Protective effect of phytochemical constituents on oxidative & non oxidative DNA damage induced by H_2O_2& aspirin

Abstract:

Aspirin is a non steroidalanti inflammatory drug used widely due to its low cost and efficacy. Aspirin alone does not cause DNA damage but at certain situations like aspirin in water can induce DNA damage. H2O2 is generally used to induce oxidative stress and in this study we used 1% Aspirin dissolved in water and three different concentrations of H2O2 like 5%,15% and 25% to induce DNA damage. We studied protective effect of 10 phytochemical constituents like *Lablab purpureus,Mangiferaindica,Vignaunguiculata,Musa paradisiaca, Oryza sativa, Vigna radiata, Vigna mungo, Azadirachta indica, Ocimum sativum and Eucalyptus*on DNA damage induced by aspirin and H2O2. In the 10 phytochemicals used *Oryza sativa*offered full protection commonly to all three concentrations of5%,15% and 25% H2O2 used. Where as*Lablab purpureus Ocimum sativum and Eucalyptus*protected commonly with 5% and 25% H2O2.

Key words: Aspirin, H2O2, Oxidative stress, NSAIDs, *Lablab purpureus,Mangiferaindica,Vignaunguiculata,Musa paradisiaca, Oryza sativa, Vigna radiata, Vigna mungo, Azadirachta indica, Ocimum sativum and Eucalyptus*

Introduction:

Aspirin is a non steroidalanti inflammatory drug used widely due to its low cost and efficacy. From the studies ofGómez-Oliván LM et al.,(2014)aspirin dissolved in water solution cause oxidative stress and DNA damage in *Daphnia magna.* Inflammation is one of the major cause for bone marrow ablation induced after irradiation and from the studies of Dorota Kacprzak , Rafał Pawliczak (2015). aspirin protects bone marrow ablation by reducing the antiinflammatory genes gene expression.

Aspirin induced asthma is a distinct disorder caused by ingestion of aspirin or other anti-inflammatory non steroidal drugs(NSAIDs). However more recent studies are carried on the study of oxidative stress induced by aspirin in the cancer cells.However the patho mechanism is not well understood (Dorota Kacprzak , Rafał Pawliczak (2015)) . From the studies of Gąsecka et al., (2025)it is proven to be oxidative stress induced due to coronary artery bypass grafting is independent of aspirin intake.

Generally H2O2 is the preferred agent to induce oxidative stress and according to the reports of Ransy C et al., (2020)high concentrations of H2O2 is required to induce oxidative stress and can be monitored using polarographic measurement and mechanism of oxidative stress by H2O2 involves production of O2 and exposure of O2 to uncoupled NOS and NADPH oxidase. According to findings of Ransy C et al., (2020) complex interaction between H2O2 and oxygen Radical generating enzymes is responsible for the oxidative stress induced by H2O2. In this current study we studied the effect of DNA damage induced by Aspirin and H2O2 and protection offered by various phytochemical constituents against DNA damage induced by aspirin and H2O2.

Methodology:

1. Isolation of DNA from chicken liver:

DNA is isolated from chicken liver using the procedure of Vijay Hemmadi (2016).

2. Separation of DNA by Agarose Gel Electrophoresis:

Seperation of DNA by agarose gel electrophoresis was done according to vlab.amritha.edu,. (2012). Agarose Gel Electrophoresis (AGE).

3. Induction of DNA damage by aspirin and 5%,15% and 25% H2O2:

1ml of isolated DNA is transferred to standard flask and made up to 100ml with distilled water and used as standard for studying the DNA damage. 1% aspirin solution is prepared by weighing and transferring of 1g of aspirin in to 100ml distilled water. Similarly 30% H2O2 is used for preparing stock solutions of 5%, 15% and 25% H2O2 and these stock solutions are used for inducing oxidative damage in DNA.

To 1ml of DNA solution, 1ml of aspirin and 5%,15% and 25% H2O2 is added to different test tubes and incubated for time periods 15', 30',45' and 60' time period and the reading is recorded at 260nm using UV Visible spectrophotometer.

