Multiversal
Technologies to impel your future

Multiversal Industries Pvt. Ltd.,
Villa 4A, Sriram Vijaya Hyyde Park, Duraisamy Layout, Avarampalayam Rd.,
Peelamedu, Coimbatore, TN 641004 Cell No. +91 90473 65548
GSTIN: ***33AAKCM9736A1ZI***

08/06/2022

To

The Principal,
JKK Munirajah Institute of Health Sciences College of Pharmacy,
TN Palayam.

Subject: Proposal for Research Collaboration

Dear Sir,

Greetings. I am writing on behalf of Multiversal Industries Pvt. Ltd., to propose a collaboration that aligns with our mutual interests and scientific objectives.

We are impressed by your institution's expertise and research capabilities, particularly in the field of pharmacology and the study of natural extracts. Our organization is keen to explore the possibility of engaging JKK Munirajah Institute of Health Sciences College of Pharmacy in conducting research on the **"Evaluation of Neuroprotective Effect of Diosgenin on Rotenone-Induced Parkinson's Mice Model; An Adjuvant Therapy for Treatment of Parkinson's Disease."**

Our interest in this project is driven by our commitment to advancing pharmaceutical research and finding innovative solutions to address the challenges posed by Parkinson's disease. Given the esteemed reputation of your institution, we firmly believe that a collaboration with JKK Munirajah Institute of Health Sciences College of Pharmacy would significantly enhance our research efforts in this specific area.

In this regard, we would like to propose that your esteemed institution undertakes the research project outlined above, with funding and logistical support provided by Multiversal Industries Pvt. Ltd., We are dedicated to ensuring the success of this project and are prepared to provide all the necessary financial resources, as well as any logistical support required for its seamless execution.

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

JKK Munirajah Institute of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt.

Multiversal Industries Pvt. Ltd.
Villa 4A, Sriram Vijaya Hyyde Park,
Duraisamy Layout, Avarampalayam Rd.,
Peelamedu, Coimbatore, TN 641 004 INDIA

Multiversal
Technologies to impel your future

Multiversal Industries Pvt. Ltd.,
Villa 4A, Sriram Vijaya Hyyde Park, Duraisamy Layout, Avarampalayam Rd.,
Peelamedu, Coimbatore, TN 641004 Cell No. +91 90473 65548
GSTIN: ***33AAKCM9736A1ZI***

To facilitate your consideration, we kindly request that you provide us with the following details:

1. **Budget Details:** A comprehensive breakdown of the estimated budget required for the research Project, including expenses for equipment, materials, personnel, and any other relevant costs.

2. **Faculty Details:** Information regarding the faculty members and researchers who will be involved in the project, including their qualifications, expertise, and experience in related fields.

We look forward to your positive response and the opportunity to collaborate on this important research endeavour.

We are excited about the potential of this collaboration and the positive impact it can have on advancing our understanding of Parkinson's disease and potential treatment options.

Thanking You

For MULTIVERSAL INDUSTRIES PVT. LTD.

Director

Sincerely,

Principal
IKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

IKK Munirajah Institutes of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt.

Multiversal Industries Pvt. Ltd.
Villa 4A, Sriram Vijaya Hyyde Park,
Duraisamy Layout, Avarampalayam Rd.,
Peelamedu, Coimbatore, TN 641 004 INDIA

JKK MUNIRAJAH INSTITUTE OF HEALTH SCIENCES
COLLEGE OF PHARMACY

(Approved by Tamil Nadu Govt. & Pharmacy Council of India - New Delhi, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai)
Thookanaickenpalayam, Gobichettipalayam (TK), Erode (DT) - 638506, Tamil Nadu.

DR. P. PERUMAL M.Pharm., Ph.D., FIC.,
Professor & Principal

13.06.2022

To

Multiversal Industries Pvt. Ltd.,
Avarampalayam Rd.,
Peelamedu, Coimbatore, TN 641004.

Subject: Response to Proposal for Research Collaboration – Reg.

Dear Sir,

Greetings. We greatly appreciate your interest in collaborating with JKK Munirajah Institute of Health Sciences College of Pharmacy for the research project titled **"Evaluation of Neuroprotective Effect of Diosgenin on Rotenone-Induced Parkinson's Mice Model; An Adjuvant Therapy for Treatment of Parkinson's Disease."**

First and foremost, we are honoured and excited about the possibility of working with **Multiversal Industries Pvt. Ltd.,** on this significant research endeavour. Your organization's dedication to advancing pharmaceutical research resonates with our mission to contribute to the field of pharmacology and improve healthcare outcomes.

We have carefully reviewed your proposal, and we are enthusiastic about the potential impact of this collaboration. The research project aligns perfectly with our expertise and ongoing efforts in the area of natural extracts and their therapeutic applications. We believe that this partnership will not only enhance our research capabilities but also foster valuable contributions to the scientific community.

We would like to express our gratitude for your willingness to provide financial support and logistical assistance for this project. We are confident that this collaboration will yield substantial results and advancements in the understanding and treatment of hyperlipidaemia.

To move forward, we propose scheduling a meeting to discuss the specific details of the collaboration, including project timelines, budget considerations, and other essential aspects. Our team is excited to engage in this research endeavour and is committed to ensuring the successful completion of the project. Please let us know your availability, and we will coordinate a meeting that accommodates your schedule. You can reach me at **principal@jkkmihscp.org** to coordinate further.

Principal
KK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

JKK MUNIRAJAH INSTITUTE OF HEALTH SCIENCES
COLLEGE OF PHARMACY

(Approved by Tamil Nadu Govt. & Pharmacy Council of India - New Delhi, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai)
Thookanaickenpalayam, Gobichettipalayam (TK), Erode (DT) - 638506, Tamil Nadu.

DR. P. PERUMAL M.Pharm., Ph.D., FIC.,
Professor & Principal

We look forward to a productive partnership and the opportunity to contribute meaningfully to the advancement of pharmaceutical research.

With reference to the letter dated 08/06/2022, JKKMIHSCP is permitting the following faculty members to do collaborative research with Multiversal Industries Pvt. Ltd., Coimbatore and a proposal on the mentioned title "Evaluation of Neuroprotective Effect of Diosgenin on Rotenone-Induced Parkinson's Mice Model; An Adjuvant Therapy for Treatment of Parkinson's Disease" is submitted along with this letter. The faculty members were assigned to do research work with Multiversal Industries Pvt. Ltd., Coimbatore.

1. Mr. GOVINDARAJ. C, Assistant Professor, Department of Pharmacology.
2. Dr. YOGAKRISHNAN. S, Assistant Professor, Department of Pharmacology.
3. Dr. NAVANEETHAKRISHNAN. S, Assistant Professor, Department of Pharmacology.

Kindly permit the above faculty members to execute the above research work. We are expecting a positive reply from your end.

Thanking you,

Principal Investigator

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

JKK Munirajah Institutes of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt.

