Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 3
Open Access

Short Title: Antioxidant Activity Of Moringa Oleifera Leaf Extract

Long Title: Free Radical Scavenging And Antioxidant Enhancing Ability Of Moringa Oleifera Leaf Extract Against Gamma Radiation-Induced Enzyme Toxicity In The Liver Of Pangasius Sutchi

GI Darul Raiyaan[1], D Sankari*[1], G Balaji[2], A Kantha Deivi[3]
*[1,] [1]*Department of Biotechnology, College of Science and Humanities,*
SRM Institute of Science and Technology, Kattankulathur, Tamil Nadu - 603203, India
[2]Department of Biotechnology, Bhupat and Jyoti Mehta School of Biosciences,
Indian Institute of Technology Madras, Chennai, Tamil Nadu – 600036, India
[3]Faculty of Sciences, Marwadi University, Rajkot, Gujarat – 360003, India
*sankarid@srmist.edu.in

Cite this paper as: GI Darul Raiyaan, D Sankari, G Balaji, A Kantha Deivi (2024) Antioxidant Activity Of Moringa Oleifera Leaf Extract. *Frontiers in Health Informatics, 13* (3), 841-852.

Abstract
Radiotherapy is the most common treatment for cancer patients, where ionizing radiation kills the tumor and healthy non-cancerous cells surrounding them. A synthetic drug, amifostine is commonly utilized to prevent the major drawback of radiotherapy. This drug may also be toxic to the individual being treated. In this aspect, *Moringa oleifera* leaf – 30% ethanol extract (MOLE) shows abundant antioxidant-rich polyphenols, neutralizing free radicals from radiation penetration into living cells' water molecules. The free radical scavenging ability of MOLE in the liver of *Pangasius sutchi* can be quantified by antioxidant enzyme assays such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), which are endogenous enzymes of our body and are involved in neutralizing free radicals. The lipid peroxidation (LPO) assay also quantifies the peroxidation level of fatty acids arising from lipoxygenases or free radical pathways. The default radioprotective effect of amifostine was high among the comparative compounds. This result was followed by MOLE and flavonoids such as kaempferol and quercetin. Thus, the study forms a base for the radioprotection activity of MOLE in replacing the chemical drug amifostine.

Keywords: Antioxidants, catalase, glutathione, free radical, lipid peroxide, superoxide dismutase.

Conflict of Interest: The authors declare that there is no conflict of interest.

INTRODUCTION:

Medical imaging techniques, including roentgen diagnostics, computed tomography, and radiotherapy, pose a risk of radiation-induced side effects to patients and medical professionals[1]. Side effects, such as nausea, loss of appetite, cancer, hair loss, infertility, and secondary cancer. [2]. When radiation penetrates the tissue, it may induce ionization and generate hydroxyl free radicals[3]. The hydroxyl radical poses the greatest risk to tissues because of its exceptional reactivity and capacity to oxidize several cellular constituents, including lipids, proteins, and carbohydrates. Oxidative stress induces harm to both mitochondrial and nuclear DNA, potentially leading to genetic mutation and the development of cancer.

Free radicals and reactive oxygen species (ROS) are protected by both endogenous and exothermic antioxidant

protection. Endogenous antioxidant enzymes, including SOD, CAT, and substances like GSH prevents excessive oxidative stress. Polyphenols are a kind of exogenous antioxidants. The combined action of naturally occurring and externally derived antioxidants helps maintain a balance between oxidative processes and antioxidant defenses, reducing the harmful consequences of oxidative stress. This equilibrium allows ROS to carry out crucial physiological roles as mediators of communication between cells.

Those who are affected by cancer and receive radiation therapy must use radio protectors to shield their healthy cells from radiation. It decreases the production of harmful molecules called free radicals, triggers a state of low oxygen levels in cells, and stimulates the activation of cellular radioprotective enzymes such as SOD. Furthermore, radio protectors assist in improving the DNA repair process and hinder the activation of death-signaling pathways[4].

Amifostine is a versatile and effective drug that protects cells throughout the body. It can safeguard almost all regular tissues against the adverse consequences of radiation or certain chemotherapy drugs. The medicine has hypotensive effects, resulting in gastrointestinal problems in both the upper and lower parts of the digestive system. This leads to negative behavioral reactions and a decline in performance[5]. While the potential of synthetic radio protectants in medicinal applications is exciting, their effectiveness is limited due to their intrinsic systemic toxicity and short biological half-lives. Therefore, alternative herbal therapy has a significant impact in substituting synthetic pharmaceuticals[6].