4. Study of protective effect of phytochemical on DNA damage induced by Aspirin and 5%,15% and 25% H2O2:

1g of phytochemical is dissolved in 10ml of distilled water and allowed to stand for 1 hr. After the time period 1ml of the filtrate of phytochemical is added to tubes with aspirin + DNA and similarly to DNA+5%,15% and 25% H2O2 separately. The reaction is stopped by addition of 0.5ml of 0.5M NaOH. Reading is recorded after 15', 30',45' and 60' time period using UV Visible spectrophotometer.

The results are tabulated and represented graphically to study the protective effect of phytochemicals on DNA damage.

10 phytochemical constituents like *Lablab purpureus,Mangiferaindica,Vignaunguiculata,Musa paradisiaca, Oryza sativa, Vigna radiata, Vigna mungo, Azadirachta indica, Ocimum sativum and Eucalyptus*are used for the study.

Calculation of molarity of H2O2:

5% H2O2: 5ml of 30% H2O2 is added to 100ml of water and the molarity of 5% H2O2 is calculated to be as 0.44M.

15% H2O2: 15ml of 30% H2O2 is added to 100ml of water and the molarity of 5% H2O2 is calculated to be as 1.32M.

25% H2O2: 25ml of 30% H2O2 is added to 100ml of water and the molarity of 5% H2O2 is calculated to be as 2.2M.

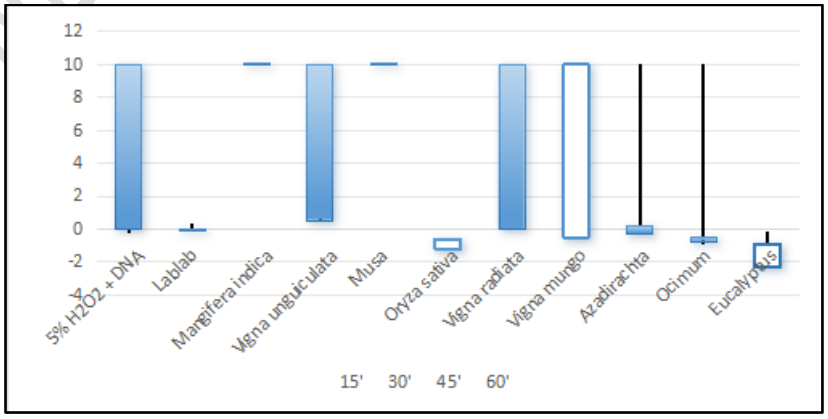
Results:

DNA is isolated form chicken liver and separated on 1% agarose gel. Oxidative and non oxidative damage of DNA is studied by inducing DNA damage with aspirin and H2O2. Protective effect of phytochemicals against DNA damage is studied using Phyto constituents like *Lablab purpureus,Mangiferaindica,Vignaunguiculata,Musa paradisiaca, Oryza sativa, Vigna radiata, Vigna mungo, Azadirachta indica, Ocimum sativum and Eucalyptus.*

Oxidative damage is studied using concentrations of 5%,15% and 25% H2O2 to induce oxidative stress and from table 1 after 15' of exposure phytochemicals except *Mangifera indica, Vigna unguiculata, Musa paradisiaca* and *Vigna radiataoffered* protection against DNA damagebut after 60min exposure *Vigna mungo* failed to offer protection against DNA damage induced by 5% H2O2..

	5% H2O2 + DNA	Lab lab	Mangifera indica	Vigna unguiculata	Musa	Oryza sativa	Vigna radiata	Vigna mungo	Azadirachta	Ocimum	Eucalyptus
15'	10	-0.0421	10	10	10	-1.2627	10	-0.5079	0.1723	-0.5279	-2.2976
30'	-0.0489	0.344	10	0.8475	10	-0.6631	0.5711	10	0.5457	10	-0.8702
45'	-0.2653	-0.0741	10	10	10	-0.6988	1.1282	10	10	-0.9265	-0.1683
60'	-0.0233	-0.1257	10	0.5217	10	-0.5957	-0.0093	10	-0.3063	-0.7631	-0.9162

Table: 1 Effect of 5% H2O2 on DNA and protection against DNA damage offered by phytochemical constituents *Lablab purpureus, Oryza sativa, Vigna radiata, Azadirachta indica, Ocimum sativum and Eucalyptus.*



