Lipopolysaccharide aggravates the restraint stress-induced behavioral deficits and hippocampal damage: Effect of Fisetin treatment

NIPER - Guwahati

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❖Introduction

❑Neuropsychiatric disorders like depression, anxiety, and dementia in humans often coexist together in certain medical conditions such as cancer, cardiovascular diseases, neurodegenerative disease which decreased the quality of life in patients and increase rate of mortality.

❑Numerous reports provided insights for implications of chronic restraint stress and lipopolysaccharide (LPS) results in hippocampal neuronal damage and various neurobehavioral anomalies in rodents [1, 2].

❑In this present study, we have tried to establish a model where 28 days chronic restraint stress mediated neurobehavioral and neurochemical changes got aggravated by a single dose of LPS triggering various neuroinflammatory cascade resulting hippocampus neuronal damage.

❑We further investigated the effect of Fisetin (3, 7, 3′, 4′-tetrahydroxyflavone) flavonol in preventing the restraint stress (RS) exposure and lipopolysaccharide (LPS) treatment induce neurobehavioral deficits and hippocampal damage.

❖Methods

All experiments were performed in accordance with the CPCSEA, Government of India guidelines. Adult male Swiss albino mice (25–30 g) were divided into following experimental groups:

Group 1: No stress/no LPS normal control

Group 2: Restraint stress exposure for 6 h per day for 28 days (RS).

Group 3: LPS injected (0.83 mg/kg, i.p.) on the 28th day (LPS control).

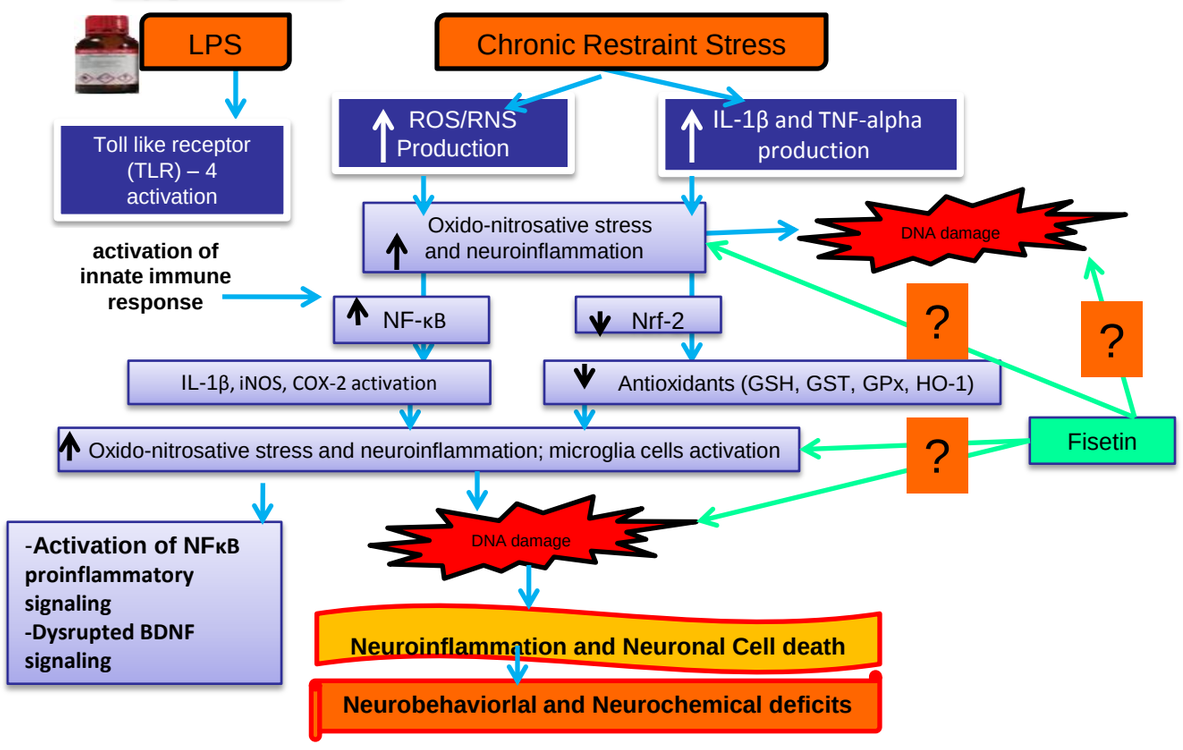
Group 4: Restraint stress (6 h per day for 28 days) and LPS injected (0.83 mg/kg, i.p.) on the 28th day (RS + LPS).

