BANARAS HINDU UNIVERSITY
FACULTY OF MEDICINE
INSTITUTE OF MEDICAL SCIENCES
VARANASI 221 005
(542/GO/ReBi/S/02/CPCSEA dated 26.5.2017)

No. Dean/2017/ CAEC/261 Dated: 21.11.2017

The Head
Electrophysiology Lab
School of Biomedical Engineering
Indian Institute of Technology
Banaras Hindu University

Dear Sir

A meeting of the Central Animal Ethical Committee of the University was held on 21.11.2017 at 03.00 PM in the Chamber of the Dean, Faculty of Medicine, IMS for animal ethical clearance of the proposal submitted by the following.

Name of the Student : Chandra Kant Singh Tekam
Title --- Role of Electromagnetotherapy in treatment of cerebral ischemia in rodents
Suggestion: The CPCSEA guidelines strictly are followed while handling the animals
Remarks: The synopsis is approved by the Central Animal Ethical Committee of the University

This is for your information and necessary action at your end.

Yours sincerely,

21-11-17

(JAI PRAKASH)
DEAN
&
CHAIRMAN
CENTRAL ANIMAL ETHICAL COMMITTEE OF THE UNIVERSITY

भारतीय प्रौद्योगिकी संस्थान
काशी हिन्दू विश्वविद्यालय
INDIAN INSTITUTE OF TECHNOLOGY
BANARAS HINDU UNIVERSITY

Department of Pharmaceutical Engineering & Technology

Regd. No. 2123/GO/Re/S/21/CPCSEA Date: 03 May, 2022

IAEC Approval Number: **IIT(BHU)/IAEC/2022/073**

CERTIFICATE

This is to certify that the project proposal entitled "**Design and development of low frequency low intensity PEMF chamber for treatment of neuroinflammation and cognitive impairment**" submitted by **Mr. Chandra Kant Singh Tekam** under supervision of **Dr. Sanjeev Kumar Mahto** has been approved/recommended by the IAEC of *Indian Institute of Technology, Banaras Hindu University, Varanasi* in its meeting dated **03/05/2022** and has been sanctioned **62 Male/Female Wistar Rats (200-250 gm)** under this proposal for a duration of **Twelve (12) months.**

Prof. Sushant Kumar Shrivastava	Dr. Vinod Tiwari	Dr. Shesh Narayan Mishra
Name & Signature	**Name & Signature**	**Name & Signature**
Chairman	**Member Secretary**	**Main Nominee of CPCSEA**

Note: The CPCSEA Guideline should be followed strictly while handling the animals

effects of ELF-PEMF exposure (1-3 mT, 20 min (twice) with 4 h gap) on cell proliferation of lung adenocarcinoma (A549), breast cancer (MCF-7), and hepatoblastoma (HepG2) cell lines. Moreover, we also determined the 50 Hz ELF-PEMF exposure induced cytotoxicity with MTT assay, supporting its potential for biomedical applications.

Finally, the key conclusions and future scope of work of the thesis are summarized in **Chapter 6**.

1.11 References

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under present experimental conditions, which acts as supportive scientific evidence for safe use of 50 Hz ELF-PEMF exposure (Table 4.5).

4.5 Conclusion

The present study demonstrates the effects of 50 Hz ELF-PEMF using *in vitro* (RFP-L929 mouse fibroblast cells) and *in vivo* (adult male Wistar rats) models. The results reveal that an exposure duration of 20 min (each) with a 4 h gap is non-destructive for RFP-L929 cells and causes mild alterations in biochemical parameters but not in organ coefficient, tissue structure, and morphology of adult Wistar rats. The results demonstrated that 50 Hz ELF-PEMF exposure did not cause any significant cellular fragmentation and changes in the morphology of mouse fibroblast cells. We observed mild alterations in biochemical parameters of rats among magnetic field exposed and control groups. Conversely, histological analysis of the selected liver, kidney, and heart sections following ELF-PEMF exposure revealed no significant changes in tissue structure and morphology. Our efforts provide conceptual and experimental support to establish a link between 50 Hz magnetic field exposure in residential and occupational environments.

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interaction of ELF-MF with biological tissues would be to carefully screen through the various exposure parameters (intensity, frequency, duration, etc.).

5.6 Conclusion

We have shown for the first time that a 50 Hz ELF-PEMF exposure (1-3 mT) (horizontal) with duration (< 1 h/day) can directly affect biological responses at the cellular level. Furthermore, we also observed that weak ELF-MF, inhibited the proliferation of A549 cancer cells depending on their strength, frequency, and duration. Our results also support earlier studies by showing that different cell lines can respond differently to exposure to the same magnetic field and that the same cell line can respond differently to fields with varying intensities. The present Helmholtz coil system constitutes a novel method of screening in the requirement for effective 50 Hz magnetic field parameters to influence cancer cell proliferation.

