The following **Research Protocols** for animal research have been approved for the experimentation by the IAEC, KU in the meeting held on 13-04-2022 and the protocols can be initiated from this date and should complete within one year i.e before 12-04-2023.

S.No	Names of the Scholar	Application No:	Research Topic	IAEC-KU-No.	Animals-Approved	Remarks
01	Names of the PhD-Scholar: **M.Madhavi** Name of the Supervisor: **Prof. Y.Narsimha Reddy**	01	Studies on pharmacodynamic and Pharmacokinetic interaction of some phytochemicals with Selected antidiabetic, antiepileptic and antihyperlipidemic Drugs	**(01/IAEC/UCPSc/KU/2022: CPCSEA 2018-23).**	Strain: Rats M&F Species: Wistar Total No.Apr: (96**)** *In Vitro* = nil Toxicity Study:Nil Chpt.1= (30**)** Chpt.2= (36**)** Chpt.3= (30**)**	Proceed with the study within a year from date of approval
02	Names of the PhD-Scholar: **R. Rajendra Prasad** Name of the Supervisor: **Dr. Shayeda**	05	Development and evaluation of nanoformulations for some drugs	**(02/IAEC/UCPSc/KU/2022: CPCSEA 2018-23).**	Strain:Rars M Species: Wistar Total No.Apr: (24**)** *In Vitro* =Nil Toxicity Study: Nil Chpt.1= (24**)** Chpt.2=Nil Chpt.3=Nil	Proceed with the study within a year from date of approval
03	Names of the PhD-Scholar: **Mr. Amma Venu** Name of the Supervisor: **Prof. Y.Narsimha Reddy**	03	Studies on Nephroprotective and Anti-oxidant activity of some Indian medicinal plants.	**(03/IAEC/UCPSc/KU/2022: CPCSEA 2018-23).**	Strain: Rats-Male Species: Wistar Total No.Apr: (120**)** *In Vitro* =Nil Toxicity Study: (**30)** Chpt.1=(**30)** Chpt.2=(**30)** Chpt.3=(**30)**	Proceed with the study within a year from date of approval
	Names of the				Strain:Rats Male	Proceed

04	PhD-Scholar: **Swathi Jakku** Name of the Supervisor: **Dr. J. Krishnaveni**	04	Development of Intranasal formulations for substances of natural origin for the treatment of neurodegenerative disorders	**(04/IAEC/UCPSc/KU/2022: CPCSEA 2018-23).**	Species: Wistar Total No.Apr: (216) *In Vitro* =Nil Toxicity Study:Nil Chpt.1= (108) Chpt.2= (108) Chpt.3=Nil	with the study within a year from date of approval
05	Names of the PhD-Scholar: **K.Rajeswari** Name of the Supervisor: **Dr.V. Swaroopa Rani**	05	Studies on bioenhancing ability of some phytochemicals on some CYP and P-gp substrate drugs	**(05/IAEC/UCPSc/KU/2022: CPCSEA 2018-23).**	Strain: Rats male Species:Wistar Total No.Apr: (90) *In Vitro* = (30) Toxicity Study:Nil Chpt.1= (30) Chpt.2= (30) Chpt.3= Nil	Proceed with the study within a year from date of approval
06	Names of the PhD-Scholar: **P.S.Malathy** Name of the Supervisor: **Prof. Y.Narsimha Reddy**	06	Pharmacokinetic and Pharmacodynamic interactions of Phytoconstituents with some conventional drugs.	**(06/IAEC/UCPSc/KU/2022: CPCSEA 2018-23).**	Strain:Mice Male Species:Swiss Albin Total No.Apr: (102) *In Vitro* =Nil Toxicity Study: Nil Chpt.1=(30) Chpt.2=(36) Chpt.3=(36)	Proceed with the study within a year from date of approval
07	Names of the PhD-Scholar: **M.Rama** Name of the Supervisor: **Prof. Y.Narsimha Reddy**	07	Amelioration in Learning and Memory of Some Indian Medicinal Plants by Neuronal Cell Injury Induced Animal Models.	**(07/IAEC/UCPSc/KU/2022: CPCSEA 2018-23).**	Strain:Mice Male Species:Swiss Albin Total No.Apr: (120) *In Vitro* =Nil Toxicity Study: (30) Chpt.1=(30) Chpt.2=(30) Chpt.3=(30)	Proceed with the study within a year from date of approval
08	Names of the PhD-Scholar: **D.Saritha** Name of the Supervisor: **Dr. J.**	08	Development and evaluation of nanocarrier based Transdermal drug delivery systems of selected drugs	**(08/IAEC/UCPSc/KU/2022: CPCSEA 2018-23).**	Strain:Rats Male Species: Wistar Total No.Apr: (216) *In Vitro* =Nil Toxicity Study:Nil Chpt.1= (72)	Proceed with the study within a year from date of approval

	Krishnaveni				Chpt.2= (72**)** Chpt.3=(72**)**	
09	Names of the PhD-Scholar: **Ms. Gouthami Thumma** Name of the Supervisor: **Dr. Shayeda**	09	Design, Characterization and Evaluation of Nano Formulations to Enhance Bioavailability of Some Drugs	**(09/IAEC/UCPSc/KU/2022: CPCSEA 2018-23).**	Strain: Rats-Male Species: Wistar Total No.Apr: (**30**) *In Vitro* =Nil Toxicity Study:Nil Chpt.1= (**30**) Chpt.2= Nil Chpt.3= Nil	Proceed with the study within a year from date of approval
10	Names of the PhD-Scholar: **Kambakam. Venkatalakshmi** Name of the Supervisor: **Dr.V. Swaroopa Rani**	10	Evaluation of some medicinal plants for antidiabetic activity	**(10/IAEC/UCPSc/KU/2022: CPCSEA 2018-23).**	Strain:Rats male Species:Wistar Total No.Apr: (126**)** *In Vitro* =Nil Toxicity Study:Nil Chpt.1= (42**)** Chpt.2= (42**)** Chpt.3= (42**)**	Proceed with the study within a year from date of approval
11	Names of the PhD-Scholar: **P.Durga** Name of the Supervisor: **Dr. N.Prasad**	11	Improvization of Anticancer activity of Polyphenols in Animals by Co crystal Technology	**(11/IAEC/UCPSc/KU/2022: CPCSEA 2018-23).**	Strain:Rats M&F Species: Wistar Total No.Apr: (72**)** *In Vitro* =Nil Toxicity Study: Nil Chpt.1= (24**)** Chpt.2=(24**)** Chpt.3=(24**)**	Proceed with the study within a year from date of approval
12	Names of the PhD-Scholar: **M.Sravanthi** Name of the Supervisor: **Dr. Shayeda**	12	Green biosynthesis, Charecterization, In Vitro Pharmacological Activities and investigational acute toxicity study of Herbal medicated Nanoparticles on animal models	**(12/IAEC/UCPSc/KU/2022: CPCSEA 2018-23).**	Strain:Rate Female Species:Wistar Total No.Apr: (30**)** *In Vitro* = Nil Toxicity Study: (30**)** Chpt.1= Nil Chpt.2= Nil Chpt.3= Nil	Proceed with the study within a year from date of approval
	Names of the				Strain: Rats male	***In vitro***

13	PhD-Scholar: **S.Anusha** Name of the Supervisor: **Dr.V. Swaroopa Rani**	13	Appraisal of some medicinal plants for the management of Diabetic complications through inhibition of aldose reductase and advanced glycation endproducts formation.	**(13/IAEC/UCPSc/KU/2022: CPCSEA 2018-23).**	Species:Wistar Total No.Apr: (115**)** *In Vitro* = (7**)** Toxicity Study:Nil Chpt.1= (54**)** Chpt.2= (54**)** Chpt.3= Nil	**Should be finished first then** Proceed with the study within a year from date of approval
14	Names of the PhD-Scholar: **S.Manjula** Name of the Supervisor: **Dr.G. Sammaiah**	14	Synthesis and evaluation of new Isatin Derivatives for their possible biological activities	**(14/IAEC/UCPSc/KU/2022: CPCSEA 2018-23).**	Strain: Rats male Species:Wistar Total No.Apr: (126**)** *In Vitro* = Nil Toxicity Study:Nil Chpt.1= (42**)** Chpt.2= (42**)** Chpt.3= (42**)**	***In vitro* Should be finished first then** Proceed with the study within a year from date of approval

By

Member Secretary
Institutional Animal Ethics Committee
KAKATIYA UNIVERSITY,
HANAMKONDA, 506009.

Protocol No :01
Name:M.Madhavi

Approval No: (01/IAEC/UCPSc/KU/2022:CPCSEA 2018-23)

Form B (per rule 8(a)* for Submission of Research Protocol (s)

Application for Permission for Animal Experiments

Application to be submitted to the CPCSEA, New Delhi after approval of Institutional Animal Ethics Committee (IAEC)

Section -I

1.	Name and address of establishment	University College Of Pharmaceutical Sciences Kakatiya University Warangal-506009(T.S)
2.	Registration number and date of registration.	1820/GO/Re/S/15/CPCSEA Date: 01-09-2015
3.	Name, address and registration number of breeder from which animals acquired (or to be acquired) for experiments mentioned in parts B & C	Vyas Labs,Amberpet,Hyderabad CPCSEA:2085/PO/RCBiBt/S/19/CPCS
4.	Place where the animals are presently kept (or proposed to be kept).	Animal house, University College of Pharmaceutical Sciences, KU, Warangal.
5.	Place where the experiment is to be performed (Please provide CPCSEA Reg. Number)	Animal house, University College of Pharmaceutical Sciences, KU, Warangal.
6.	Date and Duration of experiment.	5 Months
7.	Type of research involved (Basic Research / Educational/ Regulatory/ Contract Research)	Educational research

Signature

Name and Designation of Investigator
Prof .Y.NARSIMHA REDDY
Dean of the Pharmaceutical sciences
UCPSc, Kaktiya University.

Date:

Place:

Section -II

Protocol form for research proposals to be submitted to the Institutional Animal Ethics Committee/ CPCSEA, for new experiments or extensions of ongoing experiments using animals.

1. Project / Dissertation / Thesis Title: STUDIES ON PHARMACODYNAMIC AND PHARMACOKINETIC INTERACTION OF SOME PHYTOCHEMICALS WITH SELECTED ANTIDIABETIC, ANTIEPILEPTIC AND ANTIHYPERLIPIDEMIC DRUGS

2. Principal Investigator / Research Guide / Advisor:

   a. Name Prof. Y. Narsimha Reddy
   b. Designation: Professor and Dean of Pharmacy
   c. Dept / Div/ Lab: Pharmacology
   d. Telephone No: 9440705384
   e. E-mail Id: ynrku@yahoo.co.in
   f. Experience in Lab animal experimentation 28years

3. List of all individuals authorized to conduct procedures under this proposal.

   a. Name: M. Madhavi
   b. Designation: Research Scholar
   c. Department: Pharmacology
   d. Telephone No. 7989067254
   e. E-mail Id: haritha.madhavi@gmail.com
   f. Experience in Lab animal experimentation:12 years

4. Funding Source / Proposed Funding Source with complete address (Please attach the proof)
   AICTE sponsored QIP Ph.D.,
   AICTE,New Delhi.

5. Duration of the animal experiment.

   a. Date of initiation (Proposed) March 2022
   b. Date of completion (Proposed) July 2022

6. Describe details of study plan to justify the use of animals (Enclose Annexure)

7. Animals required

a. Species and Strain: Wistar Rats
b. Age and Weight: 200-250gms
c. Gender: Male and Female
d. Number to be used (Year-wise breakups and total figures needed to be given in tabular form) : 96

Year	No of groups	No of animals(n=6)
First year	5	30
Second Year	6	36
Third Year	5	30

e. Number of days each animal will be housed. 5 months

8. Rationale for animal usage

a. Why is animal usage necessary for these studies?

Since these were the preliminary studies, the activity of above drugs had to be confirmed by animal experiments. As the rats were easy to handle, easily available, easy to subject them for testing, and their nutrition resembled that of human, so, they were preferred for the usage in this study to determine the pharmacokinetic & pharmacodynamic interactions of some phytochemicals with selected drugs

b. Whether similar study has been conducted on *in vitro* models? If yes, describe the leading points to justify the requirement of animal experiment.
NO

c. Why are the particular species selected?

Wistar rats are reported to be used in these kind of studies

d. Why is the estimated number of animals essential?

The number of animals used in each chapter must be the minimum necessary to obtain valid and meaningful results and we consider six animals per group as adequate sample size and to get good statical result. We require 5 groups (n=6) of animals (total 5X 6 = 30) to conduct the study.

e. Are similar experiments conducted in the past in your establishment? **No**
f. If yes, justify why new experiment is required?
g. Have similar experiments been conducted by any other organization in same or other *in vivo* models? If yes, enclose the reference.

Similar experiments were not conducted.

9. Describe the procedures in detail:

a. Describe all invasive and potentially stressful non-invasive procedures that animals will be subjected to in the course of the experiments):NA
b. Furnish details of injections schedule Substances:
   Doses : 10mg/kg
   Sites : oral route
   Volumes : 0.3ml
c. Blood withdrawal Details:
   Volumes : 0.2ml
   Sites : Retro orbital
d. Radiation (dosage and schedules):NA
e. Nature of compound/Broad Classification of drug/NCE: chemical, phytochemical

10. Does the protocol prohibit use of anesthetic or analgesic for the conduct of painful procedures? If yes, justify. **No anesthetic is needed**

11. Will survival surgery be done? **Not required**

If yes, the following to be described. List and describe all surgical procedures (including methods of asepsis)
f. Names, qualifications and experience levels of personnels involved.
g. Describe post-operative care
h. Justify if major survival surgery is to be performed more thanonce on asingle animal.

12. Describe post-experimentation procedures. NA

i. Scope for Reuse: NA

j. Rehabilitation (Name and Address, where the animals are proposed to be rehabilitated) : NA

k. Describe method of Euthanasia (If required in the protocol): CO_2

l. Method of carcass disposal after euthanasia: The animals carcass is collected in

colour codet bins and sent to an authorized bio medical waste collection agency for final disposal.

13. Describe animal transportation methods if extra-institutional transport is envisaged.NA

14. Use of hazardous agents (use of recombinant DNA-based agents or potential human pathogens require documented approval of the Institutional Biosafety Committee (IBC). For each category, the agents and the biosafety level required, appropriate therapeutic measures and the mode of disposal of contaminated food, animal wastes and carcasses must be identified).
If, your project involved use of any of the below mentioned agent, attach copy of the approval certificates of the respective agencies: NA

(a) Radionucleotides (AERB)
(b) Microorganisms / Biological infectious Agents (IBSC)
(c) Recombinant DNA (RCGM)
(d) Any other Hazardous Chemical / Drugs

Investigator's declaration.

1. I certify that the research proposal submitted is not unnecessarily duplicative of previously reported research.

2. I certify that, I am qualified and have experience in the experimentation on animals.

3. For procedures listed under item 10, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.

4. I will obtain approval from the IAEC/ CPCSEA before initiating any changes in this study.

5. I certify that performance of experiment will be initiated only upon review and approval of scientific intent by appropriate expert body (Institutional Scientific Advisory Committee / funding agency / other body).

6. I certify that I will submit appropriate certification of review and concurrence for studies mentioned in point 14.

7. I shall maintain all the records as per format (Form D) and submit to Institutional Animal Ethics Committee (IAEC).

8. I certify that, I will not initiate the study before approval from IAEC/ CPCSEA received in writing. Further, I certify that I will follow the recommendations of IAEC/ CPCSEA.

9. I certify that I will ensure the rehabilitation policies are adopted (wherever required).

Signature

Name of Investigator

Date: 13/04/2022

Certificate

This is to certify that the project proposal no .....entitled … STUDIES ON PHARMACODYNAMIC AND PHARMACOKINETIC INTERACTION OF SOME PHYTOCHEMICALS WITH SELECTED ANTIDIABETIC, ANTIEPILEPTIC AND ANTIHYPERLIPIDEMIC DRUGS submitted by Dr./ Mr. / Ms. M,Madhavi has been approved/recommended by the IAEC of…UCPSC,KAKATIYA UNIVERSITY……(Organization) in its meeting held on……13/04/2022……. (date) and ……96 WISTAR RATS……(Number and Species of animals) have been sanctioned under this proposal for a duration of next ……12…….. months.

Authorized by	**Name**	**Signature**	**Date**
Chairman:	…….	……	……..
Member Secretary:	…….	……	……..
Main Nominee of CPCSEA:	…….	……	……..

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

STUDY PLAN

Title: STUDIES ON PHARMACODYNAMIC AND PHARMACOKINETIC INTERACTION OF SOME PHYTOCHEMICALS WITH SELECTED ANTIDIABETIC, ANTIEPILEPTIC AND ANTIHYPERLIPIDEMIC DRUGS.

CHAPTER-I: Pharmacodynamic And Pharmacokinetic Interaction of Phytochemical-I, Phytochemical-II, Phytochemical-III with Antidiabetic drug

METHOD-INDUCTION OF DIABETES

Wistar rats fasted over night and diabetes induced by administration of streptozotocin 50mg/kg in 0.1M sodium citrate buffer was administered I.P. rats were immediately administered with 5% dextrose to antagonize the rapid hypoglycemia effects.

Pharmacokinetic and Pharmacodynamic study:

- Group 1: Diabetic control
- Group 2: Pure antidiabetic drug will administer to rats
- Group 3: Phytochemical only will administer to rats
- Group 4: Phytochemical followed by drug will administered to rats for single dose interaction study
- Group 5: Phytochemical will administer for 7 days and on 8^{th} day phytochemical followed by drug will administered to rats for multiple dose interaction study

Blood samples will be collected from orbital puncture at time intervals between 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 hrs.
Estimation of Blood Glucose levels, Creatinine, BUN, Histopathological studies.

CHAPTER-II Pharmacodynamic And Pharmacokinetic Activity of Phytochemical-I, Phytochemical-II, Phytochemical-III with Antihyperlipidemic Drug

METHOD-INDUCTION OF HYPERLIPIDEMIA

High fat diet will be fed to rats for 28 successive days to induce hyperlipidemia

- Group 1: Normal control
- Group 2: HFD control Group: The rats will be fed HFD for 4 weeks after 4 weeks rats will be treated orally 1 ml of 0.5% CMC .

- Group 3: After 4 weeks of HFD feeding, the rats will be treated with standard drug
- Group 4: After 4 weeks of HFD feeding the rats will be treated with phytochemical
- Group 5: After 4 weeks of HFD feeding the rats will be treated with phytochemical followed by drug will administer to rats for single dose interaction study
  Group 6: After 4 weeks of HFD feeding the rats, phytochemical will administer for 7 days and on 8th day phytochemical followed by drug will administered to rats for multiple dose interaction study

Blood samples will be collected from orbital puncture at time intervals between 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 hrs.

Lipid Profile, Histopathological studies

CHAPTER-III. Pharmacodynamic And Pharmacokinetic Activity of Phytochemical-I, Phytochemical-II, Phytochemical-III with Anticonvulsant Drug

Induction of convulsions by MES method

MES method of evaluating anti-seizure activity of a drug against generalized tonic-clonic seizures (grandmal seizures). Screening of rats will used to test by subjecting to maximal electroshock using an electroconvulsiometer with an alternating current of 150 mA intensity for 0.2 seconds using trans-auricular electrodes. Rats which will give positive for MES seizures, identified by the development of characteristic tonic-clonic seizures are selected. Rats were kept in separate polypropylene cages for conditioning them to the laboratory environment for 3 days and to avoid any possible kindling effect. The 2 main parameters onset of tonic hind limb extension (THLE) and duration of THLE will be observed

- Group 1: Normal control
- Group 2: Pure drug will administer to rats
- Group 3: Phytochemical only will administer to rats
- Group 4: Phytochemical followed by drug will administer to rats for single dose interaction study
- Group 5: Phytochemical will administer for 7 days and on 8th day phytochemical followed by drug will administered to rats for multiple dose interaction study

Blood samples will be collected from retro orbital puncture at time intervals between 0, 0.5, 1, 2, 4,

6, 8, 12 and 24 hrs.

Blood CBP, SGOT, SGPT, Histopathological studies

Protocol No :02
Name:R.Rajendra Prasad

FormB (per rule8(a)*for Submission of Research Protocol(s)

Application for Permission for Animal Experiments

Application to be submitted to the CPCSEA, New Delhi after approval of Institutional Animal Ethics Committee (IAEC)

Section-I

1.	Name and address of establishment	University College of Pharmaceutical Sciences Kakatiya University Warangal – 506 009 (T.S)
2.	Registration number and date of registration.	1820/GO/Re/S/15/CPCSEA, Date: 01-09-2015
3.	Name, address and registration number of breeder from which animals acquired (or to be acquired) for experiments mentioned in parts B&C	Vyas Labs, Amberpet, Hyderabad CPCSEA:2085/PO/RCBiBt/S/19/CPCS
4.	Place where the animals are presently kept (or proposed to be kept).	Animal house, University College of Pharmaceutical Sciences, KU, Warangal
5.	Place where the experiment is to be performed (Please provide CPCSEA Reg.Number)	Animal house, University College of Pharmaceutical Sciences, KU, Warangal
6.	Date and Duration of experiment.	3 months
7.	Type of research involved (BasicResearch/Educational/Regulatory/ContractResearch)	Educational research

Date: 21-04-2022

Warangal:

Signature

Name and Designation of Investigator
Dr. Shayeda,
Asst. Professor.