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

JKK MUNIRAJAH INSTITUTE OF HEALTH SCIENCES
COLLEGE OF PHARMACY

(Approved by Tamil Nadu Govt. & Pharmacy Council of India - New Delhi, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai)
Thookanaickenpalayam, Gobichettipalayam (TK), Erode (DT) - 638506, Tamil Nadu.

DR. P. PERUMAL M.Pharm., Ph.D., FIC.,
Professor & Principal

BUDGET AND FACULTY DETAILS

Project Title: Evaluation of Neuroprotective Effect of Diosgenin on Rotenone Induced Parkinsons Mice Model; an Adjuvent Therapy for Treatment of Parkinsons Disease

Project Duration: 6 months

Project Budget:

S.No	Budget category	Amount in lakhs
1.	Animal (For testing) + Maintenance cost	*0.50*
2.	*Chemical cost* (Diosgenin, Rotenone, etc.,)	*1*
3.	Laboratory Supplies and Consumables	*0.30*
4.	Miscellaneous Costs	*0.20*
	Total Budget	***2 lakhs***

Project Team:

Principal Investigator (PI):	Mr. GOVINDARAJ. C Assistant Professor, Department of Pharmacology, JKKMIHSCP.
Co-Investigators:	Dr. YOGAKRISHNAN. S Assistant Professor, Department of Pharmacology, JKKMIHSCP. Dr. NAVANEETHAKRISHNAN. S Assistant Professor, Department of Pharmacology, JKKMIHSCP.

We kindly request an opportunity to discuss this funding application further. Your support will contribute significantly to the success of our project.

Thank you

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

Yours Sincerely,

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

04285 - 262220 jkkmihscp@gmail.com www.jkkmihscp.org

Multiversal
Technologies to impel your future

Multiversal Industries Pvt. Ltd.,
Villa 4A, Sriram Vijaya Hyyde Park, Duraisamy Layout, Avarampalayam Rd.,
Peelamedu, Coimbatore, TN 641004 Cell No. +91 90473 65548
GSTIN: ***33AAKCM9736A1ZI***

Date: 20/06/2022

To

The Principal,
JKK Munirajah Institute of Health Sciences College of Pharmacy,
T.N. Palayam, Erode, 638506.

Copy to: HOD/Principal Investigator/Co-investigator

Sub: Project Acceptance and Sanction Order – Reg.

Dear Sir/Madam,

We are greatly privileged to offer the grant of **Rs. 1,86,500/- (Rupees One lakh eighty-six thousand five hundred only)** to the project "Evaluation of Neuroprotective Effect of Diosgenin on Rotenone Induced Parkinsons Mice Model; an Adjuvent Therapy for Treatment of Parkinsons Disease". The project will be carried forward during the period of 6 months by the team members of Mr. GOVINDARAJ. C as a Principal Investigator, Dr. YOGAKRISHNAN. S, and Dr. NAVANEETHAKRISHNAN. S as a Co-investigator of JKK Munirajah Institute of Health Sciences College of Pharmacy. We would extend our continuous support throughout the implementation of the project.

Thanking You

For MULTIVERSAL INDUSTRIES PVT. LTD.

Director

Sincerely,

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

JKK Munirajah Institutes of Health Sciences College of Pharmacy
T N.Palayam
Erode Dt

JKK MUNIRAJAH INSTITUTE OF HEALTH SCIENCES
COLLEGE OF PHARMACY

(Approved by Tamil Nadu Govt. & Pharmacy Council of India - New Delhi, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai)
Thookanaickenpalayam, Gobichettipalayam (TK), Erode (DT) - 638506, Tamil Nadu.

DR. P. PERUMAL M.Pharm., Ph.D., FIC.,
Professor & Principal

PROJECT COMPLETION REPORT

Title of the project: Evaluation of Neuroprotective Effect of Diosgenin on Rotenone Induced Parkinsons Mice Model; an Adjuvent Therapy for Treatment of Parkinsons Disease

Category of the project: Research project

Date of approval of competent authority : 20/06/2022

Total cost of the project : Rs: 1,86,500/-

S.NO.	ITEMS	AMOUNT (₹)
1.	*Consumables*	*95000*
2.	*Animals, Equipment and Materials*	*40000*
3.	*Travel and Field work*	*20000*
4.	*Data Acquisition and Analysis*	*10000*
5.	*Research Literature and Documentation*	*15000*
6.	*Others (for analysis)*	*6500*
	Total	***186500***

Date of start of the project : *20/06/2022*

Date of completion of project : 16/12/2022

Name and Signature of Principal Investigator

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

Name and Signature of Co-investigator

Multiversal
Technologies to impel your future

Multiversal Industries Pvt. Ltd.,
Villa 4A, Sriram Vijaya Hyyde Park, Duraisamy Layout, Avarampalayam Rd.,
Peelamedu, Coimbatore, TN 641004 Cell No. +91 90473 65548
GSTIN: **33AAKCM9736A1ZI**

LETTER OF APPRECIATION

Date: 19.12.2022

To

The Principal,
JKK Munirajah Institute of Health Sciences College of Pharmacy,
T.N. Palayam, Erode, 638506.

Subject: Completion of project – reg.

Dear Sir,

With reference to above cited subject, Multiversal Industries Pvt. Ltd., extend sincere gratitude towards JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N. Palayam, for successfully completion of project "Evaluation of Neuroprotective Effect of Diosgenin on Rotenone Induced Parkinsons Mice Model; an Adjuvent Therapy for Treatment of Parkinsons Disease".

We also appreciate sincere efforts taken by Mr. C. GOVINDARAJ, for guiding and valuable suggestions provided for completion of this project. We look forward to the continuation of our successful partnership and to exploring new opportunities for collaboration.

Thank you

For MULTIVERSAL INDUSTRIES PVT. LTD.

Director

Sincerely,

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

T.N.Palayam
Erode Dt

JKK MUNIRAJAH INSTITUTE OF HEALTH SCIENCES
COLLEGE OF PHARMACY

(Approved by Tamil Nadu Govt. & Pharmacy Council of India - New Delhi, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai)
Thookanaickenpalayam, Gobichettipalayam (TK), Erode (DT) - 638506, Tamil Nadu.

DR. P. PERUMAL M.Pharm., Ph.D., FIC.,
Professor & Principal

UTILIZATION CERTIFICATE

Certified that out of Rs. 1,86,500/— sanctioned by **Multiversal Industries Pvt. Ltd.,** towards financial assistance for the student project titled "Evaluation of neuroprotective effect of Diosgenin on Rotenone Induced Parkinson's Mice Model; An Adjuvent therapy - parkinson disease", an amount of Rs. 1,86,500/— was utilized for the purpose for which it was sanctioned, leaving a balance of Rs. 0/— at the close of 16/12/2022 As shown in the Statement of Expenditure annexed.

