



JKIMSU

JOURNAL OF KRISHNA INSTITUTE OF MEDICAL SCIENCES UNIVERSITY

An Official Publication of Krishna Vishwa Vidyapeeth (Deemed to be University), Karad

ISSN 2231-4261

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Current Issue

JKIMSU, Vol. 10, No. 2, April-June2021

Table of Contents

A REVIEW ARTICLE :

Emerging and Re-emerging Viral Infections in the 21st Century: Microbiological and Public Health Perspectives.

Mohan Bilikallahalli Sannathimmappa, Vinod Nambiar, Rajesh Patil (page no:1-20)



B ORIGINAL ARTICLES:

1. Association of Second Hand Smoke and Biomass Cooking Fuel with the Tuberculosis: A Case-Control Study from Southern Haryana, India. Vipin Goyal, Abhishek Singh, Vikas Gupta, Sanjeet Panesar, Pawan Kumar Goel, Deepika Agrawal (page no: 21-34)



2. Body Weight Estimation from Hand Anthropometry among Minangs, an Indigenous Ethnic Group in West Sumatra, Indonesia for Person Identification.

Nataraja Moorthy T, Noor Hafizah H, Henky (page no: 35-42)



3. Behavioral Risk Factors for Coronary Artery Disease among Adults Residing in Rural Area of Belagavi District, Karnataka.

Avinash Kavi, Padmaja R. Walvekar, Rekha S. Patil (page no: 43-54)



4. Effects of Ethanol Extract of *Secamoneafzelii* (Schult) K. Schum (Asclepiadaceae) Leaves on Aluminum Chloride-induced Reproductive Toxicity in Male Wistar Rats.

Taiwo OO, Ayoka AO, Akano OP, Adeoye AD, Odukoya SOA (page no: 55-66)



5. Impact of Antenatal Maternal Morbidity on Birth Weight and Gestational Age of the Baby.

Avinash H. Salunkhe, Asha Pratinidhi, Jyoti A. Salunkhe, Satish V. Kakade (page no: 67-75)



6. Lumbosacral Transitional Vertebra-Prevalence of Different Types in South Indian Population with Low Backache.

V. Sivakumar, C. Poonam, D. Ethiraj, I. Venkatraman (page no: 76-84)



7. Molecular Characterization of Staphylococcal Cassette Chromosome mecA and Concomitant Pantone-valentine Leukocidine in Clinical Isolates of Community-acquired Methicillin-resistant *Staphylococcus aureus*.

Savita V. Jadhav, Deepali S. Desai, Shahzad Beg Mirza, Anjali D. Apte-Deshpande, Sumedh Deshpande (page no: 85-99)



8. A Cross-sectional Study on Vitamin D Status in Patients with Diabetic Nephropathy.

Bhuneswar Yadav, Shashidhar KN, Raveesha A, Muninarayana C (page no: 100-109)



9. Comparative Study of Crystal Violet Stain and Haematoxylin and Eosin Stain in the Assessment of Mitotic Figures in Dysplastic and Malignant Lesions of Oral Cavity.

Nayantrishna Nath, Surekha Ulhas Arakeri (page no: 110-122)



10. A Prospective Randomised Comparative Study on Use of Lignocaine with Zinc with Hydrocortisone Combination Ointment versus Lignocaine with Sucralfate with Metronidazole Combination Ointment in the Treatment of Acute Fissure in Ano.

Neeraj K. Dewanda, Siddesh HV, Deshraj Chawla (page no: 123-129)



11. Study of Sputum Bacteriology of Patients with Acute Exacerbation of Chronic Obstructive Pulmonary Disease.

Shaila Jay Shah, Niyati Mehta, Shruti Shah (page no: 130-138)



12. *Phoenix dactylifera* Polyphenols Ameliorates Monosodium Glutamate Induced Cell Damage in the Dentate Gyrus.

