Evaluation of Production of Reactive Oxygen Species in Chronic Myeloid Leukaemia- A Combination Treatment of Imatinib and Naringin

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ABSTRACT

Chronic myeloid leukaemia (CML) is a type of blood cancer that is characterized by the abnormal growth of myeloid cells in the bone marrow. Imatinib is a tyrosine kinase inhibitor that has been used as a standard treatment for CML, but the development of drug resistance has limited its efficacy. The combination treatment of Imatinib with flavonoids such as Naringin has shown promising results in terms of overcoming drug resistance and enhancing the therapeutic efficacy of Imatinib. The generation of reactive oxygen species such as LDH, MDA, and catalase in K562 cells treated with Imatinib and Naringin in combination suggests that this therapy may have potential as a treatment for CML. In the present study, LDH assay, MDA estimation, and catalase activity are determined to know ROS production in the cell line culture. The results of the LDH assay showed that the combination treatment of Imatinib and Naringin significantly reduced the release of LDH compared to the control group, The results of the MDA assay showed that the combination treatment of Imatinib and Naringin significantly reduced the levels of MDA compared to the control group. This suggests that the combination therapy has a protective effect on the cell membranes and the results of the catalase assay showed that the combination treatment of Imatinib and Naringin significantly increased the levels of catalase compared to the control group. The combination treatment of Imatinib and Naringin has been shown to yield promising results in terms of the generation of ROS such as LDH, MDA, and catalase in K562 cell lines.

Keywords: Imatinib, Naringin, Reactive Oxygen Species, leukemia, cancer

INTRODUCTION

Unrestricted cell proliferation, faulty cell death, and spatial-temporal abnormalities in cell physiology are only a few of the complex pathways that can result in the creation of malignant tumours with the potential to spread into cancer **[1].**With a small male preponderance, chronic myeloid leukaemia (CML)affects one person per 100,000 people annually and makes up 15% of all new cases of leukaemia in the Western Hemisphere **[2].** CML is a myeloproliferative illness in which at any stage of the progression of CML, granular leucocytes significantly proliferate in blood and bone marrow **[3].**By 2022, the American Cancer Society (ACS) predicts that 8,860 new cases of CML will have been discovered, and 1,220 people will have passed away from the condition. 15% of newly diagnosed cases of leukaemia are CML. In the USA, CML can be found in 1 in 526 people at any point in life**[4].**CML often develops between the ages of 40 and 50, however, it can start at any age and progress in both sexes. Any pluripotent stem cell with one or more genetic mutations can cause CML, which then causes the clonal expansion of altered myeloid progenitors. This will influence all cells generated from this precursor**[5].**Most CML patients are discovered in the chronic phase, which is determined by cytogenetic and bone marrow biopsy. The slow progression of the chronic phase is fairly managed and can be maintained for two to seven years **[6].**There is an urgent need for coordinated efforts to find and describe more effective treatments given the terrible loss of human lives **[7].**

Reactive oxygen species (ROS) is a crucial signalling molecule that plays a significant role in the onset and progression of many diseases, including cancer. They are persistently created by metabolising organelles, primarily mitochondria, peroxisomes, and the endoplasmic reticulum, and are extremely reactive oxygen-containing molecules **[7]**. ROS has a peculiar range of actions that seem to have both pro- and anti-malignant effects. Little levels of ROS are created and preserved in dynamic equilibrium by antioxidant effectors under normal cellular circumstances. This equilibrium can be slightly tipped in the direction of oxidative stress, which activates several signalling pathways and causes DNA damage, mutagenesis, increased cell proliferation, and an aggravation of the Warburg metabolic effect that cancer cells use **[8].**Contrary to popular belief, ROS can also activate anti-tumorigenic signalling, leading to the demise of tumour cells due to oxidative stress. To detoxify excessive ROS levels, create a redox balance, sustain pro-tumorigenic signalling, and resist apoptosis, tumour cells express higher quantities of antioxidant proteins **[9].**Because tumour cells differ from their normal counterparts in terms of their redox balance, ROS modification is recognised as a possible target for cancer therapy. Antioxidant defences' primary role is to avoid the accumulation of too much ROS and to maintain a reduction-oxidation (redox) equilibrium **[10].**The antioxidant ability of tumour cells neutralises excess ROS while preserving amounts of pro-tumorigenic ROS, allowing

the illness to advance and forgo apoptosis. In cancer, ROS generation adds to genomic instability and DNA damage, which has significant ramifications such as medication resistance and a higher frequency of a relapse. Toxic amounts of ROS generation in malignancies, however, are anti-tumourigenic and cause oxidative stress to rise and tumour cell death to be induced **[11, 12].**

