Advances in Basic and Applied Sciences 2 (2024) 92-104

Advances in Basic and Applied Sciences

journal homepage: https://abas.journals.ekb.eg/

# The Ameliorative Role of *Camellia Sinensis* Extract against Spleen's Extra-Medullary Hematopoiesis in Acute Myeloproliferative Disorder Model in Rats: Histopathological Study

Sherif R. Mohamed*, Ahmed S. Alazzouni, Mona A. Ibrahim, Mohamed A. Dkhil, Basma N. Hasan

*Department of Zoology and Entomology, Faculty of Science, Helwan University, Cairo,11795 Egypt*

**ARTICLE INFO**

**Article history:**
Received 4 November 2023
Received in revised form 1 December 2023
Accepted 26 December 2023
Available online 31 January 2024

doi: 10.21608/ABAS.2023.245441.1035

**Keywords:** Benzene; complete blood count; cyclophosphamide; *Camellia sinensis* extract; leukemialspleen.

## ABSTRACT

Leukemia represents a kind of malignancy that affects the bone marrow and blood, resulting in un-controlled proliferation of abnormal leucocytes. Cyclophosphamide (CP) chemotherapy has a number of side effects, including liver toxicity and renal toxicity. The purpose of the current study was to look into the therapeutic role of *Camellia sinensis* extract (CSE) on caused leukemia in adult male albino rats. Following induction of leukemia with benzene injection, both healthy and leukemic rats were separated into four groups of ten rats each: 1) untreated control rats, 2) induced- leukemia rats, 3) leukemia rats treated orally with (350 mg/kg) daily CSE for 4 weeks, and 4) leukemia rats treated with CP (7.5 mg/kg/48 hr.) for 4 weeks by intra-peritoneal injection. At the conclusion of the experiment, the rats were slaughtered. Hematological markers were assessed, including the total blood count. The results showed that treating leukemic rats with CSE effectively alleviated the leukemic complication signs, as evidenced by a significant reduction in WBCs count; additionally, CSE significantly ameliorated histo-pathological alterations in the spleen, restoring normal area percent of collagen fibers, PAS-stained general carbohydrates, and Myeloperoxidase-positive stained splenocytes in a benzene-induced leukemia model. It is possible to conclude that CSE has anti-leukemic potential, making it a promising treatment option for leukemia.

* **Corresponding author E-mail:** sherifrabea@science.helwan.edu.eg

.

## 1. Introduction

Leukemia represents a type of malignancy that affects the blood as well as bone marrow, causing an uncontrollable multiplication of aberrant white blood cells [1]. In terms of cell lineage, leukemia is classified as lymphoid as well as myeloid, and disease progression is classified as either acute or persistent [2]. According to Bray [3], leukemia constitutes one of the most common malignant diseases affecting the global population. According to Kassahun *et al*. [4], the global incidence of leukemia in boys and females is 6.3 along with 4.5 per 100,000 individual years, respectively.

Leukemia, which means "white blood" in the language of Latin, is a kind of cancer that interferes with the body's immunity system and erythropoiesis. Leukemia induces cell growth outside of the bone marrow, resulting in tumors in vital organs such the brain, lymph nodes, spleen, as well as liver [5]. Leukemia can occur as a result of chemically driven changes in the bone marrow caused by benzene exposure, smoking and alcohol use, radiation, viral infections, and genetic disorders [6, 7].

Benzene is a monocyclic aromatic hydrocarbon that is used as a solvent in a variety of industries, primarily as a feeder chemical in the manufacture of detergents, lubricants, rubber, dyes, and pesticides. Chronic benzene exposure, on the other hand, has been linked to a progressive decline in hematopoietic function, which may give rise to a variety of disorders such as aplastic anemia, myelo-dysplastic syndrome, and leukemia [8]. Benzene is an aromatic compound present in crude oil and gasoline [9]. Furthermore, it has been classified as a known human carcinogen by the International Agency for Research on Cancer (IARC) [10]. Myelodysplasia, aplastic anemia and leukemia, particularly acute myeloid leukemia (AML), have all been associated to chronic benzene exposure [11, 12].

Treatment with chemotherapy, surgical procedures, hormonal therapy, and radiotherapy are among cancer treatment options. Chemotherapy is one of the most common and extensively used cancer therapies; it administers anticancer drugs to patients systemic in order to halt the unrestrained development of malignant cells [13].

