Research Article

Exploring Zea mays Leaf Extracts: An In-Depth Analysis of Lipid Peroxidation in Diverse Membrane Models and DNA Protection in Various *In-Vitro* Systems

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Received: 26-12-2023; **Revised:** 03-02-2024; **Accepted:** 10-02-2024; **Published on:** 15-02-2024.

ABSTRACT

Introduction: Reactive Oxygen Species (ROS) pose a threat to cellular components, leading to oxidative stress and potential harm. The impact of oxidative and antioxidative processes on redox homeostasis affects signal proteins, and pathways, and influences various cellular processes, including apoptosis and cell proliferation. The presence of oxidative stress, marked by an imbalance between the generation and removal of reactive oxygen or nitrogen species, is linked to the onset and advancement of cancer. This underscores the importance of chemopreventive approaches that involve natural dietary compounds.

Objectives: The study focuses on Zea mays leaves, evaluating their antioxidant potential against biomolecules and investigating the effect on oxidative DNA damage.

Methodology: The methodology involves assessing lipid peroxidation in diverse membrane models including RBC ghosts, Liver homogenates, and Liver slices. The study also focuses on examining the protective effects of Zea mays leaf extracts on DNA damage using various in vitro systems (λ DNA, haploid herring sperm DNA, diploid calf thymus DNA, and intact cell DNA).

Results: Zea mays leaf extracts exhibit significant inhibition of lipid peroxidation in membrane models, with methanolic and aqueous extracts outperforming the chloroform extract. In the evaluation of DNA damage, the methanolic extract consistently provides maximum protection across different DNA types.

Conclusion: Our findings highlight the potential of Zea mays leaves, especially the methanolic extract, in protecting cellular structures and DNA from oxidative damage. This research contributes to the understanding of herbal extracts' protective effects, with implications for cancer prevention. Further exploration is warranted to translate these findings into clinical applications.

Keywords: Lipid peroxidation, Oxidative DNA damage, RBC Ghosts, Haploid Herring sperm DNA, Diploid Calf thymus DNA.

INTRODUCTION

Reactive Oxygen Species are highly reactive, which can harm DNA, proteins, and lipids, especially high ROS levels trigger apoptosis[1]. Oxidative and antioxidative processes impact the cell's redox state, influencing signal proteins and pathways. Oxidative stress regulates various processes, including antioxidant enzyme synthesis, repair mechanisms, inflammation, apoptosis, and cell proliferation, influencing malignancy[2].

The detrimental effects of ROS are counteracted by both enzymic and non-enzymic antioxidants, which donate electrons to free radicals, preventing damaging chain reactions. Despite the presence of the cell's antioxidant system, oxidative damage accumulates throughout the life cycle, contributing to age-dependent diseases such as atherosclerosis, arthritis, neurodegenerative disorders, and cancer[3,4].

Oxidative stress, characterized by an imbalance in the production and elimination of reactive oxygen or nitrogen species, has been implicated in cancer initiation and progression. This condition has various protumorigenic effects, including an elevated DNA mutation rate, DNA damage, genome instability, and increased cell proliferation[5]. The chemopreventive effects associated with natural dietary compounds encompass antioxidative and anti-inflammatory activities, along with the induction of enzymes, apoptosis, and cell cycle arrest[6].

Medicinal plants, known for their therapeutic benefits and minimal side effects, represent a valuable source of diverse bioactive compounds. Exploring and scientifically validating these compounds within India's abundant biodiversity holds great promise for addressing various diseases. By emphasizing safety, efficacy, and quality, the development of traditional medicinal systems not only preserves cultural heritage but also promotes the rational use of natural products in healthcare. In light of this, our study focused on evaluating the antioxidant potential of *Zea mays* leaves, commonly known as maize, and aimed to conduct in vitro investigations to establish the antioxidant activity against key biomolecules and determine the effect on oxidative DNA damage.