Imatinib which is a targeted medicine that selectively targets the protein causing the aberrant proliferation of white blood cells is one of the most efficient treatments for CML. It stops a BCR-ABL protein from acting in the chronic myeloid leukaemia oncogenic pathway (CML). The constitutive tyrosine kinase activity is directly inhibited by imatinib. By blocking the transfer of a phosphate group to tyrosine on the protein substrate and the consequent activation of phosphorylated protein, imatinib binds to the BCR-ABL kinase domain. As a result, leukemic cell death is brought on and proliferative signals are prevented from reaching the nucleus. Imatinib was approved by the FDA in December 2002 as the first-line treatment for CML patients who had just been diagnosed (with IRIS) **[13].**Imatinib has been widely used as the first-line treatment for CML, and it is highly effective, with a response rate of over 90%

Imatinib does have certain downsides, too. Drug resistance can arise as a result of mutations in the BCR-ABL gene, which is one of the main issues with imatinib therapy. This may result in the imatinib treatment failing and the disease getting worse. Researchers have investigated the use of combination therapy with other medications to combat drug resistance to solve this issue **[13].**

Recent research has revealed that flavonoids, a class of naturally occurring substances present in many fruits and vegetables, may be useful in boosting imatinib's effectiveness in CML. Although flavonoids' effects have been studied for a long time, their anti-inflammatory, anticarcinogenic, and antioxidant properties have only lately come to light **[14].**Plant-based phytochemicals and their derivatives may increase therapeutic efficacy and lessen side effects in cancer patients. The plant molecules are known to be safe, natural, and effective sources that work in concert with one another or through other conceivable methods. Naringin (NAR) is a 4',5,7-trihydroxy flavonone-7-rhamnoglucoside found in citrus fruits and is a chief flavanone glycoside (flavonoid).Naringin, a flavanone glycoside present in grapefruits and oranges, has been identified as one of several flavonoids that may improve Imatinib activity. In CML cells, Naringin has been demonstrated to promote imatinib's anti-cancer action. It can also increase the production of reactive oxygen species (ROS), including catalase, malondialdehyde (MDA), and lactate dehydrogenase (LDH). Combining IMT therapy and AKT targeting with flavonoid (NAR) compounds may be particularly beneficial in CML through additive processes. It modulates and interacts with several signalling pathways. Through altering several cellular signalling pathways, such as those that restrict angiogenesis, oxidative stress, and malignant cell proliferation, NAR has been demonstrated to decrease the number

of malignancies. Since ROS are known to be a major factor in the growth of cancer, their buildup can result in DNA damage and cell death.**[15].**

Combination therapy, particularly using synthetic medications and phytochemicals, would be a creative way to have the most pharmacological impact. Naringin has been demonstrated to have additional health advantages in addition to its capacity to increase the efficacy of Imatinib. It can enhance lipid metabolism and cardiovascular health and has anti-inflammatory and antioxidant qualities. Numerous illnesses, such as cancer, diabetes, and cardiovascular disease have been linked to Naringin as a potential treatment. Flavonoids, such as Naringin, have been recognised as possible Imatinib efficacy enhancers and may be a promising method to combat drug resistance. Positive health effects cannot be disputed, but further research is required to investigate their potential in the treatment of CML and other disorders.

