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Genotoxicity Analysis of PBMCS in Cells Using Comet and DNA Fragmentation Assay Methods

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ABSTRACT
Phenol is a common industrial chemical that may be harmful to human health. This study examines how phenol affects the genetic material of Peripheral Blood Mononuclear Cells (PBMC) using MTT assay, DNA fragmentation assay, and comet assay. The MTT assay measured cell survival cell viability. The IC50 value was found to be 5.078 µg/ml, indicating that phenol disrupts the normal functioning of cells, especially their mitochondria, leading to decreased survival. The DNA fragmentation assay confirmed that phenol DNA damage. This prove that phenol harms cells through oxidative stress or by directly affecting their genetic material. The comet assay provided further evidence of DNA damage. Cells exposed to phenol showed an increased number of DNA strand breaks compared to untreated cells. These results show that phenol has harmful effects on cells and DNA. More research is needed to fully understand how phenol causes this damage. Future studies should investigate oxidative stress, DNA repair mechanisms, and genes involved in cell death. Long-term studies in living organisms are also important to determine if phenol exposure could increase the risk of diseases like cancer. Understanding these effects will help create safety guidelines to reduce human exposure to phenol and its toxic effects.

KEY WORDS: Phenol, MTT, DNA damage, Oxidative Stress, Comet, Toxic effect

I. INTRODUCTION

To evaluate phenol's possible detrimental effects on genetic material—which could result in mutations, chromosomal damage, or even cancer—genotoxicity analysis is crucial. A common industrial chemical, phenol is known to pollute the environment and can be absorbed through the skin, food, or air. Understanding its genotoxic potential is essential for assessing the danger to human health because it is present in a variety of industrial processes, wastewater, and consumer items. To ascertain whether phenol causes genetic harm, this analysis uses a range of in vitro and in vivo test systems, including the Ames test, micronucleus assay, and chromosome aberration tests. Determining the genotoxicity of phenol aids in regulatory decision-making and directs the creation of safer chemicals and public health exposure limits.

PHENOL:

With the chemical formula C_6H_5OH, phenol, commonly referred to as carbolic acid, is an aromatic substance. It is made up of a hydroxyl group (-OH) linked to a benzene ring (C_6H_5). A versatile chemical, phenol finds usage in many different industrial processes, including the manufacturing of dyes, detergents, polymers, and medications. Although it can be produced synthetically from petrochemicals, it is found naturally in coal tar. At room temperature, pure phenol is a colourless or faintly pink solid, though in warmer temperatures it may take on the appearance of a liquid**.**

Because of the resonance stabilization of the phenoxide ion (C_6H_5O-), which is created when it donates a proton (H+), phenol exhibits more acidic chemical behaviour than alcohols. Because of this characteristic, phenol plays a significant role in a number of chemical processes. For instance, phenol can experience electrophilic aromatic substitution processes in which a different group takes the place of the hydrogen atom on the benzene ring. Additionally, phenol can react with bases to produce salts, and it can combine with

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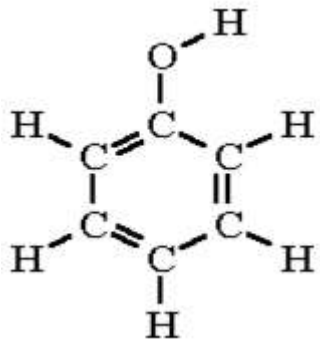
formaldehyde to create phenolic resins, which are frequently used in coatings and adhesives**.**

Phenol is poisonous and can be dangerous if swallowed, absorbed via the skin, or inhaled, even though it is used extensively in industry. Both the skin and the eyes are corroded by it, and exposure to high quantities can have serious health consequences like respiratory distress or internal organ damage. Phenol handling in production or laboratory settings requires safety precautions, and its environmental presence needs to be strictly controlled to avoid contamination and ecological damage.

The reactivity of phenol is applicable to a number of industrial processes, most notably the synthesis of phenolic resins, which are utilized in the manufacturing of paints, adhesives, and plastics. These resins, which are necessary for producing long-lasting materials, are produced when formaldehyde and phenol combine. Additionally, because of its antibacterial qualities, phenol is used for sterilizing in the manufacturing of medications, dyes, and disinfectants.



Structure of phenol

PROPERTIES OF PHENOL**:**

At room temperature, phenol is a colourless to light pink crystalline solid with a distinctly sweet, tar-like smell. It has a limited solubility in water, however as the temperature rises, solubility increases. Phenol dissolves more easily in organic solvents such as ether, alcohol, and chloroform at room temperature. It has the usual characteristics of an aromatic compound, such as a stable ring structure that enhances its chemical reactivity. The behaviour and reactivity of the benzene ring are greatly influenced by the presence of the hydroxyl group (- OH) connected to it.

The acidity of phenol is one of its main characteristics. The resonance stabilization of the phenoxide ion (C_6H_5O-), which is created when phenol loses a proton (H+), makes it more acidic than alcohols. This increases the likelihood that phenol will react with bases to generate salts such sodium phenoxide (C_6H_5ONa). Phenol's acidity also makes it a useful starting material for the synthesis of other molecules, such as organic compounds and phenolic resins. But compared to carboxylic acids, which are more likely to lose a proton, it is still less acidic.

Phenol is chemically reactive and can undergo electrophilic aromatic substitution processes, in which a number of functional groups can take the place of the hydrogen atom on the benzene ring. This can involve, among other things, reactions involving nitro, alkyl, and halogen groups. For instance, in the presence of water, phenol and bromine easily combine to generate 2,4,6-tribromophenol. Because of its reactivity, phenol is a valuable intermediate in industrial and organic synthesis processes. Furthermore, phenol and formaldehyde can combine to generate phenolic resins, which are crucial for the manufacturing of paints, adhesives, and plastics.

Phenol has certain poisonous and dangerous qualities despite its industrial application. It can cause extreme skin and eye irritation and is corrosive. Phenol can be dangerous if inhaled or consumed; symptoms include headaches, nausea, dizziness, and in severe situations, organ damage. Owing to these health hazards, handling phenol in industrial and laboratory settings requires the implementation of suitable safety protocols. It is also regarded as a possible environmental contaminant, necessitating appropriate disposal techniques to prevent soil or water pollution.

EFFECTS OF PHENOL IN HUMAN BODY:

Depending on the amount and kind of exposure, phenol is a hazardous substance that can have serious negative effects on the human body. It can irritate and harm tissues when it enters the body by the mouth, nose, or skin. Prolonged contact to phenol, particularly at high concentrations, can cause skin blistering, redness, and burns. Phenol can irritate the respiratory system if inhaled, resulting in chest discomfort, coughing.

Phenol can seriously harm the gastrointestinal system when consumed, resulting in internal burns, nausea, vomiting, and abdominal pain. Additionally, it may impact the neural system, leading to headaches, disorientation, and light-headedness. Excessive levels of phenol poisoning can result in loss of coordination, tremors, and muscle weakness.

