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Research Article

NEURO PROTECTIVE POTENTIAL OF ETHANOLIC EXTRACT OF *HYPERICUM HOOKERIANUM* IN HALOPERIDOL INDUCED SCHIZOPHRENIA IN SWISS ALBINO MICE

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ABSTRACT
Herbal drugs are playing an important role in health care all over the world, especially in developing countries because they are being cheap, locally available and consider being safe. Neuroleptic drugs used in the treatment of schizophrenia and other affective disorders are known to produce extra pyramidal side effects. *Hypericum hookerianum* (Hh) is known to possess various therapeutic properties. We have earlier characterized its phytochemical and physic chemical properties. However, their role in modulating stress-induced central changes is unexplored. Thus, the present study was aimed to investigate the brain oxidative stress induced by haloperidol in swiss albino mice and evaluate the consequent changes in brain neurotransmitters levels such as Dopamine, serotonin, nor adrenaline and GABA. Results showed that Brain neurotransmitters in this study were decreased in schizophrenic mice which may be related to oxidative stress induced by haloperidol. This is reversed by the administration of ethanolic extract of *Hypericum hookerianum* (EEHh) which seems to be a highly promising herb in protecting the schizophrenicmice against oxidative damage and preventing brain complications such as reduction of neurotransmitters. Further studies are required to understand the mechanism of action of this herb and active principle responsible for this neuroprotection.
Keywords: Schizophrenia, neurotransmitters, Haloperidol, *Hypericum hookerianum*, Neuroprotection

INTRODUCTION

Schizophrenia is a mental disorder characterized by the breakdown of thought processes and by poor emotional responsiveness[1]. It most commonly manifests itself as auditory hallucinations, paranoid or bizarre delusions, disorganized speech and thinking. It is still one of the most costliest and mysterious mental disorders in terms of human suffering and societal expenditure. Genetics, early environment, neurobiology and psychological and social processes appear to be important contributory factors; some recreational and prescription drugs appear to cause or worsen the symptoms and the precise social burden of schizophrenia is difficult to estimate[2]. Thus schizophrenia causes more loss of lives than do most cancers and physical illness. The disorder is mainly to affect cognition, but it also usually contributes to chronic problems with behavior and emotions[3]. People with schizophrenia are likely to have additional (co morbid) emotions, including the major depression and anxiety disorders, the life time occurrence of substance abuse is almost 50 %[4]. The average life expectancy of people with this disorder is 12-15 years less than those without the results of increased physical health problem and a higher suicide rate (about 5 %)[5]. Psychiatric co morbidities are common among patients with schizophrenia. Substance abuse co morbidity predominates[6]. The diagnosis of schizophrenia is associated with demonstrable alterations in brain structure and changes in dopamine neurotransmission, the latter being directly related to hallucinations and delusions. Pharmacological treatments, which block the dopamine system, are effective for delusions and hallucinations but less so for disabling cognitive and motivational impairments[7]. The brain is an intricately interconnected structure in which different regions are linked by extensive nerve cell projections. It is therefore unlikely that there is one place in the brain that is responsible for schizophrenia. Rather; it is likely that flaws in brain structure and chemistry make functioning in several parts of the brain defective. Chemical messenger systems such as dopamine, serotonin, glutamate, and perhaps others may be impaired in the many different brain regions (involved closely with emotions)[8]. Dean A Haycock-2009 noticed visible differences in structure of brain affected with schizophrenia. Indeed, there are reports that associate smaller brain mass with schizophrenia[9]. Herbal medicine is gaining popularity in developing countries. Herbal remedies are often believed to be harmless because they are natural and free of side effect[10]. Medicinal plants play major role against these neurodegenerative diseases such as Alzheimer's disease, stroke, schizophrenia and parkinson's disease are caused by neuronal cell death[11]. Gingo biloba, St. John's wort are the medicinal plants in decreasing negative side effects along using with the anti psychotics[12]. While depression and anxiety are commonly researched, the efficacy of herbal medicines in other mental disorders requires attention. Particular attention is given to clinical and safety issues with St. John's wort and Kava[13]. Certified nutritional consultant Phyllos A Balch, states that Gingo, Kava, Passion flower, 5-HTP, flax seed oil, folic acid, GABA, garlic, glutathione, L-asparagine, L-glutamic acid and L-methionine may all be helpful in treating schizophrenia[14]. *Hypericum hookerianum* (Hooker's St. Johns wort) is a small wide fully hardy perennial evergreen shrub with yellow flowers *Hypericum hookerianuim* belongs to the family of *Hyperiaceae* is a well-known plant among the 20 different species of *Hypericum* found in India[15]. It is mainly present in Asia – tropical areas, Bangladesh, Bhutan. In India *H. hookerianum* mainly in the areas of Arunachal Pradesh, Karnataka, Manipur, Meghalaya, Sikkim, Tamil Nadu mainly in Nilgris, India. Antibacterial spectrum of *Hypericum hookerianum* has been reported[16]. Anxiolytic potential of ethanolic extract of Hh in stress induced swiss albino mice was evaluated by Suba kanmani and Uma Devi in 2012[17]. *Hypericum hookerianum* stem parts possess potent antitumor activity against DLA induced tumor