METHODOLOGY

Effect of *Zea mays* leaf extracts on oxidative damage to biomolecules

Excessive ROS production harms the body by causing cell membrane disintegration, membrane protein damage, and DNA mutation[7]. Lipid peroxidation, in addition to

destroying membranes, can generate reactive products that further damage DNA, which is the ultimate target of ROS[8]. The solvents varying in their polarity used for the leaf extracts are water, methanol, and chloroform.

1. Evaluation of the effects of *Zea mays* leaf extracts on membrane lipids

The study employed three membrane model systems to assess lipid peroxidation (LPO) and the protective effects of leaf extracts. These models included the plasma membrane, internal membrane, and intact (live) cells. Plasma membranes were sourced from goat blood, fresh liver slices represented intact live cells, and internal membranes were obtained from goat liver homogenate prepared with Tris HCl buffer (40mM, pH 7.0). This variety aimed to investigate the impact of leaf extracts on LPO, considering differences in lipid composition and membrane nature.

1.1. Evaluation of LPO in RBC Ghosts

Preparation of goat RBC ghosts: Fresh goat blood (50ml) was collected and immediately defibrinated using sterile acid-washed stones. The defibrinated blood was then diluted 1:1 with sterile isotonic KCl. Red blood cells (RBCs) were pelleted through centrifugation at 3000xg for 10 minutes at 4°C. After washing the pellet thrice with isotonic KCl, it was treated with hypotonic KCl (0.5%) and allowed to lyse at 37°C for one hour. The resulting lysate was centrifuged at 5000Xg for 10 minutes at 4°C, and the pellet obtained was washed until a pale pink pellet was achieved. The final pellet was suspended in 1.5ml of TBS, and 50µl aliquots were used for the assay following Dodge et al.'s (1963)[9] protocol

1.2. Estimation of LPO in Goat Liver Homogenate

Preparation of goat liver homogenate: Fresh goat liver was obtained from a slaughterhouse and washed with Tris HCl buffer (40mM, pH 7.0). A 20% liver homogenate was prepared using a motorized Teflon homogenizer in the same buffer. The homogenate was clarified through low-speed centrifugation to remove debris, serving as the membrane source for the LPO assay based on the method of Okhawa et al. (1979)[10].

1.3. Estimation of LPO in Goat Liver Slices

The extent of inhibition of LPO in goat liver slices was estimated by the method proposed by Nichans and Samuelson (1968)[11].

2. Effect of the extracts of *Zea mays* leaves on oxidant-induced DNA damage

The study investigated DNA damage in vitro using commercially available DNA preparations and intact cells from different evolutionary hierarchies. Commercial DNA samples included viral DNA (λ DNA), herring sperm DNA, and calf thymus DNA. Intact cell DNA was sourced from human peripheral blood cells.

2.1. Estimation of Damage in λ DNA: The method proposed by Chang et al. (2002)[12] was used to determine DNA strand breaks.

2.2. Estimation of Damage in Herring Sperm and Calf Thymus DNA: The degree of DNA damage induced by hydrogen peroxide and the impact of *Zea mays* leaves were investigated using the methodology outlined by Aeschlach et al. (1994)[13].

2.3. Evaluation of the extent Of DNA damage in Intact Cells: The extent of DNA damage in intact cells was assessed using the comet assay, conducted under alkaline conditions as described by Singh et al. (1988)[14]. Peripheral blood cells were utilized, and before the assay, these cells were resuspended in Hank's Balanced Salt Solution (HBSS).

RESULTS

Lipid peroxidation (LPO) extent was examined in three systems: goat liver homogenate with intact organelles, RBC ghosts representing plasma membrane lipids, and goat liver slices inducing in vitro LPO. Leaf extracts demonstrated significant inhibition in all systems, protecting both plasma and intracellular membranes from LPO. Aqueous and methanolic extracts notably inhibited LPO, while chloroform extract exhibited lower efficacy (Figure 1). This suggests the protective impact of *Zea mays* leaf extracts on various membrane components.