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6.2 Scope for future work

The future perspectives of the current research work may involve exploring the effects of magnetic field exposure in combination with chemotherapy drugs on different cancer cells to develop an alternative therapeutic methodology for cancer treatment (Alkis et al., 2022; Chen et al., 2022; Nuccitelli et al., 2010). The fabricated device may also assist in assessing the treatment of neuroinflammation and cognitive impairment (Fu et al., 2008; Serafini et al., 2015; Téglás et al., 2018). Moreover, this device is capable of facilitating magnetic field exposure experiments on wound healing, cellular dynamics, application with magnetic nanoparticles, growth factors, etc., synapse-related studies, and cell-cell interaction, of naming a few (Ganguly et al., 2022; Goya et al., 2013; Ju et al., 2016; Nuccitelli et al., 2010). The magnetic field application as a therapeutic strategy has significant potential to be considered a breakthrough technological advancement in bioelectromagnetic.

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LIST OF PUBLICATIONS

Journals:

1. **Chandra Kant Singh Tekam[1]**, Shreyasi Majumdar[2], Pooja Kumari[1], Santosh Kumar Prajapati[2,4], Ajay Kumar Sahi[1], Saksha Shinde[3], Richa Singh[1], Puneet Kumar Samaiya[5], Ranjana Patnaik[6], Sairam Krishnamurthy[2], Sanjeev Kumar Mahto[1*]. Effects of ELF-PEMF Exposure on Spontaneous Alternation, Anxiety, Motor Co-ordination and Locomotor Activity of Adult Wistar Rats and Viability of C6 (Glial) Cells in Culture, Toxicology, 2022, 153409, ISSN 0300-483X, https://doi.org/10.1016/j.tox.2022.153409.

2. **Chandra Kant Singh Tekam[1]**, Ajay Kumar Sahi[1], Saksha Shinde[2], Pooja Kumari[1], Sanjeev Kumar Mahto*. Design and Development of an Extremely Low-Frequency Pulsed Electro magnetotherapy Chamber for *in vivo* and *in vitro* Studies. Eur. Chem. Bull. 2023, 12(Special Issue 4), 9559-9578.https://doi: 10.48047/ecb/2023.12.si4.858.

LIST OF PUBLICATIONS

Publications not related to the thesis work:

1. Mitra, N.S., Shinde, S., Sharma, N., **Chandra Kant Singh Tekam** and Maurya, M., 2020. Agranulocyte & neutrophil nucleus enhancement through 510nm wavelength of light. *Indian Journal of Clinical and Experimental Ophthalmology*, *6*(2), pp.279-281.DOI:10.18231/jijceo.2020.060

2. Gundu, S., Sahi, A.K., Kumari, P., Tekam, C.S., Allu, I., Singh, R. and Mahto, S.K., 2024. *In vivo* characterization of a luffa-based composite scaffold for subcutaneous implantation in rats. *Journal of Biomaterials Science, Polymer Edition*, *35*(12), pp.1922-1946.

Book chapters:

1. **Chandra Kant Singh Tekam*,** Shinde, S., Patnaik, R. and Mahto, S.K., 2021. Bilateral Common Carotid Artery Occlusion: Stroke Model. In *Models and Techniques in Stroke Biology* (pp. 41-56). Springer, Singapore. ISBN 978-981-336-679-4
2. **Chandra Kant Singh Tekam,** Tripathi, A.K., Kumar, G. and Patnaik, R., 2019. Emerging role of electromagnetic field therapy in stroke. In *Advancement in the Pathophysiology of Cerebral Stroke* (pp. 93-102). Springer, Singapore. ISBN 978-981-13-1453-7

International Conferences:

1. Shinde, S., Sharma, N., Bansod, P., Singh, M. and **Chandra Kant Singh Tekam***, 2020, February. Automated nucleus segmentation of leukemia blast cells: color

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Workshops and Hands-on-trainings:

1. Participated in **Faculty Development Programme** on **Material Characterization** organised by IIT Roorkee and IIT(BHU) Varanasi (23/09/2022 – 27/09/2022)
2. Participated in **1-week Hands-on Arduino Workshop on opensource Lab Development** organised by Teaching & Learning center, Indian Institute of Technology (BHU), Varanasi (02/04/2018 – 07/04/2018).
3. Participated in training programme on **Tools for Scientific Documentation: LATEX, JabRef, DocEar.** Organised by DST-CIMS, BHU (05/02/2018 – 16/02/2018).
4. Participated in symposium on **Research Methodology for Research Students & Faculty Members (RMFR-2017)** organised by Teaching & Learning center, Indian Institute of Technology (BHU), Varanasi [24/03/2017 – 29/03/2017]

Response to the Examiners' Comments

The time and efforts put forth by the examiners for reviewing the thesis is deeply appreciated. All the issues indicated in the review report have been addressed and it is believed that the revised version of the thesis can now meet the examiners' expectations. The issues are also incorporated in the revised thesis.

