EVALUATION OF NEUROPSYCHOPHARMACOLOGICAL EFFECTS OF *Hypericum hookerianum* EXTRACTS ON SWISS ALBINO MICE

*a* thesis submitted by

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under the supervision of

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DECLARATION

I, S. SUBAKANMANI hereby declare that the thesis, entitled “Evaluation of neuropsychopharmacological effects of Hypericum hookerianum extracts on Swiss Albino mice”, submitted to the Karunya University, in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Biotechnology is a record of original and independent research work done by me during the period 2009-2014, under the Supervision and guidance of Dr. S. MURUGAN, Assistant Professor (SG), School of Biotechnology and Health Sciences, Karunya University. The work contained in this thesis has not been previously submitted to meet the requirement for a degree or diploma at this or any other higher education institution.

S. SUBAKANMANI
BONAFIDE CERTIFICATE

Certified that this thesis titled “Evaluation of neuropsychopharmacological effects of Hypericum hookerianum extracts on Swiss Albino mice”, is the bonafide work of S. SUBAKANMANI who carried out the research under my supervision. Certified further, that to the best of my knowledge the work reported herein does not form part of any other thesis or dissertation on the basis of which a degree or award was conferred on an earlier occasion on this or any other scholar.

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ABSTRACT

According to WHO (2014), the burden of mental disorders continue to grow with significant impacts on health, society and economy. Anxiety, depressions are the major mood oriented disorders, whereas dementia and Parkinson’s disease are neurodegenerative disorders. The main cause for these disorders is oxidative stress and imbalance in antioxidants. The synthetic drugs that are currently available for treatment of these disorders exhibited undesirable side effects. Therefore to overcome this situation, search for novel pharmacotherapy from medicinal plants has progressed significantly in the past decade.

Hypericum hookerianum is a small shrub with yellow flowers and basically referred as ornamental plant. Currently, scientific community concentrates on neuroprotective effect of Hypericum species because of their richness in bioactive constituents like hypericin, hyperoside, anthroquinones, quercetin, rutin, quercitrin and etc.

In the first phase, powdered sample of aerial parts of H. hookerianum was standardized to ensure the quality of the crude drug. Ethanolic extract of H. hookerianum (EEHh) was prepared by successive soxhlation and Glycosidic Flavonoid enriched extract was prepared by acid hydrolysis method. Total flavonoidal estimation and HPTLC confirmed that GFHh have high concentration of flavonoids like quercetin and rutin than EEHh. In vitro antioxidant potential of EEHh and GFHh were evaluated and compared with standard quercetin by various free radical scavenging methods like 1,1-Diphenyl- 2-picrylhydrazyl (DPPH), 2, 2'-
azino bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) Super oxide (O$_2^-$)(SO), Nitric oxide (NO), 2,2-azo bis (2- amidino propane) di hydro chloride (AAPH) and hydroxyl radicals (OH) (HRSA). The results of *in vitro* antioxidant assays (DPPH, ABTS, SO, NO, AAPH and HRSA) showed that free radical scavenging effect of GFHh is higher in all assays when compared to EEHh which is attributed to higher concentration of flavonoids.

The anxiolytic like effect of EEHh (200 and 400 mg/kg) and GFHh (100 mg/kg) were evaluated in stress induced mice by Elevated plus maze (EPM) test, Open Field Test (OFT), Hole Board Test (HBT), Light dark exploration Test (LDE), Marble buried test (MBT) and Novelty Induced Feeding Latency (NIFL). Also the biochemical parameters were analyzed to study the *in vivo* antioxidant and neuroprotective effects of EEHh and GFHh on enzymic, non-enzymic antioxidants, lipid peroxidation and brain neurotransmitters (GABA, serotonin and dopamine). Diazepam (1 mg/kg) served as a standard anxiolytic drug. The experimental results showed that all the abnormal findings caused by stress (free radical generation) can be alleviated by EEHh and GFHh treatment.