in mice[18]. The wound healing potential of *H. hookerianum* leaf and stem extracts has been evaluated[19,20]. The physicochemical parameters, preliminary phytochemicals analysis and elemental analysis of plant *Hypericum hookerianum* aerial parts was already evaluated[21]. From these studies though it is clear that *Hypericum* species is having wide clinical and medicinal applications, but sofar there is no detailed authentic scientific evaluation about this plant. Literature is stating that Hh is having neuro protective potential and is being used in folk medicine in ethinic community to treat mental illnesses. But there is no scientific validation about this plant. It is in view of this that the current study was undertaken to investigate the neuroprotective potential of the ethanolic extract of *Hypericum Hookerianum* in swiss albino mice

MATERIALS AND METHODS

Collection and authentication of the Plant material

The plant material in this study was collected from the Nilgris, Western Ghats of India. The plant was authenticated by Dr. S. Rajan, Field Botanist, Survey of Medicinal Plants and Collection Unit, (Central Council for Research in Homoeopathy), and Department of AYUSH. The collected plant was subjected to shade drying for about 5 weeks. The dried plant material was crushed to powder mechanically and sieved and stored in air tight container for further analysis.

Preparation of the Extract

The shade dried aerial parts of *Hypericum hookerianum* was pulverized to get a coarse powder. A weighed quantity of powder (950 g) was sieved and subjected to hot solvent extraction at the temperature range of 40-80^{0}C, extracted with pet ether, chloroform and ethanol successively by soxhlation method, water by maceration method at room temperature and concentrated over water bath and evaporated under reduced pressure. The percentage % yield of extracts was calculated.

Experimental animal studies

Colony in bred strains swiss albino mice of either sex weighing 21-30 g were used for pharmacological studies. The animals were kept under standard conditions (day/night rhythm) 8.00 am-8.00 pm, 22±$^{\circ}$C room temperature, in poly propylene cages. The animals were purchased from KMCH college of pharmacy Coimbatore, India were fed on pelleted standard diet (KMCH Pharmacy, Coimbatore, India) and water *ad libitum.* The animals were housed for one week in poly propylene cages prior to the experiments to acclimatize to laboratory conditions. All experiments were carried out between 9.00 and 12.00 hours. It is randomly distributed into 5 different groups with 5 animals in each group under identical conditions throughout the experiments. All the experimental protocols were approved by Institutional Animals Ethics Committee (IAEC) as per provisions of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), New Delhi, India

Drug administration in animal groups (For 21 days)

Control Group

Treatment with 0.5 ml of distilled water

Induced group treated with Haloperidol

Treatment with Haloperidol (2.5 mg/kg .i p.); make a stock solution containing 0.3 mg/ml of the drug and inject 1 ml/100 g body weight of mouse[22]

Plant extract treated group I

The plant extract was weighed as per the dosage 200 mg/kg in 1 ml, administered orally

Plant extract treated group II

The plant extract was weighed as per the dosage 400 mg/kg in 1 ml, administered orally

Standard group treated with drug Scopolamine

Treatment with Scopolamine (2 mg/kg, i.p.); prepare a stock solution containing 0.4 mg/ml of drug and inject 0.5 ml/100 g body weight of animal[23,24]

Dissection and homogenization

Chronic haloperidol treated animals on day 22ndday after behavioral quantification was sacrificed by decapitation. The brain were removed and put on ice. A 10 % (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). Whole brains of one set of animals from each group were separately stored at 0°C for neurotransmitters estimation.

The potential neurotransmitters, Neuro modulators and receptor systems involved in learning memory are Glutamate with NMDA, AMPA receptors; Acetylcholine with mucsuranic and nicotinic receptors; Dopamine with D_1 and D_2 receptors; Serotonin with 5-HT_3, 5-HT_{1A} receptors; NorAdrenaline with α,β-receptors; So the neurotransmitters have been selected for the current study are:

- GABA
- Serotonin
- Nor Adrenaline
- Dopamine

Estimation of brain neurotransmitters

A fluorimetric micro method for the simultaneous determination of serotonin noradrenaline and dopamine in milligram amount of brain tissue was evaluated by the method of Schlumpf M, Lichtensteiger, 1974[25]

Statistical analysis

Data were analyzed by one-way ANOVA followed by a post hoc Dunnett test using the SPSS 11.0 program. Differences between experimental groups were considered statistically significant when $P < 0.05$.