Section-II

Protocol form for research proposals to be submitted to the Institutional Animal Ethics Committee/CPCSEA, for new experiments or extensions of ongoing experiments using animals.

1. Project/Dissertation/Thesis Title: DEVELOPMENT AND EVALUATION OF NANO FORMULATIONS FOR SOME DRUGS

2. PrincipalInvestigator/ResearchGuide/Advisor:
   a. Name: Dr. Shayeda
   b. Designation: Asst. Professor
   c. Dept/Div/Lab: Pharmaceutics
   d. Mobile No: 9849531109
   e. E-mailId: shayeda_ucpsc@yahoo.com
   f. Experience in Lab animal experimentation: 16 years

3. List of all individuals authorized to conduct procedures under this proposal.
   a. Name: R. Rajendra prasad
   b. Designation: Research Scholar
   c. Department: Pharmaceutics
   d. TelephoneNo. 8341990882
   e. E-mail: prasad.chinna225@gmail.com
   f. Experience in Lab animal experimentation: 3years

4. Funding Source/Proposed Funding Source with complete address (Please attach the proof)
   AICTE sponsored QIP-AICTE (Ph.D), New Delhi.

5. Duration of the animal experiment.
   a. Date of initiation (Proposed): April, 2022
   b. Date of completion(Proposed): June, 2022

6. Describe details of study plan to justify the use of animals (Enclose Annexure):

   Separate paper attached.

7. Animals required

a. Species and Strain: Wistar Rats
b. Age and Weight: 200-250gms
c. Gender: Male
d. Number to be used (Year-wise breakups and total figures needed to be given in tabular form) : 24
e. Number of days each animal will be housed: 1 month

8. Rationale for animal usage

a. Why is animal usage necessary for these studies?

To determine the oral bioavailability of optimized Doxepin SLN & Buspirone Hcl SLN formulations in comparison to the marketed product.

b. Whether similar study has been conducted on *in vitro* models? If yes, describe the leading point's to justify the requirement of animal experiment: **NO**

c. Why are the particular species selected?
Wistar rats are reported to be used in these kinds of studies.

d. Why is the estimated number of animals essential?

The number of animals used in each chapter must be the minimum necessary to obtain valid and meaningful results and we consider six animals per group as adequate sample size and to get good statical result. We require 4 groups (n=6) of animals (total 4X6=24) to conduct the study.

9. Are similar experiments conducted in the past in your establishment? **No**

a. If yes, justify why new experiment is required?
b. Have similar experiments been conducted by any other organization in same or other *in vivo* models? If yes, enclose the reference.
Similar experiments were not conducted.

10. Describe the procedures in detail:

a. Describe all invasive and potentially stressful non-invasive procedures that animals will be subjected to in the course of the experiments): **NA**
b. Furnish details of injections schedule Substances:
Doses: 0.4mg/kg & 2mg/kg
Sites: Oral route
Volumes: 0.3ml
c. Blood withdrawal Details:
Volumes: 0.2ml
Sites: Retro orbital

d. Radiation(dosage and schedules): NA
e. Nature of compound/Broad Classification of drug/NCE: Chemical

11. Does the protocol prohibit use of anesthetic or analgesic for the conduct Of painful procedures? If yes, justify. **No anesthetic is needed**

12. Will survival surgery be done? **Not required**

If yes, the following to be described. List and describe all surgical procedures (including methods of asepsis)

a. Names, qualifications and experience levels of personals involved.
b. Describe post-operative care
c. Justify if major survival surgery is to be performed more than once on a single animal.

13. Describe post-experimentation procedures: NA

a. Scope for Reuse: NA

b. Rehabilitation (Name and Address, where the animals are proposed to be rehabilitated): NA

c. Describe method of Euthanasia (If required in the protocol): CO_2

d. Method of carcass disposal after euthanasia: The animal's carcass is collected in color coded bins and sent to an authorized bio medical waste collection agency for final disposal.

14. Describe animal transportation methods if extra-institutional transport is Envisaged: NA

15. Use of hazardous agents (use of recombinant DNA-based agents or potential Human pathogens required documented approval of the Institutional Bio safety Committee (IBC). For each category, the agents and the bio safety level required, appropriate therapeutic measures and the mode of disposal of contaminated food, animal wastes and carcasses must be identified).

If, your project involved use of any of the below mentioned agent, attach copy of the approval certificates of the respective agencies: **NA**

(a) Radionucleotides(AERB)
(b) Microorganisms/Biological infectious Agents (IBSC)
(c) RecombinantDNA(RCGM)
(d) AnyotherHazardousChemical /Drugs

NA

Investigator's declaration.

1. I certify that the research proposal submitted is not unnecessarilyduplicativeofpreviouslyreportedresearch.

2. I certify that, I am qualified and have experience in the experimentation on animals.

3. For procedures listed under item 10, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.

4. I will obtain approval from the IAEC/CPCSEA before initiating any changes in this study.

5. I certify that performance of experiment will be initiated only upon review and approval of scientific intent by appropriate expert body(InstitutionalScientificAdvisoryCommittee/fundingagency/other body).

6. I certify that I will submit appropriate certification of review and concurrence for studies mentioned in point 14.

7. I shall maintain all the records as per format(Form D) and submit to Institutional Animal Ethics Committee (IAEC).

8. I certify that, I will not initiate the study before approval from IAEC/ CPCSEA received in writing. Further, I certify that I will follow the recommendations of IAEC/CPCSEA.

9. I certify that I will ensure the rehabilitation policies are adopted (Wherever required).

Signature

Name of Investigator

Date: 21-04-2022

Certificate

This is to certify that the project proposal no ..... entitled **Development And Evaluation of Nano Formulations For Some Drugs** submitted by Dr./Mr./Ms **R. Rajendra Prasad** has been approved/recommended by the IAEC of..... (Organization) in its meeting held on..... (Date) and ..... (Number and Species of animals) have been sanctioned under this proposal for a duration of next.....months.

Authorizedby	**Name**	**Signature**	**Date**
Chairman:	.....	.....	.....
MemberSecretary:	.....	.....	.....
Main Nominee of CPCSEA:	.....	.....	.....

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

STUDY PLAN

Objective: To conduct Pharmacokinetic studies of Doxepin loaded solid lipid nanoparticles & Buspirone loaded solid lipid nanoparticles.

Drug profile:

Doxepin is used to treat depression and anxiety. Doxepin is in a class of medications called tricyclic antidepressants.

Pharmacokinetic Parameters

Bioavailability : 30%
Half-life : 8-20 hrs
Metabolism : Hepatic

Drug profile:

Buspirone is used to treat anxiety disorders or in the short-term treatment of symptoms of anxiety.

Pharmacokinetic Parameters

Bioavailability: 5%
Half-life : 6 hrs
Metabolism : Hepatic

Protocol for pharmacokinetic study

- The male wistar rats weighing 200 to 250 gm will be divided into 2 groups, each consisting of 6 rats (2* 6 = 12). The following treatments will be given to these groups.

Group A: Doxepin marketed tablets by oral delivery
Group B : Doxepin optimized SLN formulation by oral delivery
Blood samples will be withdrawn from retro-orbital sinus at time intervals of 0, 0.5, 1, 2, 4, 6, 8, 12, 18 and 24 hrs.

- The male wistar rats weighing 200 to 250 gm will be divided into 2 groups, each consisting of 6 rats (2* 6 = 12). The following treatments will be given to these groups.

Group A: Buspirone Hcl coarse suspension by oral delivery
Group B : Buspirone Hcl optimized formulation by oral delivery
Blood samples will be withdrawn from retro-orbital sinus at time intervals of 0, 0.5, 1, 2, 4, 6, 8, 12, 18 and 24 hrs.

The optimized SLN formulations and the marketed available dosage form was to be given to each group of rats and the blood samples are to be collected from the retro orbital vein of rats.
The collected blood samples are to be centrifuged to separate the serum.
All the samples were analyzed by HPLC to estimate the amount of drug.

Approval No: (03/IAEC/UCPSc/KU/2022:CPCSEA 2018-23)

Protocol No :03
Name:Amma Venu

Amma. Venu

Form B (per rule 8(a)* for Submission of Research Protocol (s)

Application for Permission for Animal Experiments

Application to be submitted to the CPCSEA, New Delhi after approval of Institutional Animal Ethics Committee (IAEC)

Section -I

1.	Name and address of establishment	University College Of Pharmaceutical Sciences Kakatiya University Warangal-506009(T.S)
2.	Registration number and date of registration.	1820/GO/Re/S/15/CPCSEA Date: 01-09-2015
3.	Name, address and registration number of breeder from which animals acquired (or to be acquired) for experiments mentioned in parts B & C	Vyas Labs,Amberpet,Hyderabad CPCSEA:2085/PO/RCBiBt/S/19/CPCS
4.	Place where the animals are presently kept (or proposed to be kept).	Animal house, University College of Pharmaceutical Sciences, KU, Warangal.
5.	Place where the experiment is to be performed (Please provide CPCSEA Reg. Number)	Animal house, University College of Pharmaceutical Sciences, KU, Warangal.
6.	Date and Duration of experiment.	8 Months
7.	Type of research involved (Basic Research / Educational/ Regulatory/ Contract Research)	Educational research

Signature

Name and Designation of Investigator
Prof .Y.NARSIMHA REDDY
Dean of the Pharmaceutical sciences
UCPSc, Kaktiya University.

Date: 13/04/2022

Place: Warangal

Section -II

Protocol form for research proposals to be submitted to the Institutional Animal Ethics Committee/ CPCSEA, for new experiments or extensions of ongoing experiments using animals.

1. Project / Dissertation / Thesis Title: Studies on Nephroprotective and Anti-oxidant activity of some Indian medicinal plants.

2. Principal Investigator / Research Guide / Advisor:

   a. Name:prof .Y.Narsimha Reddy.
   b. Designation: Professor and Dean of the Pharmaceutical sciences
   c. Dept / Div/ Lab:Pharmacology
   d. Telephone No:9440705384
   e. E-mail Id:ynrku@yahoo.co.in
   f. Experience in Lab animal experimentation:26years

3. List of all individuals authorized to conduct procedures under this proposal.

   a. Name: Amma Venu
   b. Designation:Research scholar
   c. Department:Pharmacology
   d. Telephone No: 9396108100
   e. E-mail Id:venu.amma1966@gmail.com
   f. Experience in Lab animal experimentation: 10 years

4. Funding Source / Proposed Funding Source with complete address (Please attach the proof)
   AICTE sponsored QIP -Ph.D,
   AICTE,New Delhi.

5. Duration of the animal experiment: 8 months

   a. Date of initiation (Proposed) -June
   b. Date of completion (Proposed) -January

6. Describe details of study plan to justify the use of animals (Enclose Annexure)

7. Animals required

a. Species and Strain: **Wistar rats**
b. Age and Weight: **150-200grams**
c. Gender: **Male**
d. Number to be used (Year-wise breakups and total figures needed to begiven in tabular form): **120**

e. Number of days each animal will be housed: **21 dyas**

8. Rationale for animal usage

a. Why is animal usage necessary for these studies?
Animals are used in scientific research to help us understand our own bodies and how they work. Animals are also used to safety test potential medicines before they are tested in people and to check the safety of other chemicals.

b. Whether similar study has been conducted on *in vitro* models? If yes, describe the leading points to justify the requirement of animal experiment.- **No**

c. Why are the particular species selected?
Male Wistar rats are reported to be used in these kind of animal model studies.

d. Why is the estimated number of animals essential?
We require 5 groups (n=6) of animals (total 5X 6 = 30) to conduct the study. To have statistical significance, these are essential.

e. Are similar experiments conducted in the past in your establishment?
No.

f. If yes, justify why new experiment is required?
NA.

g. Have similar experiments been conducted by any other organization in same or other *in vivo* models? If yes, enclose the reference.

Similar experiments were not conducted.

9. Describe the procedures in detail:

a. Describe all invasive and potentially stressful non-invasive procedures that animals will be subjected to in the course of the experiments)-NA
b. Furnish details of injections schedule Substances:
   Doses :100mg/kg
   Sites :Oral route
   Volumes :0.3ml
c. Blood withdrawal Details:
   Volumes :0.3ml
   Sites : retro orbital
d. Radiation (dosage and schedules):NA
e. Nature of compound/Broad Classification of drug/NCE: Plant extract

10. Does the protocol prohibit use of anesthetic or analgesic for the conduct of painful procedures? If yes, justify.

No anaesthetic is needed

11. Will survival surgery be done? **Not required**

If yes, the following to be described.

a. List and describe all surgical procedures (including methods of asepsis)
b. Names, qualifications and experience levels of personnels involved.
c. Describe post-operative care

Justify if major survival surgery is to be performed more than once on asingle animal

12. Describe post-experimentation procedures.

a. Scope for Reuse :NA

b. Rehabilitation (Name and Address, where the animals are proposed to be rehabilitated) :NA

c. Describe method of Euthanasia (If required in the protocol) : CO_2

d. Method of carcass disposal after euthanasia. : The animals carcass is collected in colour codet bins and sent to an authorized bio medical waste collection agency for final disposal.

13. Describe animal transportation methods if extra-institutional transport is envisaged. **NA**

14. Use of hazardous agents (use of recombinant DNA-based agents or potential human pathogens requires documented approval of the Institutional Biosafety Committee (IBC). For each category, the agents and the biosafety level required, appropriate therapeutic measures and the mode of disposal of contaminated food, animal wastes and carcasses must be identified).
If, your project involved use of any of the below mentioned agent, attach copy of the approval certificates of the respective agencies: **NA**

(a) Radionucleotides (AERB)
(b) Microorganisms / Biological infectious Agents (IBSC)
(c) Recombinant DNA (RCGM)
(d) Any other Hazardous Chemical / Drugs

No hazardous agents

Investigator's declaration.

1. I certify that the research proposal submitted is not unnecessarily duplicative of previously reported research.

2. I certify that, I am qualified and have experience in the experimentation on animals.

3. For procedures listed under item 10, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.

4. I will obtain approval from the IAEC/ CPCSEA before initiating any changes in this study.

5. I certify that performance of experiment will be initiated only upon review and approval of scientific intent by appropriate expert body (Institutional Scientific Advisory Committee / funding agency / other body).

6. I certify that I will submit appropriate certification of review and concurrence for studies mentioned in point 14.

7. I shall maintain all the records as per format (Form D) and submit to Institutional Animal Ethics Committee (IAEC).

8. I certify that, I will not initiate the study before approval from IAEC/ CPCSEA received in writing. Further, I certify that I will follow the recommendations of IAEC/ CPCSEA.

9. I certify that I will ensure the rehabilitation policies are adopted (wherever required).

Signature

Name of Investigator

Date: 13/04/2022

Certificate

This is to certify that the project proposal no.....entitled **Studies on Nephroprotective and Anti-oxidant activity of some Indian medicinal plants**. submitted by Dr./ Mr. / Ms. **Amma Venu** has been approved/recommended by the IAEC of **UCPSc Kakatiya University**(Organization) in its meeting held on **13/04/2022** (Date) and **120 Wistar Rats** (Number and Species of animals) have been sanctioned under this proposal for a duration of next **12** months.

Authorized by	**Name**	**Signature**	**Date**
Chairman:	…….	……	……..
Member Secretary:	…….	……	……..
Main Nominee of CPCSEA:	…….	……	……..

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

STUDY DESIGN

Aim: Studies on Nephroprotective and Anti-oxidant activity of some Indian medicinal plants.

Method: The aim of this study was to investigate the nephroprotective properties of some medicinal plant extracts using a Gentamicin induced nephrotoxicity rat model.

The male wistar rats randomly divided into five experimental groups of six rats.

- Group 1: control: oral treatment of vehicle 10 ml/kg daily for 8 days.
- Group 2: Gentamicin alone: oral treatment of vehicle 10 ml/kg daily for 8 days with intraperitoneal injection of Gentamicin 100 mg/kg daily for 8 days.
- Group 3: oral treatment of extract 100 mg/kg body weight and intraperitoneal injection of Gentamicin 100 mg/kg 1 hr after the extract treatment daily for 8 days
- Group 4: oral treatment of extracts 200 mg/kg body weight and intraperitoneal injection of Gentamicin 100mg/kg 1 hr after the extract treatment daily for 8 days.
- Group 5: oral treatment of extracts 400 mg/kg body weight and intraperitoneal injection of Gentamicin 100 mg/kg 1 hr after the extract treatment daily for 8 days.

After dosing on the day 8, individual rats were placed in separate metabolic cages for 24 h for urine collection to determine urine creatinine content.

Biochemical Estimations

Blood samples were collected via retro-orbital puncture at the end of 24 h; the serum was rapidly separated and processed for determination of serum creatinine, serum urea, and blood urea nitrogen (BUN) using of Span Diagnostic kits. Body weight of animal was also recorded.

Histopathological Studies

Rats were sacrificed and both kidneys were isolated from each rat. The kidneys were processed for histopathological examination. The kidney were excised quickly and fixed in 10% formalin and stained with hemotoxylin and eosin and then observed under microscope for degeneration, fatty changes, necrotic changes, and evidence of nephrototoxicity if any.

Anti-oxidant activity

Free radical scavenging assays

1. DPPH assay
2. Determination of super oxide anion ($O_2^{\bullet -}$) radical scavenging activity
3. Determination of peroxide (H_2O_2) radical scavenging activity

CHAPTER-1

All the animals were divided into 5 groups containing 6 animals each (n=6) 5×6=30

S.no	Groups	Drug and Plant Extract
1	Group-I	Saline
2	Group-II	Gentamycin
3	Group-III	Plant extract 1
4	Group-IV	Plant extract 1
5	Group-V	Plant extract 1

CHAPTER-2

All the animals were divided into 5 groups containing 6 animals each (n=6)5×6=30

S.no	Groups	Drug and Plant Extract
1	Group-I	Saline
2	Group-II	Gentamycin
3	Group-III	Plant extract 2
4	Group-IV	Plant extract 2
5	Group-V	Plant extract 2

CHAPTER-3

All the animals were divided into 5 groups containing 6 animals each (n=6) 5×6=30

S.no	Groups	Drug and Plant Extract
1	Group-I	Saline
2	Group-II	Gentamycin
3	Group-III	Plant extract 3
4	Group-IV	Plant extract 3
5	Group-V	Plant extract 3

- Number of animals for acute toxicity=30

Protocol No :04
Name:Swathi Jakku

Approval No: (04/IAEC/UCPSc/KU/2022:CPCSEA 2018-23)

Form B (per rule 8(a)* for Submission of Research Protocol (s)Application for Permission for Animal Experiments

Application to be submitted to the CPCSEA, New Delhi after approval of InstitutionalAnimal Ethics Committee (IAEC)

Section -I

1.	Name and address of establishment	University College Of Pharmaceutical Sciences Kakatiya University Warangal-506009(T.S)
2.	Registration number and date of registration.	1820/GO/Re/S/15/CPCSEA Date:01-09-2015
3.	Name, address and registration numberof breeder from which animals acquired (or to be acquired) for experiments mentioned in parts B & C	Vyas Labs,Amberpet,Hyderabad CPCSEA:2085/PO/RCBiBt/S/19/CPCS
4.	Place where the animals are presently kept (or proposed to be kept).	Animal house, University College of Pharmaceutical Sciences, KU, Warangal.
5.	Place where the experiment is to be performed (Please provide CPCSEA Reg. Number)	Animal house, University College of Pharmaceutical Sciences, KU, Warangal.
6.	Date and Duration of experiment.	6 Months
7.	Type of research involved (Basic Research / Educational/ Regulatory/ Contract Research)	Educational research

Signature

Name and Designation of Investigator

Date: 13-04-2022
Place: Warangal.

J. KRISHNAVENI

Section II

Protocol form for research proposals to be submitted to the Institutional Animal Ethics Committee/ CPCSEA, for new experiments or extensions of ongoing experiments using animals.

1. Project / Dissertation / Thesis Title: Development of Intranasal formulations for substances of natural origin for the treatment of neurodegenerative disorders

2. Principal Investigator / Research Guide / Advisor:

a. Name: Dr. J. Krishnaveni
b. Designation: Associate Professor of Pharmacy
c. Dept / Div/ Lab: Pharmaceutics
d. Telephone No: 9247161127
e. E-mail Id: Krishnaveni.janapareddi@gmaill.com
f. Experience in Lab animal experimentation: 15years

2. List of all individuals authorized to conduct procedures under this proposal.

a. Name: Swathi Jakku
b. Designation: Research scholar
c. Department: Pharmaceutics
d. Telephone No:9963391339
e. E-mail Id:swathijakku@gmail.com
f. Experience in Lab animal experimentation: 2 years

3. Funding Source / Proposed Funding Source with complete address (Please attach the proof)

AICTE sponsored QIP Ph.D,
AICTE,New Delhi.