Group 5: Restraint stress (6 h per day for 28 days) and Fisetin (15 mg/kg, p.o.) treatment from 15th to 28th day.

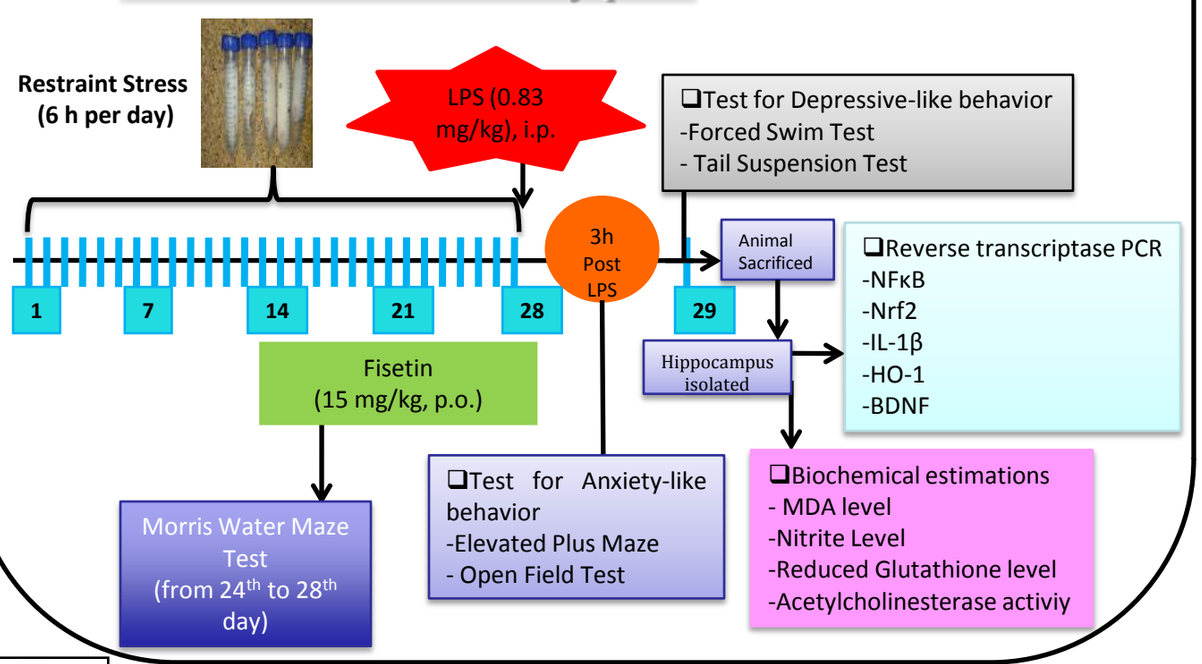
Group 6: Fisetin (15 mg/kg, p.o.) treatment from 15th to 28th day and LPS injected (0.83 mg/kg, i.p.) on the 28th day

Group 7: Restrained stress (6 h per day for 28 days) and LPS injected (0.83 mg/kg, i.p.) on the 28th day; and Fisetin (15 mg/kg, p.o.) treatment for last 14 days.