Name & Signature of the Principal Investigator

JKK Munirajah Institutes of Health Sciences College of Pharmacy TN.Palayam Erode Dt

Name & Signature of Head of Institution

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

Principal
KK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

"EVALUATION OF NEUROPROTECTIVE EFFECT OF DIOSGENIN ON ROTENONE INDUCED PARKINSON IN MICE MODEL; AN ADJUVANT THERAPY FOR PARKINSONS DISEASE"

PRINCIPAL INVESTIGATOR

Mr. C. GOVINDARAJ, M. Pharm.,

Assistant Professor,

Department of Pharmacology,

CO-INVESTIGATORS

Dr. S. YOGAKRISHNAN, Pharm. D.,

Assistant Professor,

Department of Pharmacology,

Dr. S. NAVANEETHAKRISHNAN, Pharm. D.,

Assistant Professor,

Department of Pharmacology,

T.N.Palayam
Erode Dt

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

DECEMBER-2022

JKK MUNIRAJAH INSTITUTE OF HEALTH SCIENCES
COLLEGE OF PHARMACY,
T.N- PALAYAM-638506, GOBI (TK), ERODE (DT),
TAMILNADU.

CERTIFICATE

This is to certify that the Research entitled **"EVALUATION OF NEUROPROTECTIVE EFFECT OF DIOSGENIN ON ROTENONE INDUCED PARKINSON IN MICE MODEL; AN ADJUVANT THERAPY FOR PARKINSONS DISEASE"** submitted to The **Multiversal Industries Pvt. Ltd.,** is the bonafide project work carried out in the Department of Pharmacology, JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N-Palayam, Gobi, Erode, Under the guidance of **Mr. C. GOVINDARAJ, M. Pharm., Assistant Professor, Department of Pharmacology,** JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N Palayam,Gobi, Erode. During the academic year 2022-2023.

JKK Munirajah Institutes of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt.

Place: T.N-Palayam

Date: 16.12.2022

Dr. P. Perumal. M.Pharm,Ph.D,FIC

PRINCIPAL

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

DECLARATION

This is to certify that the Research entitled **"EVALUATION OF NEUROPROTECTIVE EFFECT OF DIOSGENIN ON ROTENONE INDUCED PARKINSON IN MICE MODEL; AN ADJUVANT THERAPY FOR PARKINSONS DISEASE"** submitted to The **Multiversal Industries Pvt. Ltd.,** is the bonafide project work carried out in the Department of Pharmacology, JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N-Palayam, Gobi, Erode, Under the guidance of **Mr. C. GOVINDARAJ, M. Pharm., Assistant Professor, Department of Pharmacology,** JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N Palayam,Gobi, Erode. During the academic year 2022-2023.

JKK Munirajah Institutes of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt.

Place: T.N-Palayam

Date: 16.12.2022

Mr. C. GOVINDARAJ, M. Pharm.,
Principal Investigator

Dr. S. YOGAKRISHNAN, Pharm. D.,
Co-Investigator

Dr. S. NAVANEETHAKRISHNAN, Pharm. D.,
Co-Investigator

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

DECLARATION

The research work emboided in this work entitled "Evaluation of Neuroprotective Effect of Diosgenin on Rotenone Induced Parkinsons Mice Model; an Adjuvent Therapy for Treatment of Parkinsons Disease" was carried out by us under the direct supervision of Mr. C. GOVINDARAJ, M. Pharm., Assistant Professor, Department of Pharmacology, JKK Munirajah Institute of Health SciencesCollege of Pharmacy, T.N-Palayam, Gobi.

The Project submitted to the **Multiversal Industries Pvt. Ltd.,** during the academic year 2022-2023.

JKK Munirajah Institutes of Health Sciences College of Pharmacy T.N.Palayam Erode Dt

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

ACKNOWLEDGEMENT

First and for most we express our heartful sense of gratitude and faithfulnessto God 'grace and our family members, which has enabled us to finish our project work successfully.

With the blessing of our Founder chairman Dr. J.K.K Munirajah, M.Tech,(Bolton). D.Litt., and Secretary Mrs. Kasthuripriya Kirupakarmurali, M.B.A.,

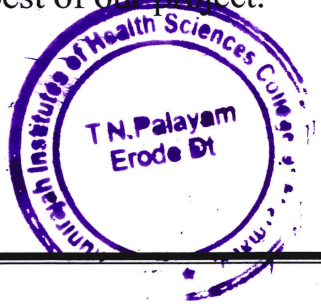
J.K.K Munirajah Institute of Health Sciences College of Pharmacy, T.N-Palayam, Gobi, Erode for providing all the facilities to carry out this work.

Our sincere gratitude to our beloved sir, Dr. P.Perumal, M.Pharm, Ph.D,FIC., Principal and Head of the Department of Pharmaceutical Chemistry, J.K.K Munirajah Institute of Health Sciences College of Pharmacy, T.N-Palayam, Gobi, Erode for his kindly support for our project work and for his encouragement and alsoproviding all facilities in this Institute to the fullest possible extent enabling us to complete this work.

With the immense pleasure and pride, we would to take opportunity in expressing our deep sense of gratitude to our beloved guide Mr. C. GOVINDARAJ, M. Pharm., Assistant Professor, Department of Pharmacology, J.K.K Munirajah Institute of Health Sciences College of Pharmacy, T.N-Palayam, Gobi, Erode under whose active guidance, innovate ideas, constant inspiration and encouragement of the work entitled "Evaluation of Neuroprotective Effect of Diosgenin on Rotenone Induced Parkinsons Mice Model; an Adjuvent Therapy for Treatment of Parkinsons Disease" has been carried out.

We also express our grateful thanks to all the teaching and non-teaching staff members of J.K.K Munirajah Institute of Health Sciences College of Pharmacy for their valuable advice and cooperation.

We express our heartful gratitude to the algmighty, for giving us the right way to achieve the best of our project.



Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

We would like to give sincere thanks to our classmates for their timelyhelp and co-operation.

We also extend our thanks to all staff members of Department of Pharmaceutical Biotechnology, Pharmaceutical Chemistry, Pharmacognosy, Pharmaceutics and Pharmacology for their co-operation.

We would like to thank Multiversal Industries Pvt. Ltd., to give a Financial and moral support to completion of the project being a successful manner on the duration of 2022-2023.

Last but not least, great thanks from the heart to our beloved MOTHER and FATHER. They are our living god, as who guided us in the rightful way to achieve all our activities. They gave the incredible effort to become a successful for bright future in this world. Thanks a lot, to my parents.

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

ABSTRACT:

This study aims to evaluate the neuroprotective effect of diosgenin on rotenone induced Parkinson animal model by analyzing behavioral changes, estimation of catecholamine levels, various endogenous antioxidant enzymes levels and histo pathological evaluation. In recent years there has been growing interest in alternative therapies, among that one of the leading choice is adjuvant therapy. Before administration of rotenone, group IV and V is diosgenin was administration to orally for a period of 7 days (200mg/kg). After administration of rotenone in mice for a period of 7 days (3mg/kg ip), the Parkinson's diseases was confirmed by various behavioral studies. To find out its neuroprotective ,effect of various behavioral studies.

biochemical estimations, neurotransmitter evaluations and histopathological studies have been performed. The results were promising like the behavioral studies the rota rod the diogenin and diosgenin with standard treated group the muscle grip strength (96±1.155, 110.7±1.764) was significantly increased compared to rotenone induced group(35±1.528). Treatment with diosgenin and diosgenin with standard results in regains the levels of endogenous antioxidant levels to normal values. Especially neuroprotective effect of diosgenin and diosgenin with standard was confirmed by histopathology. So this studyconcludes the neuroprotective effect of diosgenin.