Given all of the potential benefits of Naringin, we believed that the combination of Imatinib and Naringin would be a novel therapeutic approach for the treatment of CML with enhanced anticancer efficacy. An exhaustive literature search revealed that the combination of Imatinib and Naringin as a treatment method has yet to be tested. In this study, a lactate dehydrogenase (LDH) assay was used to quantify the amount of cell death in response to drug treatment. The LDH assay can be used to assess the cytotoxic effects of imatinib and naringin when used in combination in K562 cell lines. LDH assay measures total cell death, including both necrotic and apoptotic cell death. Therefore, additional assays, such as the Annexin V assay or TUNEL assay, may be required to determine the specific mode of cell death induced by the drug treatment.

MATERIALS AND METHODS

Imatinib and Naringin were purchased fromAcros organics, Belgium. Cell culture medium were purchased from Himedia India. K562 cell lines were purchased from NCCS, Pune, India. LDH assay reagent, trichloroacetic acid (TCA), thiobarbituric acid (TBA) from Sigma Aldrich, hydrogen peroxide from Himedia.

LDH Assay

LDH assay measures total cell death, including both necrotic and apoptotic cell death. Therefore, additional assays, such as the Annexin V assay or TUNEL assay, may be required to determine the specific mode of cell death induced by the drug treatment

In this method, K562 cells were cultured in the suitable growth medium and incubated at 37°C in a humidified incubator with 5% CO2 until they reach confluency. These K562 cells were treated with varying concentrations of Imatinib and Naringin, alone or in combination, for a predetermined length of time. As a reference, controlled group or untreated cells were used appropriately. After the

treatment period was over, the culture medium was collected and transferred into the 96-well microplate. In each well of the microplate, LDH assay reagent was added. This microplate was incubated for a suitable length of time (usually 30 minutes to 1 hour) at room temperature, protected from light.The absorbance of each well was measured using a microplate reader set to the appropriate wavelength (usually 490-540 nm).The percentage of LDH release by comparing the absorbance values of the treated cells to the control group was calculated.The formula for calculating the percentage of LDH release is mentioned below. The data was analysed by using the appropriate statistical methods like calculating IC50 values for performing a dose-response analysis.Plate mapping and Protocol summary for the LDH assay method are given in Table 1 and Table 2 respectively.

$$\% \text{ LDH Release} = \frac{(\text{Absorbance of treated cells} - \text{Absorbance of control cells})}{(\text{Absorbance of maximum LDH release} - \text{Absorbance of control cells})} \times 100$$

Table 1: Plate mapping

	1	2	3	4	5	6
A	Imatinib	Naringin	Imatinib+ Naringin	Control	DMSO	triton-x 100 treated
B	Imatinib	Naringin	Imatinib+ Naringin	Control	DMSO	triton-x 100 treated
C	Imatinib	Naringin	Imatinib+ Naringin	Control	DMSO	triton-x 100 treated

Table 2: Protocol summary of LDH determination

Test compound Concentration	1 µM (Imatinib), 30 uM(Naringin)
Triton x-100 (Control)	1%
Incubation Time	1 Hrs
No of Replicates	Three
Endpoint	LDH release content
Analysis	Absorbance at 490
Components	**Final Concentration**
INT	2 mM
NAD	3.2 mM
Lithium lactate	160 mM
PMS	0.6 mM

Estimation of malondialdehyde (MDA)

Malondialdehyde (MDA) quantification can reveal the extent of lipid peroxidation, a condition brought on by oxidative stress, in K562 cell lines treated with Imatinib and Naringin together.