❖Hypothesis

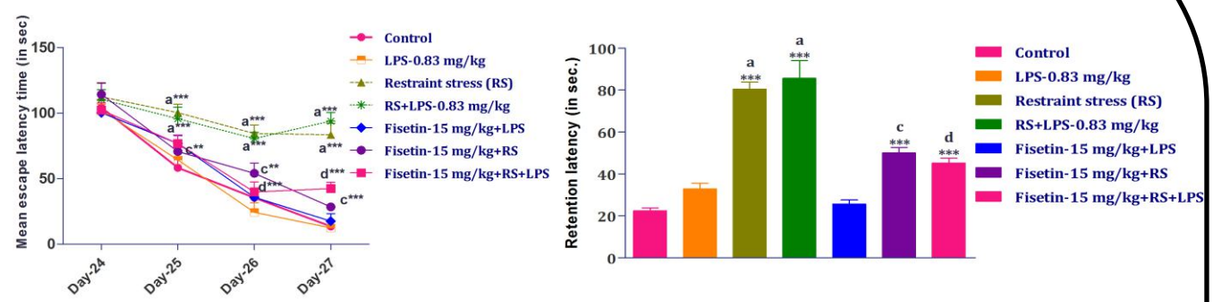


❖Illustration of the study plan



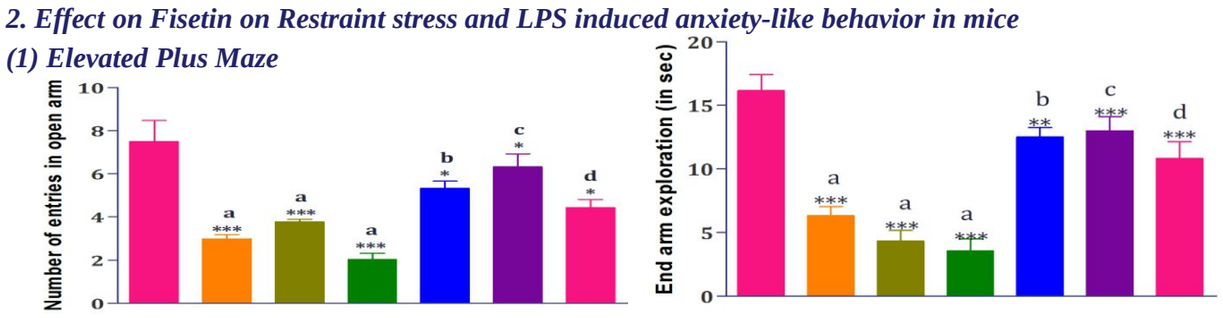
❖Results

I. 1. Effect of Fisetin on Restraint stress and LPS induced learning and memory function impairment

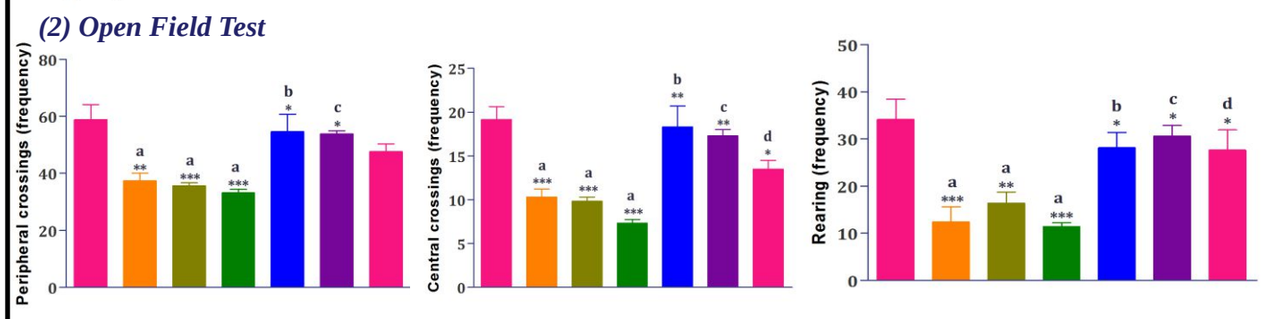


2. Effect on Fisetin on Restraint stress and LPS induced anxiety-like behavior in mice

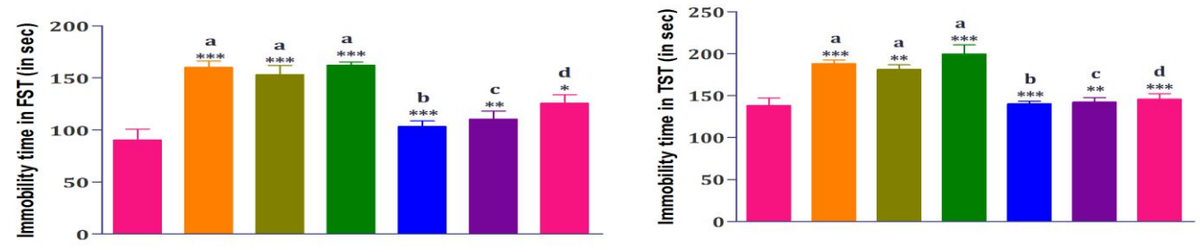
(1) Elevated Plus Maze



(2) Open Field Test



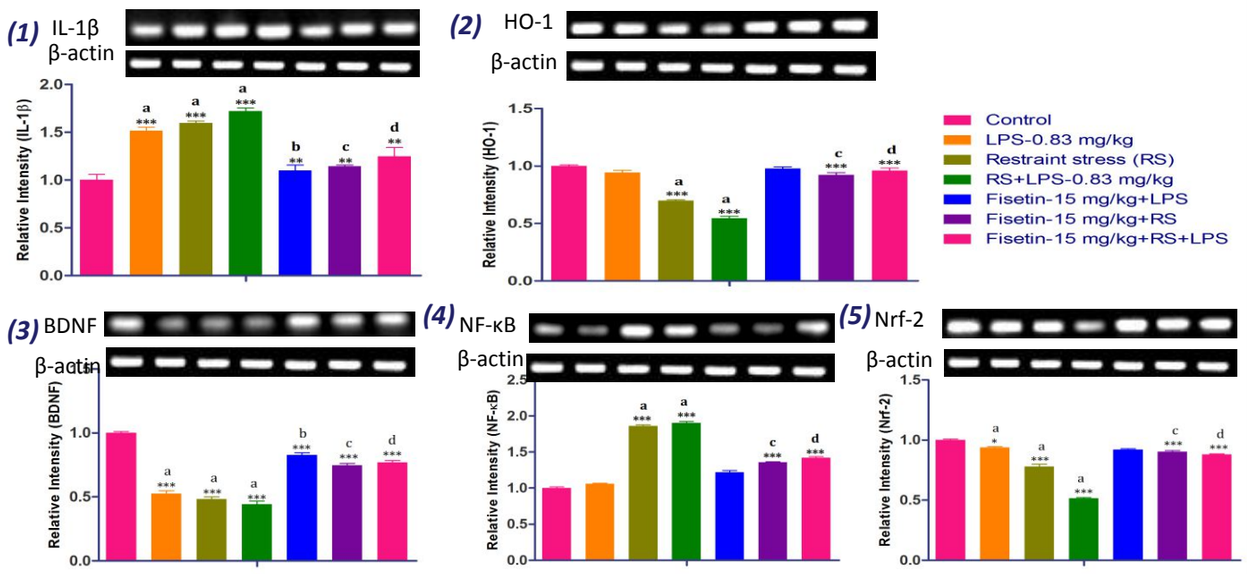
3. Effect on Fisetin on Restraint stress and LPS induce depressive-like behavior in mice



II. Effect on Fisetin treatment on Restraint stress and LPS induced Oxido-nitrosative stress, altered AchE activity in the hippocampus of mice