Ruqayyah Yetunde Usman, Fatima Ajoke Sulaimon, Akeem Ayodeji Okesina, Aminu Imam, Abubakar Lekan Imam, Moyosore Salihu Ajao (page no: 139-145)



C CASE REPORT :

1. Late-onset Pompe Disease: A Diagnostic Challenge.

Ritesh Shah, Seema Balasubramaniam (page no: 146-149)



2. Cleidocranial Dysplasia Affecting Three Generations in a Family: A Unique Case Report.

Vathsala Patil, Keerthilatha M. Pai, Yogesh Chhapparwal, Shubha Chhapparwal (page no: 150-155)



3. Meropenem Induced Severe Thrombocytopenia in an Adult Patient: A Case Report.

Idris Dawaiwala, Sunita Pawar (page no: 156-160)



4. Small Vessel Vasculitis: A Rare Presentation.

Judhajit Maiti, Somnath Maitra, Sujoy Sarkar (page no: 161-164)



D Letter To Editor

Letter to Editor on "Gollop-Wolfgang Complex in a New Born with Morton's Toe and Congenital Heart Disease".

Prerna AnupPatwa, Rohan Kumar Singh, Gaurav Vedprakash Mishra (page no: 165-166)



Old Issue >

[Vol. 10, No. 1 January-March 2021](#)

[Vol. 9, No. 4, Oct-Dec 2020](#)

[Vol. 9, No. 3, July-September 2020](#)

[Vol. 9, No. 2, April-June 2020](#)

[Vol. 9, No. 1, Jan-Mar 2020](#)

[Vol. 8, No. 4, October-December, 2019](#)

[Vol. 8, No. 3, July-Sep 2019](#)

[Vol. 8, No. 2, April-June 2019](#)

[Vol. 8, No. 1, Jan-Mar 2019](#)

[Vol. 7, No. 4, October-December 2018](#)

[Vol. 7, No. 3, July-Sep 2018](#)

[Vol. 7, No. 2, April-June 2018](#)

[Vol. 7, No. 1, Jan-Mar 2018](#)

[Vol. 6, No. 4, October-December 2017](#)

[Vol. 6, No. 3, July-Sep 2017](#)

[Vol. 6, No. 2, April-June 2017](#)

[Vol. 6, No. 1, Jan-Mar, 2017](#)

[Vol. 5, No. 4, Oct-Dec 2016](#)

[Vol. 5, No. 3, July - Sep 2016](#)

[Vol. 5, No. 2, April-June 2016](#)

[Vol. 5, No. 1, Jan-Mar, 2016](#)

[Vol. 4, No. 4, Oct-Dec 2015](#)

[Vol. 4, No. 3, July-September 2015](#)

[Vol. 4, No. 2, April-June, 2015](#)

[Vol. 4, No. 1, Jan-March 2015](#)

[Vol. 3, No. 2, July-Dec 2014](#)

[Vol. 3, No. 1, Jan-June 2014](#)

[Vol. 2, No. 2, July-Dec 2013](#)

[Vol. 2, No. 1, Jan -June 2013](#)

[Vol. 1, No. 2, July-Dec 2012](#)

[Vol. 1, No. 1, Jan-June 2012](#)

ORIGINAL ARTICLE***Phoenix dactylifera* Polyphenols Ameliorates Monosodium Glutamate Induced Cell Damage in the Dentate Gyrus**

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Abstract:

Background: Monosodium Glutamate (MSG) use is quite alarming considering the cascades of toxicity that arises from it. However, this study demonstrated the possible ameliorative activities of polyphenols of *Phoenix dactylifera* (PPD) on MSG-induced dentate gyrus degeneration in male adult Wistar rats. **Aim and Objectives:** To check the effects of PPD on MSG-induced dentate gyrus neuronal damage in adult male Wistar rats. **Material and Methods:** Groups A to D of adult male rats underwent 14-day treatment of Normal Saline (NS), 500 mg/kg PPD, 4 mg/g of MSG only, and 4 mg/g MSG and 500 mg/kg PPD (MSG+PPD) concurrently. Group E received 500 mg/kg PPD for a 14-day period prior to another 14-day of 4mg/gMSG (PPD then MSG). **Results:** PPD was able to ameliorate the toxic effect of MSG as evidence of better cellular integrity, minimal cell vacuolations, minimized dispersed Nissl bodies and deeply stained Nissl bodies were observed upon PPD administration. It was also observed that it reduced the proliferation of reactive oxygen species, proteins and DNA damage in the cells of the dentate gyrus. **Conclusion:** The study concluded that PPD was able to ameliorate the degeneration induced by MSG in the dentate gyrus of Wistar rats.