4. Duration of the animal experiment: 6 months

a. Date of initiation (Proposed) -March
b. Date of completion (Proposed) -August

5. Describe details of study plan to justify the use of animals (Enclose Annexure)

6. Animals required

a. Species and Strain: Wistar rats
b. Age and Weight: **200-250grams**
c. Gender: **Male**
d. Number to be used (Year-wise breakups and total figures needed to be given in tabular form): **216 rats**

e. Number of days each animal will be housed:1 Month

7. Rationale for animal usage

a. Why is animal usage necessary for these studies?
Humans and animals share hundreds of illnesses, so animals often act as models for the study of human disease. We can study how bodies work by running experiments in animals that would be impossible in human volunteers, and this is where most research animals are used to determine the Pharmacodynamic and Pharmacokinetic activity of the Cannabidiol, Jyotishmathi oil and Bacopa monnieri oil

b. Whether similar study has been conducted on *in vitro* models? If yes, describe the leading points to justify the requirement of animal experiment.- **No**

c. Why are the particular species selected?
Male wister rats to be used in these kind of animal model studies.

d. Why is the estimated number of animals essential?
We require 5 groups (n=6) of animals (total 5X 6 = 30) to conduct the study.
To have statistical significance,these are essential.

e. Are similar experiments conducted in the past in your establishment?
No.

f. If yes, justify why new experiment is required?
NA.

g. Have similar experiments been conducted by any other organization in same or other *in vivo* models? If yes, enclose the reference.

Similar experiments were not conducted.

8. Describe the procedures in detail:

a. Describe all invasive and potentially stressful non-invasive procedures that animals will be subjected to in the course of the experiments)-NA
b. Furnish details of injections schedule Substances: Doses
: 20mg oral, 5mg Nasal
Sites : Oral and Nasal route

Volumes : as per rat weight

c. Blood withdrawal Details:
   Volumes :0.3ml
   Sites : Retro orbital plexus
d. Radiation (dosage and schedules):NA
e. Nature of compound/Broad Classification of drug/NCE: oils from natural origin.

9. Does the protocol prohibit use of anesthetic or analgesic for the conduct of painful procedures? If yes, justify.

No anaesthetic is needed

10. Will survival surgery be done? **Not required**

If yes, the following to be described.

a. List and describe all surgical procedures (including methods of asepsis)
b. Names, qualifications and experience levels of personnels involved.
c. Describe post-operative care
d. Justify if major survival surgery is to be performed more than once on a single animal.

11. Describe post-experimentation procedures. **NA**

a. Scope for Reuse : NA

b. Rehabilitation (Name and Address, where the animals are proposed to be rehabilitated) : NA

c. Describe method of Euthanasia (If required in the protocol) :Co_2

d. Method of carcass disposal after euthanasia. : The animals carcass is collected in colour codet bins and sent to an authorized biomedical waste collection agency for final disposal.

12. Describe animal transportation methods if extra-institutional transport is envisaged. **NA**

13. Use of hazardous agents (use of recombinant DNA-based agents or potential human pathogens requires documented approval of the Institutional Biosafety Committee (IBC). For each category, the agents and the biosafety level required, appropriate therapeutic measures and the mode of disposal of contaminated food, animal wastes and carcasses must be identified).
If, your project involved use of any of the below mentioned agent, attach copy of the approval certificates of the respective agencies: **NA**

(a) Radionucleotides (AERB)
(b) Microorganisms / Biological infectious Agents (IBSC)
(c) Recombinant DNA (RCGM)
(d) Any other Hazardous Chemical / Drugs

No hazardous agents

Investigator's declaration.

1. I certify that the research proposal submitted is not unnecessarily duplicative of previously reported research.

2. I certify that, I am qualified and have experience in the experimentation on animals.

3. For procedures listed under item 10, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.

4. I will obtain approval from the IAEC/ CPCSEA before initiating any changes in this study.

5. I certify that performance of experiment will be initiated only upon review and approval of scientific intent by appropriate expert body (Institutional Scientific Advisory Committee / funding agency / other body).

6. I certify that I will submit appropriate certification of review and concurrence for studies mentioned in point 14.

7. I shall maintain all the records as per format (Form D) and submit to Institutional Animal Ethics Committee (IAEC).

8. I certify that, I will not initiate the study before approval from IAEC/ CPCSEA received in writing. Further, I certify that I will follow the recommendations of IAEC/ CPCSEA.

9. I certify that I will ensure the rehabilitation policies are adopted (wherever required).

Signature

Name of Investigator

Date:

Certificate

This is to certify that the project proposal no..... entitled **Development of Intranasal formulations for substances of natural origin for the treatment of neurodegenerative disorders** submitted by Dr./ Mr. / Ms **Swathi Jakku** has been approved/recommended by the IAEC of **UCPSc**, **Kakatiya University**(Organization) in its meeting held **on 13-04-2022** (date) and **216 rats and Male sister rats**(Number and Species of animals) have been sanctioned under this proposal for a duration of Next **1 2** months.

Authorized by	**Name**	**Signature**	**Date**
Chairman:	…….	……	……..
Member Secretary:	…….	……	……..
Main Nominee of CPCSEA:	…….	……	……..

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

STUDY PLAN

Title: Development of Intranasal formulations for substances of natural origin for the treatment of Neurodegenerative disorders

Objective: To evaluate the Pharmacodynamic and Pharmacokinetic activity of natural susbstances for Parkinson's disease and Alzheimer's disease via nasal route comparison to standard drugs through Oral route.

CHAPTER -1

Anti-Parkinson's activity

The male wistar rats weighing 300 to 350 gm will be divided into 5 groups, each group contains 6 rats. Total 6*5 = 30 Rats.

S.no	Groups	Drug	Route
1	Group -1 Normal control	PBS	
2	Group-2 Positive control	Rotenone	I.P
3	Group-3 Standard drug	Rotenone+Levodopa	Oral
4	Group-4Optimized Formulation	Rotenone+ Optimized Formulation	Oral
5	Group-5Optimized Formulation	Rotenone+Optimized Formulation	Nasal

THREE DRUGS: Cannabidiol, Jyotishmati oil and Bacopa monnieri oil 30*3=90

Rats

Pharmacokinetic study

Pharmacokinetic studies in plasma and brain of optimized microemulsion via nasal route were evaluated in comparison tothe oral route(**18 rats).**

CHAPTER -2

Anti-Alzheimer's Activity (Pharmacodynamic Study)

S.no	Groups	Drug	Route
1	Group -1 Normal control	PBS	
2	Group-2 Positive control	Aluminum chloride	I.P
3	Group-3Standard drug	Aluminumchloride+ Donepazil	Oral
4	Group-4Optimized Formulation	Aluminum Chloride +Optimized formulation	Oral
5	Group-5Optimized Formulation	Aluminum Chloride+ Optimized formulatiom	Nasal

THREE DRUGS
30*3=90 Rats

Pharmacokinetic study

Pharmacokinetic studies in plasma and brain of optimized microemulsion via nasal route were evaluated incomparison to the oral route(**18 rats**).

Protocol No :05
Name:K.Rajeshwari

Approval No: (05/IAEC/UCPSc/KU/2022:CPCSEA 2018-23)

Form B (per rule 8(a)* for Submission of Research Protocol (s)

Application for Permission for Animal Experiments

Application to be submitted to the CPCSEA, New Delhi after approval of Institutional Animal Ethics Committee (IAEC)

Section -I

1.	Name and address of establishment	University college of pharmaceutical sciences Kakatiya University, Warangal
2.	Registration number and date of registration.	1820/GO/RE/S/15/CPCSEA, Dt.01-09-2015
3.	Name, address and registration number of breeder from which animals acquired (or to be acquired) for experiments mentioned in parts B & C	Sainath agencies, Hyderabad
4.	Place where the animals are presently kept (or proposed to be kept).	University college of pharmaceutical sciences Kakatiya University, Warangal
5.	Place where the experiment is to be performed (Please provide CPCSEA Reg. Number)	Animal house, University college of pharmaceutical sciences, K U, Warangal
6.	Date and Duration of experiment.	12 months
7.	Type of research involved (Basic Research / Educational/ Regulatory/ Contract Research)	Educational research

Signature

Name and Designation of Investigator
Dr. V. SWAROOPA RANI
ASSOCIATE PROFESSOR
UCPSC Kakatiya university

Date: 13/4/2022
Place: Warangal

Section -II

Protocol form for research proposals to be submitted to the Institutional Animal Ethics Committee/ CPCSEA, for new experiments or extensions of ongoing experiments using animals.

1. Project / Dissertation / Thesis Title: STUDIES ON BIOENHANCING ABILITY OF SOME PHYTOCHEMICALS ON SOME CYP and P-gp SUBSTRATE DRUGS

2. Principal Investigator / Research Guide / Advisor:

   a. Name: Dr. V. Swaroopa rani
   b. Designation: Associate professor
   c. Dept / Div/ Lab: Pharmacognosy dept.
   d. Telephone No. 7981754379
   e. E-mail Id: swarooparanivanapatla@gmail.com
   f. Experience in Lab animal experimentation: 15 yrs

3. List of all individuals authorized to conduct procedures under this proposal.

   a. Name : K Rajeshwari
   b. Designation: Research Scholar
   c. Department: Pharmacognosy dept.
   d. Telephone No: 9908998286
   e. E-mail Id: raji.kampelli8286@gmail.com
   f. Experience in Lab animal experimentation: 14 yrs

4. Funding Source / Proposed Funding Source with complete address (Please attach the proof)

   AICTE sponsored QIP Ph.D.,
   AICTE, New Delhi.

5. Duration of the animal experiment: 12 months

   a. Date of initiation (Proposed) 15/03/2022
   b. Date of completion (Proposed) 15/03/2023

6. Describe details of study plan to justify the use of animals (Enclose Annexure)

7. Animals required

a. Species and Strain: Wistar rat
b. Age and Weight: 200-250 gm
c. Gender: Male
d. Number to be used (Year-wise breakups and total figures needed to begiven in tabular form) 90 animals

Method	Drug I	Drug II	Drug III
In-vitro	30	30	30

e. Number of days each animal will be housed. 1 Month.

8. Rationale for animal usage

a. Why is animal usage necessary for these studies?

Since these were the preliminary studies, the activity of above drugs had to be confirmed by animal experiments. As the rats were easy to handle, easily available, easy to subject them for testing, and their nutrition resembled that of human, so, they were preferred for the usage in this study to determine the pharmacokinetic interactions of some phytochemicals with selected drugs

b. Whether similar study has been conducted on *in vitro* models? If yes, describe the leading points to justify the requirement of animal experiment.
NO

c. Why are the particular species selected?
Male wistar rats are reported to be used

d. Why is the estimated number of animals essential?
The number of animals used in each chapter must be the minimum necessary to obtain valid and meaningful results and we consider six animals per group as adequate sample size and to get good statistical result. We require 5 groups (n=6) of animals (total 5X 6 = 30) for *in-vitro* and for *in-vivo* & *in-situ* (total 5X 6 = 30) and a total of 60 animals for each chapter. A total of 180 animals for 3 study protocols

e. Are similar experiments conducted in the past in your establishment? No

f. If yes, justify why new experiment is required?

g. Have similar experiments been conducted by any other organization in same or other *in vivo* models? If yes, enclose the reference:

Similar experiments were not conducted

9. Describe the procedures in detail:
In-vitro and *in-vivo* procedures that animals will be subjected to in the course of the experiments

a. Describe all invasive and potentially stressful non-invasive procedures that animals will be subjected to in the course of the experiments)
b. Furnish details of injections schedule Substances:

Name	**Drug I**	**Drug II**	**Drug III**
Dose(mg/kg)	1 mg/kg	60 mg/kg	10 mg/kg
Route/Site	Oral	Oral	Oral
Volume(ml)	As per rat weight	As per rat weight	As per rat weight
Frequency	Single dose	Single dose	Single dose
Volume(ml)	0.5 ml	0.5 ml	0.5 ml
Site	Tail vein	Tail vein	Tail vein

c. Radiation (dosage and schedules): NA
d. Nature of compound/Broad Classification of drug/NCE: Chemical, Phytochemical

10. Does the protocol prohibit use of anesthetic or analgesic for the conduct of Painful procedures? If yes, justify.
No anaesthetic is needed

11. Will survival surgery be done? **Not required**
If yes, the following to be described.
a. List and describe all surgical procedures (including methods of asepsis)
b. Names, qualifications and experience levels of personnels involved.
c. Describe post-operative care
d. Justify if major survival surgery is to be performed more than once on asingle animal.

12. Describe post-experimentation procedures.

a. Scope for Reuse No scope of reuse :

b. Rehabilitation (Name and Address, where the animals are proposed to be rehabilitated) : NA

c. Describe method of Euthanasia (If required in the protocol): Co2

d. Method of carcass disposal after euthanasia: The animals carcass is collected in colour codet bins and sent to an authorized bio medical waste collection agency for final disposal

13. Describe animal transportation methods if extra-institutional transport is Envisaged: Not applicable

14. Use of hazardous agents (use of recombinant DNA-based agents or potential human pathogens requires documented approval of the Institutional Biosafety Committee (IBC). For each category, the agents and the biosafety level required, appropriate therapeutic measures and the mode of disposal of contaminated food, animal wastes and carcasses must be identified).
If, your project involved use of any of the below mentioned agent, attach copy of the approval certificates of the respective agencies:

(a) Radionucleotides (AERB) :NA
(b) Microorganisms / Biological infectious Agents (IBSC): NA
(c) Recombinant DNA (RCGM): NA
(d) Any other Hazardous Chemical / Drugs: No Hazardous Chemicals

Investigator's declaration.

1. I certify that the research proposal submitted is not unnecessarily duplicative of previously reported research.

2. I certify that, I am qualified and have experience in the experimentation on animals.

3. For procedures listed under item 10, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.

4. I will obtain approval from the IAEC/ CPCSEA before initiating any changes in this study.

5. I certify that performance of experiment will be initiated only upon review and approval of scientific intent by appropriate expert body (Institutional Scientific Advisory Committee / funding agency / other body).

6. I certify that I will submit appropriate certification of review and concurrence for studies mentioned in point 14.

7. I shall maintain all the records as per format (Form D) and submit to Institutional Animal Ethics Committee (IAEC).

8. I certify that, I will not initiate the study before approval from IAEC/ CPCSEA received in writing. Further, I certify that I will follow the recommendations of IAEC/ CPCSEA.

9. I certify that I will ensure the rehabilitation policies are adopted (wherever required).

Signature: Swaroopa

Name of Investigator: Dr. V. Swaroopa Rani

Date: Associate professor.
UCPSC, KU, WGL.
13/4/22

Certificate

This is to certify that the project proposal no .....entitled ..... submitted by Dr./ Mr. / Ms. .....

Has been approved/recommended by the IAEC of.....(Organization) in its meeting held on..... (date) and ..... (Number and Species of animals) have been sanctioned under this proposal for a duration of next ..... months.

Authorized by	Name	Signature	Date
Chairman:	.....	.....	.....
Member Secretary:	.....	.....	.....
Main Nominee of CPCSEA:	.....	.....	.....

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

Study plan

Objective: To improve the Bioavailability of some poor bioavailable drugs by some herbal bioenhancers

Methods:

Study I

In-vitro non everted sac method

Rats were grouped up to 5 (n=6) after overnight fasting intestines were isolated under anesthesia by using thiopental sodium (50mg/kg/i.p), and ice-cold saline was used to flush. The rats were exsanguinated, the jejunum and ileum portions isolated and a segment of each was cut into a length of 10cm for preparation of the sac for the experiment.

In this a total of 30 (5x6) animals will be used for each study protocol

Group I: Control

Group II: Veerapamil

Group III: Lopinavir

Group IV: Sinomenine +Lopinavir

Group V: Veerapamil+ Lopinavir

Study II

In-vitro non everted sac method

Rats were grouped up to 5 (n=6) after overnight fasting intestines were isolated under anesthesia by using thiopental sodium (50mg/kg/i.p), and ice-cold saline was used to flush. The rats were exsanguinated, the jejunum and ileum portions isolated and a segment of each was cut into a length of 10cm for preparation of the sac for the experiment.

In this a total of 30 (5x6) animals will be used for each study protocol

In this a total of 30 (5x6) animals will be used for each study protocol

Group I: Control

Group II: Veerapamil

Group III: Buspirone

Group IV: Diosmine + Buspirone

Group V: Veerapamil +buspirone

Study III

In-vitro non everted sac method

Rats were grouped up to 5 (n=6) after overnight fasting intestines were isolated under anesthesia by using thiopental sodium (50mg/kg/i.p), and ice-cold saline was used to flush. The rats were exsanguinated, the jejunum and ileum portions isolated and a segment of each was cut into a length of 10cm for preparation of the sac for the experiment.

In this a total of 30 (5x6) animals will be used for each study protocol

Group I: Control

Group II: Veerapamil

Group III: Niaziridine

Group IV: Niaziridine +Lovastatin

Group V: Veerapamil+ Lovastatin

A total of 90 (30x3) animals will be used for all the 3 *in-vitro* study protocols.

Protocol No :06
Name:S.Malathy

P.s malathy

0Form B (per rule 8(a)* for Submission of Research Protocol (s)

Application for Permission for Animal Experiments

Application to be submitted to the CPCSEA, New Delhi after approval of Institutional Animal Ethics Committee (IAEC)

Section -I

1.	Name and address of establishment	University College Of Pharmaceutical Sciences Kakatiya University Warangal-506009(T.S)
2.	Registration number and date of registration.	1820/GO/Re/S/15/CPCSEA Date: 01-09-2015
3.	Name, address and registration number of breeder from which animals acquired (or to be acquired) for experiments mentioned in parts B & C	Vyas Labs,Amberpet,Hyderabad CPCSEA:2085/PO/RCBiBt/S/19/CPCS
4.	Place where the animals are presently kept (or proposed to be kept).	Animal house, University College of Pharmaceutical Sciences, KU, Warangal.
5.	Place where the experiment is to be performed (Please provide CPCSEA Reg. Number)	Animal house, University College of Pharmaceutical Sciences, KU, Warangal.
6.	Date and Duration of experiment.	8Months
7.	Type of research involved (Basic Research / Educational/ Regulatory/ Contract Research)	Educational research

Signature

Name and Designation of Investigator
Prof .Y.NARSIMHA REDDY
Dean of the Pharmaceutical sciences
UCPSc, Kaktiya University.

Date: 13/04/2022
Place: Warangal.

Section -II

Protocol form for research proposals to be submitted to the Institutional Animal Ethics Committee/ CPCSEA, for new experiments or extensions of ongoing experiments using animals.

1. Project / Dissertation / Thesis Title: Pharmacokinetic and Pharmacodynamic interactions of Phytoconstituents with some conventional drugs

2. Principal Investigator / Research Guide / Advisor:

   a. Name:prof .Y.Narsimha Reddy ,Dean of the Pharmacy.
   b. Designation:Professor
   c. Dept / Div/ Lab:Pharmacology
   d. Telephone No:9440705384
   e. E-mail Id:ynrku@yahoo.co.in
   f. Experience in Lab animal experimentation:26years

3. List of all individuals authorized to conduct procedures under this proposal.

   a. Name:P.S.Malathy
   b. Designation:Research scholar
   c. Department:pharmacology
   d. Telephone No:7680819110
   e. E-mail Id:psmalathy5@gmail.com
   f. Experience in Lab animal experimentation: 18years

4. Funding Source / Proposed Funding Source with complete address (Please attach the proof)
   AICTE sponsored QIP Ph.D,
   AICTE,New Delhi.

5. Duration of the animal experiment: 8 months

   a. Date of initiation (Proposed) -June 2022
   b. Date of completion (Proposed) -February2022

6. Describe details of study plan to justify the use of animals (Enclose Annexure)

7. Animals required

a. Species and Strain: **Wistar Rats**
b. Age and Weight: **150-200grams**
c. Gender: **Male**
d. Number to be used (Year-wise breakups and total figures needed to begiven in tabular form): **102**

Year	No of Groups	No of Animals(n=6)
First year	5	30
Second Year	6	36
Third Year	6	36

e. Number of days each animal will be housed: 21 days

8. Rationale for animal usage

a. Why is animal usage necessary for these studies?
Humans and animals share hundreds of illnesses, so animals often act as models for the study of human disease. we can study how bodies work by running experiments in animals that would be impossible in human volunteers and this is where most research animals are used to study the Pharmacokinetic and Pharmacodynamic interactions of Phytoconstituents with some conventional drugs

b. Whether similar study has been conducted on *in vitro* models? If yes, describe the leading points to justify the requirement of animal experiment.- **No**

c. Why are the particular species selected?
Wistar Rats are reported to be used in these kind of animal model studies.

d. Why is the estimated number of animals essential?
We require 5 groups (n=6) of animals (total 5X 6 = 30) to conduct the study. To have statistical significance,these are essential.

e. Are similar experiments conducted in the past in your establishment?
No.

f. If yes, justify why new experiment is required?
NA.

g. Have similar experiments been conducted by any other organization in same or other *in vivo* models? If yes, enclose the reference.

Similar experiments were not conducted.