In this method, K562 cells were cultured in the suitable growth medium and incubated at 37°C in a humidified incubator with 5% CO2 until they reach confluency. These K562 cells were treated with varying concentrations of Imatinib and Naringin, alone or in combination, for a predetermined length of time. As a reference, controlled group or untreated cells were used appropriately. After the treatment period, cell pellets were collected by centrifugation method at a suitable speed and for a suitable length of time (e.g., 1000 x g for 10 minutes).The cell pellet was resuspended in phosphate-buffered saline (PBS) and homogenized the cells using asonicator or a homogenizer.Centrifugation was done for the homogenized cells at a suitable speed and for a suitable length of time to remove the cellular debris.The supernatant was transferred to a new tube and an appropriate volume of 10% trichloroacetic acid (TCA) was added to precipitate the protein. The mixture was centrifuged at a suitable speed to remove precipitated proteins.Transferred the supernatant to a new tube and add an appropriate volume of thiobarbituric acid (TBA). to react with the MDA. The mixture was incubated at a suitable temperature (e.g., 95°C) for a suitable length of time (e.g., 60 minutes) to form the MDA-TBA adduct. Cooled the mixture and centrifuged it at a suitable speed and for a suitable length of time to remove any precipitated material. The Absorbance of the supernatant was measured using a spectrophotometer set to the appropriate wavelength of 532 nm.The amount of MDA in the sample was calculated using the molar extinction coefficient (usually 1.56 x 10^5 M^-1 cm^-1). The data were analysed using appropriate statistical methods, such as calculating IC50 values or performing a dose-response analysis.Plate mapping for MDA determination and Protocol summary is given in Table 3 and Table 4 respectively

Table 3: Plate mapping for MDA estimation

	1	**2**	**3**	**4**	**5**	**6**
A	Imatinib	Naringin	Imatinib+ Naringin	Control	DMSO	Blank
B	Imatinib	Naringin	Imatinib+ Naringin	Control	DMSO	Blank
C	Imatinib	Naringin	Imatinib+ Naringin	Control	DMSO	Blank

Table 4: Protocol summary of MDA estimation

Test compound Concentration	1 µM (Imitanib), 30 uM(Narinjin)
Incubation Time	30hrs
No of Replicates	Three
Endpoint	MDA content
Analysis	Absorbance at 535
Components	**Final Concentration**
Tri chloroacetic acid	4%
Conc. HCl	0.05 N
Thiobarbituric Acid	0.93%
Butylated Hydroxytoluene	0.03%

Determination of catalase activity

The enzyme catalase is essential for shielding cells from oxidative damage. Estimating the amount of catalase in K562 cell lines treated with Imatinib and Naringin together can reveal details on the antioxidant defence capacities of these cells.

In this method, K562 cells were cultured in the suitable growth medium and incubated at 37°C in a humidified incubator with 5% CO2 until they reach confluency. These K562 cells were treated with varying concentrations of Imatinib and Naringin, alone or in combination, for a predetermined length of time. As a reference, controlled group or untreated cells were used appropriately. After the treatment period, cell pellets were collected by centrifugation method at a suitable speed and for a suitable length of time (e.g., 1000 x g for 10 minutes). The cell pellet was resuspended in phosphate-buffered saline (PBS) and homogenized the cells using a sonicator or a homogenizer. Centrifugation was done for the homogenized cells at a suitable speed and for a suitable length of time to remove the cellular debris. the supernatant was transferred to a new tube and kept on ice until a catalase assay was performed. A suitable dilution of the supernatant was prepared to ensure enzyme activity within the linear range of the assay.An appropriate volume of hydrogen peroxide (H2O2) to the diluted supernatant was added to start the catalase reaction. Incubated the mixture at a suitable temperature (e.g., 37°C) for a suitable length of time (e.g., 1-5 minutes) to allow the catalase reaction to proceed. The reaction was stopped by adding an appropriate volume of a suitable stopping solution such as sodium hydroxide (NaOH) or perchloric acid (PCA).The absorbance of the sample was measured using a spectrophotometer of wavelength 240-280nm. The amount of catalase in the sample was calculated using the molar extinction coefficient and the initial rate of the reaction.The data were analysed using a statistical method like calculating IC50 values or performing a dose-response

analysis.Plate map and Protocol summary of catalase activity are given in Table 5A, 5B, and Table 6 respectively.