Keywords: *Phoenix dactylifera*, Polyphenols, Monosodium glutamate, Dentate gyrus

Introduction:

Monosodium Glutamate (MSG) is a sodium salt of the glutamic acid, which serves as a major

component of most food seasonings for example, natural flavorings, bouillon cubes, hydrolyzed vegetable protein, sodium caseinate, calcium caseinate, yeast protein, autolyzed yeast, vegetable protein extract, gelatin, fish seasoning, pepper soup sauce and so on [1]. MSG performs its normal physiological actions through the activation of two receptors namely; ionotropic and metabotropic glutamate receptors. In an extreme case, MSG can also cause over activities of the glutamate receptors, which can lead to neuronal cell death [2-3]. Progressive studies revealed similarities in the various neurodegenerative diseases at a sub-cellular level, which means similar approach can assist in many of these diseases simultaneously [4]. The dentate gyrus is part of the hippocampal formation and forms the major cortical input from the entorhinal cortex to the cornu ammonis of the hippocampus. Consequently, the adult dentate gyrus is known to be able to undergo neurogenesis [5]. However, adult neurogenesis plays an important role in learning and memory, which is evident in rats. Therefore, any form of disruption of cellular proliferation and migration in the dentate gyrus of the adult animal can lead to a decline in hippocampal learning and memory [5].

Phoenix dactylifera (*P. dactylifera*) also known as Date palm, belongs to the family Aceraceae and considered to be an important fruit tree in Asian countries [6-7]. *P. dactylifera* plant has antiulcer, anticancer, anti-diarrheal, hepatoprotective, anti-mutagenic, antioxidant, aphrodisiac, anti-inflammatory, anti-microbial, anti-hyperlipidemic, nephroprotective and neuroprotective properties [8-9]. However, the polyphenolic content of *Phoenix dactylifera* fruit extract and the antioxidant activity is said to have a linear relationship [10]. Antioxidant agents from natural sources such as plants help to compensate for degenerative diseases caused by free radicals [11].

Therefore, it is imperative to check the effects of Polyphenols of *P. dactylifera* (PPD) on MSG-induced dentate gyrus neuronal damage in adult male Wistar rats, based on the rate at which MSG is being consumed and how this event can be associated with issues of learning and memory.

Material and Methods:

Ethical Approval:

The approval for this study was obtained from the Ethics Committee of the University of Ilorin through the Faculty of Basic Medical Sciences, University of Ilorin (UERC/ASN/2016/656).

Experimental Animals:

Thirty (30) male Wistar rats (*Rattus norvegicus*), weighing between 150-180g were used for this study. The rats were gotten from the animal holdings of the Department of Zoology, University of Ilorin, and were acclimatized in the College of Health Sciences, University of Ilorin animal house, for two-weeks prior to commencement of the various treatments. The animals were housed in cages under normal light/dark cycle and at

normal room temperature/humidity. Adequate food and water were available to these animals *ad libitum* [12].

Experimental Design:

The rats were randomly divided into five (5) treatment groups, with each group containing six (6) animals and treatments were done via oral route. Normal Saline (NS) 0.8 ml was given for a 14 day period to the control group, PPD 500 mg/kg for 14-day period to positive control group, MSG 4mg/kg for 14-day period to the negative control group, MSG 4 mg/g and PPD 500 mg/kg was administered concurrently for a 14-day period (MSG+PPD) to a treatment group and PPD 500 mg/kg was given for 14 days followed by administration of 4 mg/kg of MSG for another 14-day period (PPD then MSG) to another treatment group. At the end of the various treatments i.e., Twenty-four hours later, the animals were sedated with 20 mg/kg of ketamine and perfused through the heart. At the completion of perfusion, the whole brain tissues were excised and post-fixed in 4% paraformaldehyde overnight. The whole hippocampal regions were excised and equilibrated in 30% sucrose solution. The sections were taken at 2µm on paraffin wax embedded tissue blocks and later mounted on a glass slide. Harris Hematoxylin and Eosin (H&E) was used to demonstrate the general histo-architecture of the cells of the dentate gyrus and cresyl fast to demonstrate Nissl bodies (endoplasmic reticulum and ribosome) in the cells of the dentate gyrus. All antibodies were procured from Dianova (GmbH/ Warbugstr. 45/20354 Hamburg. Also, reagents and buffers used in this study were molecular biology grade (99.9% pure) from Sigma-Aldrich.

Determination of Biochemical Parameters:

The remaining brain tissues were homogenized in 5% sucrose solution and taken to the centrifuge. The homogenate was spun for 10 minutes at 5000 revolutions per minutes and the supernatants were placed in plain bottles and taken for analysis. Oxidative stress parameters examined were Malondialdehyde (MDA), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx). These analyses were done to check the level of oxidative stress produced in the MSG treated and compared to the effects in PPD treated rats.

Isolation of Polyphenols:

Fruits of *P. dactylifera* were obtained locally in Minna, Niger State, Nigeria and were certified in the Department of Plant Biology, University of Ilorin, Nigeria, with a voucher specimen number is: UILH/001/1205. PPD was extracted by successive solvent extraction with n-hexane, ethyl acetate and methanol. *P. dactylifera* fruits were depitted, dried in air drying oven at 40°C for 24 hours and was milled manually with mortar and pestle. Five hundred gram of *P. dactylifera* fruit was soaked in a liter of each solvent successively with n-hexane, ethyl acetate and methanol respectively. Phytochemical analysis of the extracts from the three solvents was carried out. The extract from methanol, which was the last extracting solvent had flavonoids, phenols, tannins and saponins present.

Statistical Analyses:

The statistical package for social sciences (SPSS, version 20) was used to analyze the data by one-way analysis of variance. All the values were reported as Mean $\pm$ Standard Error of Mean (SEM) and $p < 0.05$, was considered statistically significant.

Results:**PPD Attenuates MSG Induced Cell Damage:**

The administration of MSG caused histopathological damage to the granule cells in the dentate gyrus of the exposed rats, evidence suggests disoriented cellular arrangement coupled with numerous necrotic cells i.e., nuclear fragmentation (Fig. 1), accompanied by presence of dispersed and disintegrated Nissl bodies i.e., chromatolytic cells and reduced cellular density (Fig. 2) within the granule cells of the dentate gyrus. PPD administration prevented the severity of the degenerative activity of MSG by restoring the integrity of the cells with visible nuclei evidence of granule cells with their nuclei membrane intact in both MSG+PPD. Fig. 1a, also there was presence of Nissl bodies in the granule cells of both MSG+PPD and MSG then PPD Fig. 1b, thereby reducing the number of cells undergoing the process of chromatolysis.



Fig. 1a: Representative Photomicrograph of the Dentate Gyrus of the Experimental Rats

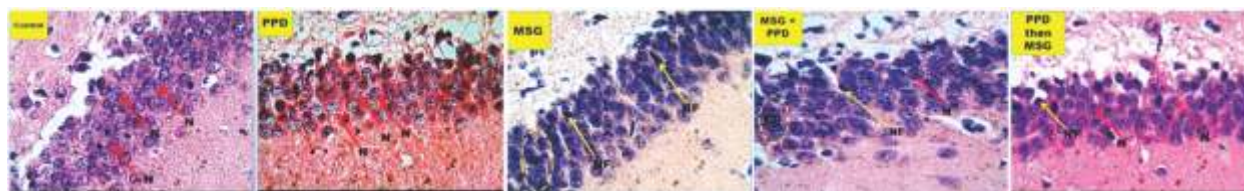


Fig. 1b: Representative Photomicrograph of the Dentate Gyrus of the Experimental Rats
Rats exposed to Normal saline, Polyphenols of *Phoenix dactylifera* (PPD), Monosodium glutamate (MSG) only, MSG and PPD (MSG+PPD), PPD then MSG (PPD-MSG). (Scale bar: 10µm)