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Even at lesser quantities, prolonged exposure to phenol can have long-term negative health effects. Dermatitis, skin pigmentation, and chronic irritation can result from prolonged skin contact. Over time, breathing in phenol fumes repeatedly can harm lung tissues and result in respiratory disorders. Furthermore, because the liver and kidneys are involved in detoxifying and eliminating phenol from the body, it is known that they have an impact on their function. Continuous exposure over time may cause organ failure or damage. Long-term exposure to phenol may also raise the chance of developing cancer because it has been connected to possible carcinogenic effects.

According to studies, those who work in businesses that handle phenol-based goods may be more susceptible to several types of cancer. Strict safety precautions, such as wearing protective gear and having adequate ventilation, are necessary while handling phenol because of these negative effects in order to reduce exposure and avoid serious health impacts.

PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCs):

The circulation contains a varied collection of immune cells called peripheral blood mononuclear cells (PBMCs), which are essential to the body's defensive systems. They are mostly composed of monocytes and lymphocytes (T cells, B cells, and natural killer cells), which are critical for immunological responses.

Unlike other blood cells like red blood cells and polymorphonuclear cells (like neutrophils), these cells have a single spherical nucleus. Because of their essential roles in the immune system, PBMCs are extensively researched in the fields of immunology, infectious illnesses, and cancer research. The main constituent of PBMCs, T cells, are in charge of cell-mediated immunity. They play a part in controlling immune responses and aid in identifying and eliminating malignant or contaminated cells. While CD8+ cytotoxic T cells target infected cells directly, CD4+ helper T cells aid in the activation of other immune cells.

Another subset of PBMC, B cells, are in charge of creating antibodies that aid in the neutralization of infections. Targeting virus-infected and tumour cells without prior sensitization is a crucial function of natural killer (NK) cells, which are also a component of PBMCs. Another important subset of PBMCs are monocytes, which present antigens and are involved in both innate and adaptive immunity. or macrophage cells, which aid in absorbing pathogens and presenting antigens to stimulate further immune cells. Monocytes are a vital component of the immune response against infections and illnesses because of these roles. PBMCs can react swiftly to infections and other immunological issues because they circulate throughout the body. PBMCs are widely employed in diagnostics and medical research. They are frequently separated from blood samples in order to research immunological responses, vaccine development, and the course of diseases like as autoimmune disorders, HIV, and cancer. To investigate drug efficacy and immunological responses and gain insight into possible treatments, scientists employ PBMCs in in-vitro research. They are also useful for long-term research and biobanking due to their cryopreserved nature. PBMCs are frequently used in clinical and laboratory settings because of their significance in immunity and disease research.

Their research aids in our comprehension of the immune system's reaction to illnesses, immunizations, and therapies. Researchers can create tailored treatments for illnesses, enhance vaccination plans, and investigate customized medicine techniques by examining PBMCs. Consequently, PBMCs remain an essential instrument in immunological and biological research.

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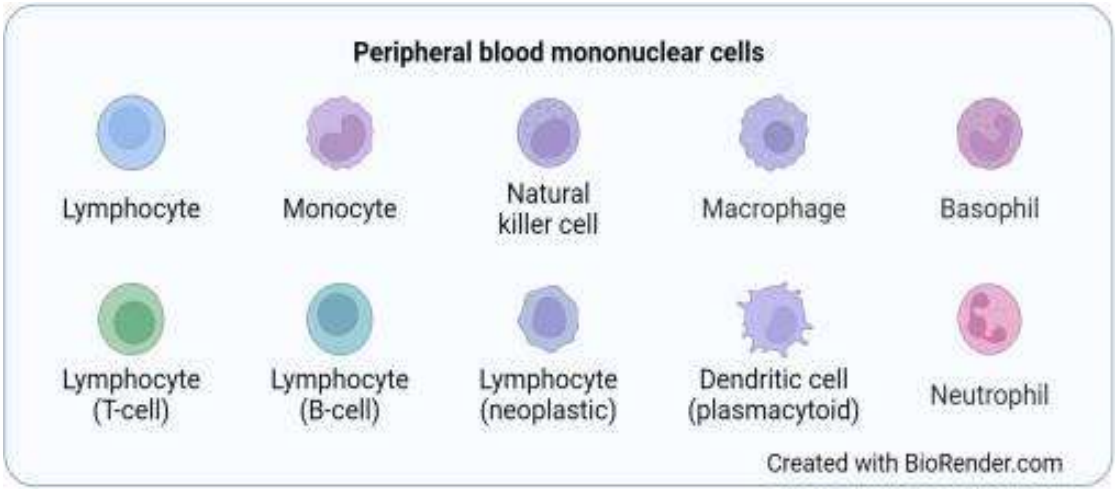

Types of PBMCs Cells

COMET ASSAY:

The Comet test, sometimes referred to as Single-Cell Gel Electrophoresis (SCGE), is a popular method for identifying single-cell DNA damage. This assay is extremely sensitive and can detect oxidative DNA damage, DNA strand breakage, and a cell's ability to repair itself. It is frequently used to assess how radiation, chemicals, and other genotoxic ants affect DNA integrity in genetic toxicology, environmental monitoring, and cancer research. The distinctive "comet-like" appearance of damaged DNA under a fluorescence microscope is the source of the assay's name.

The Comet assay's basic idea is that damaged or fragmented DNA can travel in an electric field. After being placed in an agarose gel on a microscope slide, cells are lysed with a detergent solution to extract the DNA from the membranes and proteins. Damaged DNA fragments can then move away from the nucleus when the slide is electrophoresed in neutral or alkaline conditions.

While broken DNA strands generate a tail-like shape that resembles a comet, intact DNA stays compact. There are various kinds of Comet assays, such as the neutral and alkaline Comet assays. Because it can identify alkali-labile sites and both single-strand breaks (SSBs) and double-strand breaks (DSBs), the alkaline variant (pH >13) is more frequently utilized. In contrast, the neutral Comet assay is specifically designed to identify double-strand DNA breaks. Genotoxicity testing is one of the main uses for the Comet assay. It is frequently used to evaluate novel chemicals, insecticides, medicines, and environmental contaminants for their potential to damage DNA. It is also used in cancer research to examine how radiation and chemotherapy affect DNA integrity. The assay also aids in risk assessment and regulatory decision-making by monitoring people exposed to hazardous compounds in occupational and environmental health investigations.

The Comet assay's benefits include its great sensitivity, ease of use, and capacity to examine DNA damage at the cell level. It does, however, have many drawbacks, including subjectivity in data interpretation, the requirement for standardization, and variations in assay conditions. The Comet assay is nevertheless a useful technique in toxicology, clinical research, and molecular biology despite these difficulties, helping to clarify the mechanisms underlying DNA damage and repair.

DNA FRAGMENTATION ASSAY:

A laboratory method called a DNA fragmentation assay is used to measure and identify the degree of DNA fragmentation in order to evaluate the integrity of the material. It is frequently used to investigate DNA damage brought on by a variety of causes, including oxidative stress, toxins, and radiation, as well as apoptosis, or programmed cell death. DNA cleaves into smaller fragments during apoptosis or DNA damage, and this technique can identify and examine these fragments. Understanding the causes of cell death and DNA repair processes is essential for comprehending diseases like cancer and neurological disorders.