Parameters / Experimental groups	MDA level (µM/g of tissue wt)	Nitrite level (mM/mg of protein)	Reduced glutathione (mg/g of tissue)	Acetylcholinestrase activity (micromoles/min/ mg of protein)
Control	32.48 ± 3.49	45.93 ± 5.32	62.55 ± 1.93	0.12 ± 0.01
LPS (0.83 mg/kg)	55.39 ± 4.54^{a**}	94.39 ± 3.33^{a**}	29.49 ± 4.44^{a***}	0.23 ± 0.01^{a**}
RS	65.32 ± 4.39^{a***}	99.35 ± 6.39^{a***}	32.44 ± 5.01^{a***}	0.29 ± 0.03^{a***}
RS + LPS (0.83 mg/kg)	64.32 ± 2.34^{a***}	96.34 ± 5.34^{a***}	24.39 ± 3.54^{a***}	0.33 ± 0.03^{a***}
Fisetin (15 mg/kg) + LPS	37.15 ± 1.39^{b*}	64.15 ± 4.39^{b**}	52.95 ± 2.99^{b***}	0.15 ± 0.01
Fisetin (15 mg/kg) + RS	44.39 ± 1.30^{c**}	62.39 ± 5.32^{c***}	49.95 ± 3.90^{c*}	0.17 ± 0.01^{c**}
Fisetin (15 mg/kg)+ RS +LPS	43.49 ± 6.32^{d**}	68.11 ± 2.40^{d**}	45.95 ± 2.34^{d**}	0.22 ± 0.02^{d**}