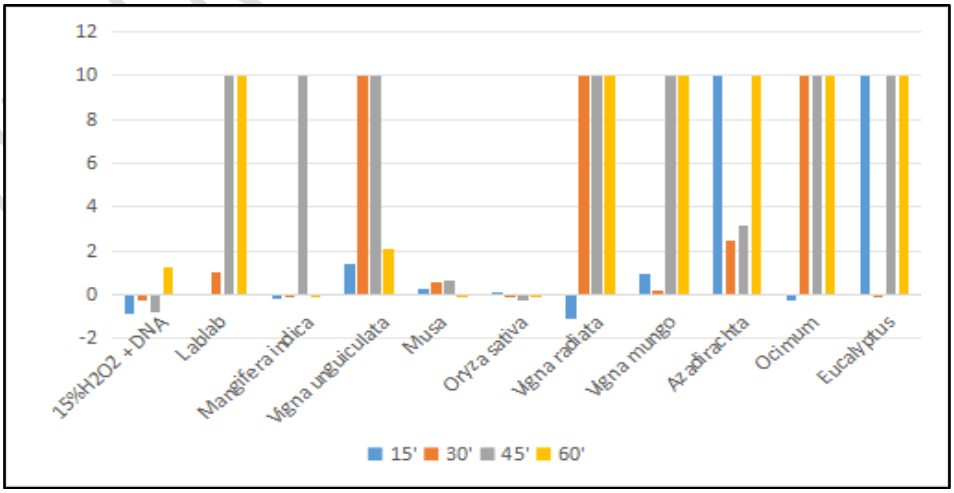
Graph: 1 Protection offered by Phytochemical constituents against DNA damage induced by 5% H2O2.

	15% H2O2 + DNA	*Lablab*	*Mangifera indica*	*Vigna unguiculata*	*Musa*	*Oryza sativa*	*Vigna radiata*	*Vigna mungo*	*Azadirachta*	*Ocimum*	*Eucalyptus*
15'	-0.8951	0.0038	-0.1653	1.4456	0.2858	0.1226	-1.0714	0.9848	10	-0.228	10
30'	-0.2962	1.0117	-0.0619	10	0.5462	-0.1123	10	0.1736	2.4774	10	-0.076
45'	-0.8195	10	10	10	0.6695	-0.2323	10	10	3.191	10	10
60'	1.2215	10	-0.0559	2.1119	-0.0051	-0.0644	10	10	10	10	10

Table:2 Effect of 15% H2O2 on DNA and protective effect of *Vigna radiata, Mangifera indica, Musa paradisiaca and Oryza sativa*on DNA damage.

15' min exposure of DNA to 15% H2O2 resulted in DNA damage and in all the 10 phytochemicals used only *Vigna radiata*offered protection against DNA damage for 15' exposure and exposure to 60' resulted in increase in DNA damage compared to 15' exposure and phytoconstituents like*Mangifera indica, Musa paradisiaca and Oryza sativa*offered protection against DNA damage induced by 15% H2O2.

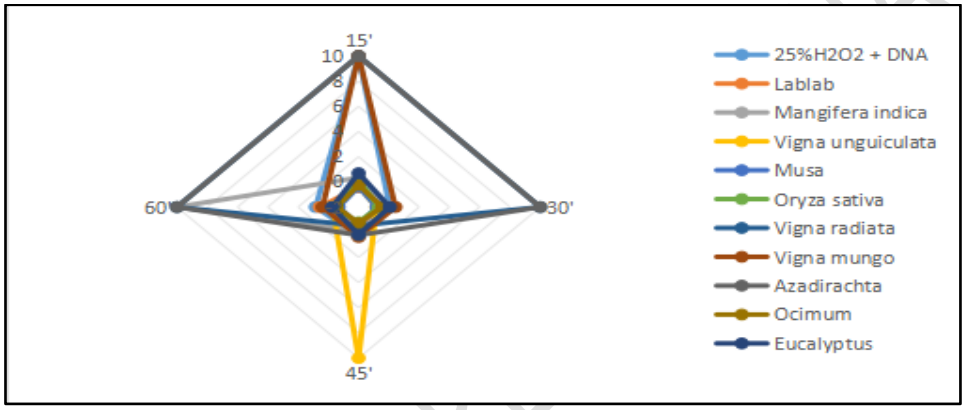
25% H2O2 induced greater DNA damage compared to 15% H2O2 and 15' min exposure resulted in highest percentage of DNA damage and almost all phytochemicals except *Vigna radiata,Vigna mungo and Azadirachta indica* offered protection against DNA damage induced by 25% H2O2. Decrease in absorbance after 60min exposure is may be due to reannealing of the repetitive DNA and phytochemicals like *Mangifera indica* elevated the DNA damage induced by 25% H2O2. *Vigna mungo*offered protection against DNA damage after 60' exposure to25% H2O2 may be due to DNA aggregation.