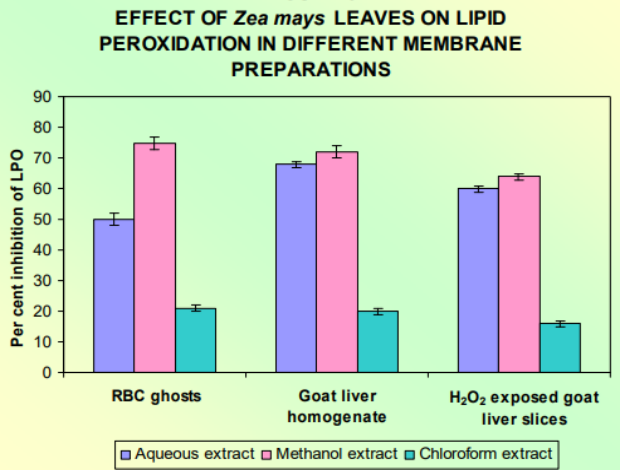

Figure 1: Graphical representation of Effect of *Zea mays* Leaf extracts on LPO in different membranes: RBC ghosts, Goat Liver, and H_2O_2 treated Goat Liver Slices

Zea mays leaf extracts were evaluated for their protective effects against oxidative damage on DNA at various hierarchical levels, including λ phage DNA, haploid herring sperm DNA, diploid calf thymus DNA, and intact cell DNA. H_2O_2-induced damage to λ DNA was evident in Figure 2, with extensive damage in lane 2. However, leaf extracts alone (lanes 3, 5, 7) did not cause DNA damage and significantly protected λ DNA (lanes 4, 6, 8).

In the study, H_2O_2 caused maximal damage to herring sperm DNA (Figure 3). All three extracts effectively reduced this damage, with the methanolic extract

demonstrating a stronger effect than the aqueous and chloroform extracts.

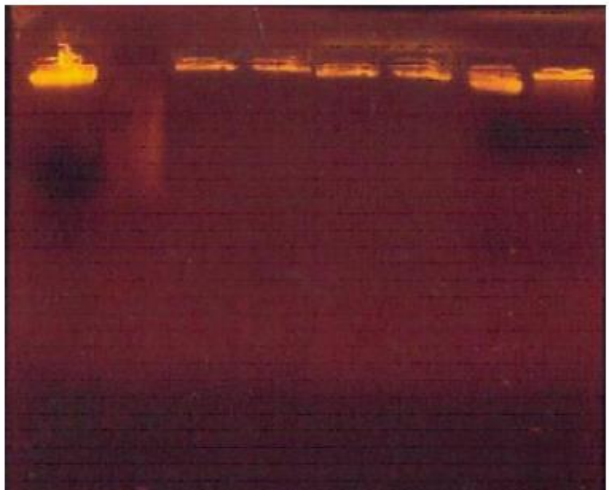

Figure 2: Migration pattern of λ DNA treated with H_2O_2 in the presence and absence *Zea mays* leaf extracts

Lane 1: Control, Lane 2: H_2O_2, Lane 3: Aqueous extract, Lane 4: H_2O_2 + Aqueous extract, Lane 5: Methanol extract, Lane 6: H_2O_2 + Methanol extract, Lane 7: Chloroform extract, Lane 8: H_2O_2 + Chloroform extract

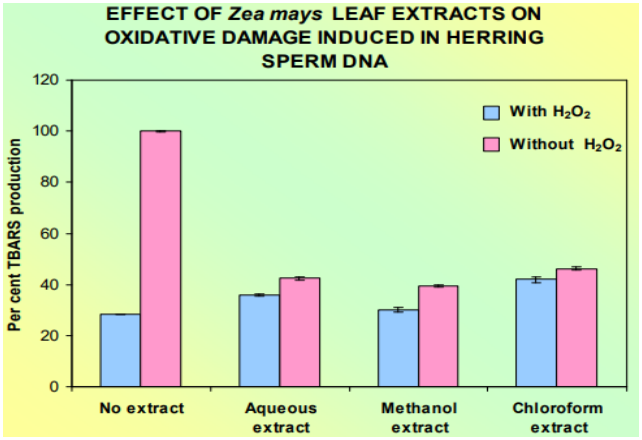

Figure 3: Graphical representation of Effect of *Zea mays* Leaf extracts on Herring Sperm DNA induced with Oxidative damage

