Examiner-I

INTRODUCTION AND SCOPE OF THE STUDY

Introduction and background of the thesis provide near complete introduction towards the topic. Though topic significance explained in the introduction section a more elaborate relevance and its application should have introduced. There is a correct focus for background information in the introduction. Research aim and hypothesis were clearly stated.

Study scope is appropriate.
The research focus, practicability and the addressed research problem are acceptable.
We thank examiner for approval of study.

Comments:-1 The brain is majorly affected by cell phone radiation- this should have been elaborated further in the introduction using available literature on electromagnetic radiation and brain functions.

Clarification:- We have elaborated the literature of electromagnetic radiation and brain functions in introduction section on page no. 3-4.

Comments:-2 Literature review of the thesis is relevant to the research issue. Using the past and present research, the topic was reviewed with adequate references. The author has used many recent research articles related to the subject to present a comprehensive review of the literature. Information was not very well integrated.
Clarification: We have edited literature review of the thesis on page no. 4, 5, 6 and 7.
Lacunae in the existing literature was very well stated
Reasonable research hypothesis and objectives
We thank examiner for appreciation
Comment:-3 References were missing for many essential statements in the review of literature.
Clarification: We have added missing references in review of literature section on page no. 2, 3,4, 5,6, 7, 9 and 10.
Comment:-4 What is the rationale behind selected pharmacology/drug effects presented in this study (only fluoxetine and ·hepatic function' and 'omeprazole' and gastric function'?

Clarification: The rationale drug effects of fluoxetine on hepatic function and omeprazole on gastric function has been explained in detail on page no. 104 and 127.

Comment:-5 The term alterations/alter' is frequently used in the hypothesis changes (increased or decreased/stimulated/inhibited)

Clarification: We have corrected hypothesis as suggested on page no. 13.

Comment:-6 What are the 'null hypotheses of the study?

Clarification:- The level of statistical significance is often expressed as a p-value between 0 and 1. In this study $p < 0.05$ were considered as statistically significant for all experimental data analysis. The lower the p-value, the greater the chances for rejection of the null hypothesis. Therefore, in our research hypothesis, there is less than a 5% probability of the null hypothesis to be correct. So there are 95% chances that our research hypothesis is true. We have mentioned on page no. 28, 64, 86-87, 110, 134 and 158.

Comment:-7 EMR on cognitive functions introduction was too short about mitochondnal dysfunction and neurodegenerative changes.

Clarification: We have explained in detailed on page no. 6.

METHODS

The study design is appropriate

Comments:-1 The sample size is adequate for the experiments, but the histopathology sample size is inadequate (In a few instances, it is only from 3 animals)

Clarification:- We have designed the experiment with the help of G-Power analysis and based on these analysis the histopathology sample size of this experiment requires 3 animal per group, added on page no., 22.

Comment: 2 Brain dissection methods for amygdala were not described.

Clarification:- Now, we described the brain dissection methods for amygdala on page no. 22

Excellent behavioural parameters were used for the study and well explained.

Comment:-3 For the electromagnetic radiation testing whether control animals were also kept in a box without radiation would expose them to the same initial stress levels as the experimental group.

Clarification:- We kept the control animal in anechoic chamber without radiation exposure for 1h daily up to 28 days. It is mentioned on page no. 22.

Comment:-4 On D-28 was the last day of the experiment, and the EPM test was done on that day- serum corticosterone would have increased on the last day alone because of the test.

Clarification:- Plasma corticosterone level was increased due to repeated exposure of EMR. We have justified on page no. 50.

Comment:5 Evaluation of cortical blood flow-method was not described in detail.

Clarification:- We have described evaluation of cortical blood flow method on page no. 62.

Comment:6 Brain dissection and collection of cortical tissue- fundamental methodology should have been included.

Clarification: We have added on page no. 61.

Comment:7 Plasma corticosterone assay method is from 1987. Elisa test was not used here.

Clarification: We have used HPLC method for Plasma corticosterone assay added on page no. 24.

Comment: 8 The free inorganic phosphate measurement using 1925 method (Fiske and subbarow)

Clarification: Yes, we have used Fiske and subbarow-1925 method for inorganic phosphate measurement, mentioned on page no.24.

Comment: 9 Hippocampal mitochondrial isolation methods were not described.

Clarification:- We have explained in detailed Hippocampal mitochondrial isolation methods on page no. 88-89.

Comment: 10 Effect of EMR on mitochondrial membrane- a few figures with membrane potential recordings from the mitochondria (Fluorescent Intensity) should have included.

Clarification: Mitochondrial membrane potential recordings is in quantitative form. It has already mentioned in page no. 38.