The "laddering" technique, which uses electrophoresis, is one of the most often used approaches in DNA fragmentation assays. This

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technique involves extracting DNA from cells and using gel electrophoresis to separate the fragmented DNA. Smaller DNA fragments flow through the gel matrix more quickly than bigger ones. DNA fragmentation in apoptotic cells usually results in a distinctive "ladder" pattern on the gel, with discrete bands appearing at regular intervals that match the size of the DNA fragments (usually multiples of 180-200 base pairs). Apoptotic DNA fragmentation is characterized by this laddering pattern.

As an alternative, terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) can be used to identify DNA fragmentation. In this assay, fluorescent or radioactive markers are applied to the free 3' hydroxyl ends of the fragmented DNA. DNA fragmentation at the cellular level can be directly seen with the TUNEL technique. By combining TUNEL assays with microscopy, it is possible to pinpoint the location of the fragmented DNA inside certain tissues or cell types, offering precise spatial data on apoptosis in tissue samples or cell cultures. Assays for DNA fragmentation are essential for comprehending a number of biological processes, such as apoptosis, DNA repair systems, and cellular reactions to injury. They are extensively employed in toxicology, cancer research, and the investigation of disorders involving defects in DNA repair and damage.

Assays for DNA fragmentation are essential for comprehending a number of biological processes, such as apoptosis, DNA repair systems, and cellular reactions to injury. They are extensively employed in toxicology, cancer research, and the investigation of disorders involving defects in DNA repair and damage. Researchers can learn a great deal about how cells react to genetic insults and environmental stressoGrs by identifying DNA fragmentation. This knowledge can then be used to develop therapeutic approaches for diseases like cancer or neurodegenerative disorders that involve aberrant cell death or DNA damage.

GRAPHPAD PRISM SOFTWARE:

A popular scientific program for data visualization, statistical analysis, and graphing is called GraphPad Prism. In biological and medical research, where precise and unambiguous data representation is essential, it is mainly utilized. GraphPad Prism, in contrast to conventional spreadsheet applications, integrates statistical analysis, data organization, and graphing capabilities into an easy-to-use interface. This software is used by scientists, researchers, and students to analyse experimental data and produce graphs that are suitable for publishing. The ability of GraphPad Prism to conduct a variety of statistical tests without necessitating a high level of statistical expertise is one of its primary characteristics. It is the perfect tool for evaluating laboratory and clinical data because it incorporates widely used tests including regression analysis, ANOVA, t-tests, and non-parametric testing.

Users may make well-informed statistical decisions by using the software's step-by-step instructions, which explain which tests to employ depending on the dataset. Data visualization is another area in which GraphPad Prism shines. It enables users to produce a variety of high- quality graphs, such as heatmaps, survival curves, bar graphs, line graphs, and scatter plots. Users can improve the clarity and presentation of their data by changing the colours, labels, and error bars using the customization options. These graphs are frequently used in scientific journals and presentations since they are made to adhere to publication requirements. The capability of GraphPad Prism to manage nonlinear regression and curve fitting is another benefit; this is especially helpful in dose-response, biochemistry, and pharmacology investigations. In drug development and toxicology research, half-maximal inhibitory concentration (IC50) and half-maximal effective concentration (EC50) values are crucial, and researchers can quickly fit their data to various models, compare fits, and produce these values. Accurate data interpretation is ensured by the software's comprehensive statistical outputs, which include goodness-of-fit metrics and confidence intervals. Additionally, GraphPad Prism provides collaboration and replication tools. Transparency and consistency in research are ensured by enabling users to store and share their analytical stages. In order to preserve the integrity of research, the software offers an audit trail that records modifications made to datasets and analyses. All things considered, GraphPad Prism is a strong and intuitive tool for academics and scientists who must effectively analyse and show data. It is a popular option in many scientific domains, especially the biological sciences, due to its straightforward workflow, graphing features, and statistical analysis. GraphPad Prism streamlines complicated data analysis, whether for clinical trials, business applications, or scholarly research, freeing users to concentrate on analysing findings and deriving insightful conclusions.

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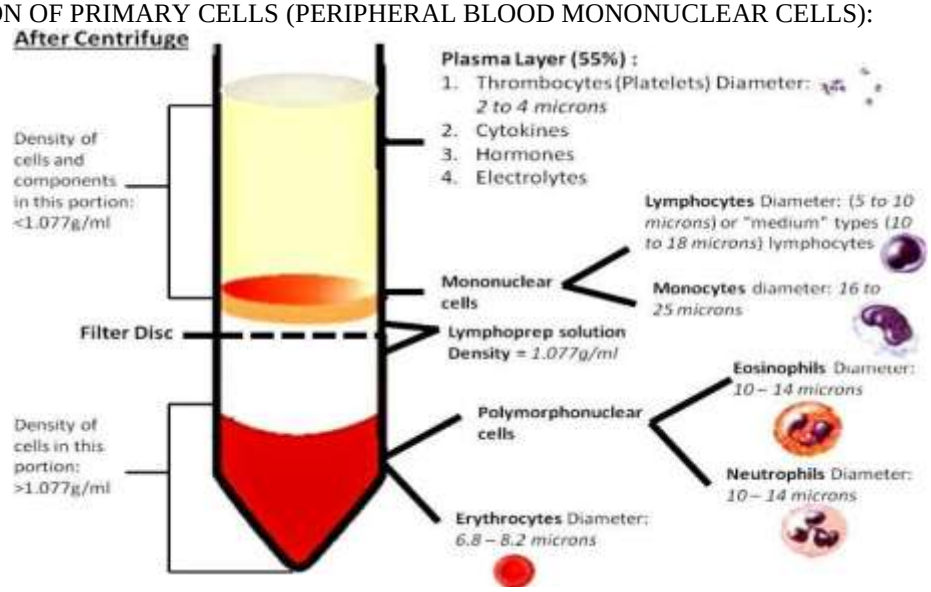
EXPERIMENTATION

Foetal Bovine Serum (FBS) and antibiotic solution were from Gibco (USA), DMSO (Dimethyl sulfoxide) and MTT (3-4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazolium bromide) (5 mg/ml) were from Sigma, (USA), RPMI medium, 1X PBS, (India). 96 well tissue culture plate and wash beaker were from Tarson (India). ETBr, agarose, Phenol, chloroform, Isoamyl alcohol, SDS, Tris HCL, EDTA, Bromophenol blue, Glacial acetic acid were from Merk, 1X PBS was from Himedia, (India). 6 well tissue culture plate and wash beaker were from Tarson (India). low melting agarose, and Normal agarose were from Merk, 1X PBS, LSM, RBC lysis buffer was from Himedia, (India). 6 well tissue culture plates and wash beakers were from Tars.

ISOLATION OF PRIMARY CELLS (PERIPHERAL BLOOD MONONUCLEAR CELLS):



Layer Separation of PBMCs Cells

Materials Required:

- Eppendorf tubes.
- Syringe.
- Blood sample.
- EDTA.
- Trypan blue.
- Ficoll/LSM.
- Distilled Water.
- Alcohol.

Instruments/Equipments:

- Centrifuge,
- Haemocytometer.