Cyclophosphamide (CP) constitutes a cytotoxic alkylating driver used to treat a variety of cancers including leukemia, prostate, carcinoma, bone, lung, and ovarian cancers, as well as Hodgkin's disease [14]. Despite its advantages, it has a number of side effects, including petechial bleeding in the small bowel, stomach discomfort, diarrhea, as well as inflammation [15]. The CP caused significant oxidative stress in the tissue as well as massive cellular damage, which raised the rate of apoptosis and death for both cancer and healthy cells [16]. Following cyclophosphamide administration, multiple complications have been reported, including bone gist repression [17], liver toxicity [18], bladder toxicity [19], renal toxicity [20], cardiovascular toxicity [21], as well as hyponatremia [22].

It is well known that natural products have a wide range of natural conditioning. Medical stores have an endless supply of anticancer medications, both in terms of variety and mode of action. Increasing evidence suggests that many naturally occurring substances parade cancer chemotherapeutic goods [23]. Green tea has become a popular beverage around the world that's produced from the leaves of *Camellia sinensis* (L.) Kuntze, a Chinese variation of tea production. It contains 3 to 6 caffeine as well as up to 30 catechins, primarily ()-epigallocatechin-3-gallate, ()-epigallocatechin-3-gallate (EGCG), ()-epigallocatechin (EGC), as well as ()-epigallocatechin (EGC) [24]. GT Catechins have anti-carcinogenic and anti-mutagenic properties in human cancers such as breast, esophageal cancer, lung cancer, stomach, small intestine cancer, colon cancer, liver cancer, and prostate cancer [25].

Tannins and alkaloids found in *Camellia sinensis* include caffeine, tristetraprolin, theobromine, and theophylline [26]. Polyphenols (catechins) are the principal active components in *Camellia sinensis* [27] Camellia sinensis is abundant in polyphenolic components that possess antimutagenic and antioxidant properties [28]. The main polyphenol in *Camellia sinensis* is epigallocatechin-3-gallate (EGCG), which plays a key role in the anticancer properties of *Camellia sinensis* polyphenols. Recent research has shown that EGCG has anticancer activity in hematopoietic cells [29] through modulation of multiple signaling cascades, EGCG has anti-fibrotic, anti-inflammatory, anti-tumor, and metabolic effects. Its ability to combat cancer has been demonstrated [30, 31].

In the left cerebral abdomen, the spleen represents a pale crimson to blue-black organ. It has a triangular-shaped, lengthy organ. It is the body's biggest secondary lymphoid immune organ, accounting for one-quarter of all lymphocytes. It is separated into two functional and morphological groups: red pulp and white pulp [32]. The activities of the spleen focus around systemic circulation. It is responsible for initiating immune responses to blood-borne antigens, cleansing the blood of foreign material and aged or damaged erythrocytes, and discarding of iron, red blood cells, and platelets. These actions are carried out by the spleen's two principal sections: the white pulp (including the peripheral zone) and the red pulp, which differ widely in architecture, vascular structure, and functioning, and cellular makeup [33].

A peroxidase enzyme called myeloperoxidase is expressed

by a gene located on a human chromosome 17. Most granulocytes, from promyelocytes to fully grown granulocytes, have it in their main granules. In more than 80% of AML blasts, it is therefore the hallmark of myeloid-lineage. MPO is not expressed by lymphoblasts or mature lymphoid cells in ALL or ALL [34]. MPO status has been used as a predictive predictor for AML patients that may benefit from bone marrow transplantation in addition to its diagnostic value [35].

Through hematological analysis and histological examination of rat spleen, the current work seeks to establish the anti-leukemic efficacy of Camellia sinensis extract (CSE) against benzene-induced leukemia in a rat model equivalent to Cyclophosphamide.

## 2. Materials and Methods

### *2,1 Drugs and chemicals*

- Benzene Chromasolv with a purity more than 99.9%, utilized in chromatographic analysis (Sigma-Aldrich, Saint Louis, Missouri, America)
- Cyclophosphamide: it is used as 200 milligrams dry powder (Baxter Oncology, Kantstrabe, Halle (Westfalen), Germany.
- Other chemicals and dyes used throughout the study were "Research grade" (El-Gomhorya Company, Cairo, Egypt).