Examiner-1 comments:

Chapter-1

1) **Figures 1.1 and 1.7 are ineligible**

   Response: I am thankful for valuable suggestions and feedback. The figures 1.1 and 1.7 have been removed as in accordance with the examiner's recommendations and the following figures serial numbers are also updated in the thesis. **(Chapter-1, page no. 02 & 13)**

2) **Units are italics, but not always, please maintain uniformity**

   Response: Examiner's suggestions are highly appreciated. The format of units is updated to be uniform throughout thesis as in accordance with the examiner's recommendations. (**Chapter-1, page no. 1-27**)

3) **Short form to be written once e.g., low frequency pulsed electromagnetic field (ELF-PEMF). Please do not repeat them throughout the thesis.**

   Response: I am thankful for the suggestions and feedback and I have updated the thesis as in accordance with the examiner's recommendations.

Chapter-2

1) **Biot-savart law, capitalize, because both are scientist names.**

   Response: We thank the examiners for their suggestions and have revised the scientists' names as in accordance with the examiner's recommendations. **(Mathematical modelling, page no. 47**)

2) **Figure 2.2, 2.6, 2.8, 2.9 is unclear/not legible. Please present this figure legibly**

   Response: We understand the concern raised by the examiner, and the images in the thesis has been accordingly revised. (**Chapter-2, page no. 51-64**)

3) **Henries was also a scientist.**

   Response: We thank the examiners for their suggestions and scientist's names are updated in thesis as in accordance with the examiner's recommendations. (**Coil inductance, page no. 55-56**)

4) **Watt is also a scientist**

   Response: We thank the examiners for their suggestions and sscientist's names are updated in thesis as in accordance with the examiner's recommendations. (**Chapter-2, page no. 70-71**)

5) **How does this present design was better than past methods available from literature?**

   Response: I agree with the examiner's perspective and feedback. We have attempted to explain the design aspects, advantages and limitations of monoaxial Helmholtz coil system in the following sections of revised thesis. **(Discussion, page no. 69-71)**

6) **What was the longevity of the set up?**

Response: I agree with the examiner's perspective and feedback. We explained the longevity aspect of our design in the following section of revised thesis. **(Discussion, page no.70-71)**

Chapter-3

1) **Maxwell is the name of scientist it can't be small letter.**

Response: We thank the examiners for their suggestions and scientist's names are updated in thesis as in accordance with the examiner's recommendations. (**Introduction, page no. 78**)

2) **Figure 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7 is unclear**

Response: We understand the concern raised by the examiner, and the images in the thesis are revised as per the recommendation of examiners. **(Chapter-3, page no. 80-92)**

3) **Equation 7 spelling mistake of weight, pl check**

Response: We understand the concern raised by the examiner. The equation number is updated according to the chapter and spelling of weight is corrected in thesis as in accordance with the examiner's recommendations.

(**Organ coefficient analysis, page no. 88**)

4) **Y maze test mentioned in section 3.2.5.5.1 can be represented in tabular form**

Response: I agree with the examiner's suggestion and I have included the schematic representation of measurement of spontaneous alternation among rats in the revised thesis. (**Y-maze test, page no. 88-89**)

5) **All the experimental methods used and their outcomes with amount of magnetic field exposure and time duration can be represented in a table for quick and precise understanding.**

Response: I agree with the examiner's suggestion. The Summary of experimental results with ELF-PEMF exposure intensity and time durations are updated in the form of table for quick and precise understanding as in accordance with the examiner's recommendations. (**Chapter-3, page no. 96-98**)

6) **State of the art studies have not been discussed to show the superiority of the experiment conducted in this thesis work.**

Response: We understand the concern raised by the examiner. We have included the state of the art of magnetic field exposure in chapter-1 of the revised thesis.

(Chapter-1, page no. 21-26)

7) **Could you please mention exactly which biomedical fields especially therapeutic, the present concept will be helpful?**

Response: I agree with the examiner's perspective and feedback. We have attempted to explain the therapeutic effectiveness of 50 Hz ELF-PEMF exposure (1-3 mT) in the following chapters of revised thesis.

(Chapter-3, page no. 101-105)

(Chapter-4, page no. 138-143)

(Chapter-5, page no. 175-177)

Chapter-4

1) **Figure 4.4, 4.5, 4.6, 4.7 is unclear/not legible.**

   Response: We understand the concern raised by the examiner, and the images in the thesis has been accordingly revised. (**Chapter-4, page no. 125-136**)

2) **What is the reproducibility of the experiment? If you have done repeatability experiment then tabulate those results in this chapter.**

Response: We understand the concern raised by the examiner regarding the reproducibility of the experiment, as it is a crucial factor in confirming the validity and reliability of research data. In the present thesis, while we have only repeated the work twice of the ELF-PEMF exposure on adult Wistar rats, we have addressed the issue of statistical power by performing a G power analysis. This analysis was conducted to ensure that our study design could detect a meaningful effect with the desired level of power, thus reinforcing the robustness of our findings. The details of the Gpower analysis, including the parameters used and the results obtained, are thoroughly explained in the following sections of thesis.