Method:

Human peripheral blood (2ml) was collected and diluted in 2ml PBS buffer to make a total volume of 4 ml. An equal volume of ficoll was taken in a centrifuge tube. The diluted blood was slowly added in layers over the ficoll/ LSM and centrifuged at 1500 rpm for 15 min at

4°C. Four layers were formed - plasma, buffy coat, mononuclear cells, and erythrocytes. The buffy coat layer in the interphase containing the mononuclear cells was gently removed. The interphase suspension was centrifuged for 10 min at 4°C and the pellet was washed in PBS.The cells were resuspended in 5 ml of RPMI medium.

MTT ASSAY:

Cell culture:

PBMC (Peripheral Blood Mononuclear Cells) were cultured in liquid medium (RPMI) supplemented 10% Foetal Bovine Serum (FBS), 100 µg/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37^{o}C.

MTT assay:

The Test sample (Phenol) was tested for in vitro cytotoxicity, using the PBMC cells by MTT assay. Briefly, the cultured PBMC cells were harvested and pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200 µL) into the 96- well tissue culture plate in RPMI medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS sample treated

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with 5 different concentrations in a serum-free RPMI medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO2 incubator for 24 h. After incubation, MTT (10 µL of 5 mg/ml) was added to each well and the cells were incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 µL) was aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a microplate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC50 value were calculated using Graph Pad Prism 6.0 software (USA).
Formula Cell viability % = Test OD/Control OD X 100

DNA FRAGMENTATION ASSAY:
Cell culture:

PBMC (Peripheral Blood Mononuclear Cells) were cultured in liquid medium (RPMI) supplemented 10% Foetal Bovine Serum (FBS), 100 µg/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37^{o}C.

DNA fragmentation Assay:

The Test sample (Phenol) was tested for in vitro cytotoxicity, using the PBMC cells by MTT assay. Briefly, the cultured PBMC cells were harvested and pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200 µL) into the 96- well tissue culture plate in RPMI medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS sample treated with IC50 concentrations in a serum-free RPMI medium incubated at 37°C in a humidified 5% CO_2 incubator for 24 h.

The samples were placed in 2mL tubes separately and then added 875µL of TE Buffer and 10% of 100µL SDS. Homogenize the sample with sterile homogenizer. And 5 µL of Proteinase K was added (20 mg/mL). The mixture was incubated at cooling temperature for 30-60 minutes (with occasional mixing/quick vortex) for easy digestion. After complete digestion, 1mL of Phenol Chloroform added and then the samples were centrifuged at 12000 rpm for 10 minutes. After that, about 500 µL of clear supernatant were collected into a new-labelled 2mL tubes. Then 100µL of 5M Sodium acetate were added and the volume make up to 2 ml with Isopropanol to precipitate the DNA. Then the samples were centrifuged at 12,000 rpm for 10 minutes. After that, the supernatant was removed and 1ml of ice-cold 70% ethanol was added to the Pellets for washing. Sample was spun at 12000 rpm, for 5 minutes. Carefully, removed the supernatant, then pipetted out excess liquid and allowed to partially dry with lid-off at room temperature. Partially dried DNA was re-suspended in 20-30µL of 1x TE buffer.

COMET ASSAY:
Cell culture:

PBMC (Peripheral Blood Mononuclear Cells) were cultured in liquid medium (RPMI) supplemented 10% Foetal Bovine Serum (FBS), 100 µg/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37^{o}C.

Comet assay:

Briefly, the cultured PBMC cells were harvested and pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200 µL) into the 96-well tissue culture plate in RPMI medium containing 10 % FBS and 1% antibiotic solution for 24- 48 hour at 37°C. The wells were treated with 5.078 µg/ml of Phenol in a serum-free DMEM medium for 24 hrs in a Co2 incubator. After incubation, the cells were collected in a 1.5 ml tube by centrifuging at 1500 rpm for 5 minutes and the supernatant was discarded and comet assay was performed based on the protocol of Nandakumar et al with slight modifications. The microscopic slides were sequentially coated with 200 µL of 0.75 % normal melting agarose as the first layer and 100 µL of 0.5% low melting agarose as the second layer. The next step was to add 20 µL cell suspensions to 60 µL of 0.5% low melting agarose, which was distributed on the slides as the third layer. Then the slides were incubated in cell lysis buffer (2.5 M NaCl, 0.2 M NaOH, 100 mM Na_2EDTA, 10 mM Tris–HCl, 1% Triton X-100 and 10% dimethyl sulfoxide, pH =10.0) for overnight at 4°C. After that, the slides were immersed in double-distilled water three times followed by 20 min incubation of unwinding solution (3 M NaOH). Subsequently, the slides were placed in a horizontal gel electrophoresis tank containing electrophoresis solution (1 mM Na2EDTA and 300 mM NaOH, pH =13). The electrophoresis was conducted at 25 V (1 V/cm, 300 mA) for 25 min. Then the slides were incubated in neutralization buffer (0.4 M Tris–HCl, pH =7.5) for 10 min followed by immersion in

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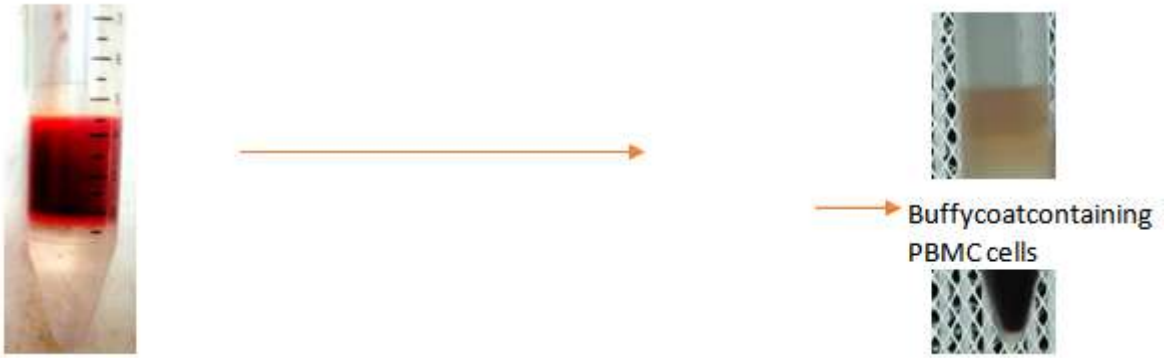
ultrapure water three times and air-drying. The cells were stained with 50 µL of ethidium bromide (5 mg/L) and observed under a fluorescent imaging system (Bio-Rad-ZOE). All steps were carried out under dim light to minimize extra DNA damage.

II. RESULT AND DISCUSSION

ISOLATION OF PMBC:



Before centrifugation

After Centrifugation

MTT:

The results indicate that as the concentration of the tested sample increases, cell viability decreases. Higher concentrations significantly reduce cell survival, while lower concentrations have a lesser impact. The IC50 value of 5.078 µg/ml suggests that this concentration reduces cell viability by 50%, demonstrating the sample's cytotoxic potential. This means the sample may be harmful to cells at higher doses. The study highlights the importance of determining safe exposure levels to minimize toxic effects. Understanding dose-dependent toxicity is essential for evaluating the sample's safety and potential applications in research or therapeutic settings. Further studies are needed for confirmation.