Graph: 2 Protective effect of phytochemicals *Vigna radiata, Mangifera indica, Musa paradisiaca and Oryza sativa* on DNA damage induced by 15% H2O2

	25% H2O2 + DNA	*Lablab*	*Mangifera indica*	*Vigna unguiculata*	*Musa*	*Oryza sativa*	*Vigna radiata*	*Vigna mungo*	*Azadirachta*	*Ocimum*	*Eucalyptus*
15'	10	-0.4305	0.3358	-0.3348	-0.3821	0.1203	10	10	10	-0.2426	0.6894
30'	0.0548	-0.0431	-0.0793	-0.8178	-0.8031	-0.6551	10	0.4329	10	-0.4553	0.0614
45'	0.2974	-0.4833	-0.0888	10	-0.569	-0.7894	-0.5178	0.383	0.2402	-0.717	0.1942
60'	0.8391	0.5698	10	-0.2756	-0.683	-0.2377	10	0.3825	10	-0.543	-0.2568

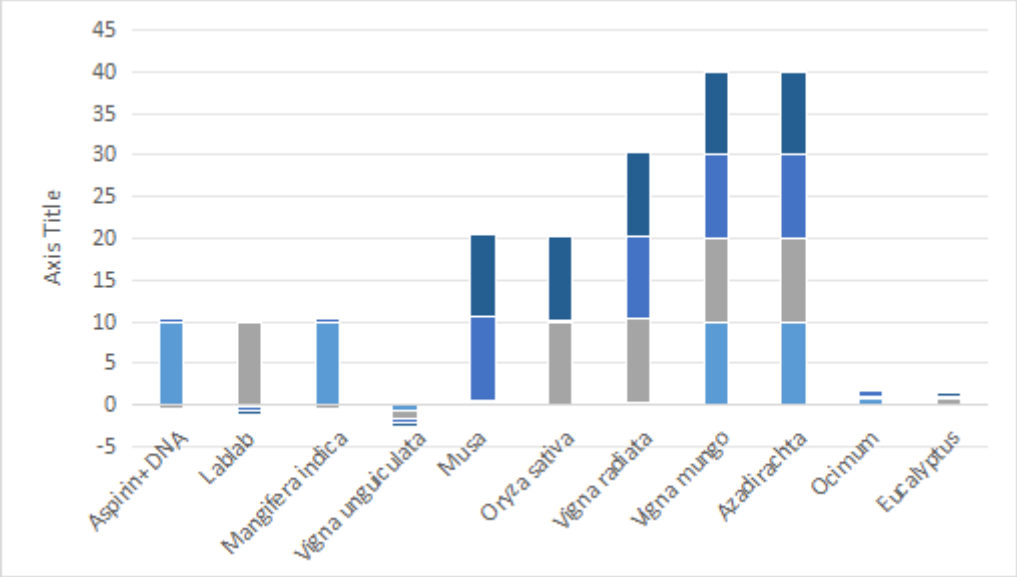
Table: 3 Protective effect of phytochemicals *Lablab purpureus,Vignaunguiculata,Musa paradisiaca, Oryza sativa, Ocimum sativum and Eucalyptus*on DNA damage induced by 25% H2O2.



Graph: 3 Protection offered against DNA damage by Phyto constituents *Lablab purpureus,Vignaunguiculata,Musa paradisiaca, Oryza sativa, Ocimum sativum and Eucalyptus.*

	Aspirin+ DNA	*Lablab*	*Mangifera indica*	*Vigna unguiculata*	*Musa*	*Oryza sativa*	*Vigna radiata*	*Vigna mungo*	*Azadirachta*	*Ocimum*	*Eucalyptus*
15'	10	-0.1943	10	-0.7688	0.2166	-0.2484	0.3629	10	10	0.7542	0.0077
30’	-0.3242	10	-0.3242	-0.825	0.3387	10	10	10	10	0.2498	0.6634
45'	0.4099	-0.438	0.4099	-0.6337	10	0.1898	10	10	10	0.6182	0.3514
60'	-0.373	-0.4482	-0.373	-0.4039	10	10	10	10	10	0.1374	0.2265

Table: 4 Protection against oxidative and non oxidative DNA damage induced by Aspirin by Phytochemical constituents like *Lablab purpureus,Vigna unguiculata*after 60’ exposure.