4. Effect on Fisetin treatment on Restraint stress and LPS modulated hippocampal gene expression levels



All the values are expressed as mean ± SEM (n = 6); $^{a***}P < 0.001$, $^{a**}P < 0.01$, $^{a*}P < 0.05$ compared with the normal control group; $^{b***}P < 0.001$, $^{b**}P < 0.01$, $^{b*}P < 0.05$ compared with the LPS group;,, $^{c***}P < 0.001$, $^{c**}P < 0.01$, $^{c*}P < 0.05$ compared with the RS group; $^{d***}P < 0.001$, $^{d**}P < 0.01$, $^{d*}P < 0.05$ compared with the LPS + RS group

❖Conclusion

The Combined paradigm of restraint stress and lipopolysaccharide represented behavioral deficits and hippocampus damage. Fisetin treatment significantly ameliorates the altered neurobehavioral and neurochemical alterations via inhibition of oxido-nitrosative stress and neuroinflammation through restoring of dysregulated inflammatory mediators gene expression level.

❖References

[1] Jangra, A., Dwivedi, S., Sriram, C.S., Gurjar, S.S., Kwatra, M., Sulakhiya, K., Baruah, C.C., Lahkar, M., 2016. Honokiol abrogates chronic restraint stress-induced cognitive impairment and depressive-like behaviour by blocking endoplasmic reticulum stress in the hippocampus of mice. Eur J Pharmacol 770, 25–32.

[2] Sriram, C.S., Jangra, A., Gurjar, S.S., Mohan, P., Bezbaruah, B.K., 2016. Edaravone abrogates LPS-induced behavioral anomalies, neuroinflammation and PARP-1. Physiol Behav 154, 135–144.

Discolosure: No potential conflict of interest

❖Acknowledgement

We would like to thank the NIPER-Guwahati, Department of Pharmaceuticals, Ministry of Chemical and Fertilizers, Government of India for financial support to carry out this work. The authors are immensely thankful to Department of Biotechnology (DBT), Government of India for awarding international travel support.

Department Of Fertilizers Ministry of Chemicals and Fertilizers Government of India

ECNP neuroscience applied

Curriculum vitae-Mohit Kwatra

2016

Nationality: Indian
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Credentials

Ph.D. (*pursuing*)	**Laboratory of Neuroscience,** Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Guwahati, Assam, India	8.93 CGPA **(Coursework)** 10 point scale
M. Pharm. (2011-2013) (Pharmacology)	**Department of Pharmacology, Jamia Hamdard (Hamdard University)**, New Delhi, India	8.43 CGPA on 10 point scale
B. Pharm (2006-2010)	**Bharati Vidyapeeth Deemed University, Poona College of Pharmacy**, Erandwane, Pune, Maharashtra, India	72 %
HSC (2006)	**Central Board of Secondary Education (CBSE), New Delhi**	67.4 %
SSC (2004)	**Central Board of Secondary Education (CBSE), New Delhi**	79.6 %