A. OD Value at 570 nm

S. No.	Tested sample concentration (µg/ml)	OD value at 570 nm (in triplicates)		
1	Control	1.574	1.497	1.569
2	100 µg/ml	0.766	0.734	0.804
3	50 µg/ml	0.802	0.764	0.783
4	25 µg/ml	0.884	0.889	0.886
5	12.5 µg/ml	0.903	0.925	0.914
6	6.25 µg/ml	0.956	0.977	0.966
7	3.15 µg/ml	0.998	1.023	1.010

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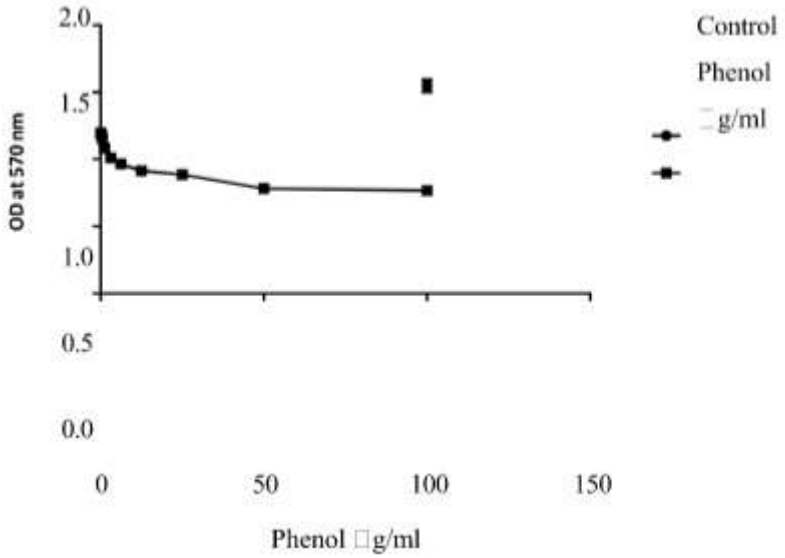
8	1.25 µg/ml	1.065	1.102	1.083
9	0.5 µg/ml	1.14	1.167	1.153
10	0.25 µg/ml	1.19	1.187	1.188
11	0.12 µg/ml	1.19	1.201	1.195



OD value for Phenol in MTT

B. Cell Viability (%)

S. No.	Tested sample concentration (µg/ml)	Cell viability (%) (in triplicates)			Mean Value (%)
1	Control	100	100	100	100
2	100 µg/ml	48.665	49.031	51.242	49.646
3	50 µg/ml	50.953	51.035	49.904	50.630
4	25 µg/ml	56.162	59.385	56.501	57.349
5	12.5 µg/ml	57.369	61.790	58.253	59.137
6	6.25 µg/ml	60.737	65.263	61.599	62.533

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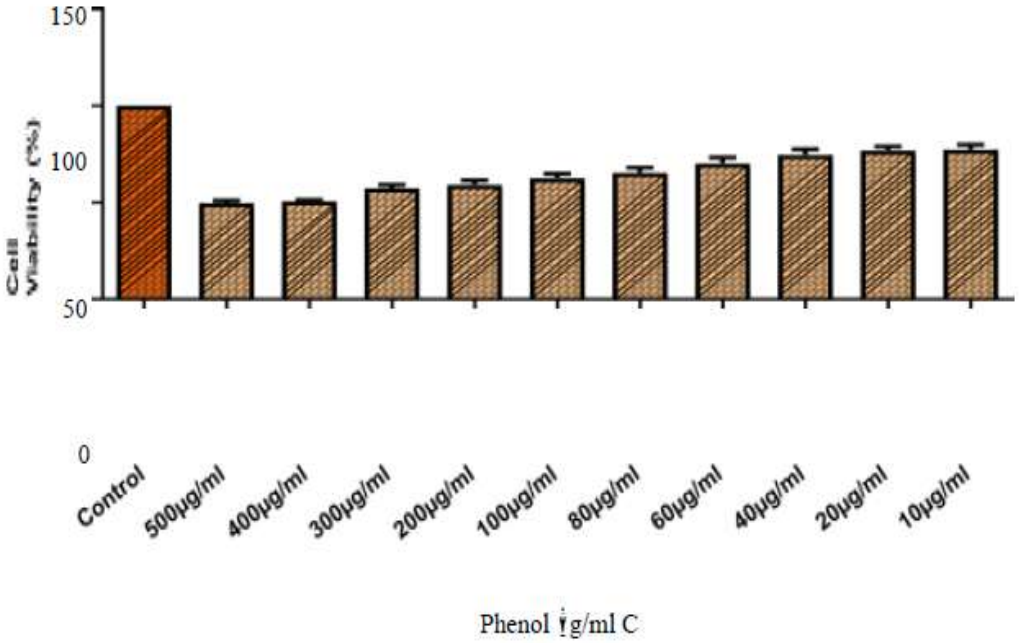
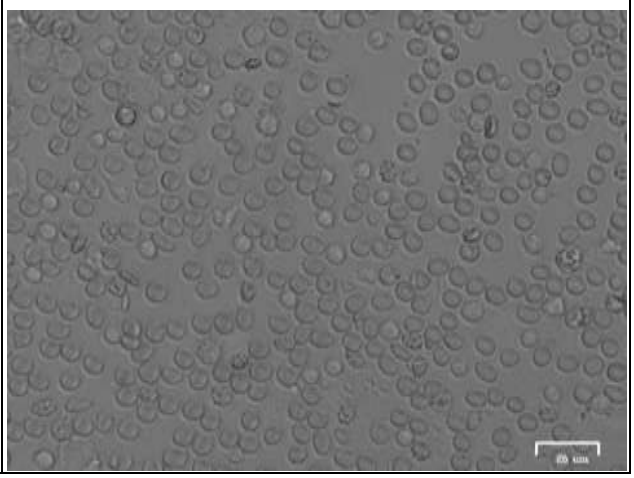
7	3.15 µg/ml	63.405	68.336	64.404	65.382
8	1.25 µg/ml	67.662	73.613	69.056	70.110
9	0.5 µg/ml	72.426	77.955	73.518	74.633
10	0.25 µg/ml	75.603	79.291	75.748	76.881
11	0.12 µg/ml	75.603	80.227	76.195	77.341