The antioxidant properties of *M. oleifera* leaf extracts are seen in both in vivo and in vitro, which may be attributed to their abundant content of phenolic acids and flavonoids[7]. Chemical compounds derived from medicinal plants serve as valuable resources to alleviate certain ailments that impact human well-being. Currently, extensive research is being conducted on extracting these bioactive chemicals, leading to identifying novel pharmaceuticals derived from natural sources using contemporary methodologies[8]. The primary bioactive constituents found in *M. oleifera* are kaempferol and quercetin. The medicinal value of kaempferol includes antibacterial, anti-inflammatory, antioxidant, anticancer, cardio protective, neuroprotective, and antidiabetic activity[9]. The in vivo antioxidant activity of quercetin shows the free radicals scavenging and prevention of lipid peroxidation[10]. Quercetin improves GSH levels and decreases malondialdehyde (MDA) and hydroxyl levels. Thus, the above-mentioned qualities of MOLE pave the way for exploring radioprotection activity in comparison with synthetic drug amifostine along with flavonoids such as kaempferol and quercetin.

MATERIAL AND METHODS:

Plant extraction procedure

The leaf of *M. oleifera* was collected from the Vellore district in Tamil Nadu, India, and the 30% ethanol extract was prepared according to Khan's methodology[11].

Adaptation of fish to new environmental conditions

The in-vivo study was conducted using fingerlings of *P. sutchi* following the guidelines of the OECD[12]. The procedures were performed according to CPCSEA[13] guidelines for experimentation on fish. Ethical clearance was given by the institutional ethical committee for animals, The IAEC No.15/IAEC/CENR/04.

Quantification of the lethal dose (LD_{30}) of gamma irradiation

The LD_{30} was measured by exposing the fish to Cobalt-60 gamma radiation using a gamma chamber-5000.

Determining the low observed effective level (LOEL) using the micronucleus (MN) test for 96 hours.
The cytotoxic tests known as MN assay developed by Bolognesi and Hayashi[14] was used to determine the LOEL level of amifostine, MOLE, kaempferol, and quercetin based on abnormalities in red blood cells (RBC) of *P. sutchi*.

Radio-protection studies using LOEL concentration of amifostine, MOLE, kaempferol, and quercetin in *P. sutchi* with LD_{30} of gamma irradiation (32 days study)

Tissue homogenate preparation

The internal organs are carefully transferred into Eppendorf tubes filled with PBS for homogenization. The tissue was promptly cut off and rinsed using chilled PBS (0.1 M, pH 7.4). It was dried by blotting paper, then weighed and finely chopped using stainless steel scissors to prepare tissue homogenate with PBS using a tissue homogenizer.

A 10% tissue homogenate was generated by mixing the tissue with a solution containing 0.1 M PBS in a ratio of 1:9. The mixture was then centrifuged at 4°C for 20 minutes at a speed of 8000 revolutions per minute. Ultimately, the liquid portion was gathered and preserved at a temperature of 20°C for further utilization in the assessment of LPO, SOD, CAT, and GSH enzymes.

Lowry's technique for protein detection

The total protein content was assessed using Lowry's protocol[15]. Protein molecules in alkaline liquids react with copper ions and the phenolic hydroxyl group that contains amino groups to generate a complex. The presence of tryptophan and tyrosine in the copper protein complex leads to a reaction with the Folin-Ciocalteau reagent, resulting in the reduction of phosphomolybdate and the formation of a blue hue. The intensity of the color is directly related to the amounts of the proteins, namely the aromatic amino acids.

A graph was created by using standard concentration on the X-axis and optical density on both sides, while blue color intensity was measured at 620 nm. The measurement of protein content and tissue level were both reported as mg/ml.

SOD activity:

Li16 elaborated on the SOD antioxidant assay at a later stage. SOD enzyme is involved in the inhibition of autoxidation, and the concentration of SOH in its 325 nm region has an impact on this.[17].

The SOD activity was calculated using the following formula:

$$\frac{\left(Absorbance\ of\ \frac{control}{T} - Absorbance\ of\ \frac{sample}{T}\right)}{Absorbance\ of\ control/T} \times 100\%$$

Where, T= 5 minutes

CAT Assay

CAT activity assay was done using the Domingues & Gravato[18] methodology. The degradation of peroxide is monitored at a wavelength of 240 nm and immediately measured to determine the rate of reaction since water and oxygen do not absorb at this specific wavelength. The presence of catalase increases the reaction rate proportionally.

Calculation: µ mol/min/mg of protein

$$Activity = \frac{(Absorbance\ of\ sample - Absorbance\ of\ blank) \times \frac{Vt}{Vs}}{Protein\ value\ \times\ \varepsilon\ \times d}$$

Vt/Vs represents the ratio of the total volume to the volume of the sample, which is 150 µL/5 µL in this instance. The amount of protein in the samples was measured in mg/mL. ε denotes the coefficient of molar extinction in hydrogen peroxide (H_2O_2), which is 0.04 mM/cm at a wavelength of 240 nm, and d represents the approximate length of the light path, which is 0.9 cm.

GSH Assay

The method used by Elman19. The compound 5-5'-dithiobis-2-nitrobenzoic acid reacts with GSH to form a chromophore with a wavelength of 412 nm, known as 5-thionitrobenzoic acid (TNB) or GS-TNB. Glutathione reductase enhances the reaction by reducing GS-TNB and releasing another TNB molecule, which is then recycled with GSH.