9. Describe the procedures in detail:

Study Design:

a. Describe all invasive and potentially stressful non-invasive procedures that animals will be subjected to in the course of the experiments)-NA
b. Furnish details of injections schedule Substances:
   Doses :10mg/ml
   Sites :Oral
   Volumes :0.3ml
c. Blood withdrawal Details:
   Volumes :0.2ml
   Sites : orbital puncture
d. Radiation (dosage and schedules):**NA**
e. Nature of compound/Broad Classification of drug/NCE: chemical, phytoconstituents

10. Does the protocol prohibit use of anesthetic or analgesic for the conduct of painful procedures? If yes, justify.

No anaesthetic is needed

11. Will survival surgery be done? **Not required**

If yes, the following to be described.

a. List and describe all surgical procedures (includingmethods of asepsis)
b. Names, qualifications and experience levels of personnels involved.
c. Describe post-operative care
d. Justify if major survival surgery is to be performed more than once on asingle animal.

12. Describe post-experimentation procedures.

a. Scope for Reuse : **NA**

b. Rehabilitation (Name and Address, where the animals are proposed to be rehabilitated) : **NA**

c. Describe method of Euthanasia (If required in the protocol) : Co_2

d. Method of carcass disposal after euthanasia. : The animals carcass is collected in colour codet bins and sent to an authorized bio medical waste collection agency for final disposal.

13. Describe animal transportation methods if extra-institutional transport is envisaged. **NA**

14. Use of hazardous agents (use of recombinant DNA-based agents or potential human pathogens requires documented approval of the Institutional Biosafety Committee (IBC). For each category, the agents and the biosafety level required, appropriate therapeutic measures and the mode of disposal of contaminated food, animal wastes and carcasses must be identified).
If, your project involved use of any of the below mentioned agent, attach copy of the approval certificates of the respective agencies: **NA**

(a) Radionucleotides (AERB)
(b) Microorganisms / Biological infectious Agents (IBSC)
(c) Recombinant DNA (RCGM)
(d) Any other Hazardous Chemical / Drugs

No hazardous agents

Investigator's declaration.

1. I certify that the research proposal submitted is not unnecessarily duplicative of previously reported research.

2. I certify that, I am qualified and have experience in the experimentation on animals.

3. For procedures listed under item 10, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.

4. I will obtain approval from the IAEC/ CPCSEA before initiating any changes in this study.

5. I certify that performance of experiment will be initiated only upon review and approval of scientific intent by appropriate expert body (Institutional Scientific Advisory Committee / funding agency / other body).

6. I certify that I will submit appropriate certification of review and concurrence for studies mentioned in point 14.

7. I shall maintain all the records as per format (Form D) and submit to Institutional Animal Ethics Committee (IAEC).

8. I certify that, I will not initiate the study before approval from IAEC/ CPCSEA received in writing. Further, I certify that I will follow the recommendations of IAEC/ CPCSEA.

9. I certify that I will ensure the rehabilitation policies are adopted (wherever required).

Signature

Name of Investigator

Date: 13/04/2022

Certificate

This is to certify that the project proposal no .....entitled **Pharmacokinetic and Pharmacodynamic interactions of Phytoconstituents with some conventional drugs** submitted by Dr./ Mr. / Ms. **P.S.Malathy** has been approved/recommended by the IAEC of **UCPSc, Kakatiya University** (Organization) in its meeting held on**13/04/2022** (date) and **102 wistar rats**(Number and Species of animals) have been sanctioned under this proposal for a duration of next 12 months.

Authorized by	**Name**	**Signature**	**Date**
Chairman:	…….	……	……..
Member Secretary:	…….	……	……..
Main Nominee of CPCSEA:	…….	……	……..

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

Study design:

Aim: To study the Pharmacokinetic and Pharmacodynamic interactions of Phytoconstituents with some conventional drugs.

Chapter-I

Method

Induction of diabetes in rats

Wistar rats fasted over night and diabetes induced by administration of streptozocin 50mg/kg in 0.1M sodium citrate buffer intraperitonialy and rats were immediately administered with 5% dextrose to antagonize the rapid hypoglycemia effects.

Pharmacokinetic and Pharmacodynamic study:

- Group 1: Diabetic control
- Group 2: Pure antidiabetic drug will administer to rats
- Group 3: Phytochemical only will administer to rats
- Group 4: Phytochemical followed by drug will administered to rats for single dose interaction study
- Group 5: Phytochemical will administer for 7 days and on 8^{th} day phytochemical followed by drug will administered to rats for multiple dose interaction study

Blood samples will be collected from orbital puncture at time intervals between 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 hrs

Chapter-II

Induction of Hyperlipidemia

High Fat Diet-Induced Hyperlipidemia

High fat diet will be fed to rats for 28 successive days to induce hyperlipidemia.

High fat diet consisted of normal feed (78.8%), cholesterol pure (1%), cholic acid (0.2%), lard (10%) and egg yolk powder (10%). Normal feed contained only freshly cooked dalia (coarsely grounded wheat). Normal and high fat diets were freshly prepared each day.

- Group 1: Normal control
- Group 2: HFD control Group: The rats will be fed HFD for 4 weeks after 4 weeks rats will be treated normal saline
- Group 3: After 4 weeks of HFD feeding, the rats will be treated with standard drug

- Group 4: After 4 weeks of HFD feeding the rats will be treated with phytochemical
- Group 5: After 4 weeks of HFD feeding the rats will be treated with phytochemical followed by drug will administer to rats for single dose interaction study
- Group 6: After 4 weeks of HFD feeding the rats, phytochemical will administer for 7 days and on 8th day phytochemical followed by drug will administered to rats for multiple dose interaction study
-

Blood samples will be collected from orbital puncture at time intervals between 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 hrs

Chapter-III

Induction of inflammation

Carrageenan induced paw edema model

A 1%w/v suspension of carrageenan is prepared freshly in normal saline and injected into subplantar region of left hind paw with dose 0.1ml to induce edema. Test drug is administered intraperitoneally according to body weight immediately half an hour before carrageenan challenge. Paw volume up to ankle joint is measured and % reduction in edema is calculated.

Group 1: Normal
Group 2: Disease control
Group 3: pure anti-inflammatory drug will be administered to rats
Group 4: Phytochemical only will administer to rats
Group 5: Phytochemical followed by drug will administered to rats for single dose interaction study
Group 6: Phytochemical will administer for 7 days and on 8th day phytochemical followed by drug will administered to rats for multiple dose interaction study

Approval No: (07/IAEC/UCPSc/KU/2022:CPCSEA 2018-23)

Protocol No :07
Name:M.Rama

M. Rama

Form B (per rule 8(a)* for Submission of Research Protocol (s)

Application for Permission for Animal Experiments

Application to be submitted to the CPCSEA, New Delhi after approval of Institutional Animal Ethics Committee (IAEC)

Section -I

1.	Name and address of establishment	University College Of Pharmaceutical Sciences Kakatiya University Warangal-506009(T.S)
2.	Registration number and date of registration.	1820/GO/Re/S/15/CPCSEA Date:01-09-2015
3.	Name, address and registration number of breeder from which animals acquired (or to be acquired) for experiments mentioned in parts B & C	Vyas Labs,Amberpet,Hyderabad CPCSEA:2085/PO/RCBiBt/S/19/CPCS
4.	Place where the animals are presently kept (or proposed to be kept).	Animal house, University College of Pharmaceutical Sciences, KU, Warangal.
5.	Place where the experiment is to be performed (Please provide CPCSEA Reg. Number)	Animal house, University College of Pharmaceutical Sciences, KU, Warangal.
6.	Date and Duration of experiment.	6 Months
7.	Type of research involved (Basic Research / Educational/ Regulatory/ Contract Research)	Educational research

Signature

Name and Designation of Investigator
Prof.Y.NARSIMHA REDDY
Dean of the Pharmaceutical Sciences
UCPSc, Kakatiya university.

Date: 13/04/2022
Place: Warangal

Section -II

Protocol form for research proposals to be submitted to the Institutional Animal Ethics Committee/ CPCSEA, for new experiments or extensions of ongoing experiments using animals.

Project / Dissertation / Thesis Title: Amelioration in Learning and Memory of Some Indian Medicinal Plants by Neuronal Cell Injury Induced Animal Models.

1. Principal Investigator / Research Guide / Advisor:

   a. Name: Prof .Y.Narsimha Reddy.
   b. Designation: Professor and Dean of the Pharmaceutical Sciences
   c. Dept / Div/ Lab: Pharmacology
   d. Telephone No: 9440705384
   e. E-mail Id:ynrku@yahoo.co.in
   f. Experience in Lab animal experimentation: 26years

2. List of all individuals authorized to conduct procedures under this proposal.

   a. Name: M Rama
   b. Designation: Research scholar
   c. Department: Pharmacology
   d. Telephone No: 8179228302
   e. E-mail Id:rama.manda6@gmail.com
   f. Experience in Lab animal experimentation: 9 years

3. Funding Source / Proposed Funding Source with complete address (Please attach the proof)
   AICTE sponsored QIP Ph.D,
   AICTE,New Delhi.

4. Duration of the animal experiment: 6 months

   a. Date of initiation (Proposed) -June
   b. Date of completion (Proposed) -November

5. Describe details of study plan to justify the use of animals (Enclose Annexure)

6. Animals required

a. Species and Strain: **Swiss albino mice**
b. Age and Weight: **20-25grams**
c. Gender: **Male**
d. Number to be used (Year-wise breakups and total figures needed tobe given in tabular form): **120**

e. Number of days each animal will be housed: **21 days**

7. Rationale for animal usage

a. Why is animal usage necessary for these studies?
Humans and animals share hundreds of illnesses, so animals often act as models for the study of human disease. we can study how bodies work by running experiments in animals that would be impossible in human volunteers, and this is where most research animals are used to determine the learning and memory activity of some Indian medicinal plants.

b. Whether similar study has been conducted on *in vitro* models? If yes, describe the leading points to justify the requirement of animal experiment.- **No**

c. Why are the particular species selected?
Swiss albino mice are reported to be used in these kind of animal model studies.

d. Why is the estimated number of animals essential?
We require 5 groups (n=6) of animals (total 5X 6 = 30) to conduct the study. To have statistical significance,these are essential.

e. Are similar experiments conducted in the past in your establishment?
No.

f. If yes, justify why new experiment is required?
NA.

g. Have similar experiments been conducted by any other organization in same or other *in vivo* models? If yes, enclose the reference.

Similar experiments were not conducted.

8. Describe the procedures in detail:

a. Describe all invasive and potentially stressful non-invasive procedures that animals will be subjected to in the course of the experiments)-NA
b. Furnish details of injections schedule Substances:
   Doses : 200mg/kg
   Sites : Oral route
   Volumes: 0.2ml
c. Blood withdrawal Details:
   Volumes : 0.2ml
   Sites : Retro orbital plexus
d. Radiation (dosage and schedules):NA
e. Nature of compound/Broad Classification of drug/NCE: Plant extract

9. Does the protocol prohibit use of anesthetic or analgesic for the conduct of painful procedures? If yes, justify.

No anaesthetic is needed

10. Will survival surgery be done? **Not required**

If yes, the following to be described.

a. List and describe all surgical procedures (includingmethods of asepsis)
b. Names, qualifications and experience levels of personnels involved.
c. Describe post-operative care
d. Justify if major survival surgery is to be performed more than once on asingle animal.

11. Describe post-experimentation procedures.

a. Scope for Reuse : NA

b. Rehabilitation (Name and Address, where the animals are proposed to be rehabilitated) : NA

c. Describe method of Euthanasia (If required in the protocol) : CO_2

d. Method of carcass disposal after euthanasia. : The animal's carcass is collected in color codet bins and sent to an authorized biomedical waste collection agency for final disposal.

12. Describe animal transportation methods if extra-institutional transport is envisaged. **NA**

13. Use of hazardous agents (use of recombinant DNA-based agents or potential human pathogens requires documented approval of the Institutional Biosafety Committee (IBC). For each category, the agents and the biosafety level required, appropriate therapeutic measures and the mode of disposal of contaminated food, animal wastes and carcasses must be identified).
If, your project involved use of any of the below mentioned agent, attach copy of the approval certificates of the respective agencies: **NA**

(a) Radionucleotides (AERB)
(b) Microorganisms / Biological infectious Agents (IBSC)
(c) Recombinant DNA (RCGM)
(d) Any other Hazardous Chemical / Drugs

No hazardous agents

Investigator's declaration.

1. I certify that the research proposal submitted is not unnecessarily duplicative of previously reported research.

2. I certify that, I am qualified and have experience in the experimentation on animals.

3. For procedures listed under item 10, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.

4. I will obtain approval from the IAEC/ CPCSEA before initiating any changes in this study.

5. I certify that performance of experiment will be initiated only upon review and approval of scientific intent by appropriate expert body (Institutional Scientific Advisory Committee / funding agency / other body).

6. I certify that I will submit appropriate certification of review and concurrence for studies mentioned in point 14.

7. I shall maintain all the records as per format (Form D) and submit to Institutional Animal Ethics Committee (IAEC).

8. I certify that, I will not initiate the study before approval from IAEC/ CPCSEA received in writing. Further, I certify that I will follow the recommendations of IAEC/ CPCSEA.

9. I certify that I will ensure the rehabilitation policies are adopted (wherever required).

Signature

Name of Investigator

Date: 13/04/2022

Certificate

This is to certify that the project proposal no…….entitled **Amelioration in Learning and Memory of Some Indian Medicinal Plants by Neuronal Cell Injury Induced Animal Models** submitted by Dr./Mr/Ms.**Manda.Rama** has been approved/recommended by the IAEC of **UCPSc, Kakatiya University** (Organization) in its meeting held on **13-04-2022** (date) and**120 Swiss albino mice** (Number and Species of animals) have been sanctioned under this proposal for a duration of next **12** months.

Authorized by	**Name**	**Signature**	**Date**
Chairman:	…….	……	……..
Member Secretary:	…….	……	……..
Main Nominee of CPCSEA:	…….	……	……..

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

STUDY DESIGN

Aim: To study of Amelioration in learning and Memory of Some Indian Medicinal plants by Neuronal Cell Injury Induced Animal Models.

Animals: For experiment Swiss albino mice procured at the age of 10 days acclimatized for 4 days weighing 22-25grams were used for the study.The animals were procured from the central animal facility at kakatiya university,Warangal.The mice were group housed in polypropylene(38×23×10cm) with not more than 5 animals per cage.They were maintained under standard laboratory conditions with a natural light dark cycle(14±1hour light;10+1hour dark) and were allowed free access to standard dry mice diet and tap water ad libitum. The mice were divided into 5 groups of 6 animals each.

Methods:

Induction of Alzheimer's: Intra cerebro ventricular (I.C.V)injection of amyloid beta(25-35)used to induce neurotoxicity by identifying bregma point in the skull.

Each animal was injected with 10µl which contain 60µg of Aβ(3mg per kg).Except group I(positive control).

Protocol:

All the animals were divided into 5 groups containing 6 animals each (n=6)

Group I **Normal control** animals will receive only phosphate buffer saline (PBS) / Saline.
Group II **Negative control** will receive only intra cerebro ventricular (i.c.v) injection of Amyloid beta on 15th day (single dose (3mg per kg)).

Group III **Positive control** animals are injected with i.c.v injection of Amyloid beta on 15th day (single dose) and treated with standard drug (Donepezil 5mg/kg(P.O)).

Group IV **Test compound low dose** animals will receive test compound in pretreatment for 14 days and Amyloid beta i.c.v injection on 15th day (single dose).

Group V **Test compound high dose** Animals will receive test compound in pretreatment for 14 days and Amyloid beta i.c.v injection on 15th day (single dose).

Treatment will be initiated 1h before Amyloid beta injection and will be continued upto day 21, in III, IV and V groups. On 22nd day, Behavioural parameters will be estimated and mices will be sacrificed and The brain tissue will be collected and stored for further analysis.

Number of plants			
Name	**Plant 1**	**plant 2**	**Plant 3**
Dose (mg/kg)	200	200	200
Route/ Sites	Oral	Oral	Oral
Volumes (ml)	As per mice weight	As per mice weight	As per mice weight
Vol. of Blood collection	0.2 ml	0.2ml	0.2ml
Site of Collection	Retro orbital plexus	Retro orbital plexus	Retro orbital plexus

- The Behavioral Parameters determined by conducting following:

  a) Jumping box test
  b) Rectangular maze test
  c) Morris water maze test
  d) Y- maze test

- The biochemical parameters determined by conducting following:
  a) Acetylcholinesterase enzyme
  b) Glutathione peroxidase.
  c) Estimation of DPPH radical Scavenging activity.
  d) Measurement of lipid peroxidation
- Histopathological Studies

CHAPTER-1

All the animals were divided into 5 groups containing 6 animals each (n=6) 5×6=30

S.no	Groups		Drug and Plant Extract
1	Group-I	Normal Control	PBS
2	Group-II	Negatve Control	Amyloid beta peptide
3	Group-III	Positive Control	Donepezil
4	Group-IV	Test compound Low dose	Plant extract 1
5	Group-V	Test compound High dose	Plant extract 1

CHAPTER-2

All the animals were divided into 5 groups containing 6 animals each (n=6) 5×6=30

S.no	Groups		Drug and Plant Extract
1	Group-I	Normal Control	PBS
2	Group-II	Negatve Control	Amyloid beta peptide
3	Group-III	Positive Control	Donepezil
4	Group-IV	Test compound Low dose	Plant extract 2
5	Group-V	Test compound High dose	Plant extract 2

CHAPTER-3

All the animals were divided into 5 groups containing 6 animals each (n=6) 5×6=30

S.no	Groups		Drug and Plant Extract
1	Group-I	Normal Control	PBS
2	Group-II	Negatve Control	Amyloid beta peptide
3	Group-III	Positive Control	Donepezil
4	Group-IV	Test compound Low dose	Plant extract 2
5	Group-V	Test compound High dose	Plant extract 2

- Number of animals for acute toxicity studies:30

Approval No: (08/IAEC/UCPSc/KU/2022:CPCSEA 2018-23)

Protocol No :08
Name:D.Saritha

Form B (per rule 8(a)* for Submission of Research Protocol (s)

Application for Permission for Animal Experiments

Application to be submitted to the CPCSEA, New Delhi after approval of Institutional Animal Ethics Committee (IAEC)

Section -I

1.	Name and address of establishment	University College of pharmaceutical Sciences, Kakatiya University, Warangal.
2.	Registration number and date of registration.	1820/GO/Re/S/15/CPCSEA Date:01-09-2015
3.	Name, address and registration number of breeder from which animals acquired (or to be acquired) for experiments mentioned in parts B & C	Vyas Labs, Amberpet, Hyderabad. CPCSEA:2085/PO/RCBiBt/S/19/CPCS.
4.	Place where the animals are presently kept (or proposed to be kept).	University College of Pharmaceutical Sciences, KU, Warangal
5.	Place where the experiment is to be performed (Please provide CPCSEA Reg. Number)	University College of Pharmaceutical Sciences, KU, Warangal
6.	Date and Duration of experiment.	Experiments will start only after IAEC approval and duration is One year
7.	Type of research involved (Basic Research / Educational/ Regulatory/ Contract Research)	Educational research

Signature
Name and Designation of Investigator
Dr. J. Krishnaveni
Associate professor

Date: 13-04-2022
Place: WGL

Section -II

Protocol form for research proposals to be submitted to the Institutional Animal Ethics Committee/ CPCSEA, for new experiments or extensions of ongoing experiments using animals.

1. Project / Dissertation / Thesis Title:

   "DEVELOPMENT AND EVALUATION OF NANOCARRIER BASED TRANSDERMAL DRUG DELIVERY SYSTEMS OF SELECTED DRUGS "

2. Principal Investigator / Research Guide / Advisor:

   a. Name: Dr. J. KRISHNAVENI
   b. Designation: ASSOCIATE PROFESSOR
   c. Department: PHARMACEUTICS
   d. Telephone No.: 9247161127
   e. E-mail Id: krishnavenij153@gmail.com
   f. Experience in Lab animal experimentation: 25 years

3. List of all individuals authorized to conduct procedures under this proposal.

   a. Name: SARITHA DUNAKA
   b. Designation: PhD SCHOLOR
   c. Department: PHARMACEUTICS
   d. Telephone No: 9160888578
   e. E-mail Id: saritharapol@gmail.com
   f. Experience in Lab animal experimentation: 10 years

4. Funding Source / Proposed Funding Source with complete address (Please attach the proof)

   AICTE Sponsored QIP, PhD.
   AICTE. New Delhi

   In-house project.

5. Duration of the animal experiment.

   a. Date of initiation (Proposed): Experiments will start only after IAEC approval.
   b. Date of completion (Proposed): within 1-2 years.