Concentration of phenol

C. Images of control cells and treated cells.

CONTROL	

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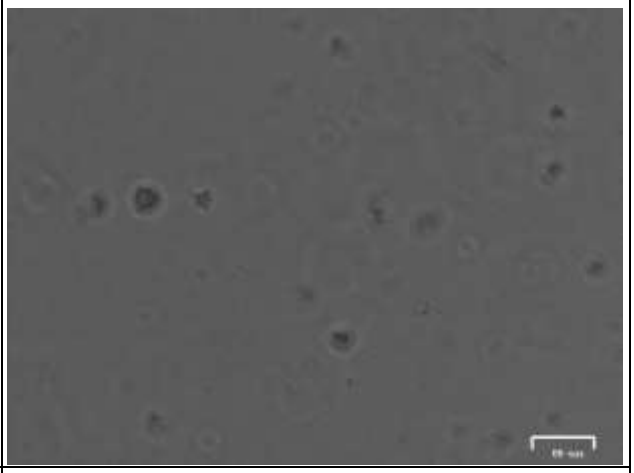
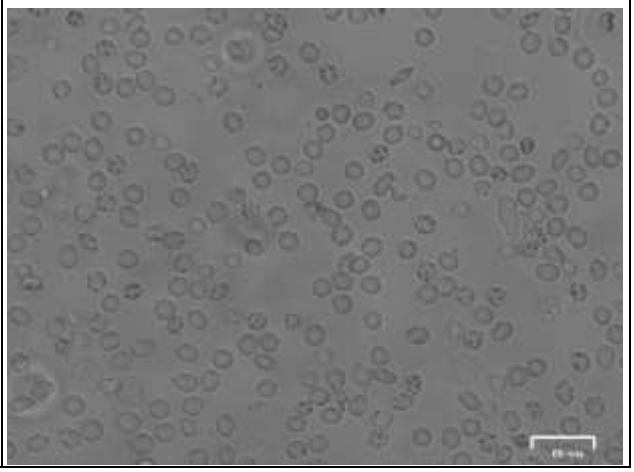
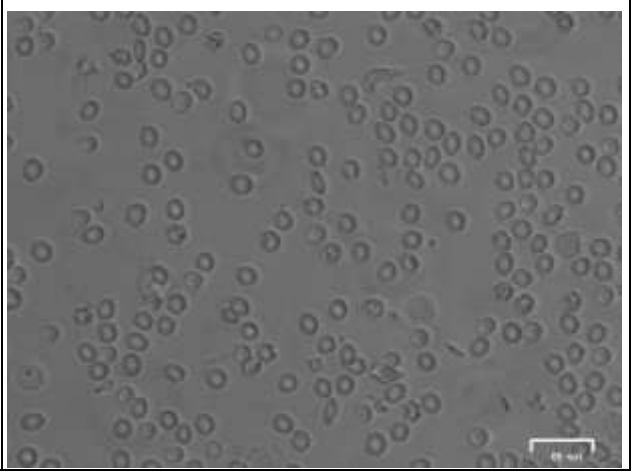
1000 µg/ml	
900 µg/ml	
800 µg/ml	

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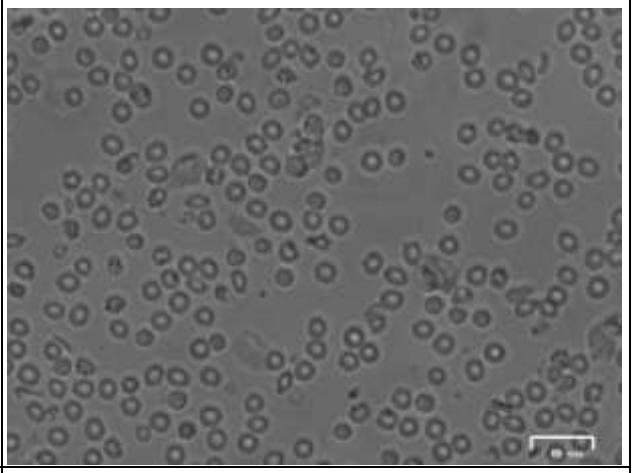
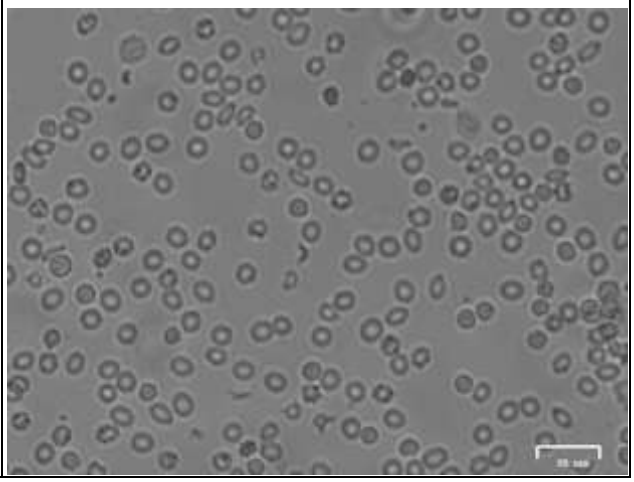
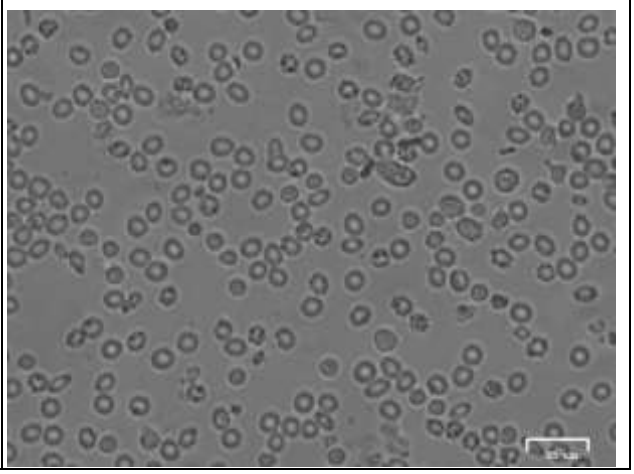
700 µg/ml	
600 µg/ml	
500 µg/ml	

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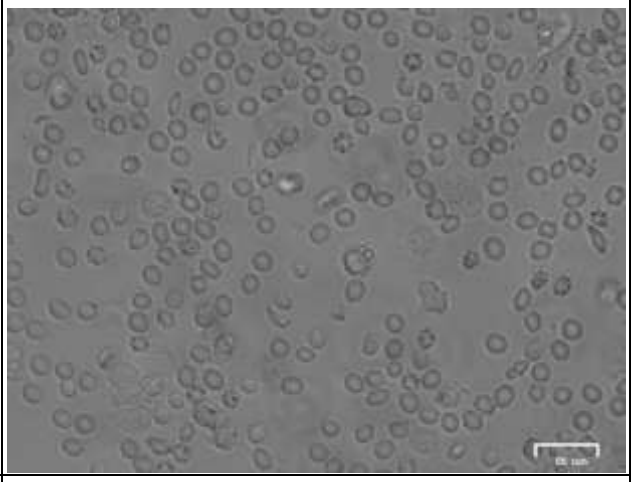
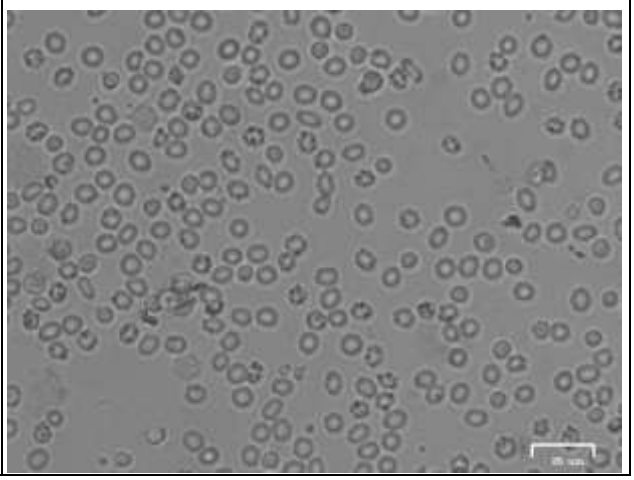
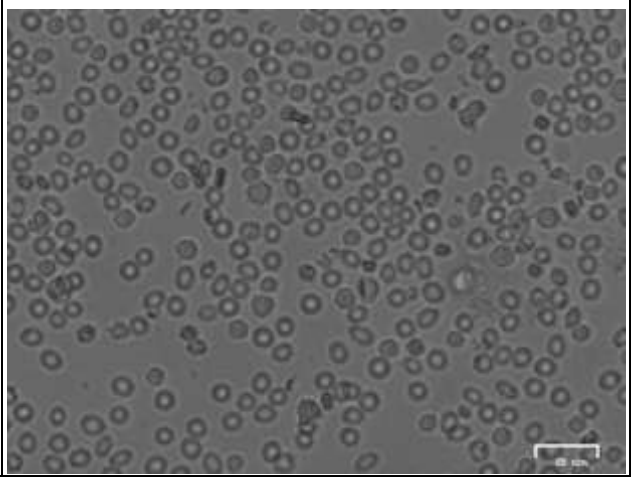
400 µg/ml	
300 µg/ml	
200 µg/ml	