(Experimental protocols, page no. 121-126)

3) **In a table, at the end, the effects on all the vital organs by the magnetic field can be summarized.**

   Response: The Summary of experimental results with amount of magnetic field exposure and time duration are updated in the form of table for quick and precise understanding as in accordance with the examiner's recommendations. (**Results, page no. 137-138**)

4) **What was the organ coefficient value? How was this value is compared with the literature?**

Response: I understand the examiners perspective and I am thankful for his valuable feedback. We have attempted to explain the rationale behind organ coefficient value and its comparison with the appropriate literature studies in the following sections of revised thesis. **(Sample collection and data analysis page no. 127)**

(*In Vivo* analysis, page no. 130-131)

(Discussion, page no. 140)

5) **There are another statistics measure such as student's t test etc. should also be performed. Please compute student t test.**

Response: I thank the examiner for his valuable feedback. The theoretical work on "t-distribution" was first done by W.S. Gosset under the name student, that is why it is called Student's t-test. Student's t-test is used when the sample size is less than 30 ($n < 30$) and population standard deviation (σ) is unknown. It can only be used when total groups in the experiment is 2 and if the groups are more than 2 then we prefer the ANOVA statistical analysis method. The details of experimental protocol are mentioned in following sections of revised thesis.

(Experimental protocol, page no.123-126)

Chapter-5

1) **Figure 5.1,5.3, 5.5 is unclear/not legible.**

Response: We understand the concern raised by the reviewer, and the images in the thesis are revised according to the examiner's recommendations. **(Introduction, page no. 160)**

(Results, page no. 166-168)

2) **Evaluate specifically what significant biological impact on cancer cells in vitro was observed in the cancer cells.**

Response: I absolutely agree with the points raised by the examiner. I have explained the effects on antiproliferative activity, cell morphologies and induced cytotoxicity in A549 cancer cells after 50 Hz ELF-PEMF exposure in the following sections of revised thesis.

(Results, page no. 166-175)

3) **Cell death occurred in normal and cancer cell with magnetism 200 hz as given in figure 5.9, so what is the benefit? Any clinical support/evidence for this claim.**

Response: I absolutely agree with the points raised by the examiner. I have tried to explain the probable cause of A549 cell death with ELF-PEMF (1-3 mT) exposure at 50 Hz (not 200 Hz) as depicted in figure 5.9 under *in vitro* conditions inside following sections of thesis. (**Discussion, page no. 177-179**)

4) **How a significant decline in the level of proliferation of A549 cells upon exposure to ELF-PEMF was observed in this experiment exclusively without the reference of literature please mention separately.**

Response: I absolutely agree with the points raised by the examiner. I have explained the significant biological impact on cancer cells under *in vitro* conditions inside following sections of thesis:

(Experimental protocol, page no. 161-162)

(Results, page no. 166-175)

5) **One drawback has been mentioned, what are the other limitations of the experiment.**

Response: I agree with the points raised by the examiner. We have attempted to precisely define the exposure parameters that could be potentially therapeutic but we have mentioned some of the limitations of experiment in following sections of revised thesis. **(Discussion, page no. 178)**

6) **Make a tabular chart of all the effects of the proposed instrument on the different types of cells and their cell behaviour, in terms of its morphology.**

   Response: I absolutely agree with the points raised by the examiner. I have summarized the ELF-PEMF exposure effects observed on various cell lines under present thesis. (**Chapter-5, page no. 175-176**)

7) **How the above experiments play a role in lung cancer treatment, please elaborate? If you have any suggesting clinical evidence, you may explain in detail.**

   Response: I absolutely agree with the points raised by the examiner. I have explained the role of present experiment in lung cancer treatment under following sections of present thesis. (**Discussion, page no. 176-179**)

Examinar-2 comments

1) **There is no information in the thesis whether the examinee has further plan to publish articles from the remaining chapters in the thesis and in the viva this point may be raised.**

   Response: I absolutely agree with the points raised by the examiner. At present the unpublished manuscripts are under review.

2) **Although the examinee has demonstrated a significant example of a Helmholtz coil model for testing cells and small animals,which is merit worthy and commendable, however an important characterization is missing from the thesis, which should be raised in the phd viva. In the design of Helmholtz coil and modelling of field,there is no consideration of the relationship between the applied magnetic field and biophysics of cell and animal tissue as media. This is important to know beforehand because when the interaction of magnetic field with a medium is considered, the magnetic magnetic permeability and susceptibility properties of the cellular and tissue media should also be known, so that the knowledge would then enable much better quatitative analysis of the field response. For example whether a chosen medium is diamagnetic,paramagnetic of ferromagnetism. As a classic example, the magnetic behaviour of haemoglobin have been reported.**

Response: I understand the examiners perspective and thankful for the feedback. I have explained the biophysical model of action of A.C. magnetic field and magnetic susceptibility of cells and tissues under following sections of thesis. **(Chapter-1, Page no. 14-16)**