The antidepressant like effect of EEHh (200 and 400 mg/kg) and GFHh (100 mg/kg) in reserpine (2mg/kg) induced mice were evaluated by Forced Swimming Test (FST), Tail Suspension Test (TST), Locomotor activity (LMA), RotoRod Test (RRT) and reserpine induced hypothermia. Also the biochemical parameters were analyzed to study the *in vivo* antioxidant and neuroprotective effects of EEHh and GFHh on enzymic, non- enzymic antioxidants, lipid peroxidation and brain neurotransmitters (serotonin, dopamine, adrenaline, nor-adrenaline, MAO A and B).
Imipramine (10 mg/kg) served as standard antidepressant drug. The experimental results showed that neurotoxic effects induced by reserpine can be alleviated by EEHh and GFHh.

The antiamnesic like effect of EEHh (200 and 400 mg/kg) and GFHh (100 mg/kg) in scopolamine induced mice were evaluated by Y Maze Test (YMT), Rectangular Maze Test (RMT), Novel Object Recognition test (NOR) and Pole Climbing Test (PCT). Also the biochemical parameters were analyzed to study the in vivo antioxidant and neuroprotective effects of EEHh and GFHh on enzymic, non-enzymic antioxidants, lipid peroxidation and brain neurotransmitters (acetylcholine esterase). Piracetam (100 mg/kg) served as a standard antiamnesic drug. The results showed that the memory impairment effect caused by scopolamine can be alleviated by EEHh and GFHh.

The antiparkinson like effect of EEHh (200 and 400) mg/kg and GFHh (100 mg/kg) in haloperidol induced mice were evaluated by Vacuum Chewing Movement (VCM), Tongue Protrusions (TP) and Orofacial Burst (OB), catalepsy by block and metal bar method, major parkinsonism symptoms, Beam Walk Assay, Wire Hang Test (WHT) and Gait analysis (GA). Also the biochemical parameters were analyzed to study the in vivo antioxidant and neuroprotective effects of EEHh and GFHh on enzymic, non-enzymic antioxidants, lipid peroxidation and brain neurotransmitters (GABA and glutamate), L-Dopa (30 mg/kg) served as a standard antiparkinson drug. The results showed that the motor dysfunction caused by haloperidol can be ameliorated by EEHh and GFHh.
In all the experiments, GFHh exhibited higher anxiolytic, antidepressant, antiamnesic and antiparkinson like effects. This is due to the higher concentration of flavonoids present in GFHh when compared to EEHh. Moreover, the neuroprotective effect exhibited by GFHh is comparable to the standard drugs studied in the respective experiments. Further investigations on the isolation and identification of flavonoid compounds in *H.hookerianum* may lead to chemical entities for clinical use.
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<td>ABTS</td>
<td>2,2- Azino-bis (3-ethylbenzo-thiazoline-6- sulphonic acid)</td>
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<td>ANOVA</td>
<td>Analysis of Variance</td>
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<td>AAPH</td>
<td>2,2-azo bis (2- amidino propane) di hydro chloride</td>
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<td>AChE</td>
<td>Acetyl Choline Esterase</td>
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<td>AD</td>
<td>Alzheimer’s Disease</td>
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<td>BBB</td>
<td>Blood Brain Barrier</td>
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<td>BDZ</td>
<td>Benzo Diazepene</td>
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<td>BWT</td>
<td>Beam Walk Test</td>
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<td>CAT</td>
<td>Catalase</td>
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<td>DPPH</td>
<td>2,2- Diphenyl-1- picryl hydrazyl</td>
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<td>EEHh</td>
<td>Ethanolic extract of <em>Hypericum hookeriaum</em></td>
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<td>EPM</td>
<td>Elevated Plus Maze</td>
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<td>GFHh</td>
<td>Glycosidic flavonoid enriched extract of <em>H. hookerianum</em></td>
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<td>FST</td>
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<td>FTIR</td>
<td>Fourier Transform Infrared Spectroscopy</td>
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<td>GABA</td>
<td>Gamma Amino Butyric Acid</td>
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<td>GAD</td>
<td>Generalized Anxiety Disorder</td>
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<td>Gm</td>
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<td>GPx</td>
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<td>GSH</td>
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<tr>
<td>i.p</td>
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<td>Kg</td>
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<td>mg</td>
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