6. Describe details of study plan to justify the use of animals (Enclose Annexure)

7. Animals required

a. Species and Strain: Wistar rats
b. Age and Weight: 8-12 weeks old
c. Gender: male
d. Number to be used- **126** rats (42 rats for PD study for each drug. For three drugs 42*3=**126** rats).
   - The rats used in PD study were utilized in PK studies after wash out period of 3weeks.
e. (Year-wise breakups and total figures needed to be given in tabular form)

s.no	Year	No of animals
1	2022 (July-September)	42
2	2022-22 (October -December)	42
3	2023 (February-June)	42

f. Number of days each animal will be housed -60 days

8. Rationale for animal usage

a. **Why is animal usage necessary for these studies?**
Require animal data for human safety. Humans and animals share hundreds of illnesses, so animals often act as models for the study of human disease. we can study how bodies work by running experiments in animals that would be impossible in human volunteers.

b. **Whether similar study has been conducted on *in vitro* models? If yes, describe the leading points to justify the requirement of animal experiment.**

To check he results obtained by ex -vivo studies

c. **Why are the particular species selected?**
Based on species selectivity Wister rats are reported to be used in these kinds of studies

d. **Why is the estimated number of animals essential?**
As per the requirement of the protocol.

e. Are similar experiments conducted in the past in your establishment?
Not conducted.
f. If yes, justify why new experiment is required?
NA
g. Have similar experiments been conducted by any other organization in same or other *in vivo* models? If yes, enclose the reference.
Not conducted.

9. Describe the procedures in detail:

a. Describe all invasive and potentially stressful non-invasive procedures that animals will be subjected to in the course of the experiment.

Description the procedures to be used:

The study will be carried out in albino male Wistar rats (PK studies), (PD studies). The animals will be procured from a standard breeder and they will be acclimatized to the laboratory conditions for a week prior to the test. The animals will be kept at a temperature (25°C) and Relative humidity (45%) controlled room with 12hours light, 12 hours dark cycle throughout the experimental period. The animals will be fasted overnight prior to the administration of test sample.

Pharmacodynamic studies (Rat model) Pharmacodynamic study was carried out using animal tail non-invasive blood pressure (BP) system (NIBP 200 A; Biopic System, Inc., Goleta, CA, USA) based on cuff tail technique. Male albino Wistar rats (200g-250g) will be used in this study and free access to food and water. Prior to study, the protocol will be approved by animal ethical committee. Before dosing the animals will keep overnight fasting. The male Wistar rats were divided into seven groups (groups A–G) of six animals each. Group A was taken as a negative control. Hypertension was induced in the remaining groups (groups B–G) by subcutaneous injection of methyl prednisolone acetate (MPA; Depo- Medrol; Pfizer, New York, NY, USA) (20 mg/kg/week) for 2 weeks. Group B served as hypertensive positive control and received no further treatment. Group C received Drug solution transdermally, Group D received Marketed formulation orally, Group E treated with Optimized formulation, Group F treated with Optimized formulation with permeation enhancer by transdermally, Group G treated with Optimized formulation in the form of gel by transdermally The rats were then placed in the restrainer, and the BP in the tail was recorded at predetermined time intervals at 0, 1, 2, 4, 6, 8, and 24 hours. Statistical significance was analyzed by one-way ANOVA using SPSS software 22.0. Percentage of reduction in BP from hypertensive control was calculated by the following equation:

Percentage reduction in BP=

BP of hypertensive positive control - BP of treated group/ BP of hypertensive positive control*100

Plasma pharmacokinetic studies (Rat model): The male Wistar rats will be selected for the phrmacokinetic study. The animals will be maintained under controlled conditions with temperature of 25°C and RH of 45% in poly propylene cages filled with stearile paddy husk. They will be fed with standard diet and water ad libitum. The rats will be divided in to five groups each containg six rats weighing between 200g to 250 gms. Animals will be fasted over night before the study. Group A (control group) will administer with drug solution by transdermal route, Group B (Standard group) will receive marketed formulation by oral route and Group C treated with the Optimized formulation, Group D treated with Optimized formulation with permeation enhancer by transdermally, Group E treated with Optimized formulation in the form of gel by transdermally. About 0.5 ml blood samples will collect in to the EDTA added centrifused tubes at pre-determined time intervels from the retro orbital vein by using heparinised cappillary tubes. Blood samples will be centrifused. The plasma will be separated from blood samples and stored at -20°C. The drug concentration in plasma will be determined by HPLC.

b. Furnish details of injections schedule Substances:
Dose : single dose
Sites : transdermal/oral
Volumes: As per rat weight

c. Blood withdrawal Details:
Volumes: 0.5 ml
Sites: Retro orbital vein

d. Radiation (dosage and schedules): **Not applicable**
e. Nature of compound/Broad Classification of drug/NCE: Antihypertensive drug

10. Does the protocol prohibit use of anesthetic or analgesic for the conduct of painful procedures? If yes, justify

No anesthetic is needed

11. Will survival surgery be done?
Not required
If yes, the following to be described,

a. List and describe all surgical procedures (including methods of asepsis)
NA

b. Names, qualifications and experience levels of personals involved.
NA

c. Describe post-operative care
NA

d. Justify if major survival surgery is to be performed more than once on a single animal.
Not done.

12. Describe post-experimentation procedures.
a. Scope for Reuse: The rats used in PD study were utilized in PK studies after wash out period of 3weeks.

a. Rehabilitation (Name and Address, where the animals are proposed to be rehabilitated):

b. Describe method of Euthanasia. (If required in the protocol): Co_2

c. Method of carcass disposal after euthanasia: The animals carcass is collected in colour codet bins and sent to an authorized biomedical waste collection agency for final disposal.

d. Describe animal transportation methods if extra-institutional transport is envisaged.
Not applicable.

e. Use of hazardous agents (use of recombinant DNA-based agents or potential human pathogens requires documented approval of the Institutional Biosafety Committee (IBC). For each category, the agents and the biosafety level required, appropriate therapeutic measures and the mode of disposal of contaminated food, animal wastes and carcasses must be identified).

If, your project involved use of any of the below mentioned agent, attach copy of the approval certificates of the respective agencies: **NO**

(a) Radionucleotides (AERB): NO
(b) Microorganisms / Biological infectious Agents (IBSC):NO
(c) Recombinant DNA (RCGM): NO
d) Any other Hazardous Chemical / Drugs: NO

Investigator's declaration.

1. I certify that the research proposal submitted is not unnecessarily duplicative of previously reported research.

2. I certify that, I am qualified and have experience in the experimentation on animals.

3. For procedures listed under item 10, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.

4. I will obtain approval from the IAEC/ CPCSEA before initiating any changes in this study.

5. I certify that performance of experiment will be initiated only upon review and approval of scientific intent by appropriate expert body (Institutional Scientific Advisory Committee / funding agency / other body).

6. I certify that I will submit appropriate certification of review and concurrence for studies mentioned in point 14.

7. I shall maintain all the records as per format (Form D) and submit to Institutional Animal Ethics Committee (IAEC).

8. I certify that, I will not initiate the study before approval from IAEC/ CPCSEA received in writing. Further, I certify that I will follow the recommendations of IAEC/ CPCSEA.

9. I certify that I will ensure the rehabilitation policies are adopted (wherever required).

Signature

Name of Investigator

Date:

(Dr. J. KRISHNAVENI)

Certificate

This is to certify that the project proposal no..... entitled submitted by Dr./ Mr. / Ms. ..... has been approved/recommended by the IAEC of.....(Organization) in its meeting held on..... (date) and .....(Number and Species of animals) have been sanctioned under this proposal for a duration of next ..... months.

Authorized by	Name	Signature	Date
Chairman:	.....	.....	.....
Member Secretary:	.....	.....	.....
Main Nominee of CPCSEA:	.....	.....	.....

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

STUDY PLAN

Objective: To evaluate plasma Pharmacokinetic (PK) parameters & Pharmacodynamic (PD) activity of optimized formulations on Rat and comparing with conventional dosage forms.

Selected drugs: (Antihypertensive Drugs)

Test formulations (containing Antihypertensive drug), Standard and Control

Protocol

The rats weighing 200 to 250 gm will be divided into different test groups, each consisting of 6 rats in group. Then the following treatments will be given

Control group

Standard group

Test formulation Group

Experimental Procedure:

Description the procedures to be used:

The study will be carried out in male Wistar rats (PK studies), (PD studies). The animals will be procured from a standard breeder and they will be acclimatized to the laboratory conditions for a week prior to the test. The animals will be kept in a temperature (25°C) and humidity (45%) controlled room with 12hours light, 12 hours dark cycle throughout the experimental period. The animals will be fasted overnight prior to the administration of test sample.

Pharmacodynamic studies (Rat model): Pharmacodynamic study was carried out using animal tail non-invasive blood pressure (BP) system (NIBP 200 A; Biopic System, Inc., Goleta, CA, USA) based on cuff tail technique. Male albino Wistar rats (200g-250g) will be used in this study and free access to food and water. Prior to study, the protocol will be approved by animal ethical committee. Before dosing the animals will keep overnight fasting. The male Wistar rats were divided into seven groups (groups A–G) of six animals each. Group A was taken as a negative control Hypertension was induced in the remaining groups (groups B–G) by subcutaneous injection of methyl prednisolone acetate (MPA; Depo- Medrol; Pfizer, New York, NY, USA) (20 mg/kg/week) for 2 weeks. Group B served as hypertensive positive control and received no further treatment.

Group C received Drug solution transdermally, Group D received Marketed formulation orally, GroupE treated with Optimized formulation transdermally, Group F treated with Optimized formulation with permeation enhancer by transdermally, Group G treated with Optimized in the form of gel by transdermally The rats were then placed in the restrainer, and the BP in the tail was recorded at predetermined time intervals at 0, 1, 2, 4, 6, 8, and 24 hours. Statistical significance was analyzed by one-way ANOVA using SPSS software 22.0. Percentage of reduction in BP from hypertensive control was calculated by the following equation:

Percentage of reduction in BP=

BP of hypertensive positive control - BP of treated group/ BP of hypertensive positive control*100

Plasma pharmacokinetic studies (Rat model): The male Wistar rats will select for the pharmacokinetic study. The animals will be maintained under controlled conditions with temperature of 25°C and RH of 45% in poly propylene cages filled with stearile paddy husk. They will be fed with a balanced diet and water ad libitum. The rats will be divided in to five groups, each containg six rats weighing about 200 to 250 gm. Animals will be fasted over night before the study. Group A (control group) treated with drug solution by transdermal route, Group B (Standard group) will receive marketed formulation by oral route and Group C treated with the Optimized formulation transdermally, Group D treated with Optimized formulation with permeation enhancer by transdermally, Group E treated with Optimized formulation in the form of gel by transdermally. About 0.5 ml blood samples will collect in to the EDTA added centrifused tubes at predetermined time intervels from the retro orbital vein by using heparinised cappillary tubes. Blood samples will be centrifused. The plasma will be separeted from blood samples and stored at -20°C. The drug concentration in plasma will be determined by HPLC.

Study Design: The study is divided into three chapters.

Furnish details of dose schedule –Transdermal administration. (Anti-hypertensive drugs)

Name	Nebivolol	Nebivolol	Lacidipine	Lacidipine	Nisoldipine (tentative)	Nisoldipine (tentative)
Dose (mg/kg)	5	5	4	4	10	10
Route/ Sites	oral	Transdermal	oral	Transdermal	oral	Transdermal
Volumes (ml)	As per rat weight	As per rat weight	As per rat weight	As per rat weight	As per rat weight	As per rat weight
Frequency	Single dose	Single dose	Single dose	Single dose	Single dose	Single dose

Dose and injection schedule for pharmacodynamic studies

Group A: normal control
Group B: Positive control – Methyl prednisolone acetate (20mg/kg/week) for two weeks subcutaneous injection.
Group C: MPA (20mg/kg) SC + Drug solution by transdermally
Group D: MPA (20mg/kg) SC + marketed formulation orally
Group E: MPA (20mg/kg) SC + Optimized formulation transdermally
Group F: MPA (20mg/kg) SC + Optimized formulation with permeation enhancer transdermally
Group G: MPA (20mg/kg) SC + Optimized formulation with permeation enhancer in the form of gel transdermally.

Dose schedule for pharmacokinetic studies

Group A: (control): Drug solution by transdermal route
Group B: (standard): marketed formulation orally
Group C: (test): Optimized formulation transdermally
Group D: (test): Optimized formulation with permeation enhancer transdermally
Group E: (test): Optimized formulation with permeation enhancer in the form of gel transdermally.

Chapter1: Development and evaluation of nanocarrier based transdermal drug delivery system of Nebivolol.

Animals required

a. Species and Strain: Wistar rats
b. Age and Weight: 8-12 weeks old
c. Gender: male
d. **Number of animals: 42** – The male Wistar rats were divided into seven groups (groups A–G). each group containing 6 rats (n=6).

- The rats used in Pharmacodynamic study were utilized in Pharmacokinetic study after wash out period of 3weeks.

Dose and injection schedule for pharmacodynamic studies

Group A: normal control
Group B: Positive control – Methyl prednisolone acetate (20mg/kg/week) for two weeks subcutaneous injection.
Group C: MPA (20mg/kg) SC + Drug solution by transdermally
Group D: MPA (20mg/kg) SC + marketed formulation orally
Group E: MPA (20mg/kg) SC + Optimized formulation transdermally
Group F: MPA (20mg/kg) SC + Optimized formulation with permeation enhancer transdermally
Group G: MPA (20mg/kg) SC + Optimized formulation with permeation enhancer in the form of gel transdermally.

Dose schedule for pharmacokinetic studies

The male Wistar rats were divided into five groups (groups A–E) of six animals each.

Group A: (control): Drug solution by transdermal route
Group B: (standard): marketed formulation orally
Group C: (test): Optimized formulation transdermally
Group D: (test): Optimized formulation with permeation enhancer transdermally
Group E: (test): Optimized formulation with permeation enhancer in the form of gel transdermally.

Chapter 2: Development and evaluation of nanocarrier based transdermal drug delivery system of Lacidipine.

Animals required

e. Species and Strain: Wistar rats
f. Age and Weight: 8-12 weeks old
g. Gender: male
h. **Number of animals: 42** – The male Wistar rats were divided into seven groups (groups A–G). each group containing 6 rats (n=6).

- The rats used in Pharmacodynamic study were utilized in Pharmacokinetic study after wash out period of 3weeks.

Dose and injection schedule for pharmacodynamic studies

Group A: normal control
Group B: Positive control – Methyl prednisolone acetate (20mg/kg/week) for two weeks subcutaneous injection.
Group C: MPA (20mg/kg) SC + Drug solution by transdermally
Group D: MPA (20mg/kg) SC + marketed formulation orally
Group E: MPA (20mg/kg) SC + Optimized formulation transdermally
Group F: MPA (20mg/kg) SC + Optimized formulation with permeation enhancer transdermally
Group G: MPA (20mg/kg) SC + Optimized formulation with permeation enhancer in the form of gel transdermally.

Dose schedule for pharmacokinetic studies

The male Wistar rats were divided into five groups (groups A–E) of six animals each.

Group A: (control): Drug solution by transdermal route
Group B: (standard): marketed formulation orally
Group C: (test): Optimized formulation transdermally
Group D: (test): Optimized formulation with permeation enhancer transdermally
Group E: (test): Optimized formulation with permeation enhancer in the form of gel transdermally.

Chapter 3: Development and evaluation of nanocarrier based transdermal drug delivery system of Nisoldipine

Animals required

Species and Strain: Wistar rats
Age and Weight: 8-12 weeks old
Gender: male
Number of animals: 42 – The male Wistar rats were divided into seven groups (groups A–G). each group containing 6 rats (n=6).

- The rats used in Pharmacodynamic study were utilized in Pharmacokinetic study after wash out period of 3weeks.

Dose and injection schedule for pharmacodynamic studies

Group A: normal control
Group B: Positive control – Methyl prednisolone acetate (20mg/kg/week) for two weeks subcutaneous injection.
Group C: MPA (20mg/kg) SC + Drug solution by transdermally
Group D: MPA (20mg/kg) SC + marketed formulation orally
Group E: MPA (20mg/kg) SC + Optimized formulation transdermally
Group F: MPA (20mg/kg) SC + Optimized formulation with permeation enhancer transdermally
Group G: MPA (20mg/kg) SC + Optimized formulation with permeation enhancer in the form of gel transdermally.

Dose schedule for pharmacokinetic studies

The male Wistar rats were divided into five groups (groups A–E) of six animals each.

Group A: (control): Drug solution by transdermal route
Group B: (standard): marketed formulation orally
Group C: (test): Optimized formulation transdermally
Group D: (test): Optimized formulation with permeation enhancer transdermally
Group E: (test): Optimized formulation with permeation enhancer in the form of gel transdermally.

Approval No: (09/IAEC/UCPSc/KU/2022:CPCSEA 2018-23)

Form B (per rule 8(a)* for Submission of Research Protocol (s)

Application for Permission for Animal Experiments

Application to be submitted to the CPCSEA, New Delhi after approval of Institutional Animal Ethics Committee (IAEC)

Section -I

1.	Name and address of establishment	University College Of Pharmaceutical Sciences Kakatiya University Warangal-506009(T.S)
2.	Registration number and date of registration.	1820/GO/Re/S/15/CPCSEA Date:01-09-2015
3.	Name, address and registration number of breeder from which animals acquired (or to be acquired) for experiments mentioned in parts B & C	Vyas Labs, Amberpet, Hyderabad CPCSEA:2085/PO/RCBiBt/S/19/CPCS
4.	Place where the animals are presently kept (or proposed to be kept).	Animal house, University College of Pharmaceutical Sciences, KU, Warangal.
5.	Place where the experiment is to be performed (Please provide CPCSEA Reg. Number)	Animal house, University College of Pharmaceutical Sciences, KU, Warangal.
6.	Date and Duration of experiment.	6 Months
7.	Type of research involved (Basic Research / Educational/ Regulatory/ Contract Research)	Educational research

Date: 21/04/22
Place: Warangal

Signature

Name and Designation of Investigator
Dr. Shayeda,
Asst. Professor.

Section -II

Protocol form for research proposals to be submitted to the Institutional Animal Ethics Committee/ CPCSEA, for new experiments or extensions of ongoing experiments using animals.

1. Project / Dissertation / Thesis Title: "Design, Characterization and Evaluation of Nano Formulations to Enhance Bioavailability of Some Drugs"
2. Principal Investigator / Research Guide / Advisor:

   a. Name : Dr. Shayeda.
   b. Designation : Assistant Professor
   c. Dept / Div/ Lab : Pharmaceutics
   d. Telephone No : 9849531109
   e. E-mail Id : syayeda_ucpsc@yahoo.com.
   f. Experience in Lab animal experimentation: 16years

3. List of all individuals authorized to conduct procedures under this proposal.

   a. Name : Ms. Gouthami Thumma
   b. Designation : Research Scholar
   c. Department : Pharmaceutics
   d. Telephone No. : 8340827224
   e. E-mail Id : gouthamithumma19@gmail.com
   f. Experience in Lab animal experimentation: 05

4. Funding Source / Proposed Funding Source with complete address (Please attachthe proof)
   AICTE Sponsored QIP Ph.D; AICTE, New Delhi.

5. Duration of the animal experiment: 6 months

   a. Date of initiation (Proposed) - After IAEC approval
   b. Date of completion (Proposed) -

6. Describe details of study plan to justify the use of animals (Enclose Annexure)
7. Animals required
   a) Species and Strain : Wistar rats
   b) Age and Weight : 200-250gm
   c) Gender : Male
   d) Number to be used : 30

   (Year-wise breakups and total figures needed to be given in tabular form)

   e) Number of days each animal will be housed: 30 days

8. Rationale for animal usage
   a. Why is animal usage necessary for these studies?
      To determine the PK-PD parameters of prepared Nano formulations and to check the bioavailability and efficacy of prepared Nano formulations
   b. Whether similar study has been conducted on *in vitro* models? If yes, describe the leading points to justify the requirement of animal experiment.- **No**
   c. Why are the particular species selected?
      Wistar rats are reported to be used in these kind of animal model studies.
   d. Why is the estimated number of animals essential?
      We require 6 groups (n=6) of animals (total 6X 5 = 30) to conduct the study.
      To have statistical significance, these are essential.
   e. Are similar experiments conducted in the past in your establishment?
      No.
   f. If yes, justify why new experiment is required?
      Not Applicable
   g. Have similar experiments been conducted by any other organization insame or other *in vivo* models? If yes, enclose the reference.
      Similar experiments were not conducted.

9. Describe the procedures in detail:
   a. Describe all invasive and potentially stressful non-invasive procedures thatanimals will be subjected to in the course of the experiments:-Animals will be sacrificed at the end of the study to check the histopathology of arthritis/or to check the drug level in synovial fluid
   b. Furnish details of injections schedule
      Substances :Complete Freund's Adjuvant
      Doses : 10mg/kg
      i. Sites : Sub plantar
      ii. Volumes : 0.2ml
   c. Blood withdrawal Details:
      Volumes :0.5ml
      Sites : Retro orbital plexus
   d. Radiation (dosage and schedules):NA
   e. Nature of compound/Broad Classification of drug/NCE: Anti-rheumatoid drugs
10. Does the protocol prohibit use of anesthetic or analgesic for the conduct of painful procedures? If yes, justify.
    No
11. Will survival surgery be done? No
    If yes, the following to be described.
    a. List and describe all surgical procedures (including methods of asepsis)
    b. Names, qualifications and experience levels of personnel's involved.
    c. Describe post-operative care
    d. Justify if major survival surgery is to be performed more than once on asingle animal.