A = unknown O.D/Standard O.D × Standard concentration inμg/volume spotted × 100/wt of tissue

Standard O.D

GABA = 11.1
Dopamine = 0.09
Nor Adrenaline = 4.12
Serotonin = 4.35
Standard Concentration = 0.1 μg
Volume = 1 ml

RESULTS AND DISCUSSION

Brain neurotransmitter analysis

Effect of chronic EEHH on Brain GABA levels in Chronic HAL treated mice

Figure 1 shows the brain GABA level (derivative of glutamate) of HAL was significantly ($p < 0.05$) increased compared with the control group. However, groups administered with EEHH (200 mg/kg) and (400 mg/kg) showed significant ($p < 0.05$) decrease in the level of GABA

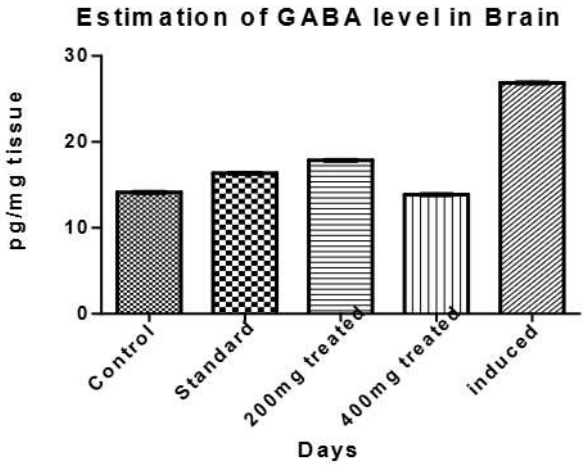

Figure 1: Effect of chronic administration of EEHH on HAL mediated brain GABA level
Values expressed as mean ± SEM, n = 5; p < 0.05 compared with HAL treated group (ANOVA followed by Dunnett's test)

Effect of chronic EEHH on Brain Serotonin levels in Chronic HAL treated mice

The brain serotonin level decreased significantly (p < 0.05) in HAL treated group compared to that of control group. However groups administered with EEHH (200 mg/kg and 400 mg/kg) showed significant (p < 0.05) increase in brain serotonin level. The effect of plant extract at dose 400 mg/kg has equitant effect as that of the standard drug scopolamine.

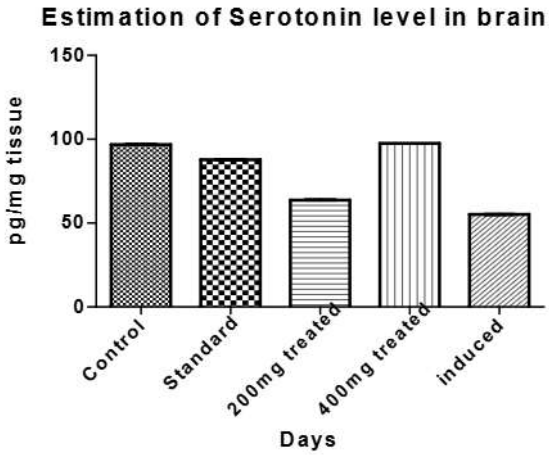

Figure 2: Effect of chronic administration of EEHH on HAL mediated brain serotonin levels
Values expressed as mean ± SEM; p < 0.05 compared with the HAL treated group (ANOVA followed by Dunnett's test)

Effect of chronic EEHH on Brain Nor Adrenaline levels in Chronic HAL treated mice

The brain Nor Adrenaline level decreased significantly (p < 0.05) in HAL treated group compared to that of control group. However groups administered with EEHH (200 mg/kg and 400 mg/kg) showed significant (p < 0.05) increase in brain noradrenalin level. The effect of plant extract at dose 400 mg/kg has equitant effect as that of the standard drug scopolamine.

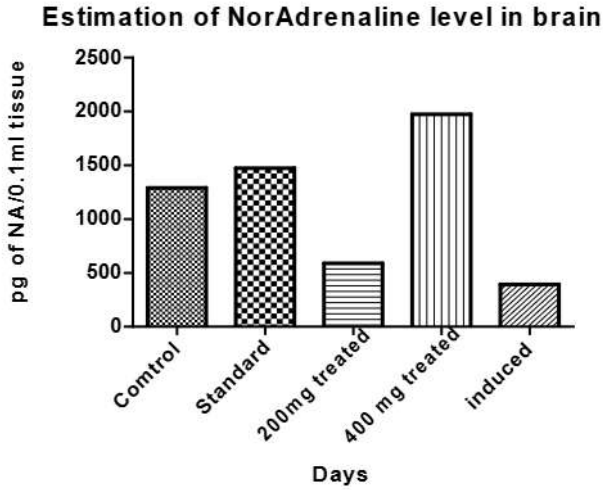

Figure 3: Effect of chronic administration of EEHH on HAL mediated brain Noradrenaline levels
Values expressed as mean ± SEM; $p < 0.05$ compared with the HAL treated group (ANOVA followed by Dunnett's test)