RESULTS

The data obtained on the effect of treatment with diosgenin and adjuvanttherapy on muscle grip strength is shown in Figures 2 and Table 5. The data reveal that induction with rotenone significantly decreases the muscle grip strength (35±1.528).Treatment with diosgenin and adjuvanttherapy significantly increases muscle grip strength (96±1.155,110.7±1.764). Moreover, theadjuvanttherapy of diosgenin shows nearly equal significant effect as that of rotenone + levodopa/selegiline treated group (126.7±3.383secs) on 21st day.

Table 5: Effect of Diosgenin and Diosgenin adjuvant therapy on muscle grip strength

Groups	Rotarod (time/sec)			
	Before		After	
	0 day	7th day	14 th day	21st day
Control	186.7±5.239	188±2.517	182±6.351	183.3±2.028
Rotenone only (3mg/kg)	185.7±2.963	36.67±0.8819***	35.33±0.667***	35±1.528***
Selegiline+	181.7±1.202	40.67±0.333***	76±2.517***	126.7±3.383***
Levodopa (12 mg/kg)				
Diosgenin (200mg/kg + Rotenone (3mg/kg)	178.7±0.6667	55.33±2.728***	61.67±1.667***	96±1.155***
Diosgenin 200mg+std + Rotenone 3mg/kg	181.3±0.8819	60.01±0.5774***	76±1.155***	110.7±1.764***

Conclusions: From the present study, it can be considered that the diosgenin and diosgenin adjuvant therapy exhibited significant neruroprotective effect on rotenone model in Parkinson mouse. All the Parameters of diosgenin and diosgenin with standard treated group animals have shown better results when compared with Rotenone- induced group and the standard L-dopa treated group.

Keywords: diosgenin, neuroprtective, rotenone, grip strength, antioxidant and adjuvant therapy.

INTRODUCTION

Parkinson's disease (PD) is a heterogeneous neurodegenerative disorder that affects an estimated 10 million sufferers worldwide. The two forms of PD include familial and sporadic, and while the etiology of PD is still largely unknown, the condition is likely to be multifactorial with genetic and environmental factors contributing to disease genesis. Diagnosis of the condition is attained through the observation of cardinal clinical manifestations including resting tremor, muscle rigidity, slowness or loss of movement, and postural instability.

Unfortunately, by the time these features become apparent extensive neurological damage has already occurred. There is no specific test for PD and most diagnoses are confirmed by a combination of clinical symptoms and positive responses to dopaminergic drug therapies. The prevalence and incidence of PD vary worldwide influenced by several factors such as age, gender, ethnicity, genetic susceptibilities, and environmental exposures.[1] Parkinson's disease (PD) is decreased nigrostriatal availability of dopamine.[2] One of many factors contributing to the development of PD is oxidative stress.

In the context of the pathogenesis of PD, oxidative stress is generated because of dysfunction of mitochondria and oxidative metabolism of dopamine.[3] Free radicals generated due to mitochondrial dysfunction could be responsible for the oxidative damage which further generates reactive oxygen species (ROS) resulting in a vicious cycle. The increase metabolism of dopamine, lipid peroxidation, and nitric oxide and reduced level of endogenous antioxidant enzymes such as glutathione (GSH) and superoxide dismutase in the brain could be responsible for neuronal death. [4]

MATERIALS AND METHODS

Evaluation of Anti-Parkinson Study of diosgenin adjuvant therapy Table 3: Selection of animal for rotenone induced Parkinson evaluation.

Species	Swiss albino mice
Age	3 months
Body weight	25-30g
Gender	Male
No of animals	30

Male Swiss albino mice 3 month of age, and 25-30 g body weight were offered by KMCH College of Pharmacy, Coimbatore. All the rats were kept at room temperature and allowed to acclimate in standard conditions less than 12 hr light/ 12 hr dark cycle in the animal house. Animals are fed with commercial pellet diet and water ad libitum freely throughout the study. The experimental procedure was approved by IAEC (Institution of Animal Ethical Committee).

JKK Munirajah Institutes of Health Sciences College of Pharmacy
T.N.Palayam
Erode Dt

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

Experimental design for rotenone induced parkinson disease

Group	Treatment	No. of Animals
Control (Saline)		6
Rotenone (10 mg/kg i.p)		6
Rotenone (10 mg/kg i.p) +Standard (levodopa (12mg/kg)+selegiline (10mg/kg))		6
Rotenone+diosgenin (200 mg/kg)		6
Rotenone+(diosgenin (200mg/kg) + Levodopa (12mg/kg)+selegiline 10mg/kg))		6

Experimental design for Rotenone induced Parkinson

Induction of parkinson disease [76]

Preparation and induction of rotenone solution

The Rotenone was purchased from sigma chemicals, Mumbai, India and was stored according to the manufacturer label (-20^0 C) to prevent its decomposition. The Rotenone solution was freshly prepared at 3 mg/kg. The Rotenone was dissolved in 2 % DMSO in normal saline and adjusts to pH 7.4 with potassium hydroxide. Rotenone injected i.p at the dose of 3 mg/kg body weight, 7 days. The solution should be used immediately after preparation. Rotenone solution is stable only for a period of 24 hours at 25˚C

Preparation of Levodopa and Selegilne

12mg/kg of levodopa suspension was freshly prepared by using 1% gum acasia and 10 mg/kg of selegilne was dissolved in distilled water. Levodopa and Selegiline was freshly prepared daily and given via oral to the standard group II for 21 days.

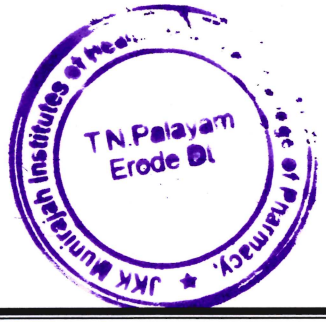
Preparation of sample

200 mg/kg of diosgenin were dissolved in 2% DMSO + Normal saline as a vehicle and it was daily prepared freshly and given via oral route to group IV and V respectively for 21 days.

Evaluation of behavioral parameters Motor Co-ordination test (Rota rod test) Principle The rota rod performance test is carried out on a rotating rod that provides forced motor activity in animals. The animals were placed on a rotating rod which is placed horizontally, suspended above a cage floor, which is high enough to induce avoidance of fall. Animals naturally try to stay on the rotating rod avoid falling to the ground. The length of time(duration) the animal stay on the rod without falling, gives a measure of their coordination, balance, physical condition and motor-planning.

Procedure Motor Co-ordination test was conducted using Rota rod apparatus. Animal was placed individually on the rotating rod and trained for 3 min trail at 25 rpm on the day before the first day of testing. A cut off time of 180s was fixed and each animal performed 3 separate trials at 5min interval. After each trial, 5 min rest period was given to alleviate stress and fatigue. Motor coordination can be tested by comparing the latency to fall on the very first trial between treatment groups. The time taken by animals to fall from the rotating rod was noted.