### *2,2 Camellia sinensis extract*

Green tea extract tablets (200 mg) obtained from Arab Company for Pharmaceuticals & Medicinal Plants, Mepaco-Pharma, Egypt. The pills were ground up and the needed amount of distilled water was dissolved. Fourier transform infrared spectrophotometry (FTIR) for *Camellia sinensis* extract was performed by PerkinElmer FT-IR spectrometers, Instrument Model: Spectrum Two, Instrument Serial Number: 109979, according to Pakkirisamy *et al*. [36], The transmittance scanning range was 4000 to 400 cm1 (the mid-IR region).

### *2.3 Animals*

A total of 40 mature male albino rats (Rattus norvigicus) totaling 180 ±20 grams at the start of the experiment were acquired from the New Veterinary Office in Giza, Egypt. Rats were kept in wired cages in a thermally controlled setting with 12-hour light/dark cycles. Water and meal water were freely provided throughout the trial.

### *2.4 Induction of Leukemia*

Following acclimation to the experimental room settings, a sufficient number of rats were subjected to leukemia induction using the Akanni *et al*. [37] method. For four weeks, the rats were injected 0.2 ml of diluted benzene chromasolv solution suspended in distilled water (1:10 ratio v/v) intravenously into the tail lateral veins (inject/48 hours). After the last injection, a blood sample was taken from each animal (by retro-orbital venous plexus puncture with sterile heparinized glass capillary tubes) for white blood cell counts and leukemia tests.

### *2.5 Experimental design*

This study employed four groups of ten rats each, in this order:

Group (1): the control group.

Group 2: includes induced-leukemic rats following method of Akanni *et al*. [37].

Group (3): consisted of leukemic rats given CSE (350 mg/kg/day) orally for 4 weeks following leukemia induction [38].

Group (4): for four weeks, leukemic rats were given an intraperitoneal injection of Cyclophosphamide (7.5mg/kg/48hr) [38].

### *2.6 Animal sacrifice and sample collection*

At the end of the trial (two months), all rats were euthanized under 6% isoflurane anesthesia, and blood was taken via ocular puncture into EDTA tubes for hematological analysis. The spleen was carefully removed and preserved in 10% formol-saline for regular histological processing.

### *2.7 Hematological assay*

Blood samples were analyzed using an automated hematology analyzer (2800 Hematology Autoanalyzer). White blood cells (WBC), red blood corpuscles (RBC), hematocrit (HCT), hemoglobin (HB), and platelet count were all measured.

### *2.8 Histopathology examination*

The spleen was dehydrated with various percentages of isopropyl alcohol (50%, 70%, 90%, and 100%), cleaned in xylene, and then impregnated in paraffin wax with a melting temperature between 55C and 56C for infiltration. After that, paraffin slices with a thickness of 5 um were put on glass slides. Suvarna *et al*. [39] employed hematoxylin and eosin (H&E) stain to highlight the overall microstructure of the spleen. Periodic Acid Schif's stain (PAS) was used to determine the presence of general carbohydrates in the cytoplasm of splenocytes [40]. The spleen was additionally stained with Masson Trichrome to reveal collagen fibers. The dyed tissue slices were then inspected under a microscope (Leica, DM 750) coupled to a camera (Leica, ICC 50).

### *2.9 Immunohistochemistry staining of Myeloperoxidase*

Approximately 4-um thick paraffin-embedded sections on

silanized glass slides were stained by hand with rabbit polyclonal anti-human anti-Myeloperoxidase antibody (ab45977; abcam corporation, the USA, diluted at 1/50, incubated for 1 hour) after heat-mediated antigen retrieval in citrate buffer pH 6.0 and blocking (5 minutes/peroxidase block and 10 minutes/protein block). Horseradish Peroxidase was utilized in the experiment. DAB was used as a chromogen, and hematoxylin was employed as a counterstain, as specified on the antibody data sheet. A suitable negative control was carried out for each staining run. Two pathologists who were not aware of the clinical data analyzed the MPO-stained slides microscopically. If the intensity was at least equivalent to the remaining myeloid series positive, the criteria of at least 3% cytoplasmic MPO expression by blasts was considered positive.

*2.10 Histomorphometry*

(H&E) stained slices were used to measure the diameter of lymphoid follicles in white pulp. 5 randomly selected fields from 5 sections from every group were used in image analysis software. The average area percent of collagen fibers, PAS positive reaction, as well as myeloperoxidase positive immunoreaction was determined and statistically assessed in ten distinct high-power areas of paraffin sections of the spleen in each group employing an image analysis program (ImageJ version 1.46, NIH, USA).