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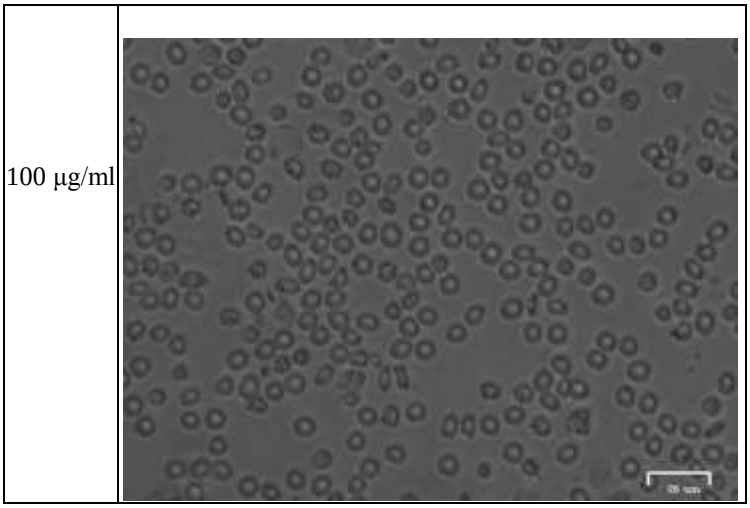

Fig 4.5 Culture Images for MTT

DNA FRAGMENTATION ASSAY:

The DNA fragmentation test shows that phenol is toxic to PBMC cells, as broken DNA was extracted and collected. This suggests that phenol causes DNA damage, and more research is needed to understand its harmful effects.

Result:

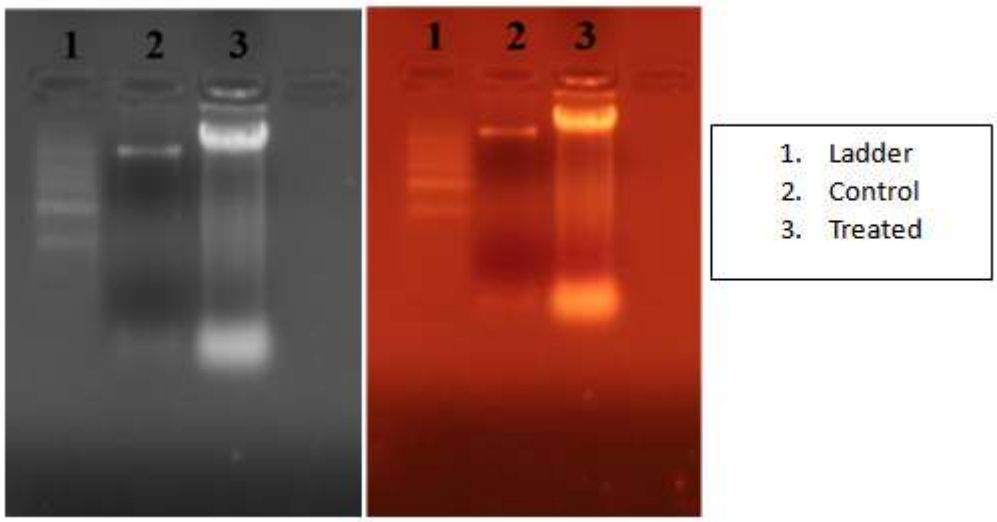

Fig 4.6 Result for DNA Fragmentation Assay

COMET ASSAY:

The comet assay results show that phenol exposure significantly damages DNA in PBMC cells compared to the control. The high number of comet events indicates DNA fragmentation, suggesting genotoxic effects. Phenol may cause cellular damage through oxidative stress or direct DNA interaction, requiring further studies on its long-term genetic impact. The control sample had no DNA damage, while treatment with 5.078 µg/ml of phenol resulted in 71.9% DNA damage, showing its harmful effects. This suggests that phenol can break DNA and potentially disrupt cell function.

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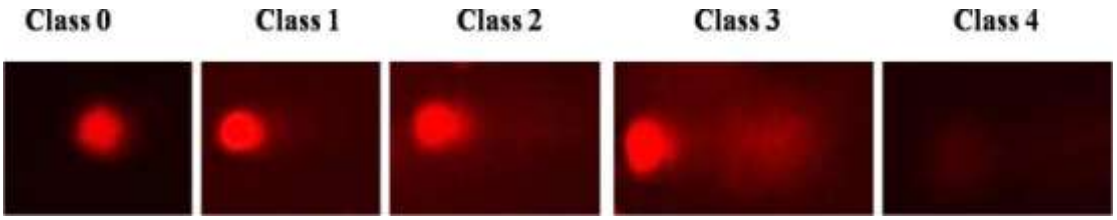
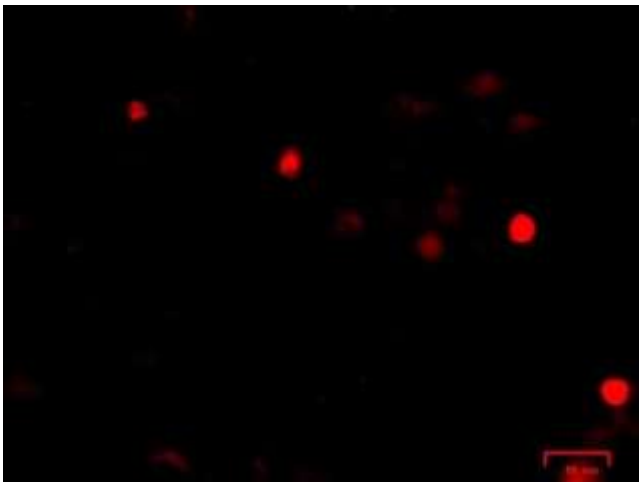
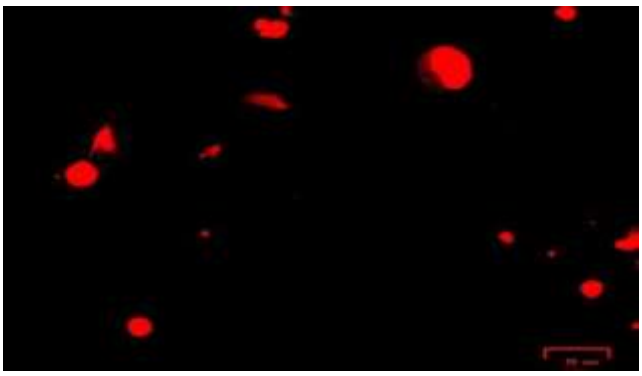
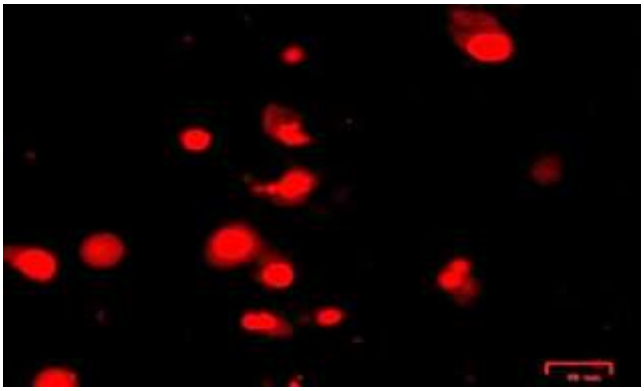