Effect of PPD on Oxidative Stress:

The mechanisms of action of MSG, can be through lipid peroxidation and generation of free radicals, which was also observed from this study as the, MDA concentration of the MSG treated rat was significantly higher than the control group (NS). PPD, significantly reduced the MDA concentration in the dentate gyrus of these animals as observed in the two groups (PPD+MSG, PPD then MSG) when compared to the MSG group were treated (Table 1).

Furthermore, of MSG introduction led to the significant reduction in SOD and GPx concentration in the dentate gyrus of the animals when compared to the MSG as seen in Table 1. However, the administration of PPD in both treatment groups (PPD+MSG, PPD then MSG) showed significant reductions in the concentrations of SOD and GPx in the dentate gyrus of these animals when compared to that group of MSG.

Table 1: SOD, GPx and MDA Activities in Brain Homogenate Solution

Groups	SOD (U/L)	GPx (U/L)	MDA (U/L)
NS	130.7±0.5871	50.67±0.7149	1.196±0.01129
MSG	124.4±4.001	35.67±1.202	1.432±0.05653
PPD	72.41±1.006 ^{ab}	14.00±0.05653 ^{ab}	7.769±0.1648 ^{ab}
PPD+MSG	97.23±1.249 ^{abc}	21.33±0.8819 ^{abc}	2.142±0.0912 ^{abc}
PPD then MSG	112.5±4.145 ^{abc}	28.67±1.520 ^{abc}	1.770±0.0594 ^{abc}

Values were expressed in Mean ± SEM, a= represents the significant level in comparison to NS at $p < 0.05$, b= represents significant level in comparison to group MSG at $p < 0.05$, c= represents the significant level in comparison to group PPD at $p < 0.05$, NS-Normal Saline, MSG- Monosodium glutamate, PPD-Polyphenols of *P. dactylifera* SOD – Superoxide dismutase; GPx – Glutathione Peroxidase; MDA - Malondialdehyde

Discussion:

Wistar rats group were treated with MSG only that showed disorientation in cellular arrangement and cell membrane distortion. As resulted change in the membrane biophysics of the cell, causing excessive influx of calcium ion which led to cell death. Furthermore, there was migration of the nucleus from the centre of the cell body. This is characteristic evidence of pyknosis which is a result of DNA fragmentation and chromatin condensation, leading to nuclear fragmentation and vacuolization in some of the granule cells of the dentate gyrus of the rats. The degenerative changes observed in this study as a result of exposure to MSG, showcase the ability of MSG to initiate cellular necrosis and cause damage to the granule cells of the dentate gyrus of the rats [13]. Nevertheless, PPD has been well-known to possess various activities such as antioxidant, anti-inflammatory and neuroprotective activities [10, 14], this is evident in this study, as the rats that received PPD followed by the administration of MSG showed that PPD was able to protect the granule cells of the dentate gyrus of rats, the cells showed normal cyto-architecture with very minimal cellular disorientation. Also, the rats treated with MSG followed by PPD revealed, that some of the granule cells found in the granule layer of the dentate gyrus have some fragmented nucleus or has undergone vacuolization while some other granule cells appeared disoriented with minimal nuclear extrusion, although, in these group are neurons showing presence of normal nucleus, intact cytoplasm and normal cellular orientation and integrity. The protective and ameliorative effects of PPD supports that *P. dactylifera* acts through enhancing memory and learning,