The conventional graph was presented with the concentration of the standard on the x-axis and optical density on the y-axis. The GSH enzyme content was expressed as µM/g tissue.

LPO Assay

The LPO assay was conducted according to the protocol described by Mani[20]. The chromogenic reagent, 2-thiobarbituric acid reacts with MDA to produce an end product of LPO. A single MDA molecule undergoes a Knoevenagel-type condensation reaction with two molecules of 2-2-thiobarbituric acid (TBA), forming a chromosphere that exhibits a maximum absorbance at 532 nm.

A conventional graph was presented with the concentration of the standard on the x-axis and optical density on the y-axis. The LPO concentration was expressed as nM MDA/mg tissue

Statistical Analysis

The statistical analysis and graphing were done using Origin Pro 2024 software. The significance of group means at 0.05 level was measured using One-way ANOVA. Mean comparison and homogeneity of variance were done by Fisher and Levene's test (Absolute Deviations) respectively.

RESULTS & DISCUSSION

Determination of LD_{30} of γ-irradiation in *P. sutchi*

The LD_{30} result was derived from the LD_{50} value of gamma irradiation using Finney's probit statistical analysis over a 32-day study period and it was found to be 13.18 Gy.

The results for LOEL concentration of compounds were given based on the MN assay showing mean percentage of abnormalities in RBCs

The LOEL concentrations were determined to be 80 mg/Kg body weight (B.W) for amifostine (Figure 1), 130 mg/Kg B.W for MOLE (Figure 2), and 20 mg/Kg B.W for both kaempferol and quercetin (Figure 3). These findings were calculated using the erythrocytic abnormalities, which were found to be around 4 to 5% in the MN test. Any value below this percentage was deemed to indicate a null impact or the normal apoptotic phase of cells. The presence of erythrocytic abnormalities, in addition to the previously specified percentage, indicates that the medications may have potentially fatal consequences due to an increase in concentration. As the doses increased the erythrocytic abnormalities such as micro nucleated, binucleated, vacuolated cells

and apoptotic cells increased.

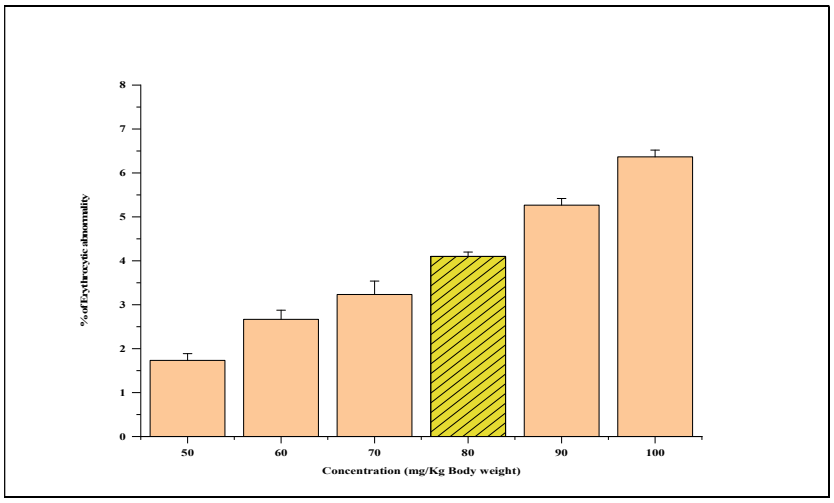

Figure 1. The mean percentage of abnormalities in RBCs showing LOEL amount of amifostine with ± standard deviation.

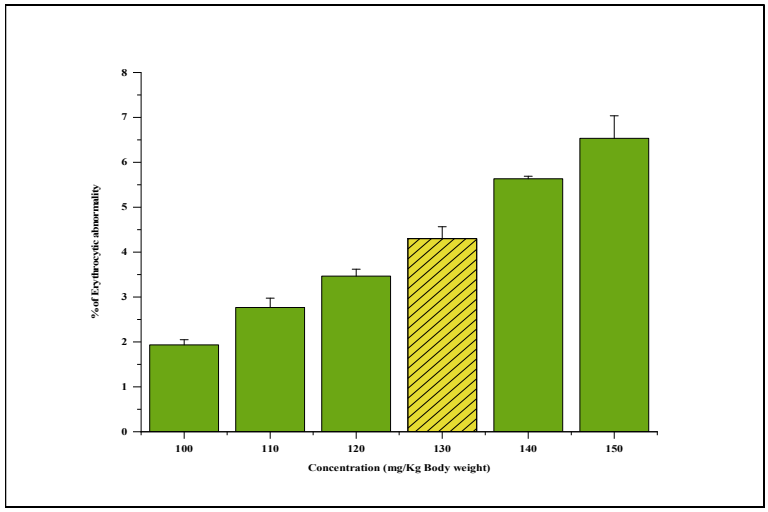

Figure 2. The mean percentage of abnormalities in RBC's showing LOEL amount of MOLE with ± standard deviation.