3) **The examiner appreciates the time and effort spent on the challenges in the measurements and viability analysis of biological cells and tissues using a magnetic field, the examinee should endeavour to mention whther the magnetic properties of cell lines and tissue types are know or not known in the literature. This part is missing form the literature review.**

Response: I understand the examiners perspective and thankful for the feedback. We added the brief details of cell lines, magnetic behaviour and reason to include under current work in following sections: **(Chapter-1, Page no. 14-18)**

4) **Under the section on the thesis supervision and roles of research supervisor, there is no expert contributing as co supervisor in magnetism and magnetic materials from either physics/engineering or biophysics/ material science.**

Response: In present thesis work, Dr. R.K Shrivastava, Professor, Department of Electrical Engineering, Indian Institute of technology (Banaras Hindu University) Varanasi has guided and helped me in the design and development process of the device and I have acknowledged his support and contributions in my previous publications (Tekam et al., 2023). Dr. K. Sairam, Professor, Department of Pharmaceutical Engineering and Technology, Indian Institute of technology (Banaras Hindu University) Varanasi has done extensive work on the EMR exposure and its health effects (Gupta et al., 2019, 2018). Dr. Sairam has guided and supported in the animal studies presented in this thesis.

5) **The thesis shows the ethics approval document from the university authorities, however there is no protocol document appended which may be helpful for designing experiments in the future**

Response: I absolutely agree with the points raised by the examiner. The ethical approval document and protocol are included in thesis as in accordance with the examiner's recommendations. **(Page no. 190-191)**

6) **In medicine research, it is recommended that the experimental methodology may be include the gender and genetic preponderance of a medical condition (for example the breast cancer happens in both genders but it is more dominant in females). Similarly the social cross sectional studies are important, for example in the case of oral cancer, the smoking and oral hygine are common features. It is unclear from the**

text whether wistar rats selection was based on gender balance and age. These data are also important for relating the preponderance of specific disease conditions e.g. cancer.

Response: I understand the concern raised by the reviewer. In current thesis work, the ELF-PEMF exposure studies are performed on adult Wistar rats (male, 8 weeks old) to better understand the responses toward novel treatment. Moreover, we also planned to perform the ELF-PEMF exposure studies to observe the changes in gene expression and more under *in vitro* and *in vivo* conditions but due to covid restrictions we could not. We have successfully observed the biological effects of 50 Hz ELF-PEMF (1-3 mT, 20 min (twice) with 4 h gap) under *in vitro* and *in vivo* conditions. Moreover, we successfully determined the PEMF intensities (1 mT < B < 3 mT) with exposure duration (< 1 h/day) which are effective on A549 cancer cells. In future, we will continue our work to better understand the molecular pathways and gene expression to relate the preponderance of specific disease conditions.

(Chapter-3, page no. 85-87)

(Chapter-4, page no. 118)

7) **Amongst the analytical methods, the examinee has only adpted the methods which are widely used in the biology. However, the analytical methods namely raman and IR spectroscopic analyses are not considered for identifying the biomarkers and metabolites. These analytical tools are invaluable and complementary.**

Response: I absolutely agree with the points raised by the examiner.

In present thesis, we have focused our work on the design and development of extremely low frequency pulsed electromagnetic field chamber for biomedical applications. We have successfully performed the ELF-PEMF exposure studies to determine harmful effects on

cell proliferation, cognitive abilities and biochemical parameters on rats under *in vitro* and *in vivo* conditions respectively. Moreover, we also performed the ELF-PEMF exposure on different cancer cell lines (A549, MCF-7 and HepG2). we were not able to perform the molecular studies to determine the molecular mechanism behind the of observed effects due to covid restrictions and non-availability of experimental facilities. But, in future we will perform the above-mentioned studies to understand the molecular mechanisms.

Suggested modifications

1) **The thesis requires a major revision for removing grammatical and syntax errors. Frequently the pronouns are written with small letters.**

   Response: I am thankful for the examiners for suggestions and feedback. I have corrected the grammatical and syntax errors in the thesis.

2) **In cell culture studies, there is little information on the selection of specific cell lines and the reason for selection. The descriptions of cell lines are important and should also be covered in the literature review. This is an important omission which should be addressed.**

   Response: I understand the examiners perspective and thankful for the feedback. I have included the information about the cell lines and reason for selection of specific cell lines under following sections of thesis. **(Chapter-1, Page no. 17-18)**

3) **The reason for not including FTIT and Raman spectroscopy when cell lines were tested in the magnetic field remains unclear. Also, the effects of magnetic field and accompanying genetic response should have been included by under taking q-PCR analysis in selected case studies.**

Response: I absolutely agree with the points raised by the examiner. In present thesis, we have focused our work on the design and development of extremely low frequency pulsed electromagnetic field chamber for biomedical applications. We have successfully performed the ELF-PEMF exposure studies to determine harmful effects on cell proliferation, cognitive abilities and biochemical parameters on rats under *in vitro* and *in vivo* conditions respectively. Moreover, we also performed the ELF-PEMF exposure on different cancer cell lines (A549, MCF-7 and HepG2). We were not able to perform the molecular studies to determine the molecular mechanism behind the of observed effects due to covid restrictions and non-availability of experimental facilities. But, in future we will perform the above-mentioned studies to understand the molecular mechanisms.