Locomotor activity on actophotometer Principle The locomotor activity (horizontal activity) can be easily measured using an actophotometer which operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photocell is cut off by the animal, a count is recorded. An actophotometer could have either circular or square area in which the animal moves.



Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

Procedure The spontaneous locomotor activity of each animal was recorded individually, using Actophotometer. The apparatus was placed in a sound attenuated and ventilated room during the testing period. All the animals were placed individually in the activity cage for 3 min to habituate them before starting actual locomotor activity task for the next 3 min. the basal activity score was noted. The units of the activity counts were arbitrary and based on the beam breaks by movement of the animal. Counts/3 min is used as an index of locomotor activity.

Grip strength activity on grip strength meter Principle The grip strength meter is positioned horizontally and the subjects are held by the tail and lowered towards the apparatus. The animals (rat or mouse) are allowed to grab the metal grid or triangular pull bar and are then pulled backwards in the horizontal plane. Grip Strength Test allows the study of neuromuscular functions by determining the maximal peak force developed by a rodent.

Procedure Gently pull the mice back by its tail ensuring the mice grips the top portion of the grid and the torso remains horizontal and record the maximal grip strength value of the mouse that is displayed on the screen. Repeat this procedure twice more to obtain 3 forelimb grip strength can be recorded and displayed in grams, newtons, or lbs.

Estimation of brain neurotransmitter Estimation of Serotonin, GABA and Dopamine[80,81,82] Preparation of tissue extractsReagents ⎪ HCl – Butanol solution: (0.85 ml of 37% hydrochloric acid in one-litre n-butanol) ⎪ Heptane ⎪ 1 M HCl: (0.85 ml conc. HCl up to 100 ml H2O)

Procedure At the end of experiment, rats were sacrificed and the whole brain was dissected out. 0.25 g of tissue was weighed and was homogenized in 5 mL HCl–butanol with motor driven Teflon coated homogenizer for about 1 min. The sample was then centrifuged for 10 min at 2000 rpm. An aliquot supernatant phase (1 mL)

JKK Munirajah Institutes of Health Sciences College of Pharmacy T.N.Palayam Erode Dt

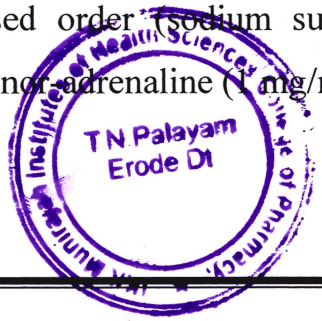
Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

was removed and added to centrifuge tube containing heptane (2.5 mL) and 0.1 M HCl (0.31 mL). After 10 min of vigorous shaking, the tube was centrifuged under the same conditions as above in order to separate the two phases, and the overlaying organic phase was discarded. The aqueous phase was then taken either for 5-HT or NA and DA assay.

Biochemical estimation

Estimation of dopaminereagents ⎪ 0.4 M HCl: 0.34 ml conc. HCl up to 10 mL H2O ⎪ Sodium acetate buffer (pH 6.9): 0.72 mL of 1 M acetic acid (6 µL of glacial acetic acid up to 1000 µL with distilled water) + 6.84 mL of 0.3 M sodium acetate (0.408 g of sodium acetate in 10 mL distilled water) and volume were made up to 25 mL with distilled water. pH was adjusted with sodium hydroxide solution. ⎪ 5 M sodium hydroxide: 5 g of NaOH pellets dissolved in distilled water and volume was made up to 25 mL with distilled water. ⎪ 0.1M Iodine solution (in Ethanol): 1 g of potassium iodide + 0.65 g of iodine dissolvedin ethanol and volume was made up to 25 mL. ⎪ Sodium thiosulphate solution: 0.625 g Na2SO3 in 2.5 mL H2O + 22.5 mL 5 M NaOH ⎪ 10 M Acetic acid: 14.25 mL of glacial acetic acid dissolved in distilled water and madeup
to 25 mL.

Procedure To 1 mL of aqueous phase, 0.25 mL 0.4 M HCl and 0.5 mL of Sodium acetate buffer (pH 6. 9) were added followed by 0.5 mL iodine solution (0.1 M in ethanol) for oxidation. The reaction was stopped after 2 min by the addition of 0.5 mL Na2SO3 solution. 0.5 mL Acetic acid was added after 1.5 min. The solution was then heated to 100°C for 6 min. When the sample reached room temperature, excitation and emission spectra were read from the spectrofluorometer. The readings were taken at 330-375 nm for dopamine. Blanks for the assay were prepared by adding the reagents of the oxidation step in reversed order (sodium sulphite before iodine). Different concentration of dopamine and nor-adrenaline (1 mg/ml) was used as standard



Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam.
Gobi (Tk), Erode (Dt) - 638 506

Estimation of serotonin

The serotonin content was estimated by the OPT method. Reagents ⎪ O-phthaldi aldehyde (OPT) reagent: (20 mg in 100 ml conc. HCl) Procedure To 1.4 mL aqueous extract, 1.75 mL of OPT reagent was added. The fluorophore was developed by heating to 100°C for 10 min. After the samples reached equilibrium with the ambient temperature, readings were taken at 360-470 nm in the spectrofluorometer. Concentrated HCI without OPT was taken as blank. Serotonin (1 mg/mL) at different concentration was used as standard.

Estimation of brain GABA content Preparation of tissue homogenate Animals were sacrificed by decapitation and the whole brain was rapidly removed. 0.5 g tissue was weighed and placed in 5 mL of ice-cold TCA (10% w/v). The tissue was then homogenized and centrifuged at 10,000 rpm for 10 min at 0°C. The supernatant was used for estimation of GABA content.

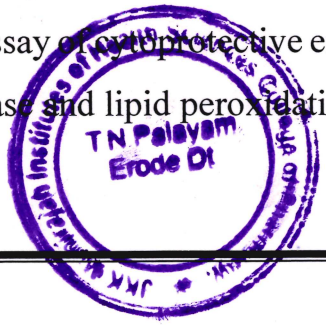
Reagents

Carbonate-bicarbonate buffer, 0.5 M (pH 9.95): 1.0501 g sodium bicarbonate and 1.3249 g sodium carbonate dissolved in distilled water and made up to 25 ml. pH adjusted to 9.95 if necessary

⎪ 0.14 M ninhydrin solution: 499 mg ninhydrin dissolved in 0.5 M carbonate bicarbonate buffer and made up to 20 ml. ⎪ Copper tartarate reagent: 0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid

Procedure 0.1 mL of tissue homogenate was placed in 0.2 mL of 0.14 M ninhydrin solution in 0.5 M carbonate-bicarbonate buffer (pH 9.95), and kept in a water bath at 60°C for 30 min. It was then cooled and treated with 5 mL of copper tartrate reagent. After 10 min fluorescence at 377/455 nm in a spectofluorimeter was recorded.