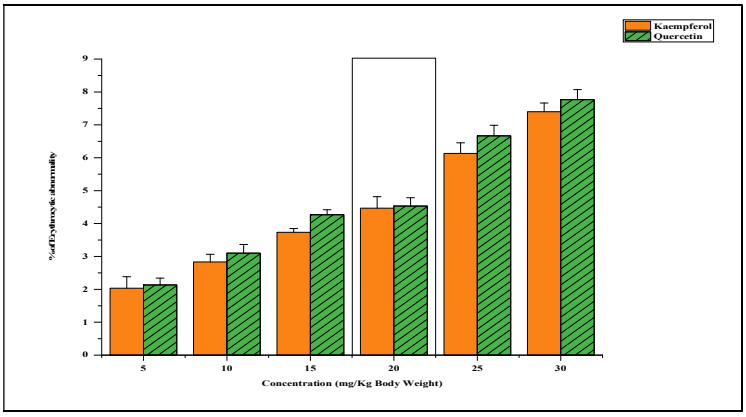

Figure 3. The mean percentage of abnormalities in RBCs showing LOEL amount of kaempferol and quercetin with ± standard deviation.

Protein analysis

The amount of protein in the animal tissue is directly proportional to the radioprotective effect of that compound. The protein level in the liver of the sham group was measured to be 74.62 mg/ml tissue on the 2nd day and 77.89 mg/ml tissue on the 32nd day. The amifostine treatment group had the highest protein content, with 65.49 mg/ml tissue on the 2nd day and 69.29 mg/ml tissue on the 32nd day. The MOLE group had the second highest protein content, with 62.62 mg/ml tissue on the 2nd day and 66.36 mg/ml tissue on the 32nd day. The kaempferol group had a protein content of 59.89 mg/ml tissue on the 2nd day and 63.96 mg/ml tissue on the 32nd day. The quercetin group had the lowest protein content, with 58.22 mg/ml tissue on the 2nd day and 60.42 mg/ml tissue on the 32nd day. The LD_{30} group had a significantly reduced protein content, with values of 52.76 mg/ml tissue on the 2nd day and 49.62 mg/ml tissue on the 32nd day. The findings are given in Figure 4.

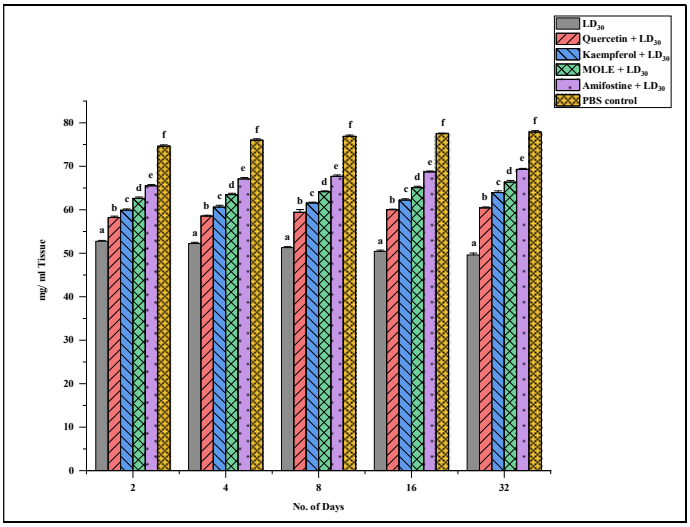

Figure 4. The amount of protein shows the radioprotective efficacy of amifostine, MOLE, kaempferol, and quercetin in the liver of *P. sutchi* exposed to LD_{30} of gamma irradiation. The one-way ANOVA using Fisher's test shows that the means of groups that do not share a similar alphabet at $p \leq 0.05$ level. The Homogeneity

of variances using Levene's test – Absolute deviations show the group variances at $p \leq 0.05$ level.

SOD assay

SOD is an enzyme that maintains the amount of superoxide by converting it into H_2O_2 and cellular oxygen[21]. It is present in all living cells exposed to oxygen[17]. SOD activity in the liver of the sham group was measured to be76% on the second day and 38.46% on the 32nd day... The group treated with amifostine exhibited the highest SOD activity, with percentages of 36.26 on the 2nd day and 36.73 on the 32nd day. This was followed by the MOLE group, which had percentages of 35.32 on the 2nd day and 35.75 on the 32nd day. The kaempferol group had percentages of 33.82 on the 2nd day and 34.64 on the 32nd day, respectively. Lastly, the quercetin group had percentages of 32.23 on the 2nd day and 32.75 on the 32nd day. The LD_{30} group had significantly reduced SOD activity, measuring 31.35% on the 2nd day and 30.84% on the 32nd day. The findings are shown in Figure 5.