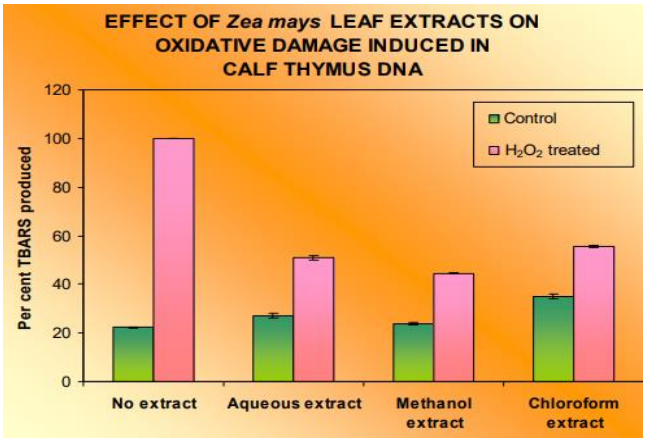

Figure 4: Graphical representation of Effect of *Zea mays* Leaf extracts on calf Thymus DNA induced with Oxidative damage

Calf thymus DNA damage, quantified spectrophotometrically (Figure 4), showed the methanolic extract offering the highest protection, followed by the aqueous extract. The chloroform extract exhibited a differential effect on the two DNA sources, and TBARS formation was higher in herring sperm DNA than in calf thymus DNA.

The investigation of H_2O_2-induced DNA damage in peripheral blood cells revealed a significant increase in comet cells, indicating severe damage in the positive control. *Zea mays* leaf extracts significantly reduced comet incidence, demonstrating protection. Simultaneous treatment with leaf extracts and H_2O_2 decreased the number of cells expressing DNA damage, with values remaining below the untreated group in the extract-exposed groups. These findings highlight the leaf extracts' ability to protect peripheral blood cell DNA from oxidative damage, as depicted in Figure 5 and Table 1.