Reference image for COMET Assay


Control image for COMET Assay





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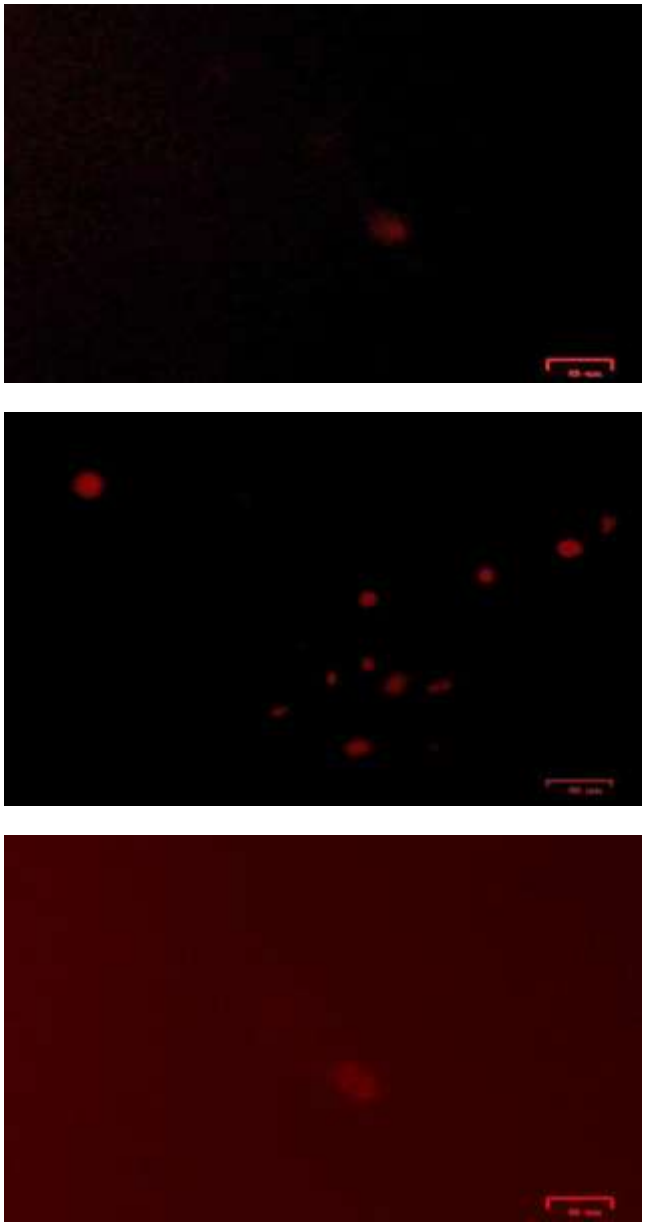

Treated with 5.078 µg/ml of Phenol sample

Test sample	Number of comet events										DNA damage events (%)
	Class 0		Class 1		Class 2		Class 3		Class 4		
Control	100	100	0	0	0	0	0	0	0	0	0
Treated with 5.078 µg/ml of Phenol sample	2	3	16	11	37	39	7	3	38	44	71.9%

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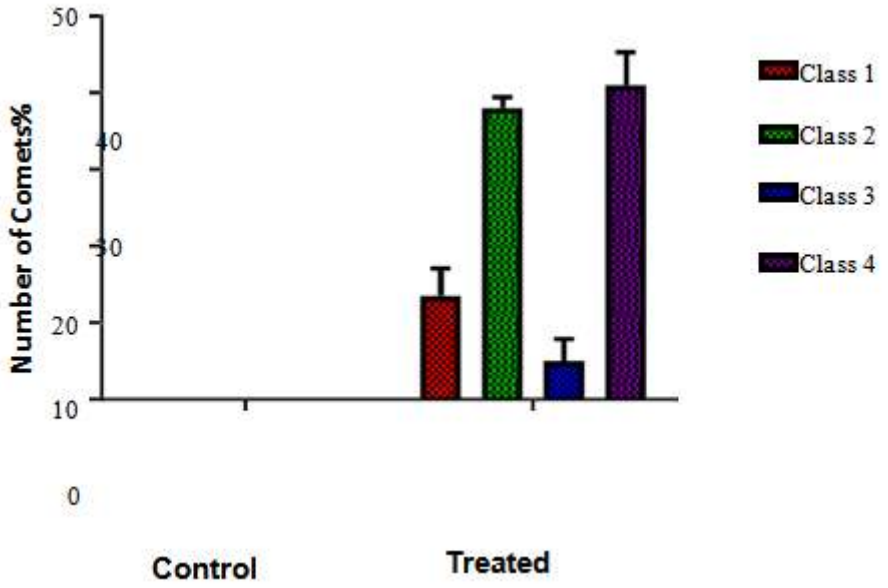

Percentage of DNA damage in COMET Assay

III. CONCLUSION

This study examined how phenol affects human blood cells (PBMC) using MTT, DNA fragmentation, and comet tests. The results show that phenol is toxic to cells and damages their DNA. The MTT test revealed that as the amount of phenol increased, cell survival decreased. The IC50 value was 5.078 µg/ml, meaning this concentration of phenol reduced cell survival by half. This suggests that phenol harms cell energy production, likely affecting the mitochondria, leading to cell damage. The DNA fragmentation test further confirmed phenol's harmful effects. The treated cells showed broken DNA, which is a sign of cell death and stress on genetic material. This suggests that phenol might cause damage through oxidative stress or direct interaction with DNA. The comet assay also showed clear signs of DNA damage. Cells exposed to phenol had more DNA breaks than the control group. The broken DNA moved towards the positive side during electrophoresis, proving severe genetic damage. Future research should focus on how phenol causes this damage at the molecular level. Studying oxidative stress, DNA repair, and cell death processes will help. Long-term animal studies are also needed to understand phenol's potential to cause diseases like cancer.

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