Table 5 A: Plate map for catalase activity

Cycle Nr.	1	2	3	4	5	6	7	**Delta Absorbance**
Time [s]	0	30	60	90	120	150	180	--
Temp. [°C]	22.7	22.7	22.6	22.7	23	22.9	23	--
G6	3.64	3.21	2.91	2.50	1.94	1.68	1.36	2.29
G7	3.66	3.17	2.57	1.87	1.17	0.87	0.65	3.00
G8	3.60	3.26	2.86	2.46	2.26	1.97	1.65	1.95
G9	3.59	3.16	2.26	1.76	1.06	0.60	0.36	3.22
G10	3.63	3.16	2.46	1.57	0.96	0.54	0.32	3.31

Table 5 B: Plate map for catalase activity

Cycle Nr.	1	2	3	4	5	6	7	**Delta Absorbance**
Time [s]	0	30	60	90	120	150	180	--
Temp. [°C]	22.1	22.1	22.1	22.4	22.3	22.4	22.5	--
E6	3.67	3.26	2.94	2.46	1.96	1.65	1.36	2.31
E7	3.59	3.16	2.56	1.83	1.26	1.15	0.96	2.62
E8	3.53	3.36	2.84	2.56	2.16	1.86	1.62	1.91
E9	3.57	3.16	2.26	1.79	1.18	0.66	0.57	3.00
E10	3.53	3.15	2.43	1.56	1.05	0.58	0.36	3.17

Table 6: protocol summary of catalase activity

Test compound Concentration	1 µM (Imitanib), 30 uM(Narinjin)
Incubation Time	Kinetic assay
No of Replicates	Three
Endpoint	Catalase activity
Analysis	Absorbance at 240 nm
Components	**Final Concentration**
INT	2 mM
NAD	3.2 mM
Lithium lactate	160 mM
PMS	0.6 mM

RESULT AND DISCUSSION

LDH Assay

The absorbance of each well was analysed at a wavelength of 490nm and the absorbance found is shown in Table 4. Plate mapping is shown in Table 1. Results for the % LDH release are shown in Table 8 where release for Imatinib was found to be 45.21 % while for Naringin was found to be 36.68. However, % release for the combination of Imatinib and Naringin was found to be increased i.e. 70.05 % which is 25 folds increase in value.During cell injury and necrosis, the cytoplasmic enzyme LDH is released into the extracellular environment. When Imatinib and Naringin are used together to treat K562 cell lines, the estimation of LDH serves as a gauge of the cytotoxicity and cell damage. The LDH assay results revealed that Imatinib and Naringin treatment dramatically decreased LDH release as compared to the control group, indicating a decrease in cell damage and necrosis. This may help to slow the evolution of CML by suggesting that combination therapy has a protective impact on K562 cells. The results for the LDH assay are represented in Table 6

Table 7: Absorbance of samples

	Absorbance of samples					
A	0.392	0.319	0.586	0.116	0.177	0.870
B	0.382	0.320	0.599	0.150	0.160	0.824
C	0.384	0.300	0.609	0.162	0.173	0.869

Table 8: Results for % LDH release

S.no	**Sample Details**	**Absorbance at 490 nm**			**% LDH release**			**Average**	**SE**
		Rep 1	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**		
1	Imatinib	0.392	0.382	0.384	45.05	46.35	44.21	45.21	0.62
2	Naringin	0.319	0.320	0.300	36.68	38.83	34.53	36.68	1.24
3	Imatinib + Naringin	0.586	0.599	0.609	67.39	72.67	70.08	70.05	1.53
4	Control	0.116	0.150	0.162	13.33	18.21	18.66	16.73	1.71
5	DMSO	0.177	0.160	0.173	20.36	19.42	19.91	19.90	0.27
6	Triton X-100	0.870	0.824	0.869					

Estimation of malondialdehyde (MDA)

The Absorbance of the samples was measured at 535 nm. Absorbance for the samples is given in Table 9 and plate mapping is given in Table 3. Results for the MDA content (nM/L) are given in Table 10. In this estimation, the MDA content for Imatinib was found to be 32.87nM/L while for Naringin it was 14.01 nM/L. For a combination of Imatinib and Naringin, it was found to be 42.30 nM/L.