12. Describe post-experimentation procedures. **NA**

a. Scope for Reuse : NA

b. Rehabilitation (Name and Address, where the animals are proposed to be rehabilitated) : NA

c. Describe method of Euthanasia (If required in the protocol) :CO_2

d. Method of carcass disposal after euthanasia. : The animal's carcass is collected in colour codet bins and sent to an authorized biomedical waste collection agency for final disposal.

13. Describe animal transportation methods if extra-institutional transport is envisaged. **Not Applicable**

14. Use of hazardous agents (use of recombinant DNA-based agents or potential human pathogens requires documented approval of the Institutional Biosafety Committee (IBC). For each category, the agents and the biosafety level required, appropriate therapeutic measures and the mode of disposal of contaminated food, animal wastes and carcasses must be identified).
If, your project involved use of any of the below mentioned agent, attach copy of the approval certificates of the respective agencies: **NA**

(a) Radionucleotides (AERB)
(b) Microorganisms / Biological infectious Agents (IBSC)
(c) Recombinant DNA (RCGM)
(d) Any other Hazardous Chemical / Drugs

No hazardous agents

Investigator's declaration.

1. I certify that the research proposal submitted is not unnecessarily duplicative of previously reported research.

2. I certify that, I am qualified and have experience in the experimentation on animals.

3. For procedures listed under item 10, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.

4. I will obtain approval from the IAEC/ CPCSEA before initiating any changes in this study.

5. I certify that performance of experiment will be initiated only upon review and approval of scientific intent by appropriate expert body (Institutional Scientific Advisory Committee / funding agency / other body).

6. I certify that I will submit appropriate certification of review and concurrence for studies mentioned in point 14.

7. I shall maintain all the records as per format (Form D) and submit to Institutional Animal Ethics Committee (IAEC).

8. I certify that, I will not initiate the study before approval from IAEC/ CPCSEA received in writing. Further, I certify that I will follow the recommendations of IAEC/ CPCSEA.

9. I certify that I will ensure the rehabilitation policies are adopted (wherever required).

Date: 21/04/22

Signature

Name of Investigator

Certificate

This is to certify that the project proposal no .....entitled "Design, Characterization and Evaluation of Nano Formulations to Enhance Bioavailability of Some Drugs" submitted by **Ms. Gouthami Thumma** has been approved/recommended by the IAEC of Kakatiya University (Organization) in its meeting held on……. (date) and ……(Number and Species of animals) have been sanctioned under this proposal for a duration of next ……..months.

Authorized by	**Name**	**Signature**	**Date**
Chairman:	…….	……	……..
Member Secretary:	…….	……	……..
Main Nominee of CPCSEA:	…….	……	……..

(Kindly make sure that minutes of the meeting duly signed by all theparticipants are maintained by Office)

Aim: The aim of the present study is to investigate the PK-PD parameters of prepared Nano formulations for Anti-Arthritic Activity

Study Design:

Animals: For experiment Wistar rats procured at the age of 3-6 months acclimatized for 4 days weighing 200-250grams will be used for the study. The animals will be procured from the central animal facility at Kakatiya University, Warangal. The rats will be group housed in polypropylene (38×23×10cm) cages with not more than 2 animals per cage. They will be maintained under standard laboratory conditions with a natural light dark cycle (14 ±1 hr light; 10+1 hr dark) and will be allowed free access to standard dry rat diet and tap water *ad libitum*. The rats will be divided into 6 groups of 6 animals each.

Methods:

Rheumatoid arthritis induction will be performed by using Freund's complete Adjuvant (0.1 ml Sub Plantar region) for 22 days. Simultaneously treatment will be given using optimized formulation I & II, and conventional formulation. Evaluated for anti- arthritic activity. After the 22nd day, animals were euthanized for evaluation of biochemical and Histopathological studies.

Protocol:

All the animals will be divided into 5 groups containing 6 animals each (n=6)

Group I: Vehicle control - animals will be not applied with any treatment.

Group II: Arthritis was induced by injecting Freund's Complete Adjuvant (0.1 ml) in a single dose into the sub plantar region of right hind paw

Group III: Arthritic rats were treated with conventional Formulation (Positive Control)

Group IV: Arthritic rats were treated with optimized Nano Formulation-I

Group V Arthritic rats were treated with optimized Nano Formulation-II

Treatment will be initiated after confirmation of arthritis and will be continued up to 21 days, in III, IV, and V groups. On 21st day, plasma samples will be collected at 0, 0.5, 1, 2, 4, 8, 12 and 24 hours and all rats will be sacrificed knee joint tissue will be collected from three rats from each group for histopathology and synovial fluid will be collected from three rats from each group and stored for further analysis.

References:

1. I S A, Krishnan S, Peter J, Sabu V, Helen A. Scientific validation of anti-arthritic effect of Kashayams - A polyherbal formulation in collagen induced arthritic rats. *J Ayurveda Integr Med*. 2021;12(1):20-27.
2. Aloke C, Ibiam UA, Orji OU, et al. Anti-arthritic potential of ethanol and aqueous extracts of stem bark of Cleistopholis patens on complete Freund's adjuvant-induced rheumatoid arthritis in rats. *J Ayurveda Integr Med*. 2021;12(1):28-34.

Chapter: Biological Evaluation of Methotrexate-Curcumin and Methotrexate-Quercetin Loaded Nanoemulsion Formulation for the Effective Treatment of Rheumatoid Arthritis

Aim: The aim of the present study is to investigate the PK-PD parameters of prepared Methotrexate-Curcumin and Methotrexate-Quercetin Loaded Nanoemulsion formulation for Anti-Arthritic Activity

Study Design:

Animals: For experiment Wistar rats procured at the age of 3-6 months acclimatized for 4 days weighing 200-250grams will be used for the study. The animals will be procured from the central animal facility at Kakatiya University, Warangal. The rats will be group housed in polypropylene (38×23×10cm) cages with not more than 2 animals per cage. They will be maintained under standard laboratory conditions with a natural light dark cycle (14 ±1 hr light; 10+1 hr dark) and will be allowed free access to standard dry rat diet and tap water ad libitum. The rats will be divided into 6 groups of 6 animals each.

Methods:

Rheumatoid arthritis induction will be performed by using Freund's complete Adjuvant (0.1 ml Sub Plantar region) for 22 days. Simultaneously treatment will be given using optimized formulation I & II, and conventional formulation. Evaluated for anti- arthritic activity. After the 22nd day, animals were euthanized for evaluation of biochemical and Histopathological studies.

All the animals will be divided into 5 groups containing 6 animals each (n=6)

Group I: Vehicle control - animals will be not applied with any treatment.

Group II: Arthritis was induced by injecting Freund's Complete Adjuvant (0.1 ml) in a single dose into the sub plantar region of right hind paw

Group III: Arthritic rats were treated with conventional Formulation (Positive Control)

Group IV: Arthritic rats were treated with optimized Nano Formulation-I (Methotrexate-Curcumin)

Group V: Arthritic rats were treated with optimized Nano Formulation-II (Methotrexate-Quercetin)

Treatment will be initiated after confirmation of arthritis and will be continued up to 21 days, in III, IV, and V groups. On 21st day, plasma samples will be collected at 0, 0.5, 1, 2, 4, 8, 12 and 24 hours and all rats will be sacrificed knee joint tissue will be collected from three rats from each group for histopathology and synovial fluid will be collected from three rats from each group and stored for further analysis.

Form B (per rule 8(a)* for Submission of Research Protocol (s)

Application for Permission for Animal Experiments

Application to be submitted to the CPCSEA, New Delhi after approval of Institutional Animal Ethics Committee (IAEC)

Section -I

1.	Name and address of establishment	University college of pharmaceutical sciences Kakatiya University, Warangal
2.	Registration number and date of registration.	1820/GO/RE/S/15/CPCSEA, Dt.01-09-2015
3.	Name, address and registration number of breeder from which animals acquired (or to be acquired) for experiments mentioned in parts B & C	Sainath agencies, Hyderabad
4.	Place where the animals are presently kept (or proposed to be kept).	University college of pharmaceutical sciences Kakatiya University, Warangal
5.	Place where the experiment is to be performed (Please provide CPCSEA Reg. Number)	Animal house, University college of pharmaceutical sciences, K U, Warangal
6.	Date and Duration of experiment.	8 months
7.	Type of research involved (Basic Research / Educational/ Regulatory/ Contract Research)	Educational research

Signature

Name and Designation of Investigator
Dr. V. SWAROOPA RANI
ASSOCIATE PROFESSOR
UCPSC Kakatiya university

Date: 13/4/2022
Place: Warangal

Section -II

Protocol form for research proposals to be submitted to the Institutional Animal Ethics Committee/ CPCSEA, for new experiments or extensions of ongoing experiments using animals.

1. Project / Dissertation / Thesis Title: **"EVALUATION OF SOME MEDICINAL PLANTS FOR ANTIDIABETIC ACTIVITY"**
   Principal Investigator / Research Guide / Advisor:

   a. Name: Dr.V.Swaroopa rani
   b. Designation:Associate Professor
   c. Dept / Div/ Lab: Pharmacognosy
   d. Telephone No: 7981754379
   e. E-mail Id:swarooparanivanapatla@gmail.com
   f. Experience in Lab animal experimentation: 17years

2. List of all individuals authorized to conduct procedures under this proposal.

   a. Name: Kambakam.Venkatalakshmi
   b. Designation: Research scholar
   c. Department: Pharmacognosy
   d. Telephone No:8985199236
   e. E-mail Id:venky.lucky13@gmail.com
   f. Experience in Lab animal experimentation: 9 years

3. Funding Source / Proposed Funding Source with complete address (Please attach the proof)
   AICTE sponsored QIP Ph.D,
   AICTE,New Delhi.

4. Duration of the animal experiment: 6months

   a. Date of initiation (Proposed) -After IAEC approval
   b. Date of completion (Proposed) -

5. Describe details of study plan to justify the use of animals (Enclose Annexure)

6. Animals required

a. Species and Strain: **Wistar Albino Rats**
b. Age and Weight: **150-200grams**
c. Gender: **Male**
d. Number to be used (Year-wise breakups and total figures needed tobe given in tabular form): **126**

e. Number of days each animal will be housed: 30 days

7. Rationale for animal usage

a. Why is animal usage necessary for these studies?

To determine the Pharmacological evaluation of some indigenous medicinal plants for anti-diabetic activity

b. Whether similar study has been conducted on *in vitro* models? If yes, describe the leading points to justify the requirement of animal experiment.- **No**

c. Why are the particular species selected?

Wistar Albino Rats are reported to be used in these kind of animal model studies.

d. Why is the estimated number of animals essential?

I require 7 groups (n=6) of animals (total 7X 6 = 42) animals for one plant extract and 84 animals for other two plant extracts. So, totally I require 126 animals to conduct the study. To have statistical significance, these are essential.

e. Are similar experiments conducted in the past in your establishment?
No.

f. If yes, justify why new experiment is required?
NA.

g. Have similar experiments been conducted by any other organization in same or other *in vivo* models? If yes, enclose the reference.

Similar experiments were not conducted.

8. Describe the procedures in detail:

a. Describe all invasive and potentially stressful non-invasive procedures that animals will be subjected to in the course of the experiments)-NA
b. Furnish details of injections schedule Substances:
Doses : Streptozocin 45mg/kg
Sites : IP route
Volumes: as per rat weight
c. Blood withdrawal
detailsVolumes: as per
rat wt.
Sites : Retro orbital plexus
d. Radiation (dosage and schedules):NA
e. Nature of compound/Broad Classification of drug/NCE: Plant extract

9. Does the protocol prohibit use of anesthetic or analgesic for the conduct of painful procedures? If yes, justify.

No anaesthetic is needed

10. Will survival surgery be done? **Not required**

If yes, the following to be described.

a. List and describe all surgical procedures (includingmethods of asepsis)
b. Names, qualifications and experience levels of personnels involved.
c. Describe post-operative care
d. Justify if major survival surgery is to be performed more than once on a single animal.

11. Describe post-experimentation procedures.

a. Scope for Reuse : NA

b. Rehabilitation (Name and Address, where the animals are proposed to be rehabilitated) : NA

c. Describe method of Euthanasia (If required in the protocol) : CO_2

d. Method of carcass disposal after euthanasia. : The animals carcass is collected in colour codet bins and sent to an authorized biomedical waste collection agency for final disposal.

12. Describe animal transportation methods if extra-institutional transport is envisaged. **NA**

13. Use of hazardous agents (use of recombinant DNA-based agents or potential human pathogens requires documented approval of the Institutional Biosafety Committee (IBC). For each category, the agents and the biosafety level required, appropriate therapeutic measures and the mode of disposal of contaminated food, animal wastes and carcasses must be identified).
If, your project involved use of any of the below mentioned agent, attach copy of the approval certificates of the respective agencies: **NA**

(a) Radionucleotides (AERB)
(b) Microorganisms / Biological infectious Agents (IBSC)
(c) Recombinant DNA (RCGM)
(d) Any other Hazardous Chemical / Drugs

No hazardous agents

Investigator's declaration.

1. I certify that the research proposal submitted is not unnecessarily duplicative of previously reported research.

2. I certify that, I am qualified and have experience in the experimentation on animals.

3. For procedures listed under item 10, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.

4. I will obtain approval from the IAEC/ CPCSEA before initiating any changes in this study.

5. I certify that performance of experiment will be initiated only upon review and approval of scientific intent by appropriate expert body (Institutional Scientific Advisory Committee / funding agency / other body).

6. I certify that I will submit appropriate certification of review and concurrence for studies mentioned in point 14.

7. I shall maintain all the records as per format (Form D) and submit to Institutional Animal Ethics Committee (IAEC).

8. I certify that, I will not initiate the study before approval from IAEC/ CPCSEA received in writing. Further, I certify that I will follow the recommendations of IAEC/ CPCSEA.

9. I certify that I will ensure the rehabilitation policies are adopted (wherever required).

Signature

Name of Investigator

Date:

Certificate

This is to certify that the project proposal no entitled **"EVALUATION OF SOME MEDICINAL PLANTS FOR ANTIDIABETIC ACTIVITY** submitted by Dr./ Mr. / Ms. **K.Venkatalakshmi** has been approved/recommended by the IAEC of **UCPSc, Kakatiya University** (Organization) in its meeting held on…… (date) and ……(Number and Species of animals) have been sanctioned under this proposal for a duration of next ……..
months.

Authorized by	**Name**	**Signature**	**Date**
Chairman:	…….	……	……..
Member Secretary:	…….	……	……..
Main Nominee of CPCSEA:		…….	……
		…	……
		…..	

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

Evaluation of some medicinal plants for anti-diabetic activity

Study plan:

A male wistar albino rats, weighing between 150-200g, were needed in the study. They were maintained under standard laboratory conditions at ambient temperature. They were feed with standard pellet diet and water ad libitum. The food was withdrawn from the animal cages 12 hrs before experiment and during experiment.

Method:

Induction of diabetes in rats:

Wistar rats were made diabetic by a single intraperitoneal injection of streptozotocin (45 mg/kg). streptozotocin was first weighed individually for each animal according to the body weight and then solubilized with 0.2 ml saline (154 mM NaCl) just prior to injection. Two days after streptozotocin injection, rats with plasma glucose levels of 140 mg/dl were included in the study. Treatment with plant extracts was started 48 h after streptozotocin injection.The rats were randomly divided in to 7 groups with each containing 6 animals (n=6).

- Group I : Normal
- Group II : Diabetic control
- Group III : Standard Glibenclamide(10mg/kg)
- Group IV : Fraction
- Group V : Fraction
- Group VI : Fraction
- Group VII : Fraction

Blood samples will be collected from the Retro orbital plexus at time intervals. Blood glucose levels will be determined using an electronic glucometer.

Furnish details of injections schedule:

Chapter 1(42 animals required)

Name	Streptozotocin	Plant 1 extracts	Glibenclamide
Doses (mg/kg)	45mg/kg	As per rat weight	10mg/kg
Routes/sites	I.P	Oral	Oral
Blood volume to be withdrawn (ml)	As per rat weight	As per rat weight	As per rat weight
With drawl sites	Retro orbital plexus	Retro orbital plexus	Retro orbital plexus

Chapter 2 (42 animals required): Groups were same as in chapter 1

Name	**streptozotocin**	**Plant 2 extracts**	**Glibenclamide**
Doses (mg/kg)	45mg/kg	As per rat weight	10mg/kg
Routes/sites	I.P	oral	oral
Blood volume to be withdrawn (ml)	As per rat weight	As per rat weight	Asper rat weight
With drawl sites	Retro orbital plexus	Retro orbital plexus	Retro orbital plexus

Chapter 3 (42 animals required): Groups were same as in chapter 1

Name	**streptozotocin**	**Plant 3 extracts**	**Glibenclamide**
Doses (mg/kg)	45mg/kg	As per rat weight	10mg/kg
Routes/sites	I.P	oral	oral
Blood volume to be withdrawn (ml)	As per rat weight	As per rat weight	As per rat weight
With drawl sites	Retro orbital plexus	Retro orbital plexus	Retro orbital plexus

Protocol No :11
Name:P.Durga

Approval No: (11/IAEC/UCPSc/KU/2022:CPCSEA 2018-23)

P. Durga

Form B (per rule 8(a)* for Submission of Research Protocol (s)Application for Permission for Animal Experiments

Application to be submitted to the CPCSEA, New Delhi after approval of InstitutionalAnimal Ethics Committee (IAEC)

Section -I

1.	Name and address of establishment	University College Of Pharmaceutical Sciences, Warangal ,Telangana.
2.	Registration number and date of registration.	1820/GO/RE/S/15/CPCSEA DATE: 1/9/2015
3.	Name, address and registration number of breeder from which animals acquired (or to be acquired) for experiments mentioned in parts B & C	Vyas Labs , Hyderabad. CPCSEA:2085/PO/RCBiBt/S/19/CPCS
4.	Place where the animals are presently kept (or proposed to be kept).	Animal house, Univerity College of Pharmaceutical Sciences ,Warangal.
5.	Place where the experiment is to be performed (Please provide CPCSEA Reg. Number)	Animal house ,University College Of Pharmaceutical Sciences,Warangal.
6.	Date and Duration of experiment.	Experiments will start only after IAEC approval and duration is One year
7.	Type of research involved (Basic Research / Educational/ Regulatory/ Contract Research)	Educational Research

Signature

Name and Designation of Investigator

Date/Place: 13/04/2022

~~18/4/2022~~

UCPSc, Wgl.

Dr. NEERATI PRASAD
M.Pharm.,Ph.D., PDF(USA)
Associate Professor of Pharmacy
University College of Pharm Sciences
KAKATIYA UNIVERSITY
WARANGAL-506 009 T.S. INDIA.

Section -II

Protocol form for research proposals to be submitted to the Institutional Animal Ethics Committee/ CPCSEA, for new experiments or extensions of ongoing experiments using animals.

1. Project / Dissertation / Thesis Title: Improvising Anticancer Activity Of Polyphenols In Animals By Cocrystal Technology

2. Principal Investigator / Research Guide / Advisor:

   a. Name: Dr. N Prasad
   b. Designation: Associate professor
   c. Dept / Div/ Lab: DMPK Division, Department of Pharmacology,
   d. Telephone No.9494812120
   e. E-mail Id: prasadneerati@gmail.com
   f. Experience in Lab animal experimentation 15 years

3. List of all individuals authorized to conduct procedures under this proposal.

   a. Name: Durga Polati
   b. Designation: Research Scholar
   c. Department: Pharmacology
   d. Telephone No.9492110008
   e. E-mail Id: durga1620@gmail.com
   f. Experience in Lab animal experimentation:7 years

4. Funding Source / Proposed Funding Source with complete address (Please attachthe proof) NA

5. Duration of the animal experiment.

   a. Date of initiation (Proposed): Experiments will start only after IAEC approval.
   b. Date of completion (Proposed): within 1-2 years.

6. Describe details of study plan to justify the use of animals (Enclose Annexure) : Enclosed

Annexure 1

Study title: Improvising Anticancer Activity of Polyphenols in Animals by Cocrystal Technology .

Aim of the study: This research is aimed to synthesize Polyphenols cocrystals by the Cocrystallization techniques intending to improve the bio availability of Polyphenols.

Objectives of the study:

- To enhance the bioavailability of selected bioactive Polyphenols by inhibiting glucuronidation and constructing co crystals using other polyphenols as coformers.
- To characterize the formulated cocrystals of Polyphenols.
- To improve the anticancer activity of Polyphenols.

Literature review:

1. **Liu et al., (2016)** prepared the cocrystal of myricetin with proline by solution crystallization. Crystal structure was characterized by differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), and scanning electron microscopy (SEM). This study proved that the dissolution rate of myricetin in the cocrystal was 7.69 times higher than that of coarse myricetin. The oral bioavailability of the cocrystal was approximately 3.03 times higher than that of myricetin (1).
2. **Michał Sowa et al., (2014)** established cocrystalline phase with combination of genistein and caffeine leads to a 1:1, which was identified by means of a solvent-drop grinding experiment and isolated afterwards in a solution-evaporation approach. Dissolution studies in a 50:50 v/v ethanol–water medium revealed that the maximum solubility of the cocrystalline phase reached 0.861 mg/mL after 8 h, revealing some degree of enhancement as compared to parent genistein, maximum solubility of which was also reached after 8 h and equalled 0.588 mg/mL (2).