Additions questions/queries from the marked thesis

Chapter-1

1) **What about the mobile phones and 2G to 5G signals and antenna? (section 1.6.2.2, page no 12)**

   Response: I absolutely agree with the points raised by the examiner and I have updated the data for mobile phones (2G-5G) signals and antenna as suggested by the examiners. **(Electrical appliances, page no.11- 12)**

2) **Apoptosis of healthy or cancer cells? (section 1.8, page no 15)**

   Response: I understand the examiners perspective and thankful for the feedback. I have updated the literature review section of the chapter 1 according to the examiner's recommendations. **(Chapter-1, page no. 18-24)**

3) **Therapeutic benefits may be described in terms of dose and *in vivo* and *in vitro* studies? (section 1.8, page no 16)**

Response: I absolutely agree with the points raised by the examiner and I have included the *in vivo* and *in vitro* studies results. I have also included the literature in the form of table to assess the therapeutic effectiveness of ELF-PEMF exposure as recommended by the examiners. **(Chapter-1, page no 18-24)**

Chapter-2

1) **Correct the 1 st line of second paragraph (revise paper to chapter) (page no 35)**

Response: I thank the examiner for the valuable comments and feedback. I have corrected the mistake in updated thesis. **(Introduction, page no.46)**

2) **Are these author's data or from the literature? (page no 39)**

Response: I thank the examiner for the valuable comments and feedback. The data presented in the respective section is author's data based on the computational and real time analysis of magnetic field. The detailed analysis is included in the upcoming sections of this chapter.

(Chapter-2, page no. 59-60)

3) **The units and dimensions are not used in explaining each equation. Each equation should have SI units (page no 41)**

Response: I thank the examiner for the valuable comments and feedback. I have included the SI units and dimensions in explanation of each equation as recommended by examiner.

4) **Are the lines in the figure fitted curve from a model? Or the lines are as guides for curve visualization? (page no 42)**

Response: I absolutely agree with the points raised by the examiner. The lines in the figures are result to the process of constructing a curve or mathematical function, that has the best fit to a series of data points.

(Chapter-2, page no. 52-55)

5) **What the difference one should be looking for? Not clear (page no 52)**

Response: I absolutely agree with the points raised by the examiner. In the respective section, we have displayed the computation analysis of magnetic field overlay between monoaxial Helmholtz coil system at different supply voltages and current. We have also attempted to explain in detail in following sections of thesis.

(Chapter-2, page no. 60-63)

6) **Correct conclusion 1st line (revise paper to chapter) (page no 55)**

Response: I thank the examiner for the valuable comments and feedback. I have corrected the mistake as recommended by examiner.

(Chapter-2, page no. 71)