Effect of chronic EEHH on Brain Dopamine levels in Chronic HAL treated mice

The brain Dopamine level decreased significantly ($p < 0.05$) in HAL treated group compared to that of control group. However groups administered with EEHH (200 mg/kg and 400 mg/kg) showed significant ($p < 0.05$) increase in brain serotonin level. The effect of plant extract at dose 400 mg/kg has equitant effects as that of the standard drug scopolamine in increasing the level of Dopamine.

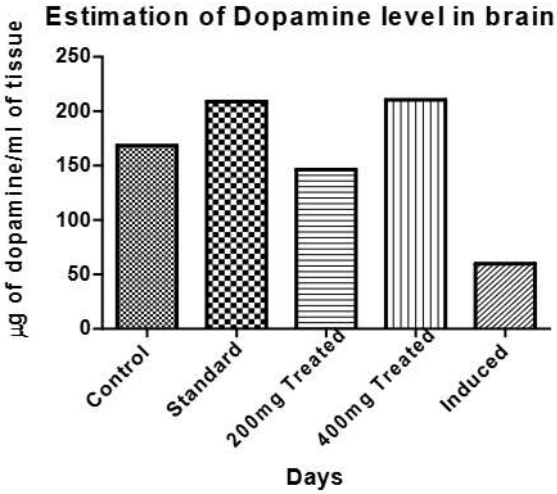

Figure 4: Effect of chronic administration of EEHH on HAL mediated brain Dopamine level
Values expressed as mean ± SEM; $p < 0.05$ compared with the HAL treated group (ANOVA followed by Dunnett's test)

Serotonin level can be modulated by oxygen level in blood; more oxygen: more serotonin[26]. Role of Serotonin in learning and memory has been receiving greater attention. Stimulation of Serotonergic neurotransmission disrupts behavioral performance, while inhibition enhances performance in experimental animals. 5-HT_3 receptor antagonist shown promise in enhancing cognitive performance. Kulkarni 1999[27]; In the present study the brain dopamine, serotonin, NA level got declined in the HAL induced animals which was recovered by the EEHh treatment. Elevation of GABA level in Brain was seen in the HAL induced group animals diminished the level by treatment with EEHH in the current study. Neuroleptics act by blocking dopamine receptors[28] and increase catecholamine turnover, which leads to excessive free radical generation. Increased metabolisms of catecholamines produce large amounts of free radicals which are cytotoxic[29]. Chronic HAL treatment increased lipid peroxidation and also nucleic acid peroxidation[30]. Results of our current findings showed that catatonic schizophrenia developed by chronic administration of Haloperidol is associated with increase in glutamate followed by Calcium influx[31]. The estimated glutamate level in the current study revealed a dose dependent reduction in glutamate level in EEHH treated animals. Increased density of Dopamine receptors and decreased level of Dopamine is observed in Chronic HAL treatment. The decreased level of dopamine may contribute to the production of excess free radicals. EEHH has shown an increase in dopamine level, which may suppress the development of super sensitivity due to increased level of Dopamine receptor. EEHH has shown 15 % and 35 % increases in learning and memory retrieval[32]. In the present study, co administration of EEHH with

Haloperidol showed a significant memory enhancement which might be due to increased level of neurotransmitters. The ability to restore Dopamine, Nor adrenaline, Serotonin in *Substantia nigra* increased the complex I activity and phytoconstituents like flavonoid, saponins and terpenes present in *Hypericum hookeranium* may contribute to its neuroprotective effect. Although the further studies are needed to explain how *Hypericum hookeranium* decreased the cataleptic and catatonic score with increased level of GABA observed in the present study.

CONCLUSION

From the study it is clear that *Hypericum hookeranium* have wide range of potential against haloperidol induced brain damage. The major phytoconstituents like flavanoids, polyphenols, saponins etc present in the plant is believed to have the neuroprotective effect. It has shown a wide improvement in memory enhancement, which might be due to the increased level of neurotransmitters. GABA level is seen to elevate with the treatment of plant; the major transmitter for post synaptic inhibition of cells in CNS. The learning deficits in HAL induced animals were rectified by the treatment of EEHH which is believed to enhance the functioning of limbic system and frontal lobe of the brain structure.

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