Table 9: Absorbance of samples

	Absorbance of sample					
A	0.5531	0.2494	0.797499999	0.1678	0.1525	0.0677
B	0.615	0.3476	0.662100002	0.1697	0.1514	0.0743
C	0.587	0.2752	0.736670001	0.1715	0.1551	0.0746

Table 10: Results of MDA estimation

S.no	Sample Details	Absorbance at 535 nm			MDA Content (nM/Lit)			Average	SE
		Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3		
1	Imatinib	0.553	0.615	0.587	31.12	34.66	32.85	32.87	1.02
2	Naringin	0.249	0.348	0.275	11.65	17.52	12.86	14.01	1.79
3	Imatinib + Naringin	0.797	0.662	0.737	46.78	37.68	42.44	42.30	2.63
4	DMSO	0.168	0.170	0.171	6.42	6.12	6.21	6.25	0.09
5	Control	0.153	0.151	0.155	5.44	4.94	5.16	5.18	0.14
6	Blank	0.068	0.074	0.075	0.00	0.00	0.00		

MDA is an indicator of lipid peroxidation, a process that generates free radicals and causes oxidative damage to cell membranes. The amount of oxidative damage to the cell membranes is shown by the estimate of MDA in K562 cell lines treated with Imatinib and Naringin together. According to the MDA assay results, Imatinib and Naringin therapy dramatically lowered MDA levels as compared to the control group. This shows that the combination medication has a protective impact on the K562 cells' cell membranes, which may help to slow the development of CML.

Determination of catalase activity

Protein content for the sample given in Table 11 was found to be linear (Figure 1) and shown in the graph. Samples absorbance, assay volume, catalase activity, and % inhibition is shown in tables 12A, B, C. Catalase activity for Imatinib was found to be 302.08, for Naringin, it was 353.48 and for combination, it was, 271.46 units/mg of protein. % inhibition for a combination of Imatinib and Naringin was found to be 27.707 %

Table 11: Protein content for catalase activity

Compound details	OD1	OD2	OD3	Protein conc 1	Protein conc 2	Protein conc 3	Average protein conc (mg/ml)
Imatinib	0.562	0.583	0.545	0.475	0.492	0.461	0.476
Naringin	0.544	0.567	0.585	0.460	0.479	0.494	0.478
Imatinib +Naringin	0.524	0.513	0.551	0.443	0.434	0.466	0.448
Control	0.595	0.591	0.603	0.503	0.499	0.509	0.504
DMSO	0.589	0.593	0.585	0.497	0.501	0.494	0.497