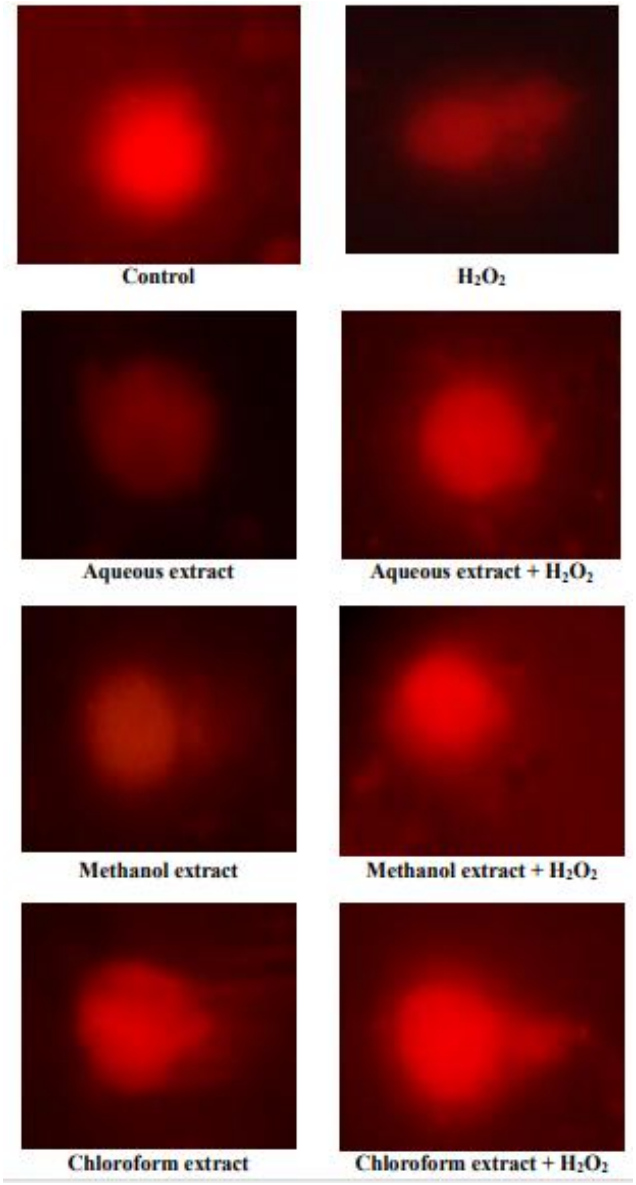

Figure 5: Representation the ability of *Zea mays* Leaf extracts to protect peripheral blood cell DNA from oxidative damage

Table 1: Effect of *Zea mays* Leaf extracts on DNA damage induced by H_2O_2 in peripheral blood cells

Sample	Number of cells with comets / 100 cells	
	Control	H_2O_2 treated
No extract	11±1	23±1 [a]
Aqueous extract	4±2 [a]	7±2 [a,b]
Methanol extract	3±2 [a]	4±1 [a,b]
Chloroform extract	6±1 [a]	8±2 [b]

The values are Means±SD of triplicates CD value = 3.77

a - Statistically significant (P<0.01) compared to untreated control

b - Statistically significant (P<0.01) compared to H_2O_2 alone treated group

c - Statistically significant (P<0.01) compared to the respective plant extract-treated group

DISCUSSION

Free radicals can oxidize intracellular molecules, leading to cell structure changes such as alterations in lipids, DNA, and proteins. Lipid peroxidation (LPO) disrupts biomembranes, transforms LDL into proatherogenic forms, and generates potentially toxic products. LPO products are mutagenic and carcinogenic. Investigating the influence of standard oxidants and *Zea mays* leaves on lipid oxidative damage is essential. ROS in aerobic organisms primarily target cellular biomembranes, inducing lipid peroxidation and adversely affecting membrane structure and function. This process generates malondialdehyde (MDA), leading to cytotoxic and genotoxic effects[15].

In our study, we examined the influence of oxidants on membrane lipids sourced from RBC ghosts, liver homogenate, and liver slices. The leaf extracts of *Zea mays* exhibited substantial protection against oxidative damage in all tested membrane model systems. The methanolic and aqueous extracts showed superior inhibition of lipid peroxidation compared to the chloroform extract. The protection was most pronounced in RBC ghosts, followed by goat liver homogenate and goat liver slices. This suggests that certain components in the leaf extracts might not readily permeate membranes, as evidenced by the lower extent of LPO in the liver slices.

Numerous studies have demonstrated the inhibitory effects of various plant extracts on lipid peroxidation. Hsu (2008)[16] reported the strong antioxidant activity of the ethanol extract of *Pyrrosia petiolosa*, while phenyl propanoid glycosides were found to inhibit lipid peroxidation and LDL oxidation[17]. Gugliucci and Menni (2002)[18] observed that extracts of *Achyrocline satureoides* inhibited human LDL oxidation across different systems. Additionally, extracts from *Adhatoda vasica, Amaranthus paniculatus, Brassica compestris, Mentha piperita,* and *Spirulina fusiformis* demonstrated inhibitory effects on lipid peroxidation in the liver [19].

Perilla leaves reversed t-BHP-induced lipid peroxidation in rat livers[20], and a methanol extract of *Curculigo orchioides Gaertn* rhizomes exhibited potent inhibition in rat liver homogenate[21]. Fenugreek extract inhibited LPO in the urinary bladder of mice[22], and the alcoholic bark extract of *Butea monosperma* reduced lipid peroxidation[23]. Seaweeds effectively suppressed TBARS formation in H_2O_2-induced lipid peroxidation in RBC[24], and the essential oil from *Chaerophyllum libanoticum Boiss. et Kotschy* fruits inhibited lipid peroxidation[25].

Our study aligns with these findings, indicating that the methanolic extract of *Zea mays* leaves provided more effective protection against lipid damage in both plasma membrane and internal organelles compared to the aqueous and chloroform extracts.

