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 neurotransmitters. Hersaponin isolated from Bacopa monniera was found to decrease the brain nor-epinephrine 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,
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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). Evolulus 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. Evolulus alsinoides reduced the stress induced perturbations similar to Panax quinquefolium (100 mg/kg p.o.), a well known adaptogen (Kiran, 2005).

Caesalpinia bonduc Roxb. (Caesalpiniaeaceae) 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

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Scientific Name</th>
<th>Family</th>
<th>Part used</th>
<th>Extraction</th>
<th>Dose for 15 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trema orientalis(Goal)</td>
<td>Ulmaceae</td>
<td>Root and stem</td>
<td>90% ethanol</td>
<td>80 mg/kg/day</td>
</tr>
<tr>
<td>2</td>
<td>Hippophae rhamnoides</td>
<td>Eleagnaceae</td>
<td>Fruit</td>
<td>do</td>
<td>65 mg/kg/day</td>
</tr>
<tr>
<td>3</td>
<td>Nardostachys jatamansi</td>
<td>Valerianace</td>
<td>Rhizome</td>
<td>do</td>
<td>15 mg/kg/day</td>
</tr>
</tbody>
</table>

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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 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% ZnSO₄ (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 75ºC 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.


RESULTS AND DISCUSSION

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Average 5-HT in μg/gm of wet tissue (Mean±S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypothalamus</td>
</tr>
<tr>
<td>Control group (N=10)</td>
<td>1.570±0.2128</td>
</tr>
<tr>
<td>Immobilization Stress group (N=10)</td>
<td>2.638±0.3786</td>
</tr>
<tr>
<td>Immobilization Stress + test drug</td>
<td>1.820±0.3812</td>
</tr>
<tr>
<td>(N=10)</td>
<td></td>
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</tbody>
</table>

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>
<thead>
<tr>
<th>Groups</th>
<th>Average 5-HT in µg/gm of wet tissue (mean ±S.D.)</th>
<th>Hypothalamus</th>
<th>Thalamus</th>
<th>Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (N=10)</td>
<td>1.562±0.2102</td>
<td>1.036±0.1262</td>
<td>0.763±0.1962</td>
<td></td>
</tr>
<tr>
<td>Immobilization Stress group (N=10)</td>
<td>2.682±0.3680</td>
<td>1.288±0.2622</td>
<td>1.284±0.2764</td>
<td></td>
</tr>
<tr>
<td>Immobilization Stress + test drug group (N=10)</td>
<td>1.812±0.3612</td>
<td>0.986±0.1632</td>
<td>0.786±0.1920</td>
<td></td>
</tr>
</tbody>
</table>

Serotonin was measured in control group different parts of brain and average Serotonin was found 1.562±0.2102 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**


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