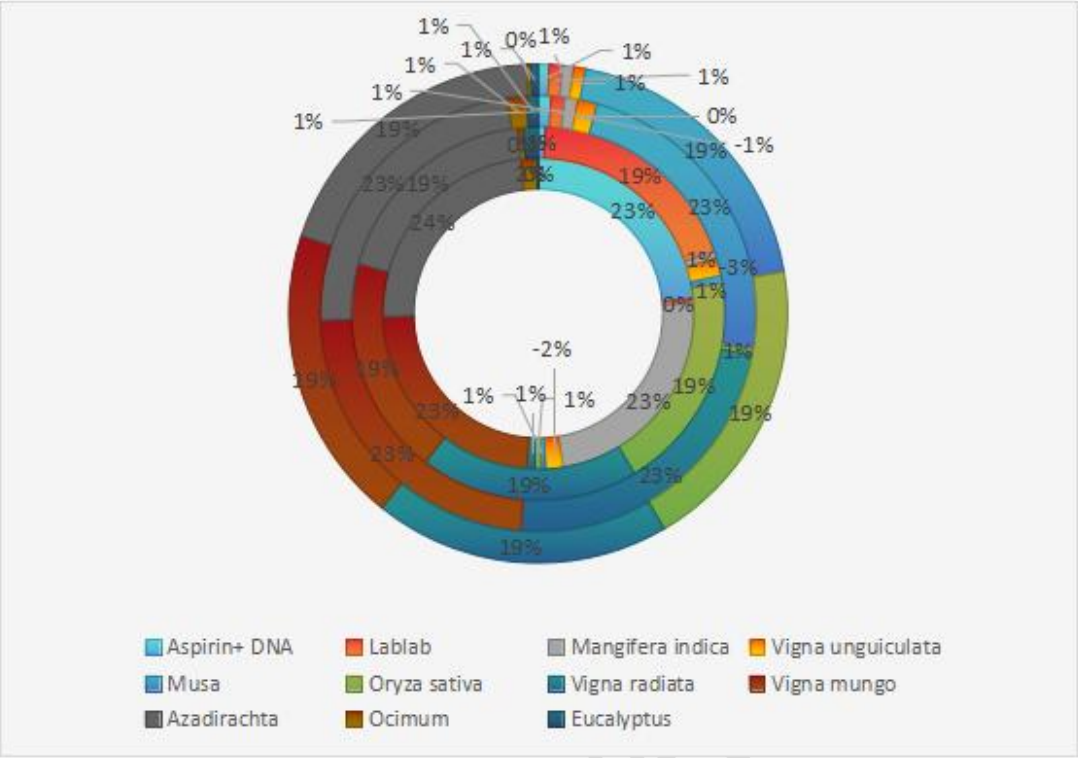


Graph: 4: Bar graph on protective effect of phyto constituents *Lablab purpureus,Vigna unguiculata*on DNA damage induced by Aspirin