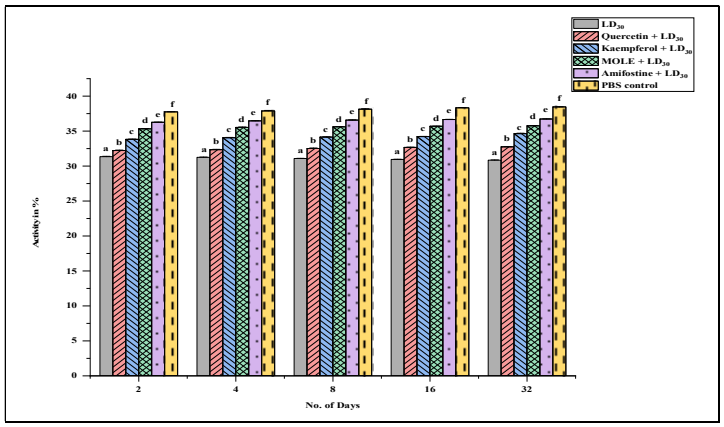

Figure 5. The SOD activity showing radioprotective efficacy of amifostine, MOLE, kaempferol, and quercetin in the liver of *P. sutchi* exposed to LD_{30} of gamma irradiation. The one-way ANOVA using Fisher's test shows that the means of groups that do not share a similar alphabet are at $p \leq 0.05$ level. The Homogeneity of variances using Levene's test – Absolute deviations show the group variances are at $p \leq 0.05$ level.

CAT assay

CAT is a ubiquitous enzyme present in almost all living species. It plays a crucial role in defense against oxidative stress by inhibiting the generation of harmful free radicals. CAT efficiently decomposes H_2O_2 into water and oxygen after SOD converts superoxide into H_2O_2. This process allows CAT to break down millions of H2O2 molecules per second rapidly[21]. The quantity of CAT in the liver of the sham group for 2nd and 32nd day was measured to be 0.99 and 1.04 µ mol/min/mg of protein respectively. The amifostine treatment group exhibited the highest concentration of CAT for 2nd day and 32nd day with values of 0.96 and 0.99 µ mol/min/mg of protein respectively. This was followed by MOLE, with values of 0.91 and 0.93 µ mol/min/mg of protein on the 2nd and 32nd day, respectively. Kaempferol had values of 0.87 and 0.89 µ mol/min/mg of protein on the 2nd and 32nd day, respectively, while quercetin had values of 0.85 and 0.86 µ mol/min/mg of protein on the 2nd and 32nd day, respectively. The LD_{30} group had a significantly low concentration of CAT, with values of 0.83 and 0.82 µ mol/min/mg of protein on the 2nd and 32nd day, respectively, Figure 6 displays the results.

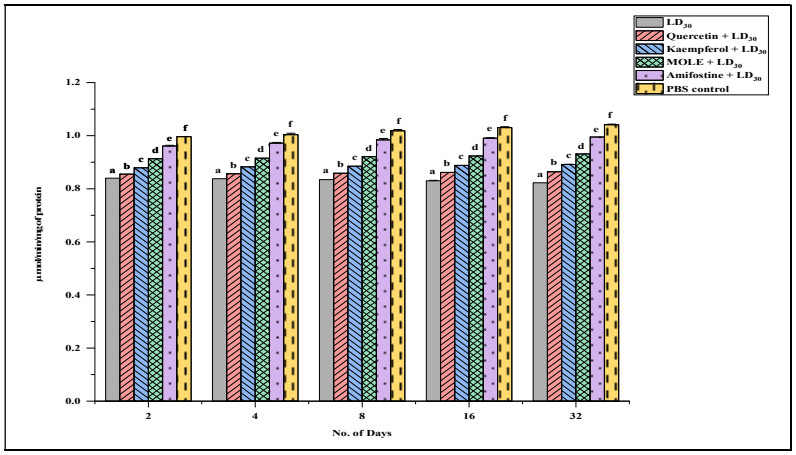

Figure 6. The concentration of CAT shows the radioprotective efficacy of amifostine, MOLE, kaempferol, and quercetin in the liver of *P. sutchi* exposed to LD_{30} of gamma irradiation. The one-way ANOVA using Fisher's test shows that the means of groups that do not share a similar alphabet are at $p \leq 0.05$ level. The Homogeneity of variances using Levene's test – Absolute deviations show the group variances, $p \leq 0.05$ level.

A deficit or mutation of CAT has been associated with a range of illnesses and abnormalities[21]. Elevated CAT activity signifies an augmented presence of H_2O_2 inside the body, indicating exposure to higher quantities of free radicals. This exposure might potentially result in oxidative stress.

GSH assay

GSH, an intracellular reducing agent, is present in all animal cells and acts as a protective factor against free radicals, peroxides or other damaging substances. It can restore antioxidants, such as ascorbic acid and tocopherol. It also plays a role in the repair of lipids, proteins, DNA, and RNA that have been damaged by peroxidation. Additionally, it helps maintain sulfhydryl protein groups in their reduced form. GSH maintains redox equilibrium by functioning as both a direct antioxidant and a substrate for glutathione peroxidase, an enzyme that scavenges free radicals[21].