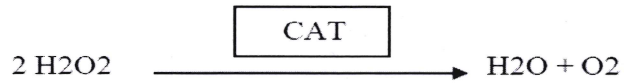
Estimation of endogenous anti oxidant enzyme levels in mice brain[83] Procedure 100 mg of the brain tissue was weighed and homogenate was prepared in 10ml tris hydrochloric acid buffer (0.5M; pH 7.4) at 4°C. The homogenate was centrifuged and the supernatant was used for the assay of cytoprotective enzymes namely catalase, superoxide dismutase, glutathione reductase and lipid peroxidation.



Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam
Gobi (Tk), Erode (Dt)

Catalase: [Cat]

Principle



In presence of CAT, H2O2 shows a continual decrease in absorbance when measured in UV range. The decomposition of H2O2 can be followed directly by the decrease in absorbance at 240nm (E240=0.00394±0.0002litres mmol-1mm-1). The difference in Absorbance (ΔA 240) per unit time is a measure of the CAT activity.

Reagents 1. Preparation of Phosphate Buffer (PB): ⎫ KHPO4 (Potassium dihydrogen phosphate): 1.703 g made upto 250 ml ⎫ Na2HPO4 (Disodium hydrogen phosphate): 1.773 g made upto 250 ml 100mL of KH2PO4 solution and 150 ml of Na2HPO4 was mixed & pH was adjusted to7.

2. Preparation of PB- H2O2 solution: 50 ml of PB + 500 µlH2O2

Critical step

The absorbance of PB- H2O2 solution was checked and it should be between 0.3-0.5. If the absorbance lies below this range, then H2O2 should be added to increase absorbance and if the absorbance lies above this range, PB was added to decrease absorbance. Time course between 0 & 60 second was selected. Auto zero was selected at 240 nm with PB.

Procedure • 3ml of H2O2 PB solution was added to 50µl of tissue homogenate. • The above solution was kept in cuvette and absorbance was taken at 240nm.

Superoxide dismutase [Sod]

Principle

T.N.Palayam Erode Dt

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

The activity of SOD was determined by the method based upon the ability of SOD to inhibit the auto-oxidation of epinephrine to adrenochrome at alkaline pH. Inhibition of the chromogen formation by superoxide dismutase is linear with increase in enzyme concentration.

Reagents ⎫

Sodium carbonate buffer 0.1 M (pH 10.2): 1.05g of Na2CO3 in 100 mL of distilled water. ⎫ Adrenaline (bitartrate) (final concentration- 250 µM)

Procedure

- 1.85ml of sodium carbonate buffer was taken in the cuvette and 50µl of tissue homogenate was added followed by 100µl of Add directly to the cuvette kept in the UV cuvette holder.
- Absorbance was read at 295 nm. The SOD activity (U/mg of protein) was calculated using the standard plot. (Photometric method).

Glutathione reductase [GSH]

Principle

GSH is a non-protein compound containing sulfhydryl group in its structure. Ellman's reagent (5, 5'-dithiobis-(2-nitrobenzoic acid) or DTNB is reduced by sulfhydryl compounds to an intensely yellow colour compound. The absorbance of the reduced chromogen is measured at 412 nm and is directly proportional to the GSH concentration.

Reagents

⎫ 5% TCA (Trichloro acetic acid): 5g in 100 ml of distilled water. ⎫ PBS (0.2M) (pH=8.0): 0.218g NaH2PO4 + 2.641g of Na2HPO4 in 100ml distilledwater ⎫ DTNB (0.6mM): 20mg in 50ml of phosphate buffer

Procedure

500 µl TCA solution was added to 500µl of tissue homogenate and then it was centrifuged. 500 µl of supernatant was Incubated with 3 ml of PBS and 500 µl of DTNB for 10min at room temperature. Absorbance was read at 412 nm.

JKK Munirajah Institute of Health Sciences College of Pharmacy T.N.Palayam Erode Dt

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

Lipid peroxidation

Principle

Malondialdehyde, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of peroxidation reaction. Malondialdehyde reacts with thiobarbituric acid to form red colour species (TBARS), which is measured at 535 nm.

Formula copy the lipid peroxidation

Reagents ⎪ TBA-TCA-HCl reagent: [15%w/v TCA, 0.375%w/v TBA and 0.2ml of 0.25N HCl]this solution was mildly heated to assist the dissolution of TBA.

Procedure

1ml of liver homogenate was combined with 2ml of TCA-TBA-HCl reagent and mixed thoroughly the solution was heated for 15min in a boiling waterbath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10min. The absorbance of the supernatant was measured at 532nm against a blank that contains all the reagents minus the liver homogenate. The malondialdehyde concentration of the sample can be calculated using an extinction coefficient of 1.56 x 105 M-1cm-1

Estimation of proteins requirements ⎪ Alkaline copper reagent ⎪ Solution A: 2% sodium carbonate in 0.1 N NaOH ⎪ Solution B: 0.5% copper sulphate in 1% sodium potassium tartarate .50 ml of solution A was mixed with 1 ml of solution B just before use. ⎪ Folin's phenol reagent (commercial reagent, 1:2 dilutions), ⎪ Bovine serum albumin (BSA).

Principle

This method is a combination of both Folin-ciocalteau and biuret reaction which involves two steps

Step: 1 Protein binds with copper in alkaline medium and reduces it to Cu++

Step: 2 The cu++ formed catalyzes the oxidation reaction of aromatic amino acid by reducing phosphomolybdotungstate to heteropolymolybdanum, which leads to the

JKK Munirajah Institutes of Health Sciences College of Pharmacy T.N.Palayam Erode Dt

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

formation of blue color and absorbance was measured at 640 nm.

Procedure

To 0.1 ml of the homogenate, 0.9 ml of water, 4.5 ml of alkaline copper sulphate reagent were added and allowed to stand at the room temperature for 10 minutes. To this 0.5 ml of folin'reagent was added. After 20 minutes, the color developed was measured at 640 nm. The level of protein present was expressed as mg/g/ tissue or mg/dl.

Histopathological studies

After behavioural and biochemical studies, the brains of different groups were perfusionfixed with 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were removed and post fixedin the same fixative overnight at 48°C. The brains were embedded in paraffin and stained with Hematoxylin-Eosin. The hippocampus lesions were assessed microscopically at 40 magnifications.

Fixation

Kept the tissue in fixative for 24-48 hours at room temperature The fixation was useful in the following ways:

a) Serves to harden the tissues by coagulating the cell protein,

b) Prevents autolysis, c) Preserves the structure of the tissue, and d) Prevents shrinkage

Common fixatives: 10% Formalin

Hematoxylin and eosin method of staining: Deparaffinize the section by xylol 5 to 10 minutes and remove xylol by absolute alcohol. Then cleaned the section in tap water and stained with hematoxylin for 3-4 minutes and again cleaned under tap water. Allow the sections in tap water for few minutes and counter stained with 0.5% eosin until section appears light pink (15 to 30seconds), and then washed in tap water. Blotted and

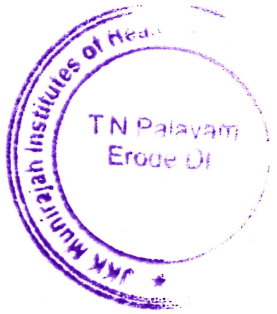
T.N.Palayam Erode Dt

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

dehydrated in alcohol and cleared with xylol (15 to 30 seconds). Mounted on a Canada balsam or DPX Moutant and kept the slide dry and remove air bubbles.