neuroprotective, anti-inflammatory, antioxidative and regenerative roles on the brain in the emerging data [9, 14-16]. The recovery and protection noticed in the granule cells of the dentate gyrus of rats in this study as resulted of the influence of PPD treatment before and after the use of MSG in the experimental groups respectively, which has established that *P. dactylifera* is able to protect and rescue neurons from insult that might have compromised the integrity of the cells in various parts of the brain of rats [14-15]. Also, it was revealed that the animals that received MSG only showed disintegrated and dispersed Nissl bodies in the cytoplasm of most neurons in the granule layers of the dentate gyrus. This implies chromatolysis, a process that most times leads to apoptosis of the neuron; furthermore, there was great cellular disorientation in the granule layer of the dentate gyrus of these rats. The rats that received MSG followed by PPD revealed that some of the neurons found in the granule layers still possess densely stained Nissl bodies in their cytoplasm. This suggests the influence of PPD on the toxic effect of MSG on the granule cells. Some of these neurons showed presence of abnormal cytoplasm and palely stained Nissl bodies. The rats that received PPD followed by the administration of MSG showed that PPD was able to protect the granule cells of rats, as they have normal cyto-architecture and densely stained Nissl substance of the neurons that was observed in the granule layer of the dentate gyrus. The degenerative changes observed in the dentate gyrus of the rat in this study, on exposure to MSG, which led to dispersed and disintegration of Nissl bodies suggests abnormal gene expression due to disrupted protein synthesis

in the neurons [17]. PPD showed the ability to mitigate damage to neurons and protect against intoxication in this study by mitigating the toxic effect of MSG on the Nissl bodies of the neurons and also protecting some of the Nissl bodies from MSG intoxication [9, 14-15].

The decrease in the SOD activity may be associated with the elevation of the intracellular concentrations of Hydrogen Peroxide (H_2O_2) or oxidative inactivation of the enzyme due to excessive Reactive Oxygen Species (ROS) generation [18] as seen in animals treated with MSG alone. Although, PPD was able to regulate the activities of SOD against MSG induced oxidative stress in the dentate gyrus of rats and it shows more efficiency when PPD was administered before MSG. Therefore, it means that PPD regulated the activities of SOD enzyme by increasing the dismutation of superoxide ion produced by MSG into oxygen molecule and hydrogen peroxide (which is less damaging) in the dentate gyrus of these rats [9,13]. Furthermore, this study suggested that PPD was able to regulate the activities of GPx against MSG induced oxidative stress in the dentate gyrus of the rats and shows more efficiency when PPD was administered before MSG. This study suggests that PPD helps regulate the activity of the GPx enzyme by detoxification of peroxides produced by MSG in the dentate gyrus of rats [19-20]. Glutathione

reacts with ROS and nucleophilic compounds, the reaction is catalyzed by GPx [21]. Lastly, PPD regulated the activities of malondialdehyde in the dentate gyrus of the rats against the MSG induced oxidative stress. It implied that PPD was able to inhibit neuronal injuries from propagating chain reaction of lipid peroxidation caused by MSG in the dentate gyrus of the rats by enhancing cell signalling, reduce protein and DNA damage [22-23].

Conclusion:

This study demonstrated the activities of PPD and MSG on the dentate gyrus of Wistar rats. PPD acts as an ameliorative agent against the insult or damaging effects of MSG by restoring the cytoarchitectural integrity of cells, reduced the process of chromatolysis, increasing the dismutation of superoxide ion oxygen molecule and H_2O_2 , detoxification of peroxides, enhancing cell signalling, reduce protein and DNA damage. This study thus suggests that *P. dactylifera* was able to amend the damage of cells induced by MSG in the dentate gyrus of the Wistar rats.

Acknowledgements:

We wish to appreciate the members of staff of the Faculty of Pharmacy and the Department of Histopathology, University of Ilorin, Nigeria, for their technical support during this work.

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How to cite this article:

Usman RY, Sulaimon FA, Okesina AA, Imam A, Imam AL, Ajao MS. *Phoenix dactylifera* Polyphenols Ameliorates Monosodium Glutamate Induced Cell Damage in the Dentate Gyrus. *J Krishna Inst Med Sci Univ* 2021; 10(2):139–145

Submitted: 05-Feb-2021 Accepted: 10-Mar-2021 Published: 01-Apr-2021