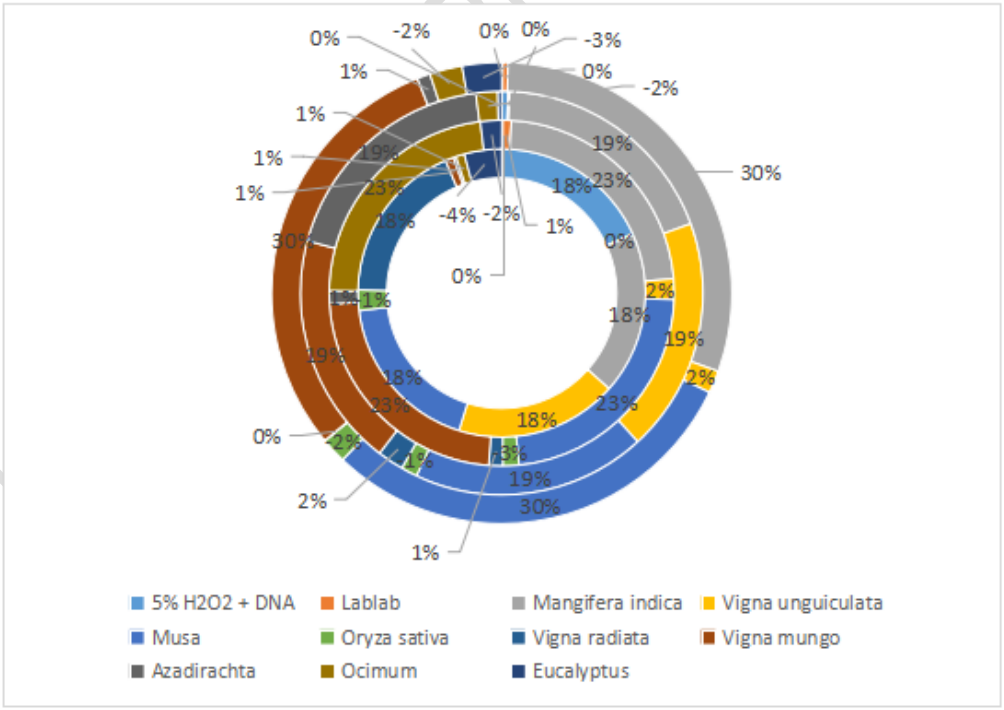
*Lablab purpureus, Vigna unguiculata, Musa paradisiaca, Oryza sativa,Vignaradiata,Ocimum sativum and Eucalyptus*offered protection against DNA damage after 15' and 60' min exposure of DNA to the drug aspirin, but *Musa paradisiaca, Oryza sativa,Vignaradiata,Ocimum sativum and Eucalyptus*failed to protect DNA against damage by aspirin after 60 'min exposure.

From the graph 5 the DNA damage caused by aspirin is found to be about 23% and most of the phytochemicalslike *Lablab purpureus, Vigna unguiculata*showed protective effect with percentage of 81% and 99%.

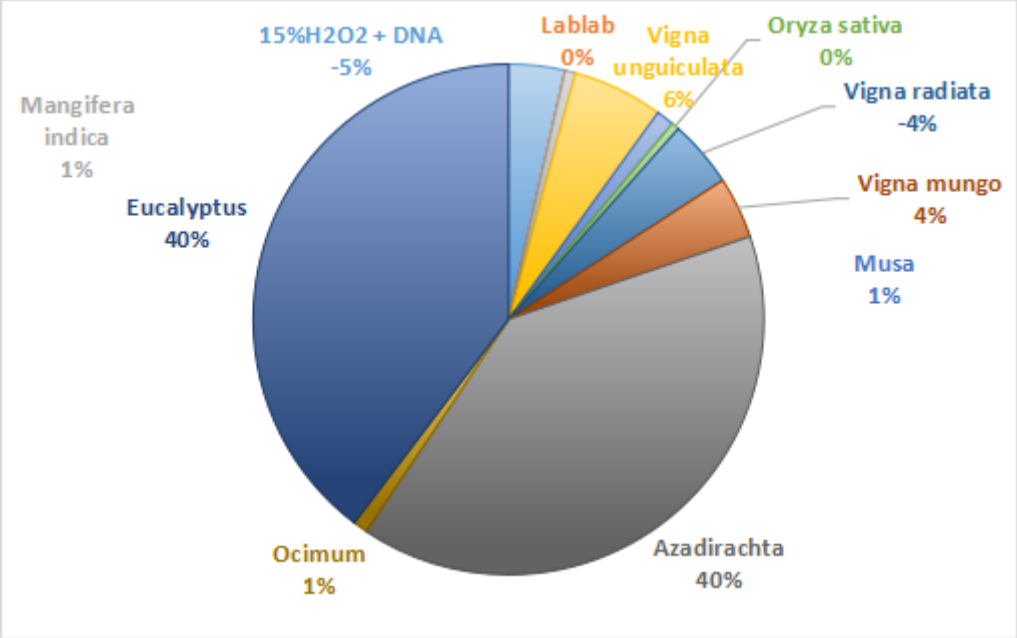
From the graph 6 DNA damage induced by 5% H2O2, 15% H2O2 and 25% H2O2 is found to be 18%, -5% and 23%.