Memberships

1. Registered Pharmacist at **Delhi Pharmacy Council, Delhi, India**

Academic achievements

1. Qualified **Graduate Aptitude Test in Engineering (GATE) – Life Sciences 2016** with All India Rank – 1548 and 84.96 percentile **organized by Indian Institute of Science (IISc) Bangalore, India**
2. **Qualified NIPER-PhD entrance with All India Rank 11, organized by NIPER Mohali**
3. **Qualified Graduate Pharmacy Aptitude Test (GPAT) – 2011** Exam with **All India Rank 423 and 98.94 percentile conducted by MS University of Baroda**, Gujarat, India
4. **Qualified GPAT- 2010 Exam** with **All India Rank 5094 and 84.36 percentile conducted by MS University of Baroda, Gujarat, India**
5. Won "**First prize" in Flow Cytometry quiz** conducted as part of **Basic and clinical flow cytometry course** and also obtained "**Professor Awtar Krishan Prize"** as certificate on 11th Nov 2014 **organised by Cachar Cancer Hospital and Research centre, Silchar, Assam, India**

Awards and Honours

1. Awarded International Travel Award (2016) by **Department of Biotechnology (DBT)**, Govt. of India, for attending " 29th ECNP (European College of Neuropsychopharmacology)" Congress held at Vienna Austria
2. Awarding scholarship of INR 28,000 + 20% HRA per month from **Ministry of Chemical and Fertilizers**, Govt. of India for Ph.D. degree at National Institute of Pharmaceutical Education and Research (NIPER), Guwahati, India
3. Awarded scholarship of INR 8000 per month from **All India Council of Technical Education (AICTE)**, New Delhi, Govt. of India for M.Pharm degree (July 2011-July 2013) at Faculty of of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi, India
4. Awarded **Senior Research Fellow (DBT Project) fellowship** of INR 18000 + 30% HRA for 6 months while working at CSIR-IGIB, New Delhi, India from Jan 2014-June 2014

Academic Research and Industrial Experience

Name of the firm	Profile and Duration	Experience
National Institute of Pharmaceutical Education and Research (NIPER) Guwahati, Guwahati, Assam, India	**Ph.D. Research Scholar Laboratory of Neuroscience Dept- Pharmacology & Toxicology** under supervision of **Dr. Mangala Lahkar** (Chief Academic co-ordinator NIPER Guwahati & HOD of Department of Pharmacology, Gauhati Medical College, Guwahati)	✦ *In-vitro* Biology, *In-vitro* model of Neurotoxicity in SHSY-5Y cells, PC12 and U87-MG cells ✦ Screening of small molecules *in-vitro* cells for specific targets activity potential ✦ *In-vivo* Pharmacological animal models (Depression, anxiety, Alzheimer's disease, Parkinson's disease) ✦ Molecular biology and Pharmacological techniques Ongoing Project: **Exploration of the redox-signaling mediators along with regulator of G-protein signaling (RGS) in in-vitro and in-vivo model of Parkinson's disease: Implication of Pharmacological Interventions**
CSIR-Institute of Genomics and Integrative Biology (IGIB) New Delhi, India	**Senior Research Fellow (Project) Department of Structural Biology** (6 months)	✦ Worked on Non-Viral peptide based DNA delivery vectors for targeting **vitiligo** ✦ Formulations of nanocomplexesand characterization
Daiichi-Sankyo Life Science Research Center in India (RCI) Ranbaxy Research Laboratories, R & D - 3, Gurgaon, Haryana, India	**Research Trainee** ***In-vitro*-Pharmacology, Department of Biology** (6 months)	✦ Worked on Drug Discovery and Development area of airway Inflammation COPD and/or asthma of target PI3 Kinase signaling Pathways and their Inhibitors. ✦ **Experience in *In-vitro* Molecular Biology techniques** ✦ ***In-vivo* Pharmacology techniques**

Professional Skills

***Animals/In-vivo* experimental experience:** Expertise in animal experiments such as neurobehavioural (Morris water maze, Passive avoidance, Forced swimming test, Tail Suspension test, open field, rota rod, Light-dark box test etc.