Comment 11:- How hippocampus was identified and dissected out? Methods are missing.

Clarification:- We have discussed the detailed methods for identification and dissection for Hippocampus on page no. 82-83.

RESULTS

Results analysis and interpretation are acceptable.

Findings were presented very well in the results section.

Figures, tables, and graphs were precise and had clear headings, clear delineation of statistical significance and descriptions.

Comment 1: Histology figure (2.2.15) is not having the magnifications [10X/100X /200X)

Clarification : We have used 25X magnification for histological study and also added on Page no. 45.

Comment 2: Figure 3.3 A & B - bars are too crowded, arid it is challenging to compare a,b, c, d e,x,y,z etc. (colour bar charts would have helped to separate the individual groups and comparisons/ significance)

Clarification : We have used colour bars to visualize individual groups on page no. 111.

Comment 3: Omeprazole alone treatment would influence the parietal cells in the healthy rats/control groups. Omeprazole only groups need to be included for the comparison.

Clarification : We have clarified this issue on page no. 131.

DISCUSSION

Comment 1: Some of the important statements in the discussion section doesn't have references.

Clarification: - We have mentioned the reference in discussion section.

Comment 2: A few sections of the discussions of different chapters appeared like a repetition of the results, where only research findings highlighted, instead of discussing the study observation using available literature. Possible mechanisms should have included under every parameter discussed in different chapters. Mechanisms of changes with EMR, especially on the brain, liver, and oxidative parameters shou1d have discussed in detail. A separate, common discussion section would have been good to bring everything together to explain the overall effect of EMR on the body systems using the results and the available literature.

Clarification: - We have edited the discussion section of this study and added possible mechanisms discussed in different chapters.

Comment 3: EMR induces liver dysfunction.'Liver dysfunction' is a broader statement. What changes in the metabolism may happen if there is a significant effect of EMR on the liver functions?

Clarification: Repeated exposure of EMR decreased CYP2D6 based metabolite in liver. This alters the pharmacokinetic parameters of fluoxetine. These changes lead to deposition of fluoxetine in rats of EMR exposed rats, added on page no. 121.

Summary and conclusion - very well done.

Comment 4: Summary and conclusion only mentioned EMR 2450 MHz. Though the other frequencies did not show statistically significant changes this also should have been stated under conclusions to highlight that the substantial changes in the nervous system and organ systems is because of high-frequency electromagnetic waves and not the lower frequencies.

Clarification: We have mentioned and highlighted the effect of lower frequencies on nervous system and organ system on page no. 168.

Comment 5: The frequencies of 3G, 4G and 5G and wireless fidelity should have been included under the discussion or conclusion section

Clarification: We have added the frequencies of 3G, 4G, 5G and Wi-Fi in discussion as well as conclusion section.

Comment 6: Nothing much has been mentioned about the application or significance of the findings. What about the remedial to avoid high-frequency magnetic radiations induced effects on the central nervous system?

Clarification: We have added the research significance of the findings on page no. 168.

Comment: 7 For the conclusion - a revisit to the research hypothesis and stating the individual research hypothesis in conclusion would have given the clear picture of the thesis outcome.

Clarification: We have edited summary with original hypothesis on page no. 166-68.

Comment 8: The research limitations were not stated.

Clarification: We have stated research limitations on page no. 170.

Comment 9: There are no research recommendations based on the present research results.

Clarification: We have added research recommendations based on the present research results on page no. 170.

References

Comment 1: References are mostly relevant to the chosen topic and seamlessly integrated.

Comment 2: Appropriate peer-reviewed references were used to support arguments, suggestive of thorough research.

Comment 3: The thesis has an appropriate number of references to support key arguments many useful review articles cited'

Comment 4: There are many references without the journal names

Clarification 1, 2, 3, 4: We made all the changes in the reference that are without journal names.

GENERAL COMMENTS AND RECOMMENDATIONS

Thesis structure is sound. Chapters, chapter sequencing Presentation of subsection contents and flow of topics are acceptable. All tables and figures labelled. There are no repetition of table data with the figure.

The study has included a variety of parameters (Behavioural and non-behavioural) and Iooked at the EMR effects on the body using different approaches. I commend the effort of the author for all the hard work undertaken to the study these critical parameters in this research.

Though the thesis demonstrates a good grasp of standard writing conventions, there are occasional noticeable minor errors. The reader can understand what the author means.