Study Design: The study is divided into three chapters. Three Phyto-phenols with poor bio availability are selected for constructing cocrystals

Chapter1: Improvisation of Anticancer activity of Curcumin with Cucumin -Piperine Cocrystal in Bladder Cancerous Female Rats

Proposed Methodology

1. Procurement of Curcumin and Piperine (coformer) and other chemicals.
2. Preparation of Co crystals using solvent evaporation technique.
3. Preparation of Nano cocrystals of Curcumin and Piperine
4. To design the characterization studies of co crystal and nano cocrystals.
   - DSC, PXRD, FTIR, SEM and/or TEM.
5. Selection and procurement of Experimental animals
6. Induction of cancer in experimental animals.

- **Bladder Cancer:** The rat models of bladder cancer were established by infusing N-methyl-nitroso-urea (MNU,10 mg/kg every 2 weeks for 8 weeks) into the bladder. (3)

7. Improvisation of anticancer activity of Curcumin.
   - Estimation of UDP-glucuronyl transferase enzyme inhibition by Piperine (coformer).
   - Evaluation of pharmacokinetic parameters of formulated cocrystals.
   - Comparison of anticancer activity of formulated Co crystals to that of powder mixture of Curcumin and Piperine.

Number of animals:24

Animals divided into 4 groups containing 6 animals each

Group 1: Cancer Control
Group 2: CUR+PIP Coarse powder
Group 3: CUR+PIP Cocrystal
Group 4: CUR+PIP Nano Cocrystal

CUR. - Curcumin
PIP - Piperine.

CHAPTER 2 Improvisation of Anticancer activity of Resveratrol with Resveratrol-Quercetin Cocrystals in Colon cancerous Rats.

Proposed Methodology

1.Procurement of Resveratrol and Quercetin (coformer) and other chemicals.

2.Preparation of Co crystals using solvent evaporation technique.

3.Preparation of nano cocrystals of Resveratrol and Quercetin

4.To design the characterization studies of co crystal.

- DSC, PXRD, FTIR, SEM and/or TEM.

5.Selection and procurement of Experimental animals

6.Induction of cancer in experimental animals.

- **Colorectal cancer:** The administration of 1,2- dimethylhydrazine [DMH] to rodents provides a reliable and consistent means of inducing colorectal cancer (4). Azoxy methane 15mg /kg once a week, up to three weeks and dextran sulphate sodium can also be used to induce colorectal cancer.

7.Improvisation of anticancer activity of Resveratrol.

- Estimation of UDP-glucuronyl transferase enzyme inhibition by Quercetin.
- Evaluation of pharmacokinetic parameters of formulated cocrystals.
- Comparison of anticancer activity of formulated Cocrystals to that of powder mixture of Resveratrol and Quercetin.

Number of animals:24

Animals divided into 4 groups containing 6 animals each

Group 1: Cancer control group

Group 2: RES+ QUER Coarse Powder

Group 3: RES+ QUER Co crystals

Group 4: RES+ QUER Nano Cocrystals

RES-Resveratrol, QUER-Quercetin

CHAPTER 3 Improvisation of Anticancer activity of Genistein with Genistein-Chrysin Cocrystals in Hepatic cancerous Rats

Proposed Methodology

1.Procurement of Genistein and Chrysin (coformer) and other chemicals.

2.Preparation of Co crystals using solvent evaporation technique.

3.Preparation of Nano cocrystals of Genistein and Chrysin

4.To design the characterization studies of co crystal.

- DSC, PXRD, FTIR, SEM and/or TEM.

5.Selection and procurement of Experimental animals

6.Induction of cancer in experimental animals.

- Liver cancer: Hepatic cancer can be induced with N Methyl nitroso Urea at the dose of 200 mg/kg BW along with bisphenol A (5).

7.Improvisation of anticancer activity of Genistein.

- Estimation of UDP-glucuronyl transferase enzyme inhibition by Chrysin
- Evaluation of pharmacokinetic parameters of formulated cocrystal.
- Comparison of anticancer activity of formulated Co crystals to that of powder mixture of Genistein and Chrysin.

Number of animals:24

Animals divided into 4 groups containing 6 animals each

Group 1: Cancer control.

Group 2: GEN+ CHRY Coarse powder

Group 3: GEN+ CHRY Cocrystals

Group 4: GEN+ CHRY Nano Cocrystals

GEN-Genistein, CHRY-Chrysin

References

1.Liu, M., et al., Development of a pharmaceutical cocrystal with solution crystallization technology: Preparation, characterization, and evaluation of myricetin-proline cocrystals. European Journal of Pharmaceutics and Biopharmaceutics, 2016. **107**: p. 151-159.

2.Sowa, M., K. Ślepokura, and E.J.J.o.M.S. Matczak-Jon, Solid-state characterization and solubility of a genistein–caffeine cocrystal. 2014. **1076**: p. 80-88.

3.Liu, D., Pan, F., Li, B. et al. Intervention of nicotine on MNU-induced bladder cancer in rats. J. Huazhong Univ. Sci. Technol. [Med. Sci.] 31, 103–106 (2011).

4.Gilbert, J.M., Experimental colorectal cancer as a model of human disease. Annals of the Royal College of Surgeons of England, 1987. 69(2): p. 48-53.

5. Afzal, M., et al., Thiamine potentiates chemoprotective effects of ibuprofen in DEN induced hepatic cancer via alteration of oxidative stress and inflammatory mechanism. 2017. 623: p. 58-63.

7. Animals required

a. Species and Strain: Rats(wistar)
b. Age and Weight: Rats- 6 weeks, Wt- 250 gms-274gms

c. Gender: Male and Female
d. Number to be used (Year-wise breakups and total figures needed to be given in tabular form): 72

Year	No of groups	No of animals(n=6)
First year	4	24
Second year	4	24
Third year	4	24

e. Number of days each animal will be housed. 4-5 weeks

8. Rationale for animal usage
   - Why is animal usage necessary for these studies?
     Animal models are powerful tools to elucidate multistage mechanisms for cancer development and to gain further insights into the biological roles of various cancer-related genes in *in vivo* situations as well as help full to screen anticancer properties of investigational compounds.
   - Whether similar study has been conducted on *in vitro* models? If yes, describe the leading points to justify the requirement of animal experiment. Yes. To observe and evaluate the effect of physiological parametres on potential of selected Phyto-phenols as anticancer drugs
   - Why are the particular species selected?
     Rodents have been one of the most widely utilized animal models to dissect the molecular events implicated in the development of cancer models
   - Why is the estimated number of animals essential?
     To meet the objectives of the study, animals will be divided into twelve groups and each group consisting of 6 animals. 12*6=72
   - Are similar experiments conducted in the past in your establishment? No
   - If yes, justify why new experiment is required?

   - Have similar experiments been conducted by any other organization in same or other *in vivo* models? If yes, enclose the reference. No

9. Describe the procedures in detail:
   - Describe all invasive and potentially stressful non-invasive procedures that animals will be subjected to in the course of the experiments)

Anticancer effects of co crystals of Polyphenols will be assessed by

observation of following parameters.

i. Percentage increase in weight as compared to day-0 weight
ii. Median survival time and increase in lifespan [% ILS]
iii. Hematological parameters
iv. Histopathological studies (Miao He et al.,2019)

- Furnish details of injections schedule Substances:

Doses: Azoxy methane -15mg /kg once a week, upto three weeks,
N Methyl nitroso Urea at the dose of 200 mg/kg BW along with bisphenol A.
Sites : s.c, oral
Volumes : As per dilutions

- Blood withdrawal Details:
- Volumes:
- Sites :

Animals	Volume	Sites
Mice	More than 0.1 ml	Retro orbital sinus/tail vein
Rat	More than 1ml	Retro orbital sinus/Tail vein
Rabbit	5ml	Marginal ear vein

- Radiation (dosage and schedules): NA
- Nature of compound/Broad Classification of drug/NCE: Phyto -poly phenols

10. Does the protocol prohibit use of anesthetic or analgesic for the conduct of painful procedures? If yes, justify.NO

11. Will survival surgery be done? NO

If yes, the following to be described

List and describe all surgical procedures (including methods of a sepsis)

- Names, qualifications and experience levels of personnels involved.
- Describe post-operative care
- Justify if major survival surgery is to be performed more than once on asingle animal.

12. Describe post-experimentation procedures.

- Scope for Reuse NO :
- Rehabilitation (Name and Address, where the animals are proposed to be rehabilitated): NA
- Describe method of Euthanasia (If required in the protocol): Physical methods
- Method of carcass disposal after euthanasia: The animal carcass is collected in colour codet bins and sent to an authorized bio medical waste collection agency for final disposal

13. Describe animal transportation methods if extra-institutional transport is envisaged. NA

14. Use of hazardous agents (use of recombinant DNA-based agents or potential human pathogens requires documented approval of the Institutional Biosafety Committee (IBC). For each category, the agents and the biosafety level required, appropriate therapeutic measures and the mode of disposal of contaminated food, animal wastes and carcasses must be identified).
If, your project involved use of any of the below mentioned agent, attach copy of the approval certificates of the respective agencies: NA

(a) Radionucleotides (AERB)
(b) Microorganisms / Biological infectious Agents (IBSC)
(c) Recombinant DNA (RCGM)
(d) Any other Hazardous Chemical / Drugs

Investigator's declaration.

1. I certify that the research proposal submitted is not unnecessarily duplicative of previously reported research.

2. I certify that, I am qualified and have experience in the experimentation on animals.

3. For procedures listed under item 10, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.

4. I will obtain approval from the IAEC/ CPCSEA before initiating any changes in this study.

5. I certify that performance of experiment will be initiated only upon review and approval of scientific intent by appropriate expert body (Institutional Scientific Advisory Committee / funding agency / other body).

6. I certify that I will submit appropriate certification of review and concurrence for studies mentioned in point 14.

7. I shall maintain all the records as per format (Form D) and submit to Institutional Animal Ethics Committee (IAEC).

8. I certify that, I will not initiate the study before approval from IAEC/ CPCSEA received in writing. Further, I certify that I will follow the recommendations of IAEC/ CPCSEA.

9. I certify that I will ensure the rehabilitation policies are adopted (wherever required).

Signature

Name of Investigator

Date:

Dr. NEERATI PRASAD
M.Pharm.,Ph.D., PDF(USA)
Associate Professor of Pharmacy
University College of Pharm Sciences
KAKATIYA UNIVERSITY
WARANGAL-506 009 T.S. INDIA.

Certificate

This is to certify that the project proposal no entitled **Improvising Anticancer Activity of Polyphenols in Animals by Cocrystal Technology** submitted by Dr./ Mr. / Ms. **Polati Durga** has been approved/recommended by the IAEC of.....(Organization) in its meeting held on..... (date) and .....(Number and Species of animals) have been sanctioned under this proposal for a duration of next .....

months.

Authorized by	Name	Signature	Date
Chairman:	.....	.....	.....
Member Secretary:	.....	.....	.....
Main Nominee of CPCSEA:	.....	.....	.....

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

Protocol No :12
Name:M.Sravanthi

Approval No: (12/IAEC/UCPSc/KU/2022:CPCSEA 2018-23)

M. Sravanthi

Form B (per rule 8(a)* for Submission of Research Protocol (s)

Application for Permission for Animal Experiments

Application to be submitted to the CPCSEA, New Delhi after approval of Institutional Animal Ethics Committee (IAEC)

Section -I

1.	Name and address of establishment	University College Of Pharmaceutical Sciences Kakatiya University Warangal-506009(T.S)
2.	Registration number and date of registration.	1820/GO/Re/S/15/CPCSEA Date:01-09-2015
3.	Name, address and registration number of breeder from which animals acquired (or to be acquired) for experiments mentioned in parts B & C	Vyas Labs,Amberpet,Hyderabad CPCSEA:2085/PO/RCBiBt/S/19/CPCS
4.	Place where the animals are presently kept (or proposed to be kept).	Animal house, University College of Pharmaceutical Sciences, KU, Warangal.
5.	Place where the experiment is to be performed (Please provide CPCSEA Reg. Number)	Animal house, University College of Pharmaceutical Sciences, KU, Warangal.
6.	Date and Duration of experiment.	6Months
7.	Type of research involved (Basic Research / Educational/ Regulatory/ Contract Research)	Educational research

Signature

Name and Designation of Investigator

Dr. Shayeda
Assistant professor

Date: 07/04/2022
Place: Warangal

Section -II

Protocol form for research proposals to be submitted to the Institutional Animal Ethics Committee/ CPCSEA, for new experiments or extensions of ongoing experiments using animals.

1. Project / Dissertation / Thesis Title: : Green Biosynthesis, Characterization, In Vitro Pharmacological activities and Investigational Acute Toxicity Study of Herbal mediated Silver Nanoparticles on Animal models.

2. Principal Investigator / Research Guide / Advisor:

   a. Name: Dr. Shayeda
   b. Designation: Assistant professor
   c. Dept / Div/ Lab: DMPK Division, Department of Pharmaceutics,
   d. Telephone No.9849531109
   e. E-mail Id: shayeda_ucpsc@yahoo.com
   f. Experience in Lab animal experimentation 15 years

3. List of all individuals authorized to conduct procedures under this proposal.

   a. Name: Sravanthi.Mandala
   b. Designation: Research Scholar
   c. Department: Pharmacognosy
   d. Telephone No.7306829762
   e. E-mail Id: sravanthireddy2011@gmail.com
   f. Experience in Lab animal experimentation:3 years

4. Funding Source / Proposed Funding Source with complete address (Please attach the proof)
   AICTE sponsored QIP Ph.D,
   AICTE,New Delhi.

5. Duration of the animal experiment: 6 months

   a. Date of initiation (Proposed)
   b. Date of completion (Proposed)

6. Describe details of study plan to justify the use of animals (Enclose Annexure)

7. Animals required

a. Species and Strain: **Wistar rats**
b. Age and Weight: **120-150grams**
c. Gender: **Female**
d. Number to be used (Year-wise breakups and total figures needed to begiven in tabular form): **30**

e. Number of days each animal will be housed:14 days

8. Rationale for animal usage

a. Why is animal usage necessary for these studies?
Humans and animals share hundreds of illnesses, so animals often act as models for the study of human disease. To determine the highlights of the safety and bio compatability of herbal mediated silver nano particles with in a cell.

b. Whether similar study has been conducted on *in vitro* models? If yes, describe the leading points to justify the requirement of animal experiment.- No

c. Why are the particular species selected?
Albino rats are reported to be used in these kind of animal model studies.

d. Why is the estimated number of animals essential?
We require 5 groups (n=6) of animals (total 5X6 = 30) to conduct the study. To have statistical significance,these are essential.

e. Are similar experiments conducted in the past in your establishment?
No.

f. If yes, justify why new experiment is required?
NA.

g. Have similar experiments been conducted by any other organization in same or other *in vivo* models? If yes, enclose the reference.

Similar experiments were not conducted.

9. Describe the procedures in detail:

a. Describe all invasive and potentially stressful non-invasive procedures that animals will be subjected to in the course of the experiments)-NA
b. Furnish details of injections schedule Substances:
Doses :400mg/kg of Herbal
extract,carbontetra chloride(0.5ml/kg)
Sites :Oral route ,intraperitonial
Volumes :0.5ml
c.Blood withdrawal Details:
Whole blood will be
collected.
d. Radiation (dosage and schedules):NA
e. Nature of compound/Broad Classification of drug/NCE:plant extract

10. Does the protocol prohibit use of anesthetic or analgesic for the conduct of painful procedures? If yes, justify.
No anaesthetic is needed

11. Will survival surgery be done? **Not required**

If yes, the following to be described.

a. List and describe all surgical procedures (includingmethods of asepsis)
b. Names, qualifications and experience levels of personnels involved.
c. Describe post-operative care
d. Justify if major survival surgery is to be performed more than once on asingle animal.

12. Describe post-experimentation procedures. **NA**

a. Scope for Reuse : NA

b. Rehabilitation (Name and Address, where the animals are proposed to be rehabilitated) : NA

c. Describe method of Euthanasia (If required in the protocol) : Co_2

d. Method of carcass disposal after euthanasia. : The animals carcass is collected in colour codet bins and sent to an authorized biomedical waste collection agency for final disposal.

13. Describe animal transportation methods if extra-institutional transport is envisaged. **NA**

14. Use of hazardous agents (use of recombinant DNA-based agents or potential human pathogens requires documented approval of the Institutional Biosafety Committee (IBC). For each category, the agents and the biosafety level required, appropriate therapeutic measures and the mode of disposal of contaminated food, animal wastes and carcasses must be identified).
If, your project involved use of any of the below mentioned agent, attach copy of the approval certificates of the respective agencies: **NA**

(a) Radionucleotides (AERB)
(b) Microorganisms / Biological infectious Agents (IBSC)
(c) Recombinant DNA (RCGM)
(d) Any other Hazardous Chemical / Drugs

No hazardous agents

Investigator's declaration.

1. I certify that the research proposal submitted is not unnecessarily duplicative of previously reported research.

2. I certify that, I am qualified and have experience in the experimentation on animals.

3. For procedures listed under item 10, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.

4. I will obtain approval from the IAEC/ CPCSEA before initiating any changes in this study.

5. I certify that performance of experiment will be initiated only upon review and approval of scientific intent by appropriate expert body (Institutional Scientific Advisory Committee / funding agency / other body).

6. I certify that I will submit appropriate certification of review and concurrence for studies mentioned in point 14.

7. I shall maintain all the records as per format (Form D) and submit to Institutional Animal Ethics Committee (IAEC).

8. I certify that, I will not initiate the study before approval from IAEC/ CPCSEA received in writing. Further, I certify that I will follow the recommendations of IAEC/ CPCSEA.

9. I certify that I will ensure the rehabilitation policies are adopted (wherever required).

Signature Shayeda

Name of Investigator Dr. Shayeda Asst. professor

Date: 29/04/2022

Certificate

This is to certify that the project proposal no..... entitled Green Biosynthesis, Characterization, In Vitro Pharmacological activities and Investigational Acute Toxicity Study of Herbal mediated Silver Nanoparticles on Animal models.
submitted by Dr./ Mr. / Ms. ...SRAVANTHI.MANDALA...
has been approved/recommended by the IAEC of.....(Organization) in its meeting held on..... (date) and .....(Number and Species of animals) have been sanctioned under this proposal for a duration of next ..... months.

Authorized by	**Name**	**Signature**	**Date**
Chairman:	.....	.....	.....
Member Secretary:	.....	.....	.....
Main Nominee of CPCSEA:	.....	.....	.....

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

Approval No: (13/IAEC/UCPSc/KU/2022:CPCSEA 2018-23)

Protocol No :13
Name:S.Anusha

S. Anusha

Form B (per rule 8(a)* for Submission of Research Protocol (s)

Application for Permission for Animal Experiments

Application to be submitted to the CPCSEA, New Delhi after approval of Institutional Animal Ethics Committee (IAEC)

Section -I

1.	Name and address of establishment	University college of Pharmaceutical sciences, Kakatiya university, Warangal.
2.	Registration number and date of registration.	1820/GO/Re/S/15/CPCSEA and Date: 01-09-2015
3.	Name, address and registration number of breeder from which animals acquired (or to be acquired) for experiments mentioned in parts B &C	Vyas Labs.Amberpet, Hyderabad CPCSEA: 2085/PO/RCBiBt/S/19/CPCSEA
4.	Place where the animals are presently kept (or proposed to be kept).	Animal house, University College of Pharmaceutical Sciences, KU, Warangal.
5.	Place where the experiment is to be performed (Please provide CPCSEA Reg. Number)	Animal house, University College of Pharmaceutical Sciences, KU, Warangal
6.	Date and Duration of experiment.	3 months
7.	Type of research involved (Basic Research / Educational/ Regulatory/ Contract Research)	Educational

Signature

Name and Designation of Investigator

Date: 13/04/2022

Place: Warangal

Dr. V. SWAROOPA RANI
ASSOCIATE PROFESSOR
University College of Pharmaceutical Sciences
KAKATIYA UNIVERSITY, Warangal.

Section -II

Protocol form for research proposals to be submitted to the Institutional Animal Ethics Committee/ CPCSEA, for new experiments or extensions of ongoing experiments using animals.