In-vitro

Expertise in Cell culture and molecular biological techniques, worked on various cell lines (SHSY-5Y, PC12, U87-MG, A549, H292, U937, THP-1, RBL-2H3, Hacat, CHO-K1), Cell free (Enzyme kinase selectivity [PI 3 α, β, γ, δ]) assays, Cell based assays, Electrophysiology, Neuronal cell culture, Brain stem cell culture, Cell imaging, DNA cloning, Cell Selex, FACS, Chromatographic techniques

Ex-vivo: Animals models: Thorough experience on various animals models for research on type 1 and type 2 diabetes, Depression, neurotoxicity and for screening of various NCE for central nervous system activity

Experimental techniques*:* Hand on experience for western blotting, ELISA, Reverse trascriptase PCR, Real-time PCR, HPLC (Waters), UV Visible Spectroscopy (Shimadzu), Histopathological slide preparation, Immunohistochemistry; Cell imaging using Phase contrast and fluorescent and confocal Microsopy

Softwares handled*:* Proficient handling of various scientific software packages such as Sci-finder, Chem Draw, ImageJ, Sigma Plot and Graph Pad Prism for data processing and presentation. Expertise in computer skill for efficient usage of Microsoft office tools and various bioinformatics tools

Literature search and scientific writing*:* Sound knowledge of filing proposals for research grants, project report and manuscript writing, Aware of various National and International Drug regulatory guidelines

Research Papers

•International

1. **Kwatra M**, Jangra A, Mishra M, Sharma Y, Ahmed S, Ghosh P, Kumar V, Vohora D, Khanam R (2016) Naringin and Sertraline Ameliorate Doxorubicin-Induced Behavioral Deficits Through Modulation of Serotonin Level and Mitochondrial Complexes Protection Pathway in Rat Hippocampus. **Neurochemical Research (Springer US)**; 41(9): 2352-2366. doi: 10.1007/s11064-016-1949-2,
2. **Kwatra M**, Kumar V, Jangra A, Mishra M, Ahmed S, Ghosh P, Vohora D, Khanam R. (2016) Ameliorative effect of naringin against doxorubicin-induced acute cardiac toxicity in rats. **Pharmaceutical Biology (Taylor and Francis, UK)**. 54(4):637-47. doi: 10.3109/13880209.2015.1070879,
3. Jangra A, **Kwatra M**, Singh T, Pant R, Kushwah P, Sharma Y, Saroha B, Datusalia AK, Bezbaruah BK (2016) Piperine augments the protective effect of curcumin against liopolysaccharide-induced Neurobehavioural and Neurochemical anomalies in mice. **Inflammation (Springer US)**. 39(3):1025-38. doi: 10.1007/s10753-016-0332-4
4. Ahmed S, Mundhe N, Borgohain M, Chowdhury L, **Kwatra M**, Bolshette N, Ahmed A, Lahkar M. Diosmin modulates the NF-κB signal transduction pathways and down regulation of various oxidative stress markers in Alloxan - induced Diabetic Nephropathy. **Inflammation (Springer US)**. doi:10.1007/s10753-016-0413-4
5. Jangra A, Dwivedi S, Sriram CS, Gurjar SS, **Kwatra M**, Sulakhiya K, Baruah CC, Lahkar M. (2015) Honokiol abrogates chronic restraint stress-induced cognitive impairment and depressive-like behaviour by blocking endoplasmic reticulum stress in the hippocampus of mice. **European Journal of Pharmacology (Elsevier).** 27;770:25-32. doi: 10.1016/j.ejphar.2015.11.047,
6. Jangra A, **Kwatra M**, Singh T, Pant R, Kushwah P, Ahmed S, Dwivedi D, Saroha B, Lahkar M. (2016) Edaravone alleviates cisplatin-induced neurobehavioral deficits via modulation of oxidative stress and inflammatory mediators in the rat h i p p o c a m p u s , **European Journal of Pharmacology (Elsevier)**. doi: 10.1016/j.ejphar.2016.08.003
7. Jangra A, Kasbe P, Pandey SN, Dwivedi S, Gurjar SS, **Kwatra M**, Mishra M, Venu AK, Sulakhiya K, Gogoi R, Sarma N, Bezbaruah BK, Lahkar M. (2016). Hesperidin and Silibinin Ameliorate Aluminum-Induced Neurotoxicity: Modulation of Antioxidants and Inflammatory Cytokines Level in Mice Hippocampus. **Biological Trace Element Research (Springer US)**. 168(2):462-71. doi: 10.1007/s12011-015-0375-7
8. **M Kwatra**, A Jangra, Chandershaker, Sr, B Saroha, M Lahakar (2014) Mangiferin Protects Against Aluminium Chloride-Induced Liver Injury by Inhibition of Oxidative Stress in Mice. **INDIAN JOURNAL OF PHARMACOLOGY**, 2014, 46, S61-S61. (Published)
   Oral Papers: TOX-8: Mangiferin Protects Against Aluminium Chloride-Induced Liver Injury by Inhibition of Oxidative Stress in Mice]