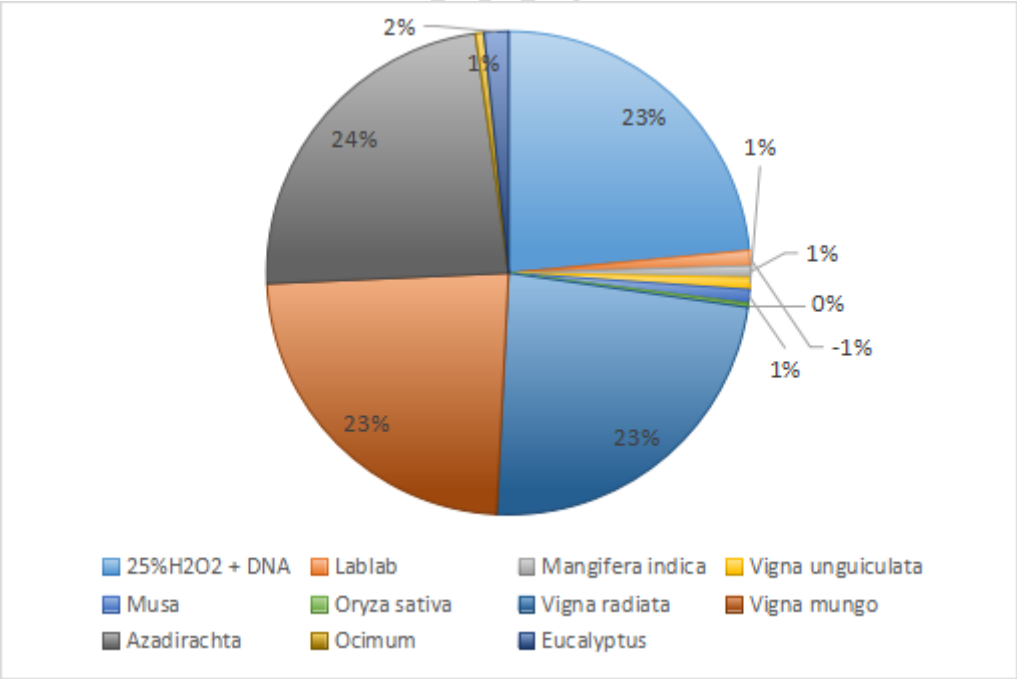
Graph: 5: % of DNA protection offered by various phytochemicals after treatment with Aspirin.



Graph: 6 Percentage of protection against DNA Damage by Phytochemicals constituents after treatment with 5% H2O2



Graph:7 Percentage of protectiveeffect offered against DNA damage induced by 15% H2O2 by various phytochemicals like *Lablab purpureus,Mangiferaindica,Vignaunguiculata,Musa paradisiaca, Oryza sativa, Vigna radiata, Vigna mungo, Azadirachta indica, Ocimum sativum and Eucalyptus.*



Graph: 8: Protection percentage of DNA offered by phytochemicals like*Lablab purpureus,Mangiferaindica,Vignaunguiculata,Musa paradisiaca, Oryza sativa, Vigna radiata, Vigna mungo, Azadirachta indica, Ocimum sativum and Eucalyptus*against DNA damage induced by 25% H2O2.

Discussion and Conclusion:

H2O2 is one of the key inducer of oxidative stress mediated intracellularlythrough redox signaling leading to loss of cellular integrity. From the findings of Saputra et al., (2024) H2O2 induced oxidative stress in zebra fish causes impairment of embryonic development and hatching and can be negotiated by the addition of antioxidants like GSH. H2O2 exposure can reduce the cell viability in meningothelial cells and from the studies of Xin et al.,(2022) H2O2 exposure affects meningothelial cells by increasing oxidative stress, decreasing antioxidant enzymes, increasing level of free radicals and decrease in mitochondrial membrane potential compared to unexposed cells and responsible for toxicity in the cells.

Aspirin can induce oxidative stress and DNA damage at certain conditions when the exposure is for greater period. From the findings of Jiang et al.,(2023) inhibition of glutathione synthesis increases the cytotoxic effect of aspirin and however exposure to N-acetyl cysteine can augments the cytotoxicity caused (HaiderRaza,AnnieJohn (2012)). From the current study oxidative stress caused by H2O2 and the oxidative and non oxidative DNA damage induced by aspirin can be augmented by certain phytoactive components*Oryza sativa,Lablab purpureus Ocimum sativum and Eucalyptus*used in the study.

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