The concentration of GSH in the liver of the sham group was measured to be 25.87 and 27.74 µM/g tissue on the 2nd and 32nd day, respectively. The amifostine treatment group had the highest GSH content, with concentrations of 23.23 µM/g tissue on the 2nd day and 24.56 µM/g tissue on the 32nd day. The MOLE group had slightly lower GSH content, with concentrations of 22.07 µM/g tissue on the 2nd day and 22.79 µM/g tissue on the 32nd day. The kaempferol group had GSH concentrations of 19.86 µM/g tissue on the 2nd day and 21.89 µM/g tissue on the 32nd day. The quercetin group had GSH concentrations of 19.31 µM/g tissue on the 2nd day and 20.59 µM/g tissue on the 32nd day. The LD_{30} group had a significantly low concentration of GSH, measuring 17.95 µM/g tissue on the 2nd day and 16.54 µM/g tissue on the 32nd day. The results are given in Figure 7.

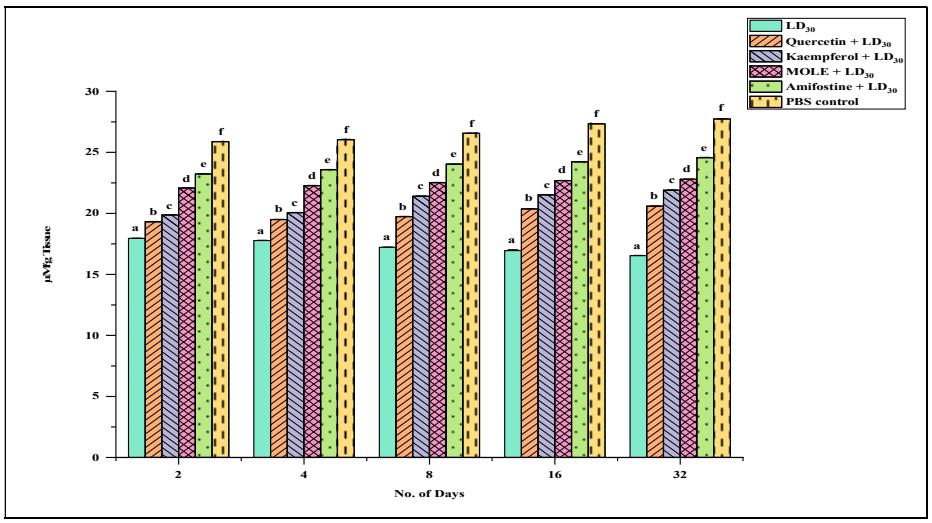

Figure 7. The quantity of GSH showing radioprotective efficacy of amifostine, MOLE, kaempferol, and quercetin in the liver of *P. sutchi* exposed to LD_{30} of gamma irradiation. The one-way ANOVA using Fisher's test shows that the means of groups that do not share a similar alphabet are at $p \leq 0.05$ level. The Homogeneity of variances using Levene's test – Absolute deviations show the group variances at $p \leq 0.05$ level.

GSH plays a crucial role in xenobiotic biotransformation by detoxifying electrophiles and damaging radicals. Several harmful chemicals are excreted in the urine as derivatives of mercapturic acid or as conjugates with bile in the form of GSH.

LPO assay

The present study demonstrated that pretreatment of MOLE protects hepatic function from radiation-induced LPO. The level of LPO in the liver of the sham group was measured to be 3.56 and 3.49 nM MDA/mg tissue on the 2nd and 32nd day, respectively. The amifostine treatment group had the highest LPO content, with levels of 3.94 and 3.84 nM MDA/mg tissue on the 2nd and 32nd day, respectively. This was followed by the MOLE group, with levels of 4.17 and 4.09 nM MDA/mg tissue on the 2nd and 32nd day, respectively. The kaempferol group had levels of 4.44 and 4.33 nM MDA/mg tissue on the 2nd and 32nd day, respectively, while the quercetin group had levels of 4.92 and 4.74 nM MDA/mg tissue on the 2nd and 32nd day, respectively. The LD_{30} group had a very low LPO content, with values of 5.15 and 5.65 nM MDA/mg tissue on the 2nd and 32nd day, respectively. The LPO results are shown in Figure 8.