Chapter-3

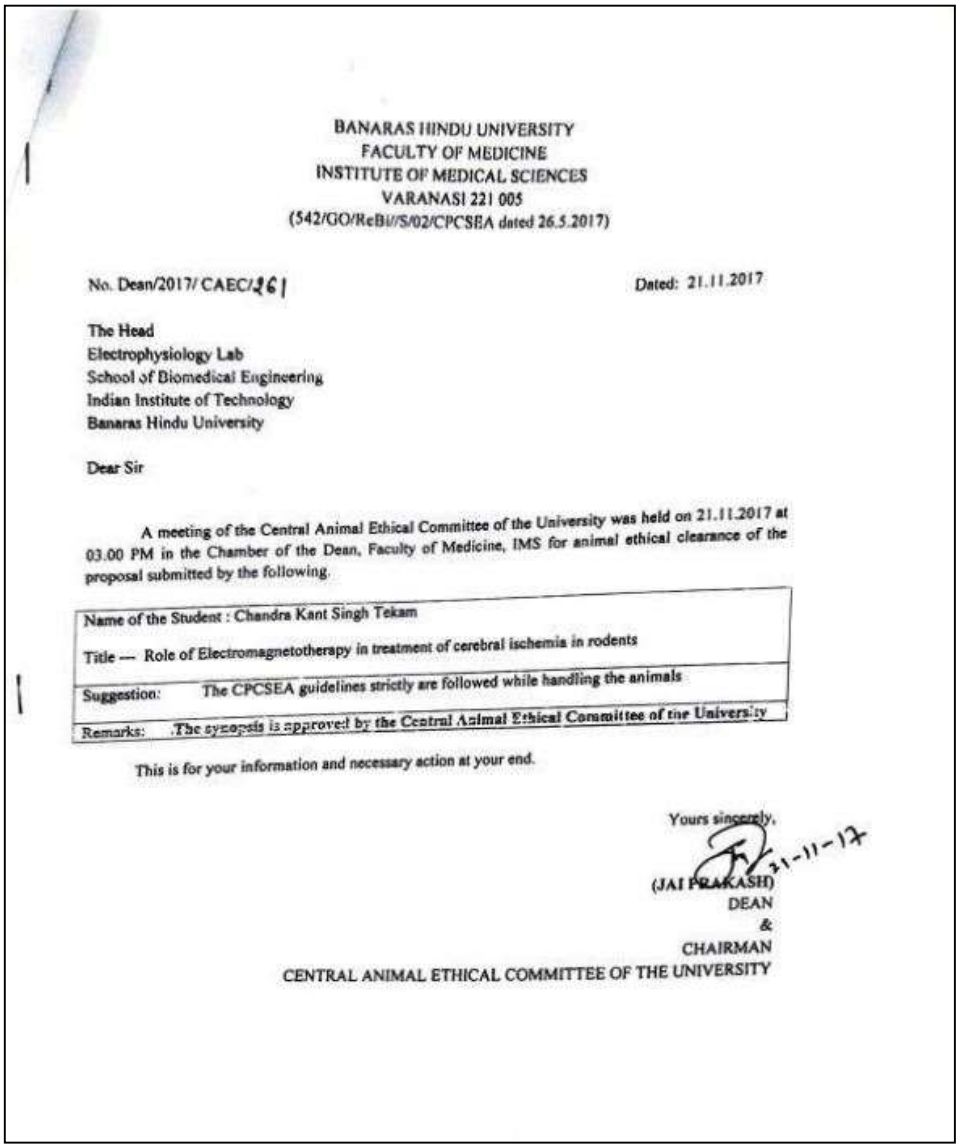
1) **Should be glial cell culture and preparation. There is no description of glial cells. There are 6 different types of glial cells and different morphologies and functional features.Since the magnetic field and complementary current pathway are dependent on the shape and morphologies features.It would be beneficial to characterize the cell morphology. (page no 67)**

Response: I thank the examiner for the valuable comments and feedback. We updated the section of the thesis as recommended by examiner. **(Glial cell culture preparation, page no. 83-84)**

2) **Ethical approval for animal studies and protocols for animal care. (page no 69)**

Response: I thank the examiner for the valuable comments and feedback. I have included the ethical certificate in the thesis as recommended by examiner.

(Page no. 190-191)



BANARAS HINDU UNIVERSITY
FACULTY OF MEDICINE
INSTITUTE OF MEDICAL SCIENCES
VARANASI 221 005
(542/GO/ReBi/S/02/CPCSEA dated 26.5.2017)

No. Dean/2017/ CAEC/261 Dated: 21.11.2017

The Head
Electrophysiology Lab
School of Biomedical Engineering
Indian Institute of Technology
Banaras Hindu University