Statistical Analysis

For in-vivo, the values are expressed as mean ± standard error of mean (SEM) of samples. All data were analyzed by one-way ANOVA followed by Dunnett's test using graph pad prism version 8.0software. The difference between the control and experimental groups were considered significant if, $p<0.05$.



Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

RESULT

Rota rod test

The data obtained on the effect of treatment with diosgenin and adjuvanttherapy on muscle grip strength is shown in Figures 2 and Table 5. The data reveal that induction with rotenone significantly decreases the muscle grip strength (35±1.528).Treatment with diosgenin and adjuvanttherapy significantly increases muscle grip strength (96±1.155,110.7±1.764). Moreover, theadjuvanttherapy of diosgenin shows nearly equal significant effect as that of rotenone + levodopa/selegiline treated group (126.7±3.383secs) on 21st day.

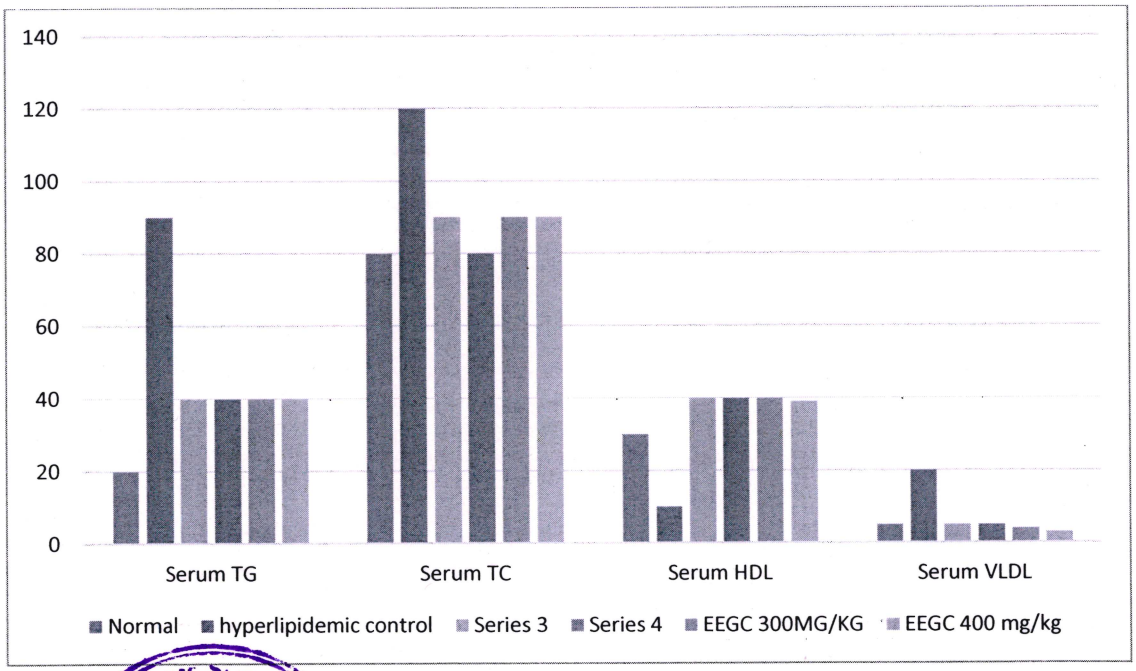


Figure 1 : Effect of Eeca on Serum Tg, Serum Tc, Hdl, Ldl And Vldl

DISCUSSION

This study aims to evaluate the neuroprotective effect of diosgenin on rotenone induced Parkinson animal model by analyzing behavioral changes, estimation of catecholamine levels, various endogenous antioxidant enzymes levels and histopathological evaluation. PD is the second most neurodegenerative disease mainly occurred due to the degeneration of dopaminergicnigrostriatal pathway, which is cumulative effect of glutathione depletion, iron deposition, increased lipid peroxidation, oxidative DNA damage, mitochondrial dysfunction, and alterations in antioxidant enzymes activities. Although the etiology of PD is unknown, environmental factors, genetic predisposition, and genetic mutations have been shown to play an important role in the origin and development of the disease. It leads to develop the symptoms like the muscular incoordination, rigidity, tremor, bradykinesia, dyskinesia. Above symptoms of Parkinson disease are easily able to demonstrate on the Parkinson animal model. The existing conventional strategies that are target the PD are associated with numerous side effects and economic burden also. In recent years there has been growing interest in alternative therapies, among that one of the leading choice is adjuvant therapy. In adjuvant therapy used to cure neurodegenerative diseases mainly Parkinson's disease and its associated symptoms. In this context, diosgenin which already reported for neuroprotective effect, free radical scavenging activity and anti-inflammatory activity is one of the promising for treating parkinsons disease mainly for reducing neurodegenration has been explored in the present study. Before administration of rotenone, group IV and V is diosgenin was administration to orally for aperiod of 7 days (200mg/kg).

After administration of rotenone in mice for a period of 7 days (3mg/kg ip), the Parkinson's disease was confirmed by various behavioral studies. Here group IV and V mice significantly increase muscle grip compared with untreated diogenin group II an

III. Then based on protocol the various doses of diosgenin were administrated. Rota rod test results were promising. As it is a standard apparatus for evaluation the muscle grip strength and muscle coordination. The loss of muscle grip is an indication of muscle weakness, which is classical symptom of Parkinson's disease. The differences in the fall off time from the rotating rod between the vehicle controls, rotenone treated control group and diosgenin treated group taken as a index of muscle strength.

Control group possess 100% of muscle grip strength. Rotenone treated group muscle grip strength is 8.3%, on 7th day. Diosgenin treated group muscle grip strength is 17%, on 7th day thisis a clear indication of muscle strength with rotenone. Treatment with standard (levodopa and selegiline), diosgenin and standard with diosgenin, percentage of muscle grip strength is significantly increased compared with rotenone 80%, 44% and 76%, resp.

The actophotometer test is helpful for modeling spontaneous locomotor activity in animals, in this test an spontaneous locomotor activity like state may be reflected by an increase in motion behaviors. After evaluation of the actophotometer test readings the percentage of behavior on Control group is 100%. In rotenone treated group there was a decreased percentage of exploration behavior 4.9%, Treatment with standard (levodopa and selegiline), diosgenin and standard with diosgenin, percentage of exploration behavior is significantly increased compared with rotenone 63%,43% and 59% resp.

Grip strength test is the screening test for evaluation of grip strength activity. After evaluation of grip strength test readings the percentage of grip strength activity of Control group was 100%. In rotenone treated group there was a decreased percentage of grip strength activity 5%, Treatment with standard(levodopa and selegiline) diosgenin and standard with diosgenin, percentage of grip strength potential was significantly increased compared with rotenone 53%, 46% and 88% resp.