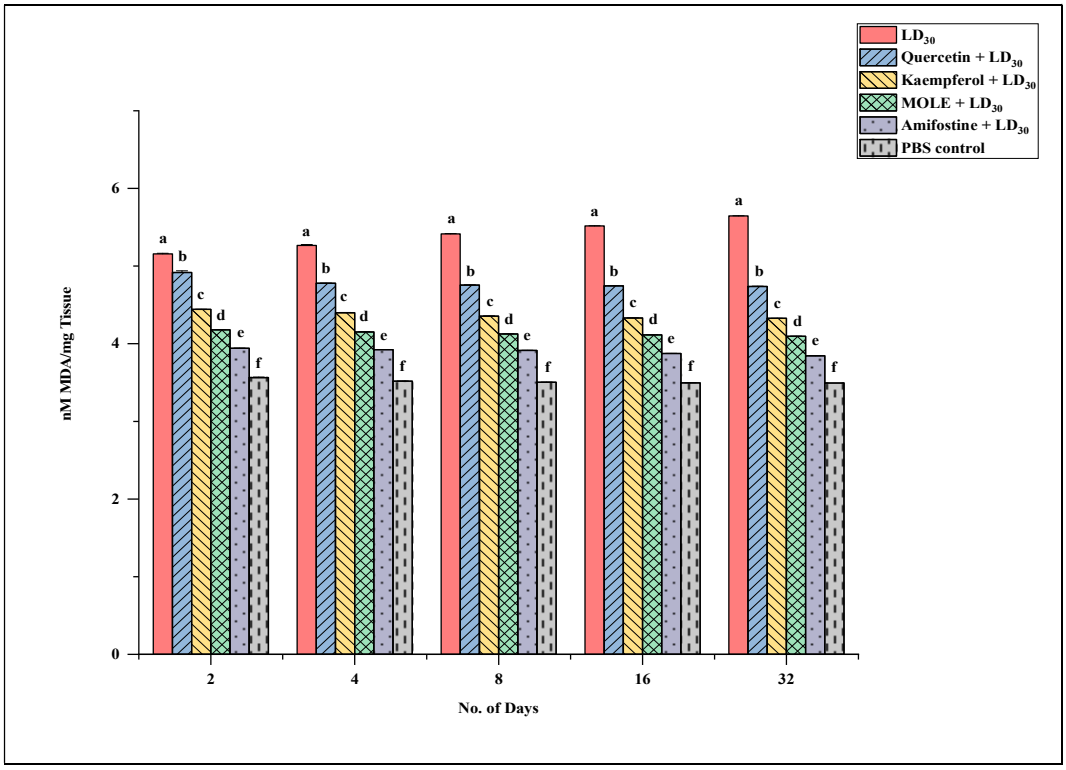

Figure 8. The amount of LPO showing radioprotective efficacy of amifostine, MOLE, kaempferol, and quercetin in the liver of *P. sutchi* exposed to LD_{30} of gamma irradiation. The one-way ANOVA using Fisher's test shows that the means of groups that do not share a similar alphabet are at $p \leq 0.05$ level. The Homogeneity of variances using Levene's test – Absolute deviations show the group variances at $p \leq 0.05$ level.

Lipid hydro peroxides are generated in living organisms by the free radical mechanisms of ROS. Additionally, they appear as byproducts when lipoxygenases work on polyunsaturated fatty acids in the presence of oxygen[17]. LPO is a key process through which ROS exerts its hazardous effects. This process involves the destruction of membranes through radical reactions, resulting in alterations in membrane permeability and fluidity[22]. Additionally, ROS has the potential to interact with DNA directly.

A decrease in protein, CAT, GSH content, SOD activity, and an increase in LPO levels was observed in the LD_{30} (Negative control) group as the days went on, which resembles permanent damage in the liver. Simultaneously an increase in protein, CAT, GSH content, SOD activity, and a decrease in LPO levels was observed in other treatment groups from the 2nd day to the 32nd day. It may be due to the protective effects of amifostine, MOLE, kaempferol, and quercetin and their cell rejuvenation activity. The lower values of protein, SOD, CAT, GSH, and elevated levels of LPO in the PBS control group during the early days of the study period are due to external stress while traveling for irradiation. As time progresses, the stress diminishes, resulting in elevated protein, SOD, CAT, GSH, and depleted LPO levels in subsequent days.

CONCLUSION:

The oxidative injury, evidenced by the elevated LPO levels, depleted GSH, CAT, protein levels, and SOD activities were noticed in this present study. The impairments in endogenous enzymes of the liver exposed to γ- irradiation were improved by MOLE pretreatment. Thus, the results emphasized the free radical scavenging

of MOLE in comparison with amifostine, kaempferol, and quercetin.

Acknowledgment

I am grateful to SRM IST for the support in conducting my research work, especially to the faculty, technicians, and scholars of the Molecular Biology and Immunology Division. I also acknowledge the Radiological Safety Division of Indira Gandhi Centre for Atomic Research for providing radiation facilities.