THESIS MAY BE ACCEPTED FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY

Examiner-II

Experiments are well planned and seem to be neatly executed' However, it is surprising to see that with the small number of animal group n=6, behavioral experiments show only small variance - one normally expects variations in such assays are done with a larger sample size. In addition, some section of the thesis draft is poorly written as listed below. There are multiple issues with the references that have been cited. For example, although the name of the author being cited is the same as in the references list, the study is entirely irrelevant to the present topic of the thesis. Multiples references are missing from the list etc. The candidate needs to look at these issues before the thesis is accepted for the award of the degree.

Comment:1 However, it is surprising to see that with the small number of animal group n=6, behavioral experiments show only small variance - one normally expects variations in such assays are done with a larger sample size.

Clarification: We have set the sample size in our experimental design through G-power analysis which shows that per group n=6 animal is required for behavioural experiments, G-power analysis sheet included in thesis page number-190.

Comment:-2 few of the irrelevant references cited are listed below:

1. L. Dogan, R. l. & Lu, Z. An inference method for disease name normalization.20l2 AAAI Fall Symposium Series, 2012.
2. Poole, D. N., Lurie, M. J. P. O.2013. A cross-sectional study to assess HPV knowledge and HPV vaccine acceptability in Mali. 8, e56402'
3. Karioidrs. I. r., pleisch' G., Brandeis, D.2i18. Simulating reading acquisition:rhe link between reading outcome and multimodal brain signetures of letter-speech sound learntng ln Prereaders. 8' 71'2I'
4. Faria, M., Bjornmalm, M., Thurecht, K. J., 2018. Minimum information reporting in bio-nano experimental literature. 13' $_{777}$ $^{-7}$ 85
5. Johansson,B.J.S.,Development,I.A.A.l.J.D.T.T.,ApplicationofTechniquesForTheAnalysis - of Surfaces,t.&Films,T.2006.ToF-slMSimagingof lipidsincell membranes.38, 1'4QI-14L2
6. Ismail,H.,Mirza,B.J.B.C.&MEDlClNE,A.2Ol5.Evaluationofanalgesic,anti-inflammatory, anti-depressant and anti-coagulant properties of Lactuca sativa (CV. Grand Rapids) plant tissues and cell suspension in rats. 15, 1-99'
7. Shapiro,M.H.,Melvin,J.D.,Whitcomb,J.H lg8l.Relationship of the 1979 southern California Radon Anomaly to a possible regional strain event. Journal of Geophysical Research: solid Earth, 86,1725-1730.
8. Shehu, N. Y., ojeh, V., Agbaji, o. J. J. o. M. C. R. 2016. A 33-year-old patient with human immunodeficiency virus on antiretroviral therapy with Efavirenz-induced complex partial seizures: a case rePort 10,93
9. Mostafa, K. M. J. C. p. 2003 Evaluation of nitrogen containing starch and hydrolyzed starch derivatives as a size base materiaI for cotton yarns.51,63.68.
10. Naravanan, R., Coss, C. C. & Dalton, I T.2018 Development of selective androgen receptor modulators (sARMs). Molecular and cellular Endocrinology, 465, 134-L42.

11. Ota, M., Noda, T., Sato, N., Hattori, K. 2014. Characteristic distributiohs of regional cerebral blood flow changes in major depressive disorder patients: A pseudo-continuous arterial spin labelling (pCASL)study. Journal of Affective Disorders,165, 59-63.
12. Recher, M., Burns, S. O., Lang, P. A. J. B. 2OI2.B cell-intrinsic deficiency of the Wiskott-Aldrich syndrome protein (WASp) cause severe abnormalities of the peripheral B-cell compartment In mice. 119, 281'9-2828.
13. Maskev,M.,Lowrv,J.,Rodgers,J.,2Ol.4. Reducing Specific Phobia/Fear in Young People with Autism Spectrum. Plosone
14. Lai 1992 (RNA recombination in animal and plant viruses. Microbiological Reviews).
15. Muruganandam et al., 2002 (Selection of phage-displayed llama single-domain antibodies that transmigrate across human blood brain barrier endothelium).
16. Ishikawa et al., 1992 Effect of molecular mass of poly-3- alkylthiophene on electrical properties.
17. Hao et al., 2015 Drought characterization from a multivariate perspective: Areview, journal of hydrology.
18. Infante et al., (Infante, Carlos R., Mihala Alexandra G. 2015 Shared Enhamcer activity in the limbs and phallus and functional divergence of a limb-genital cis-regulatory element in snakes)
19. Khalifeh et al., 2017 (Optimizing the beacon and superframe orders in IEEE 802.15.4 for realtime notification in wireless sensor networks)
20. Shapiro et al., 1979 Relationship of the 1979 southern California radon activity to a possiblr strain event. Journal of Geophysical
21. Monaco, D. Fatnassi, M. Padallio, B. 2016. Effect of alpha amylase, papain, and spermfluid treatments on viscosity and semen parameters of dromedary camel ejaculates. Research in Veterinary Science, 105, 5-9.