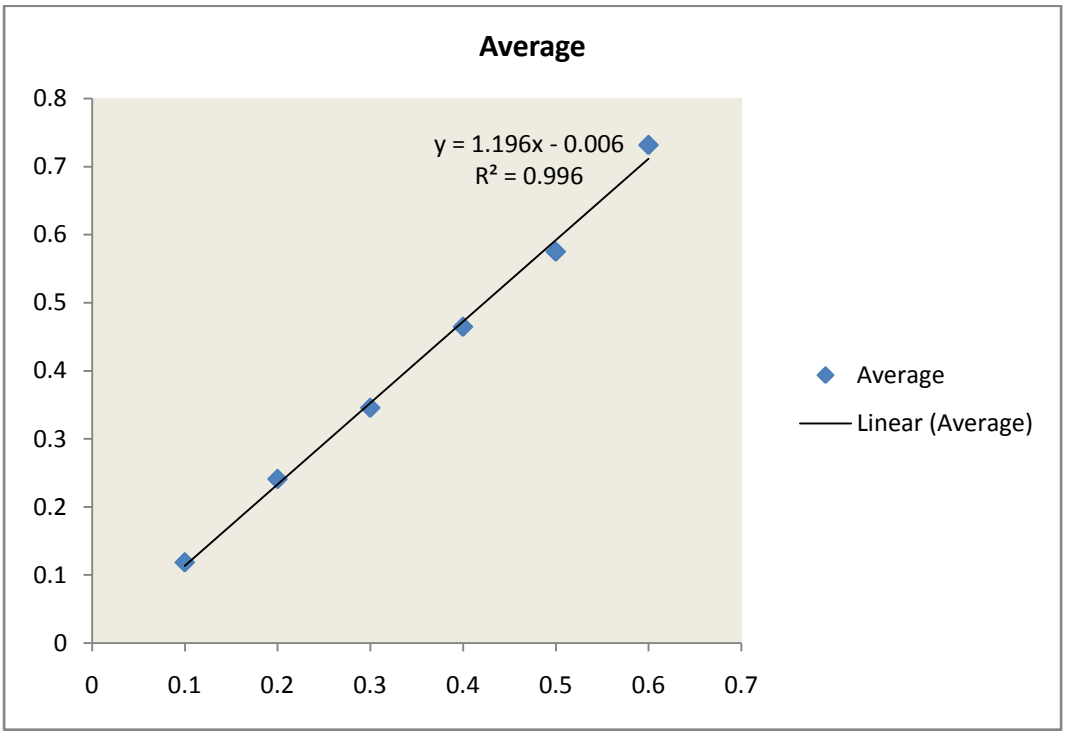

Figure 1: graph for protein content

Table 12 A: Results for Catalase activity

S.no	Sample Details					Delta Absorbance at 240 nm		
		Absorbance time (Min)	Assay volume	Average protein conc (mg/ml)	Avg protein conc per 25ul of sample	Rep 1	Rep 2	Rep 3
1	Imatinib	3	0.2	0.476	0.012	2.286	2.306	2.462
2	Naringin	3	0.2	0.478	0.012	3.004	2.623	2.656
3	Imatinib + Naringin	3	0.2	0.448	0.011	1.949	1.907	2.103
5	DMSO	3	0.2	0.504	0.013	3.223	3.002	3.060
4	Control	3	0.2	0.497	0.012	3.308	3.175	3.120

Table 12B: Results of Catalase activity

S.no	Sample Details	Catalase activity (units/mg of protein)			Average	SE
		Rep 1	Rep 2	Rep 3		
1	Imatinib	293.66	296.29	316.29	302.08	7.14
2	Naringin	384.61	335.78	340.05	353.48	15.62
3	Imatinib + Naringin	266.35	260.61	287.42	271.46	8.15
5	DMSO	391.39	364.54	371.63	375.85	8.03
4	Control	406.66	390.31	383.64	393.54	6.84

Table 12C: Results of catalase activity

S.no	Sample Details	% Inhibition			Average % inhibition	SE
		Rep 1	Rep 2	Rep 3		
1	Imatinib	24.97064	18.7215	14.8921	19.52809	2.94
2	Naringin	1.732509	7.89027	8.49847	6.040415	2.16
3	Imatinib + Naringin	31.94945	28.5106	22.6609	27.707	2.71
5	DMSO	-	-	-	-	-
6	Control	-	-	-	-	-

To protect cells from oxidative damage, the enzyme catalase converts hydrogen peroxide into water and oxygen. The amount of antioxidant defence in these cells is estimated by measuring the catalase levels in K562 cell lines treated with a combination of Imatinib and Naringin. The results of the catalase assay demonstrated that, in comparison to the control group, the combined treatment of Imatinib and Naringin considerably boosted the levels of catalase. This shows that the combo therapy strengthens K562 cells' antioxidant defence mechanisms, which may help slow the development of CML.

CONCLUSION

Myeloid cell development that is abnormally accelerated in the bone marrow is the hallmark of chronic myeloid leukaemia (CML) which is a form of blood cancer. Tyrosine kinase inhibitor i.e. Imatinib has been the go-to treatment for CML, although the emergence of medication resistance has reduced its effectiveness. Imatinib has demonstrated encouraging outcomes in overcoming drug resistance and improving the therapeutic efficiency of Imatinib when used in conjunction with flavonoids like Naringin. Imatinib and Naringin therapy has been demonstrated to improve antioxidant defence mechanisms in K562 cells and lessen oxidative stress, which is a significant contributor to the onset and progression of CML. The production of reactive oxygen species in K562 cells treated with Imatinib and Naringin together shows that this therapy may have potential as a treatment for CML. These reactive oxygen species include LDH, MDA, and catalase.Additional research is required to examine the therapeutic potential of Imatinib and Naringin combination treatment in preclinical and clinical settings. In order to combat drug resistance and improve the therapeutic efficacy of Imatinib, flavonoids like Naringin may be used in combination with Imatinib. The treatment of CML and other cancers may take a new turn with the help of this combination medication.

Conflict of interest:

The authors declare that there is no conflict of interest

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