References

1. Nuszkiewicz J, Woźniak A, Szewczyk-Golec K. Ionizing Radiation as a Source of Oxidative Stress-the Protective Role of Melatonin and Vitamin D. International Journal of Molecular Sciences. 2020; 21(16), 1-22.
2. Checker R, Patwardhan RS, Jayakumar S, Maurya DK, Bandekar M et al. Chemical and Biological Basis for Development of Novel Radioprotective Drugs for Cancer Therapy. In Free Radical Research. 2021; 55(5), 595-625). Taylor and Francis Ltd.
3. Fathur Rachman R, Rohim Tualeka A, Ardyanto DY, Jalaludin J, Rahmawati P. Systematic Review: Detoxification of Free Radicals from Ionizing Radiation with Glutathione Intake. In Journal of Positive School Psychology. 2022; 6(11), 2564-2576.
4. Kamran MZ, Ranjan A, Kaur N, Sur S, Tandon V. Radioprotective Agents: Strategies and Translational Advances. Medicinal Research Reviews. 2016; 36(3), 461-493.
5. Singh VK, Seed TM. The Efficacy and Safety of Amifostine for the Acute Radiation Syndrome. In Expert Opinion on Drug Safety. 2019; 18(11), 1077-1090. Taylor and Francis Ltd.
6. Cinkilic N, Tüzün E, Çetintaş SK, Vatan Ö, Yilmaz D et al. Radio-protective Effect of Cinnamic Acid, a Phenolic Phytochemical, on Genomic Instability Induced by X-rays in Human Blood Lymphocytes in vitro. Mutation Research - Genetic Toxicology and Environmental Mutagenesis. 2014; 770, 72-79.
7. Mrudula K, Hemant U. Chemoprevention of DMH Induced Colon Carcinogenesis by Combination Administration of Lactobacillus acidophilus, Calcium and Moringa oleifera Leaves Extract. International Journal of Pharmaceutical Quality Assurance. 2023;14(4):1002-1010.
8. Gómez-Martínez M, Ascacio-Valdés JA, Flores-Gallegos AC, González-Domínguez J, Gómez-Martínez S. Location and Tissue Effects on Phytochemical Composition and In vitro Antioxidant Activity of Moringa oleifera. Industrial Crops and Products. 2020; 151.
9. Shraddha RS, Kumar B, Neeraj SK, Manmohan S, Swati A. In-vitro Analysis of Antioxidant, Anti-Inflammatory, Antidiabetic and Antimicrobial Studies of Kaempferol Ethosomes. International Journal of Drug Delivery Technology. 2024;14(1):118-125.
10. Bhimanwar AA, Nikam AP, Dhamane SP, Barangule SP. Study of the Combined Effect of Silymarin, Quercetin, and Hesperidin on 3-nitro Propionic Acid-induced Rat Model of Huntington's Disease. International Journal of Pharmaceutical Quality Assurance. 2023;14(4):959-967.
11. Khan W, Parveen R, Chester K, Parveen S, Ahmad S. Hypoglycemic Potential of Aqueous Extract of Moringa oleifera Leaf and In vivo GC-MS Metabolomics. Frontiers in Pharmacology. 2017;

8(SEP).

12. OECD. Effects on Biotic Systems, Fish Acute Toxicity Testing, OECD Guidelines for the Testing of Chemicals. 2019.
13. CPCSEA. Guidelines of CPCSEA for Experimentation on Fishes. Government of India Ministry of Fisheries, Department of Animal Husbandry and Dairying. 2020.
14. Bolognesi C, Hayashi M. Micronucleus Assay in Aquatic Animals. Mutagenesis. 2011; 26(1), 205-213.
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein Measurement with the Folin Phenol Reagent. The Journal of Biological Chemistry. 1951; 193(1), 265-275.
16. Li X. Improved Pyrogallol Autoxidation Method: A Reliable and Cheap Superoxide-Scavenging Assay Suitable for All Antioxidants. Journal of Agricultural and Food Chemistry. 2012; 60(25), 6418-6424.
17. Nilsson R, Liu NA. Nuclear DNA Damages Generated by Reactive Oxygen Molecules (ROS) Under Oxidative Stress and their Relevance to Human Cancers, Including Ionizing Radiation-Induced Neoplasia Part I: Physical, Chemical and Molecular Biology Aspects. Radiation Medicine and Protection. 2020; 1(3), 140-152. KeAi Communications Co.
18. Domingues I, Gravato C. Oxidative Stress Assessment in Zebra Fish Larvae. Methods in Molecular Biology. 2018; 1797, 477 - 486. Humana Press Inc.
19. Ellman GL. Tissue sulfhydryl groups. In Archives of Biochemistry and Biophysics. 1959; 82.
20. Mani R, Balasubramanian S, Raghunath A, Perumal E. Chronic Exposure to Copper Oxide Nanoparticles Causes Muscle Toxicity in Adult Zebra Fish. Environmental Science and Pollution Research. 2020; 27(22), 27358-27369.
21. Mendonça J da S, Guimarães R de CA, Zorgetto-Pinheiro VA, Fernandes CDP, Marcelino G. Natural Antioxidant Evaluation: A Review of Detection Methods. Molecules. 2022; 27(11). 123-135.
22. Ogunwole GA, Abiya SE, Amaeze NH, Eze CTG. Antioxidant Markers in Gills, Liver and Muscle Tissue of the African Sharptooth Catfish (Clarias gariepinus) Exposed to Sub Chronic Levels of Ibuprofen and Dibutyl phthalate. Scientific African. 2021; 12.