Clarification: - We have deleted the irrelevant references mentioned above from reference section chapter 7.

Comment 3: And their references which are cited in main text but are missing from the list.
Missing References,

Page no. 26: Junior et al., 2014, Debrun and Dejager, 1994

Page no. 47: Kong et al., 2006

Page no. 48: Pederson et al., 1978

Page no. 48: Huang et al., 2002

Page no. 49, 110: Griffith and Houghton et al., 1974

Page no. 50: Mishra and singh 2009

Page no.71: Kalueff et al., 2016, Sestakova et al., 2017

Page no. 99 Kalogeris et al., 2012

Page no., 109 Pederson et al., 1978

Page no., 110 Sally et al., 1989

Page no., 110 Storrie et al., 2004

Page no., Celada et al., 2004

Clarification: - We have added all the missing references in reference section chapter -7

Comment 4: Another issue is that in the reference list there is that in the reference there are multiple references given with the same name and year but different studies. Out of many cited references one could relate and others irrelevant, which inevitable leads to the question.

1. Gupta D, Julka A, Jain S, Aggarwal T, Khanna A, Arunkumar N, de Albuquerque VH. Optimized cuttlefish algorithm for diagnosis of Parkinson's disease. Cognitive systems research. 2018 Dec 1;52:36-48.
2. Gupta SK, Mesharam MK, Krishnamurthy S. Electromagnetic radiation 2450 MHz exposure causes cognition deficit with mitochondrial dysfunction and activation of intrinsic pathway of apoptosis in rats. Journal of biosciences. 2018 Jun 1;43(2):263-76.
3. Gupta SK, Patel SK, Tomar MS, Singh SK, Mesharam MK, Krishnamurthy S. Long-term exposure of 2450 MHz electromagnetic radiation induces stress and anxiety like behavior in rats. Neurochemistry international. 2019 Sep 1;128:1-3.
4. Mahdavi S, Jalali M, Afkhami A. Heavy metals removal from aqueous solutions by Al 2 O 3 nanoparticles modified with natural and chemical modifiers. Clean technologies and environmental policy. 2015 Jan 1;17(1):85-102.
5. Mahdavi SA, Jafari SM, Ghorbani M, Assadpoor E. Spray-drying microencapsulation of anthocyanins by natural biopolymers: A review. Drying technology. 2014 Apr 4;32(5):509-18.
6. Mahdavi SM, Sahraei H, Yaghmaei P, Tavakoli H. Effects of electromagnetic radiation exposure on stress-related behaviors and stress hormones in male wistar rats. Biomolecules & therapeutics. 2014 Nov;22(6):570.

7. Maskey D, Kim MJ. Immunohistochemical localization of brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor in the superior olivary complex of mice after radiofrequency exposure. Neuroscience letters. 2014 Apr 3;564:78-82.
8. Maskey M, Lowry J, Rodgers J, McConachie H, Parr JR. Reducing specific phobia/fear in young people with autism spectrum disorders (ASDs) through a virtual reality environment intervention. PloS one. 2014 Jul 2;9(7):e100374.

Clarification 5: We excluded irrelevant references (red in colour) and references of the same name and year were cited based on the studies.

Comment 3: Or it is the same reference listed thrice: Page no 212 & 213

Moradi M, Naghdi N, Hemmati H, Asadi-Samani M, Bahmani M. Effect of ultra high frequency mobile phone radiation on human health. Electronic physician. 2016 May;8(5):2452.

Moradi M, Naghdi N, Hemmati H, Asadi-Samani M, Bahmani M. Effect of ultra high frequency mobile phone radiation on human health. Electronic physician. 2016 May;8(5):2452.

Moradi M, Naghdi N, Hemmati H, Asadi-Samani M, Bahmani M. Effect of ultra high frequency mobile phone radiation on human health. Electronic physician. 2016 May;8(5):2452.

Pall ML. Microwave frequency electromagnetic fields (EMFs) produce widespread neuropsychiatric effects including depression. Journal of Chemical Neuroanatomy. 2016 Sep 1;75:43-51.

Pall ML. Microwave frequency electromagnetic fields (EMFs) produce widespread neuropsychiatric effects including depression. Journal of Chemical Neuroanatomy. 2016 Sep 1;75:43-51.

Clarification: We have corrected the additional reference listed.