DNA mutation plays a pivotal role in carcinogenesis, with elevated levels of oxidative DNA lesions observed in various tumors, suggesting a strong link to cancer etiology. The implicated DNA damage is primarily associated with the initiation process[26,27]. To explore the DNA protective effects of *Zea mays* leaf extracts, assays were conducted in different in vitro systems. They are testing both intact cell DNA and commercially available DNA aimed to understand if the extracts trigger endogenous factors for protection.

In our study, we examined *Zea mays* leaf extracts for their protective effects against oxidative damage in both purified DNA preparations and DNA within intact cells. Using commercially available, purified DNA samples from various sources (λDNA, haploid herring sperm DNA, diploid calf thymus DNA, and intact cell DNA), we found that exposure to H_2O_2 resulted in extensive DNA damage. However, *Zea mays* leaf extracts significantly reduced this damage in various DNA types, with the methanolic extract providing maximum protection across all tested DNA varieties. Importantly, the extracts themselves did not induce DNA damage.

Numerous studies highlight the protective effects of various herbal extracts against oxidative DNA damage. For instance, irradiating DNA (λ phage, bovine spleen DNA, pUC 19 plasmid) with UV light in the presence of methyl resorcinol or hexyl resorcinol resulted in insignificant DNA destruction[28]. Low molecular weight chitosan and chitooligosaccharides obtained through persulfate-induced depolymerization of chitosan offered protection against calf thymus DNA damage[29]. Hydroxylated 4-thiaflavan effectively protected herring sperm DNA against oxidation damage[30].

The rhizome extract of *Dioscorea alata* exhibited radical scavenging activity and protected calf thymus DNA and plasmid DNA[31]. The alcohol: water extract of curry leaves showed high antioxidant activity[32]. Green tea consumption was associated with decreased DNA damage, regardless of hOGG1 genotype, among GSTM1-positive smokers[33]. Methanolic extracts of *Celastrus paniculatus*, *Picrorhiza kurroa*, and *Withania somnifera* displayed significant

protective capability against H_2O_2-induced DNA damage in human fibroblasts[34].

*Jurka*t T-lymphocytes pre-incubated with EGCG or quercetin were less susceptible to DNA damage induced by reactive oxygen or nitrogen species[35]. Aqueous, methanol, and ethyl acetate extracts of *Acacia salicina* leave protected human lymphoblast cells K562 against H_2O_2-induced DNA damage[36]. The current study's findings indicate that *Zea mays* leaves can safeguard DNA from oxidative damage, both in purified DNA and within intact cells.

CONCLUSION

Our study underscores the harmful effects of free radicals on cell structures, emphasizing the role of lipid peroxidation and DNA damage in carcinogenesis. Our study specifically focused on the influence of oxidants on membrane lipids derived from various sources, including RBC ghosts, liver homogenate, and liver slices. *Zea mays* leaf extracts demonstrated robust protection against oxidative damage in all tested membrane model systems. The methanolic and aqueous extracts exhibited superior inhibition of LPO compared to the chloroform extract, with the most pronounced protection observed in RBC ghosts. This suggests potential membrane impermeability of certain components in the leaf extracts, as evidenced by lower LPO in liver slices. Our findings align with numerous studies, indicating that the methanolic extract of *Zea mays* leaves provides more effective protection against lipid damage in both plasma membrane and internal organelles compared to the aqueous and chloroform extracts.

Our research focused on evaluating the protective effects of *Zea mays* leaf extracts against oxidative DNA damage. Through the examination of purified DNA and intact cell DNA, the extracts, particularly the methanolic extract, significantly reduced H_2O_2-induced DNA damage. These findings contribute to existing research on herbal extracts' protective effects against oxidative DNA damage, highlighting the potential significance of *Zea mays* leaves in cancer prevention. Further investigation is needed to fully understand and apply these protective effects in clinical contexts.

ACKNOWLEDGEMENT

We sincerely thank the late Dr. P R. Padma for her significant contributions to our project. Her dedication and expertise were truly invaluable. We remember her with deep respect and appreciation, and her legacy will always be cherished.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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