at border line ($p<0.05$) in *Hippophae rhamnoides* treated group (Table - 3, Fig. 2).

Conclusion

In my study, it was observed that organic extract of *H. rhamnoides* exert significant effect on *5-HT*, which was reduced in thalamus and cortex of *H. rhamnoides* treated stressed group. Similarly organic extract of *T. orientalis* were reduced. The levels of *5-HT* in different parts of the brain of the rats of the drug treated stressed groups in comparison to stressed rats. As the safety and efficacy profile of all the five drugs have been studied on scientific parameters and none of the drug has shown any adverse effect. Therefore, these drugs may be advocated for human use as a single or poly herbal multi-ingredient therapeutic measure as an *anti-stress* on *anti-anxiety* agent.

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REFERENCES

Arora RB, Singh M and Chandrakanta (1962a). Tranquillizing activity of jatamansone. *Life Science* **11** 571.

Bhakuni DS, Dhar ML, Dhar MM, Dhawan BN and Mehrotra BM (1969). *Indian Journal of Experimental Biology* **7** 250.

Bhattacharya SK, Goel RK, Kaur R and Ghoshal S (1987). *Phytotherapy Research* **1**(1) 32-37.

Devi U (2001). Radioprotective, anticarcinogenic and antioxidant properties of the IndianHoly Basil, Ocimum sanctum (Tulsi). *Indian Journal of Experimental Biology* **39** 185-190.

Kannur DM *et al.*, (2006). Adaptogenic activity of Caesalpiniabonduc seed extracts in rats **108**(3) 327–331.

Kasture VS *et al.*, (2000). Anticonvulsive activity of Albizzia lebbeck, Hibiscus rosa sinesis and Butea monosperma in experimental animals. *Journal of Ethnopharmacology* **71**(1-2) 65-75.

Kientsch U *et al.*, (2001). St. John's wort extract Ze 117 (Hypericum perforatum) inhibits norepinephrine and serotonin uptake into rat brain slices and reduces 3-adrenoceptor numbers on cultured rat brain cells. *Pharmacopsychiatry* **34**(Suppl 1) S56-60.

Kiran Babu Siripurapu *et al.*, (2005). Adaptogenic and anti-amnesic properties of Evolvulus alsinoides in rodents **81**(3) 424–432.
Naila S *et al.*, (2007). Effect of Bacopa monniera on stress induced changes in plasma corticosterone and brain monoamines in rats **111**(3) 671–676.
Perveen T *et al.*, (2012). Antistress effect of Mentha piperita in rats and the role of brain serotonin and dopamine, *Asian Journal of Pharmaceutical and Biological Research* **2**(1).
Rege NN, Thatte UM and Dahanukar SN (1999). *Phytotherapy Research* **13** 275-291.
Roger D Porsolt, Guy Anton, Nadine Blavet and Miaurice Jalfree (1978). Behavioural Despair in rats : A new model sensitive to antidepressant treatments. *European Journal of Pharmacology* **47** 379.
Shah ZA *et al.*, (2003). Ginkgo biloba normalises stress-elevated alterations in brain catecholamines, serotonin and plasma corticosterone levels **13**(5) 321–325.
Singh S (1999). Evaluation of the gastric antiulcer activity of fixed oil of Ocimum sanctum (Holy Basil) *Journal of Ethnopharmacology* **65** 13-19.