•Presentations at the Conferences (National and International)

1. Poster presented under the theme "Pharmaceutical research" in **International R&D conference on Overcoming the Bottlenecks in Drug Discovery and Development which was held in Gurgaon, Haryana, India from 20-21 March 2014 organised by "The Royal Society of Chemistry (RSC), Daiichi Sankyo India Pharma Private Limited (DSIN) and Ranbaxy Laboratories Limited (RLL)".** Entitled: Determination of biochemical-behavioral cross talk and ameliorative effect of Naringin in doxorubicin-induced neurotoxicity in rats
2. An oral presentation and poster was presented in "**The 38th Annual Japanese Neuroscience Meeting, Port Island, Kobe, Japan**" in which I was assigned as co-author. Work Entitled "1,5-Isoquinolinediol Ameliorates Alcohol-Induced Cognitive Deficits in Rats Fed High Fat Diet via Inhibition of Poly (ADP-Ribose) Polymerase-1 Activation" Ashok Jangra, SR Chandershaker, **Mohit Kwatra**, Babul Kumar Bezbaruah, Mangala Lahkar
3. Poster was presented, entitled as**: Naringin and Sertraline Ameliorates Doxorubicin-Induced Anxiety and Depression-like Behavior via mitigation of Oxidative Stress along with Modulation of Serotonin level and Mitochondrial Complexes Protection in Rat Hippocampus**. **Mohit Kwatra**, Ashok Jangra, Murli Mishra, Yogita Sharma, Tavleen Singh, Sahabuddin Ahmed, Rajat pant, Razia Khanam, in **29th Annual meeting of Society for Neurochemistry, India (SNCI) and National Workshop and Conference on "Advances in Computational Neurochemistry and Neurobiology" (SNCI-ACNN 2015) December 16th – 21st, 2015**
4. Poster was presented, entitled as: Edaravone ameliorates cisplatin-induced neurobehavioural and neurochemical deficits via inhibition of oxidative stress and neuroinflammation in rat hippocampus. Rajat Pant, Ashok Jangra, **Mohit Kwatra**, Tavleen Singh, Yogita Sharma, Babul Kumar Bezbaruah, in **6th International Conference on Metals in Genetics, Chemical Biology and Therapeutics (ICMG-2016), Indian Institute of Science (IISc) Bangalore, 17th – 20th Feb 2016**
5. Poster was presented entitled as **"PPAR-γ agonist Pioglitazone exerts its neuroprotective activity in Reserpine induced Parkinson -depression triads by evaluating the endoplasmic reticulum stress markers and inflammatory cytokine levels in rat brain".** Sahabuddin Ahmed, Nityanand Bolshette, **Mohit Kwatra**, Anwaruddin Ahmed, Yogita Sharma in which I was assigned as co-author contributor presented in the **14th Meeting of the Asian-Pacific Society for Neurochemistry (APSN 2016) held from 27th – 30th August 2016 at Kuala Lumpur, Malaysia.** The abstract was published in the supplementary issue of journal "***Frontiers of Cellular Neuroscience***"
6. An Oral Presentation entitled as **"Perillyl alcohol ameliorated restraint stress-induced neurobehavioral deficits in mice: prevention of neuroinflammatory cascade in the hippocampus"** will be given on the **49th Annual conference of Indian Pharmacological Society (IPSCON 2016) on 20th-23rd Oct 2016 to be held at PGIMER, Chandigarh, India**

ECNP neuroscience applied