1. Project / Dissertation / ThesisTitle: Appraisal of some medicinal plants for the management of Diabetic complications through inhibition of aldose reductase and advanced glycation endproducts formation.
2. Principal Investigator / Research Guide /Advisor:

   a. Name: Dr. V. SwaroopaRani
   b. Designation: Associate Professor
   c. Dept / Div/Lab: Pharmacognosy
   d. Telephone No: 7981754379
   e. E-mailId: swarooparanivanapatla@gmail.com
   f. Experience in Lab animalexperimentation: 17years

3. List of all individuals authorized to conduct procedures under thisproposal.

   a. Name : S.Anusha
   b. Designation: Research Scholar
   c. Department: Pharmacognosy
   d. Telephone No:9701932527
   e. E-mailId: anushasatla6789@gmail.com
   f. Experience in Lab animalexperimentation: 4years

4. Funding Source / Proposed Funding Source with complete address (Please attach the proof)

5. Duration of the project:
   a. Number of months: 3
   b. Date of initiation: April 2022
   c. Date of completion: July 2022

6. Describe details of study plan to justify the use of animals (EncloseAnnexure) Annexure(Study plan) enclosed.

7. Animalsrequired

a. Species andStrain: Wistar rats
b. Age andWeight:150-200gms
c. Gender: Male
d. Number to be used (Year-wise breakups and total figures needed to be given in tabularform): 115 (7+54+54)
e. Number of days each animal will be housed: 3 months

8. Rationale for animalusage

a. Why is animal usage necessary for thesestudies?
Rats are easily available.
b. Whether similar study has been conducted on *in vitro* models? If yes, describe the leading points to justify the requirement of animal experiment-No
c. Why are the particular speciesselected?
Rats are reported animal model for this type of study.
d. Why is the estimated number of animalsessential?
To have statistical significance, these are essential
e. Are similar experiments conducted in the past in yourestablishment?- No
f. If yes, justify why new experiment isrequired?- Not applicable
g. Have similar experiments been conducted by any other organization in same or other *in vivo* models? If yes, enclose thereference.
Similar experiments were not conducted.

9. Describe the procedures indetail:

a. Describe all invasive and potentially stressful non-invasive procedures that animals will be subjected to in the course of the experiments: NA
b. Furnish details of injections schedule substances:
Doses: 10 mg/kg, 100mg/kg
Sites : Oral
Volume: 1 ml
c. Blood withdrawl details :
Volume: 0.5 ml
Sites: Retro orbital plexus
d. Radiation (dosage and schedules) : NA
e. Nature of compound / Broad classification of drug/ NCE: Plant extract

10. Does the protocol prohibit use of anesthetic or analgesic for the conduct of painful procedures? If yes,justify.- NA

11. Will survival surgery bedone? If yes, the following to be described.- No

a. List and describe all surgical procedures (including methods ofasepsis)
b. Names, qualifications and experience levels of personnel'sinvolved.
c. Describe post-operativecare
d. Justify if major survival surgery is to be performed more than once on a singleanimal.

12. Describe post-experimentationprocedures-

a. ScopeforReuse : NA

b. Rehabilitation (Name and Address, where the animals are proposed to be rehabilitated): NA

c. Describe methodofEuthanasia. : Co_2

d. Method of carcass disposalaftereuthanasia. : The animals carcass is collected in colourcodet bins and sent to an authorized bio medical waste collection agency for final disposal.

13. Describe animal transportation methods if extra-institutional transport is envisaged.- NA

14. Use of hazardous agents (use of recombinant DNA-based agents or potential human pathogens requires documented approval of the Institutional Biosafety Committee (IBC). For each category, the agents and the biosafety level required, appropriate therapeutic measures and the mode of disposal of contaminated food, animal wastes and carcasses must beidentified).
If, your project involved use of any of the below mentioned agent, attach copy of the approval certificates of the respectiveagencies:

(a) Radio nucleotides (AERB)
(b) Microorganisms / Biological infectious Agents(IBSC)
(c) Recombinant DNA(RCGM)
(d) Any other Hazardous Chemical /Drugs

No hazardous agent is involved in the study.

Investigator's declaration.

1. I certify that the research proposal submitted is not unnecessarily duplicative of previously reportedresearch.

2. I certify that, I am qualified and have experience in the experimentation onanimals.

3. For procedures listed under item 10, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.

4. I will obtain approval from the IAEC/ CPCSEA before initiating any changes in thisstudy.

5. I certify that performance of experiment will be initiated only upon review and approval of scientific intent by appropriate expert body (Institutional Scientific Advisory Committee / funding agency / otherbody).

6. I certify that I will submit appropriate certification of review and concurrence for studies mentioned in point14.

7. I shall maintain all the records as per format (Form D) and submit to Institutional Animal Ethics Committee(IAEC).

8. I certify that, I will not initiate the study before approval from IAEC/ CPCSEA received in writing. Further, I certify that I will follow the recommendations of IAEC/CPCSEA.

9. I certify that I will ensure the rehabilitation policies are adopted (whereverrequired).

Signature

Swaroopa

Name ofInvestigator

Date: 29/04/2022

Dr. V. SWAROOPA RANI
ASSOCIATE PROFESSOR
University College of Pharmaceutical Sciences
KAKATIYA UNIVERSITY, Warangal

Certificate

This is to certify that the project proposal no. ..... entitled "**Appraisal of some medicinal plants for the management of Diabetic complications through inhibition of aldose reductase and advanced glycation endproducts formation"** submitted by Dr./ Mr./Ms. S.Anusha.has been approved/recommended by the IAEC of.....(Organization) in its meeting held on..... (date) and .....(Number and Species of animals) have been sanctioned under this proposal for a duration of next ..... months.

Authorized by	**Name**	**Signature**	**Date**
Chairman:	.....	.....	.....
Member Secretary:	.....	.....	.....
Main Nominee of CPCSEA:	.....	.....	.....

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

Study Plan

Aim

To evaluate some medicinal plants for the management of Diabetic complications through inhibition of aldose reductase and advanced glycation endproducts formation.

Objective :

The present study was aimed to evaluate the aldose reductase (AR) and advanced glycation end product (AGE) inhibitory potential of plant extracts and their phytoconstituents for its possible role in the treatment of diabetic complications

Study plan:

Male Wistar rats, weighing between 150-200g were needed in the study. They were maintained under standard laboratory conditions at ambient temperature. They were feed with standard pellet diet and water *adlibitum*.

Chapter 1:

In vitro ARI activity:

Seven Rats were sacrificed by cervical dislocation followed by removal of eyes and kidneys.

Isolation of crude AR from rat lens and kidneys.

Chapter 2:

Galactosemic rat model

Male Wistar albino rats weighing 160-200 g were divided into nine groups (n = 6), Group I and Group II served as naïve and control respectively. Naive animals were treated orally with vehicle (1% gum accacia). Group III epalrestat(1mg/kg/day) and group IV ,V, VI received plant extracts (100mg/kg) and VII, VIII, IX received phytochemicals(10 mg/kg/day), respectively, for a period of 3 weeks by oral administration. The control and test groups were fed orally with galactose at a dose of 20 mg/kg body weight throughout the experimental period. Blood was withdrawn from all the animals through retro-orbital plexus and blood glucose was estimated at every 7 day interval. All the animals were then sacrificed on the 21st day by decapitation followed by isolation of sciatic nerves and both eye balls.

Grouping of Animals: n=6

Group	Treatment
I	Negative control
II	Positive control (Galactose-20mg/kg,)
III	Epalrestat 1mg/kg
IV	*Areca bakeri* 100 mg/kg
V	*Ammi majus*100mg/kg
VI	*Solanum nigrum* 100mg/kg
VII	Arborniol 10mg/kg
VIII	Visnagin 10mg/kg
IX	Solasonine 10mg/kg

Chapter 3:

STZ induced diabetic complications

Diabetes was induced in all the male Wistar rats (160–200g) except a group of six animals which were treated as naive (group I) by intraperitoneal administration of STZ (45 mg/kg) dissolved in freshly prepared citrate buffer (pH 4.5). The animals were fasted for 12 h before STZ administration and supplemented with 10% glucose for 48 h after STZ administration. One week after STZ administration, blood glucose was estimated and the animals with more than 300 mg/dL were treated as diabetic and after a period of 6 weeks, the animals were divided into eight groups (n = 6): Group II served as diabetic control where as group III received epalrestat (1mg/kg), and groups IV, V, VI received plant extracts (100mg/kg) and groups VII, VIII,IX received phytochemicals (10mg/kg) by oral administration, for a period of 3 weeks. Blood was withdrawn from the retro orbital plexus followed by collection of 24 h urine samples, after which the animals were sacrificed , kidneys and sciatic nerves are isolated.

Grouping of Animals: n=6

Group	Treatment
I	Negative control
II	Toxic control(STZ -45mg/kg)
III	Epalrestat 1mg/kg
IV	*Areca bakeri* 100 mg/kg
V	*Ammi majus*100mg/kg
VI	*Solanum nigrum* 100mg/kg
VII	Arborniol 10mg/kg
VIII	Visnagin 10mg/kg
IX	Solasonine 10mg/kg

S. Manjula ; Pharmaceutical Chemistry

Protocol No :14
Name:S.Manjula

Approval No: (14/IAEC/UCPSc/KU/2022:CPCSEA 2018-23)

Form B (per rule 8(a)* for Submission of Research Protocol (s)Application for Permission for Animal Experiments

Application to be submitted to the CPCSEA, New Delhi after approval of Institutional Animal Ethics Committee (IAEC)

Section -I

1.	Name and address of establishment	University College Of Pharmaceutical Sciences Warangal, Telangana.
2.	Registration number and date of registration.	1820/GO/Re/S/15/CPCSEA DATE:01-09-2015
3.	Name, address and registration number of breeder from which animals acquired (or to be acquired) for experiments mentioned in parts B & C	Vyas Labs , Hyderabad CPCSEA:2085/PO/RCBiBt/S/19/CPCS
4.	Place where the animals are presently kept (or proposed to be kept).	Animal House, University College Of Pharmaceutical Sciences.
5.	Place where the experiment is to be performed (Please provide CPCSEA Reg. Number)	Animal House, University College Of Pharmaceutical Sciences
6.	Date and Duration of experiment.	Experiments will start only after IAEC approval and duration is six months
7.	Type of research involved (Basic Research / Educational/ Regulatory/ Contract Research)	Educational Research

Signature

Name and Designation of Investigator

Dr. G. Sammaiah
Associate professor
UCPSC, University College of Pharmacy, Kakatiya University Warangal

Date: 18/04/2022
Place: UCPSC, Kakatiya University, Warangal

Section -II

Protocol form for research proposals to be submitted to the Institutional Animal Ethics Committee/ CPCSEA, for new experiments or extensions of ongoing experiments using animals.

1. Project / Dissertation / Thesis Title: **Synthesis and Evaluation of New Isatin Derivatives for their Possible Biological Activities**

2. Principal Investigator / Research Guide / Advisor:

   a. Name: Dr. Gade Sammaiah
   b. Designation: Associate Professor
   c. Dept / Div/ Lab : Pharmaceutical Sciences(Pharmaceutical Chemistry)
   d. Telephone No: 9849500471
   e. E-mail Id: g.sammaiah@gmail.com
   f. Experience in Lab animal experimentation:20 years

3. List of all individuals authorized to conduct procedures under this proposal.

   a. Name:S.Manjula
   b. Designation:PhD Scholar
   c. Department: Pharmaceutical Chemistry
   d. Telephone No.8700604836
   e. E-mail Id: nampallymanjula1985@gamil.com
   f. Experience in Lab animal experimentation:3 years

4. Funding Source / Proposed Funding Source with complete address (Please attach the proof) AICTE Sponsered QIP Programme 2020-21

5. Duration of the animal experiment.6 months

   a. Date of initiation (Proposed)
   b. Date of completion (Proposed)

6. Describe details of study plan to justify the use of animals (Enclose Annexure):Enclosed

ANNEXURE

Study title: Synthesis and Evaluation of New Isatin Derivatives for their Possible Biological Activities

Back ground : Isatin (2, 3-dioxidole) is an important class of heterocyclic compounds & is an indole derivative. Isatin derivatives are synthetically important substrates, which can be used for the synthesis of large variety of heterocyclic compounds, and as raw material for drug synthesis. Recently Isatin derivatives have attracted strong interest in organic and medicinal chemistry due to their potent biological and pharmacological activities. Isatin and its derivatives process numerous biological properties like antitumor, antimicrobial, anti-inflammatory, analgesic, anti-mycobacterial, antiviral, anthelmintic, anti-HIV, anti-oxidant, CNS depressant& amp; anti-Alzheimer's activities (1).

Literature survey:

1. **Amal M. Youssef et al., (2010)** synthesized a series of novel pyrazolyl-2,4-thiazolidinediones and evaluated for their anti-inflammatory and neuroprotective properties in vitro. . Among the series, Compounds 1,3-diphenyl and 4-chlrophenyl derivative of the allyl compound showed anti-neurotoxic activity and 4-chlorophenyl showed anti-inflammatory at concentrations below their cytotoxic range

2. **Senthil kumar et al., (2018)** designed and synthesized a new series of sixteen thiazolidine-2,4-dione derivatives with azole heterocyclic compounds via Knoevenagel condensation and evaluated them for their inhibitory activity against α-amylase and α -glucosidase. Among the evaluated compounds with 2- trifluoromethoxy phenyl amino, 4-fluorophenylamino and 3-trifluoromethylamino derivatives showed better inhibition in the range of 35–40% at 250 µg/ml concentration on α -amylase and α –glucosidase

Objective: According to Literature Isatin and its different analogs are performing wide spectrum of biological activities. So the main objective of this project is to design, synthesize a drug molecule and also to identify the target site for drug action that can effectively prevent inflammatory disease to protect our body from invaders.

Proposed Methodology: Synthesis of the Isatin derivatives like sunitinib malate as a kinase inhibitor will be carried out systematically based on the schemes. Initially oxindoles and its analogs will be synthesized. The condensation of the Isatins or indurubin with active methylene heterocycles gives a desired compound for which recrystallization will be carried out for the synthesized products. Elemental analysis for the obtained derivatives would be carried out using various spectral studies

such as UV, IR, NMR & MASS. Characterization of the compounds for their physical properties such as solubility, melting point, etc. will be carried.

Docking studies for the synthesized compounds will be performed with the help of Python Molecule Viewer (Pymol) and GLIDE- Schrodinger software.

Biological Evaluation

1. Design and Synthesis of Heterocyclic Compounds for COX-2 Enzyme Interactions.
2. Use of animal models for Anti Inflammatory Activity.
3. Drug design and targeting inflammatory diseases.
4. Estimation of COX-2 inhibitory action of designed drugs.

References:

1. Mishra P, Mishra A , Bahe A ,Das R synthesis of Isatin and its derivatives containing Heterocyclic compounds.JOTCSA.2021;8(4):1089-98.
2. Senthil kumar, N., Vijayakumar, V., Sarveswari, S., Gayathri, G. A. & Gayathri, M. Synthesis of New Thiazolidine-2,-4-dione-azole Derivatives and Evaluation of Their α-Amylase and α-Glucosidase Inhibitory Activity. *Iran. J. Sci. Technol. Trans. A Sci.* 43, 735–745 (2019)
3. Youssef, A. M., Sydney White, M., Villanueva, E. B., El-Ashmawy, I. M. & Klegeris, A. Synthesis and biological evaluation of novel pyrazolyl-2,4-thiazolidinediones as anti-inflammatory and neuroprotective agents. *Bioorganic Med. Chem.* 18, 2019–2028
4. Watson D. J., Harper S. E., Zhao P.-L., Quan H., Bolognese J. A., Simon T. J. Gastrointestinal tolerability of the selective cyclooxygenase-2 (COX-2) inhibitor rofecoxib compared with nonselective COX-1 and COX-2 inhibitors in osteoarthritis. Archives of Internal Medicine. 2000;160(19):2998 3003. doi: 10.1001/archinte.160.19.2998. - DOI -PubMed

7. Animals required

a. Species and Strain: Wister Rats
b. Age and Weight.. , 200-250gms
c. Gender:Male
d. Number to be used (Year-wise breakups and total figures needed to begiven in tabular form):126

Year	No of groups	No of animals(n=6)
First year (scheme 1)	7	42
Second year (scheme 2)	7	42
Third year (scheme 3)	7	42

e. Number of days each animal will be housed:5-6 weeks

8. Rationale for animal usage

a. Why is animal usage necessary for these studies?
To evaluate the Anti-inflammatory activity of newly synthesized Isatin derivatives.
b. Whether similar study has been conducted on *in vitro* models? NO
If yes, describe the leading points to justify the requirement of animal experiment.
c. Why are the particular species selected?
Wistar rats are reported to be used in these type of studies
d. Why is the estimated number of animals essential?
Animals will be divided into seven groups ,includes one group for control, one group for standard and remaining five groups for determination of the activity of test compounds n=6, 7*6=42.This work is divided into three schemes. Total is 3*42=126
e. Are similar experiments conducted in the past in your establishment? **No**
f. If yes, justify why new experiment is required?
g. Have similar experiments been conducted by any other organization insame or other *in vivo* models? If yes, enclose the reference. **No**

9. Describe the procedures in detail:

a. Describe all invasive and potentially stressful non-invasive procedures thatanimals will be subjected to in the course of the experiments)

Screening procedure for Evaluation of anti inflammatory action of synthesized compounds

Wister strain albino rats weighing between 200-250gm fasted 24 hours before the test were divided into 7 groups each contain six animals the volume of the right hind paw was measured using a plethysmometer. This constituted the initial reading. Compounds were tested in the dose of 100m per kg body weight. Diclofenac 20mg per kg was used as standard. The compounds were administered as suspensions in sodium CMC(0.1% w/v) intraperitoneally 1 hour before the injection carrageenan. control group of animals received a suspension of sodium CMC only. 0.1 ml of 1% w/v carrageenan suspension in normal saline was injected into the plantar region (aponeurosis) of the right hind paw. The swelling produced after injection of the phlogistic agent as measured at howerly interval for four

hours percentage inhibiton edema was calculated by using the formula give below

% inhibition of edema= $\frac{\text{mean edema of control group-mean edema of treated group}}{\text{Mean edema of control group}} \times 100$

Furnish details of injections schedule substances

Name	Test compound	Diclofenac sodium	1% w/v Carrageenan
Doses (mg/kg)	100mg/kg	20mg/kg	100mg/kg
Route/ Sites	Intra-peritonial	Intra-peritonial	Intra-peritonial
Volumes (ml)	As per rat weight	As per rat weight	As per rat weight
Frequency	Single dose	Single dose	Single dose

Blood withdrawal Details:Volumes :
Sites:

Volumes (ml)	0.5 ml	0.5 ml	0.1 ml
Sites	planter region of paw	planter region of paw	planter region of paw

b. Radiation (dosage and schedules):NA

10. Nature of compound/Broad Classification of drug/NCE: **ISATIN(indole 2,3-diones) Derivatives**
11. Does the protocol prohibit use of anesthetic or analgesic for the conduct of painful procedures? If yes, justify. **No anaesthetic is needed.**

Will survival surgery be done? NA

If yes, the following to be described.

a. List and describe all surgical procedures (including methods of asepsis)
b. Names, qualifications and experience levels of personnels involved.
c. Describe post-operative care
d. Justify if major survival surgery is to be performed more than once on asingle animal.

12. Describe post-experimentation procedures.

a. Scope for Reuse:NO :

b. Rehabilitation (Name and Address, where the animals are proposed to berehabilitated) : NA

c. Describe method of Euthanasia (If required in the protocol): Administration of inhalant gas of CO_2 in a sealed container

d. Method of carcass disposal after euthanasia: The animal carcass is collected in colour codet bins and sent to an authorized bio medical waste collection agency for final disposal

13. Describe animal transportation methods if extra-institutional transport is envisaged.NA

14. Use of hazardous agents (use of recombinant DNA-based agents or potential human pathogens requires documented approval of the Institutional Biosafety Committee (IBC). For each category, the agents and the biosafety level required, appropriate therapeutic measures and the mode of disposal of contaminated food, animal wastes and carcasses must be identified).NA

If, your project involved use of any of the below mentioned agent, attach copy of the approval certificates of the respective agencies:

(a) Radionucleotides (AERB)
(b) Microorganisms / Biological infectious Agents (IBSC)
(c) Recombinant DNA (RCGM)
(d) Any other Hazardous Chemical / Drugs

Investigator's declaration.

1. I certify that the research proposal submitted is not unnecessarily duplicative of previously reported research.

2. I certify that, I am qualified and have experience in the experimentation on animals.

3. For procedures listed under item 10, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.

4. I will obtain approval from the IAEC/ CPCSEA before initiating any changes in this study.

5. I certify that performance of experiment will be initiated only upon review and approval of scientific intent by appropriate expert body (Institutional Scientific Advisory Committee / funding agency / other body).

6. I certify that I will submit appropriate certification of review and concurrence for studies mentioned in point 14.

7. I shall maintain all the records as per format (Form D) and submit to Institutional Animal Ethics Committee (IAEC).

8. I certify that, I will not initiate the study before approval from IAEC/ CPCSEA received in writing. Further, I certify that I will follow the recommendations of IAEC/ CPCSEA.

9. I certify that I will ensure the rehabilitation policies are adopted (wherever required).

Signature

Date: 18/04/2022

Name of Investigator

Dr. G. Sammaiah

Associate professor

UGPSC, University College of Pharmacy, Kakatiya University, Warangal.

Certificate

This is to certify that the project proposal no..... entitled **Synthesis and Evaluation of New Isatin Derivatives for their Possible Biological Activities** submitted by Dr./ Mr. / Ms. ...**MANJULA SAMUDRALA**..... has been approved/recommended by the IAEC of.....(Organization) in its meeting held on..... (date) and .....(Number and Species of animals) have been sanctioned under this proposal for a duration of next ..... months.

Authorized by	**Name**	**Signature**	**Date**
Chairman:	.....	.....	.....
Member Secretary:	.....	.....	.....
Main Nominee of CPCSEA:	.....	.....	.....

(Kindly make sure that minutes of the meeting duly signed by all theparticipants are maintained by Office)