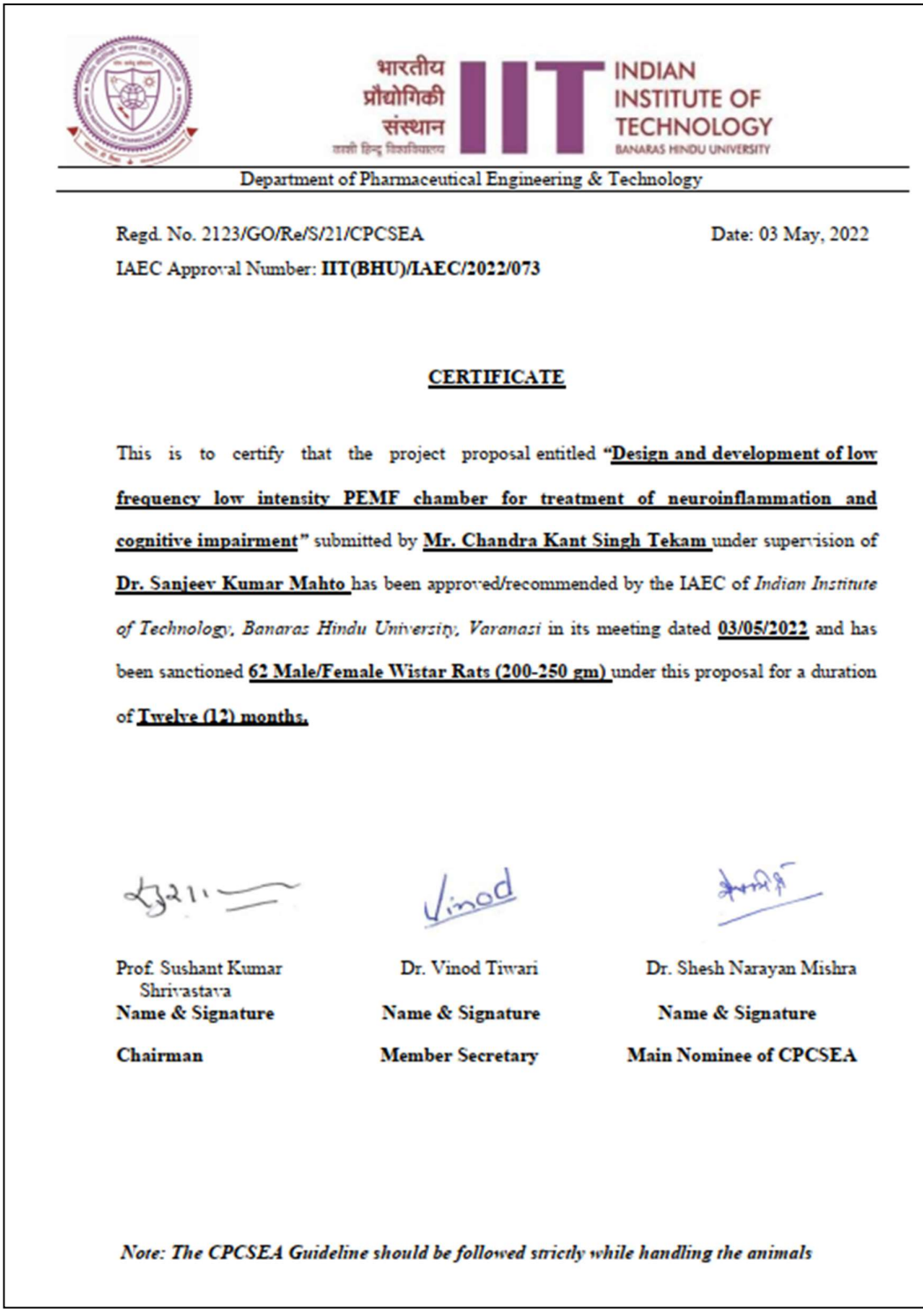
Dear Sir

A meeting of the Central Animal Ethical Committee of the University was held on 21.11.2017 at 03.00 PM in the Chamber of the Dean, Faculty of Medicine, IMS for animal ethical clearance of the proposal submitted by the following.

Name of the Student : Chandra Kant Singh Tekam
Title — Role of Electromagnetotherapy in treatment of cerebral ischemia in rodents
Suggestion: The CPCSEA guidelines strictly are followed while handling the animals
Remarks: The synopsis is approved by the Central Animal Ethical Committee of the University

This is for your information and necessary action at your end.

Yours sincerely,
21-11-17
(JAI PRAKASH)
DEAN
&
CHAIRMAN
CENTRAL ANIMAL ETHICAL COMMITTEE OF THE UNIVERSITY



भारतीय प्रौद्योगिकी संस्थान
काशी हिन्दू विश्वविद्यालय
IIT INDIAN INSTITUTE OF TECHNOLOGY
BANARAS HINDU UNIVERSITY

Department of Pharmaceutical Engineering & Technology

Regd. No. 2123/GO/Re/S/21/CPCSEA Date: 03 May, 2022

IAEC Approval Number: **IIT(BHU)/IAEC/2022/073**

CERTIFICATE

This is to certify that the project proposal entitled "**Design and development of low frequency low intensity PEMF chamber for treatment of neuroinflammation and cognitive impairment**" submitted by **Mr. Chandra Kant Singh Tekam** under supervision of **Dr. Sanjeev Kumar Mahto** has been approved/recommended by the IAEC of *Indian Institute of Technology, Banaras Hindu University, Varanasi* in its meeting dated **03/05/2022** and has been sanctioned **62 Male/Female Wistar Rats (200-250 gm)** under this proposal for a duration of **Twelve (12) months.**

Prof. Sushant Kumar Shrivastava	Dr. Vinod Tiwari	Dr. Shesh Narayan Mishra
Name & Signature	**Name & Signature**	**Name & Signature**
Chairman	**Member Secretary**	**Main Nominee of CPCSEA**

Note: The CPCSEA Guideline should be followed strictly while handling the animals

3) **Equation no should be in accordance with the chapter number. (page no 71)**

Response: I thank the examiner for the valuable comments and feedback. I have corrected the equation serial no. as recommended by examiner.

(*in-vivo* analysis, page no.88)

Chapter-4

1. **Describe RFP-L929 cell line and its role in the thesis, it is difficult to find the data on penetration depth of magnetic field between 1 and 3 mT in various cell culture media and tissues examined.**

Response: I agree with the examiner's suggestion and I have included the brief details and the role of RFP-L929 cell line in present thesis as per the recommendation by examiner.

(Chapter-1, Section 1.8.2.3, page no. 17)

(Chapter-4, Section 4.2.5.1, page no. 121-122)

2. **Why not investigate the sources of biomarkers themselves in controlled environment (page no 104)**

Response: I agree with the examiner's perspective. We could not perform the above-mentioned studies due to COVID restrictions and the limited availability of experimental facilities. But, in the future, we will perform the studies in controlled environment.