Comment 6: In page no 23-24: Author writes Previous report on human has suggested that brain is majorly affected by the cell phone radiation Mishra and Keshri, 2013). however, the cited reference ' is not a human study but, something which is irrelevant in present context of the thesis. Mishra, B' K. & Keshri, N. J. A. M. M. 2013. Mathematical model on the transmission of worms in wireless sensor network. 37, 4tO3-4LIl.

Clarification: - We have replaced reference with "Leung, S., Croft, R. J., McKenzie, R. J., Iskra, S., Silber, B., Cooper, N. R., ... & Simpson, D. (2011). Effects of 2G and 3G mobile phones on performance and electrophysiology in adolescents, young adults and older adults. *Clinical Neurophysiology*, *122*(11), 2203-2216" on Page No., 2

Comment 7: Page no 50: In western blotting protocol, author writes, after detection of the desired antibodies, perhaps it should be 'after detection of desired proteins,
Clarification: We have corrected as suggested on Page no. 27.

In Chapter 2

Comment 8: Page no 54:2.1.5.2 Long term exposure of EMR caused behavioral changes in open Field Test (OFT) ' Data not shown?

Comment 9: Page no 56:2.1.5.3 Repeated exposure of EMR exhibited anxiety-like behavior in Hole Board Test (HBT) Data not shown? For the 2nd set authors give the statistical significance for the analysed data, however mention that 'data not shown" it could be a good idea to show the data.
Clarification 8 & 9: As per examiner suggestion we have added the data of second set of experiment on page no. 32 and 35.
Comment 10: 2.1.6 Discussion Missing references include Kalueff eL al., 2A1,6, Sestakova et al., 2013, Coste et al., 2000; Jankord and Herman, 2008, Schulkinet al., 2005, de Quervain et a:.,2017, Hollis et al., 2015
Clarification: We have cited missing references in 2.1.6 discussion section

Summary

Comment 11: Figure: 2.3.10 Summary of hypothesis, the inhibition symbol placed after EMR and before neuropathophysiological changes Seems to be misplaced! Page no 129

Clarification: We have corrected the figure 2.3.10 on page no. 101.

Comment 12: Page no 163:Table:4.1what is the title for the table?

Clarification: We have added the title for the table on page no. 135.

Comment 13: Page no 165: Table: 4.2 what is the title for the table?

Clarification: We have added the title for the table on page no. 137-38.

Comment 14: Page no 169: Table: 4.3 what is the title for the table?

Clarification: We have added the title for the table on page no. 142.

Comment 15: Other minor issues that need to be fixed are: ln abbreviations list the following need to be corrected: MPTP, 5-.HT,

Clarification: We have corrected in the list of abbreviation section

Comment 16: Chapter 2.2 page no 7:2.2.3.11 Quantification of BDNF mRNA through reverse transcriptase-PCR (qRT-PCRt Total RNA (5 pg) of each individual was subjected to reverse transcription Here it should be each animal!

Clarification: We have corrected as per examiner suggested on page no. 63.

Comment 17: Page no: 99: Author writes, Necrosis and apoptosis is a cell death process that plays a key role during both physiological as well as pathological conditions, it should have been "Necrosis and apoptosis are cell death processes that play a key role during both physiological as well as pathological conditions",

Clarification: we have corrected on page no. 75.

Comment 18: In general, there is formatting issue. Table of contents and List of figures, and list of tables is poorly formatted.

Clarification: We have formatted the table of contents, list of figures and list of tables.

Comment 19: Page no 97: font size seems to be changed from 3rd paragraph

Clarification: We have changed the font size on page no 72.

Comment 20: page no 123: Author writes, "ln consonant with our result,a report"-'" Probably author meant to write in consent with or in agreement with our results!

Clarification: We have corrected on page no. 98.

Comment 21: Since a PhD thesis is a valuable resource, such mistakes jeopardise the work even if the hypothesis is good and may be experiments are carried out well with lot of

determination and hard work which one puts for the PhD work. Thesis is a resource, which next generation of researchers would refer' to get insights, derive the new research hypothesis, and in general, to understand the topic in the context. These errors also pose question on the conceived hypothesis since reader cannot have correct access to literature which is cited and based on which the study is said to be planned' Further few of them include references for the methodology that has been followed to conduct the experiment in the thesis'

It is very important that, the hypothesis, objectives, methodology and results along with thoughtful discussion are communicated clearly and more importantly correctly' It is equally important that one not only performs experiment and derives results, but also writes the work in a correct manner or communicates it well.

Clarification:- We have improved our thesis. The thesis has been thoroughly edited and revised.