Rotenone selectively damage the mitochondrial complex I, which cause changes in dopaminergicnigrostriatal system, resulting in changes in dopamine metabolites like

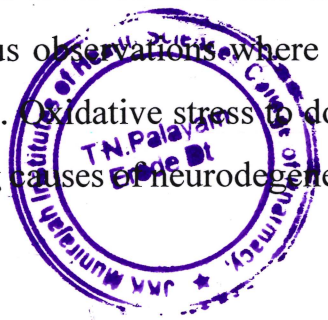
JKK Munirajah Institute of Health Sciences College of Pharmacy T.N. Palayam Erode Dt

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

DOPAC and homovanilic acid (HVA). So this causes the onset of motor symptoms, and there is a direct relationship between extent of dopamine loss and motor dysfunction. Many studies have revealed impaired behavioural responses within a short span of rotenone lessoned animals. Dopamine neurotransmitter was more affected in Parkinson's disease. Whereas other brain amines like norepinephrine, epinephrine and serotonin were much less affected than dopamine in Rotenone treated group.

The neurotoxic effects of rotenone are mediated mainly through mitochondrial complex I (CI) of the electron transfer chain, on microtubules, and on proteasomes. CI of the mitochondrial respiratory chain is a critical initiator of the energy production process; deficits in this complex usually result in excessive generation of reactive oxygen species (ROS). It is a cardinal hallmark of PD. However, excess ROS production, as may occur with rotenone exposure, has the potentialto cause cellular damage and may aid in generating further reactive species and initiating radical chain reactions. The motor deficits in Parkinsonian rat have been attenuated by antioxidant supplementation. One of the universally accepted etiologies of PD is the imbalance between free radical formation and the maintenance of the neuronal integrity through the endogenous antioxidant defense system resulting in oxidative stress. A surplus amount of free radical generation is thought to be the key module of neuronal damage in the brain. ROS threaten neuronal survival by their ability to propagate the initial attack on lipid rich membranes of the brain to cause lipid peroxidation.

Cell damage can be prevented by detoxification of free radicals, which eventually prevent the progress of LPO. In the present study we have observed an elevated level of LPO accompanied by a depleted GSH level in Rotenone induced PD mice brain. Our experimental finding reveals that treatment with various dose of SG reversing the elevated level of LPO and the depleted level of GSH which is concomitant with the previous observations where antioxidants were used as a remedy in experimental PD models. Oxidative stress to dopaminergic neurons of SNpc is believed to be one of the leading causes of neurodegeneration in PD. Oxidative stress promotes lipid peroxidation



Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

and alters the antioxidant defense system in the brain. The Rotenone treated group animal had5.667±0.333 nmoles of MDA/mg protein increased LPO levels when comparedto the control group when compared to the control group on 21 ST day. Treatment with standard drug(levodopa and selegiline), diosgenin and diosgenin with standard, percentage significantly decreased compared with rotenone 2.667±0.334, 4±0.58 and3.333±0.33 nmoles of MDA/mg protein resp.

GSH is a tripeptide in which thiol residue plays a major role on membrane protection. Its significantly depleted level may trigger the formation of lipid peroxidation and consequently disrupt the hemostat. A reduction in GSH content may also impair H2O2 and promote *OH formation. It has been further suggested that the decrease in GSH availability in the brain can promote mitochondrial damage by free radicals, and may result in selective inhibition of the complex-I activity. GSH plays a predominant role in removing excess of free radicals and hydro peroxidases and is a major defense system against oxidative stress in the brain. The Rotenone treated group animal had 2.2±0.13 μ M/mg of brain tissue protein decreased the GSH levels whencompared to the control group on 21 ST day. Treatment with standard drug (levodopa and selegiline), diosgenin and diosgenin with standard, percentage significantly decreased compared with rotenone 5.033±0.0334, 3.1±0.058 and 4.733±0.120μM/mg of brain tissue resp.

The catalase, which was found at a very low level of activity in the brain, detoxifies H2O2 to H2O. The loss of GSH and the formation of protein glutathione mixed disulfide (PrSSG) in the brain results in various membrane dysfunctions, such as inhibition on Na+K+-ATPase activity. The Rotenone treated group animal had 3.667±0.343U/mg of brain tissue protein decrease the CAT levels when compared to the control group on 21 ST day. Treatment with standard drug (levodopa and selegiline), diosgenin and diosgenin with standard, percentage significantly increased compared with rotenone 7.667±0.881, 5.667±0.333 and 6.333±0.333U/mg of brain resp.

SOD is upregulated in cells when O2− is produced in excessive levels. This

T.N.Palayam

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,

observation suggests that SOD may play a role in the toxicity observed following acute treatment of rotenone, although ROS formation may not play a major role in rotenone - induced toxicity. The Rotenone treated group animal had 2.567±0.034U/mg of brain tissue protein decrease the SOD levels when compared to the control group on 21 ST day. Treatment with standard drug (levodopa and selegiline), diosgenin and diosgenin with standard, percentage significantly increased compared with rotenone 1.067±0.067, 1.4±0.153 and 0.5667±0.033U/mg of brain tissue resp., This study reports the therapeutic effect of diosgenin and diosgenin with standard on acute rotenone induced behavioral and oxidative stress alterations in mice. 3. Results of this study suggest diosgenin is aneffective compound for protection against rotenone induced motor impairment, ROS generation, cellular stress, and oxidative damage in the mouse brain.

Histopathology results confirmed its effect on neuroinflammation. Necrosis, cerebral cortex, molecular purkinjicec cell layer and parenchyma layer were clearly observed in rotenone induced brain tissue. But diosgenin and diosgenin with standard treated group brain tissues were perfectly normal. So this gives a clear confirmation that diosgenin with standard adjuvant theraphy can be used for treatment of Parkinson disease.

T.N.Palayam
Erode Dt

Principal
JKK Munirajah Institute of Health Sciences
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

Multiversal
Technologies to impel your future

Multiversal Industries Pvt. Ltd.,
Villa 4A, Sriram Vijaya Hyyde Park, Duraisamy Layout, Avarampalayam Rd.,
Peelamedu, Coimbatore, TN 641004 Cell No. +91 90473 65548
GSTIN: ***33AAKCM9736A1ZI***

TO WHOMSOEVER IT MAY CONCERN

This is to certify that **Mr. PRAVEEN V,** S/O Venkatachalam P, a Second year B.Pharmacy, Student from **JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N.Palayam, Erode.** He has successfully completed Internship training in our organization for the period of **01.05.2022 to 30.05.2022**.

He is found to be hardworking, sincere and diligent. We wish him all the best for his future.

Principal
JKK Munirajah Institute of Health Science
College of Pharmacy, T.N.Palayam,
Gobi (Tk), Erode (Dt) - 638 506

For MULTIVERSAL INDUSTRIES PVT. LTD.

Director

For Multiversal Industries Pvt. Ltd.

JKK Munirajah Institutes of Health Sciences College of Pharmacy
T N.Palayam
Erode Dt.