*2.11 Statistical analysis*

All numerical data that was evaluated statistically was expressed using the mean standard error (SEM). The level of statistical significance was set at p 0.05 using a one-way analysis of variance test. Tukey's post hoc test was used to do further comparisons across groups using the Statistical Package for the Social Sciences (SPSS) version 20.

## 3. Results

*3.1 Extract analysis*

The results of an infrared spectrum examination of CSE are depicted in Figure (1) and Table (1). However, few researches have used FTIR to assess CSE. The overwhelming majority of frequencies represent group frequencies that indicate if a sample comprises certain functional groups. O-H stretching, C-H stretching, C-O stretching, O-H bending, N-H bending, C=C stretching, C=C bending, and S=O stretching are all included in the CSE description. As a result, alkane, alkene, alcohols, phenols, amine, and sulfoxide-compounds are likely to be present in the extract.

*3.2 Hematological result*

In terms of the leukemic marker, this study found a substantial rise in white blood cells (WBCs) count in the leukemia group when compared to the control group. Treatment of leukemia rats with either CSE or CP showed a substantial drop in WBC count when compared to the leukemia group (Table 2). When comparing the leukemia group to the control group, the current study found a substantial drop in RBC count, Hb content, hematocrit%, and platelet count. When compared to the leukemia group, treatment either with CSE or CP resulted in a substantial increase in RBC count, Hb content, hematocrit%, and platelet count (Table 2).

**Table (1)**: Fourier transform infrared analysis of Camellia sinensis extract (CSE)

| Absorption ($cm^{-1}$) | Appearance | Transmittance (%) | Group | Compound Class |
|---|---|---|---|---|
| 3279.28 | Strong broad | 23.77 | O-H stretching | alcohol |
| 2917.73 | medium | 21.15 | C-H stretching | alkane |
| 2850.34 | medium | 20.66 | C-H stretching | alkane |
| 1605.42 | medium | 11.65 | C=C stretching | conjugated alkene |
| | | | C=C stretching | cyclic alkene |
| | | | N-H bending | amine |
| 1316.54 | medium | 9.54 | O-H bending | Phenol |
| 1052.66 | strong | 7.63 | C-O stretching | Primary alcohol |
| | | | S=O stretching | sulfoxide |
| 772.77 | | 5.60 | C=C bending | alkene |

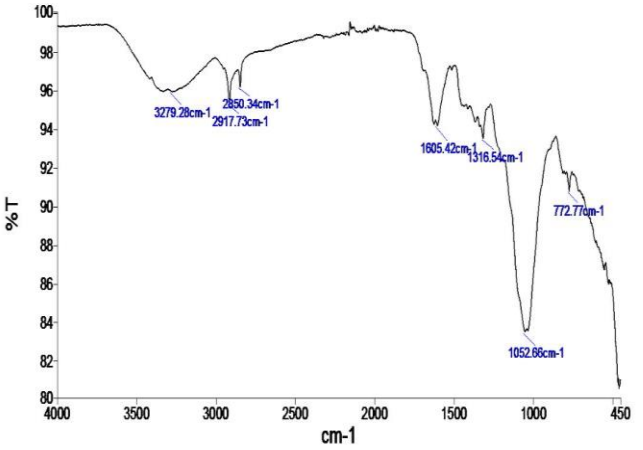



**Fig. (1):** Fourier transform infrared analysis of *Camellia sinensis* extract (CSE)

When compared with the control group, the total blood count indicated a substantial rise (P 0.05) in WBC counts (marked leukocytosis) and a significant drop (P 0.05) in RBC counts, Hb content, HCT%, and platelet count in the

leukemia group. These results demonstrated that intravenous injections of benzene Chromasolv (0.2 mL twice weekly) efficiently produced leukemia in albino rats. WBC counts fell in rats given (350 mg/kg) CSE and (7.5 mg/kg/48 h) CP, whereas RBC counts, Hb content, hematocrit, and platelet count rose. The findings reveal that blood parameters improved in both treatment groups, suggesting that this natural substance, like anti-leukemic pharmaceuticals (CP), has a potential role as an anti-leukemic agent.

**Table (2):** Mean ± SEM of complete blood count in the study groups.

| Parameters | Groups | | | |
|---|---|---|---|---|
| | Control | Leukemia | Leuk+CSE | Leuk+CP |
| WBCs ($x10^3$/µl) | 9.92±0.746 | 23.42±0.429* | 12.30±0.290*# | 8.92±0.541# |
| RBCs ($x10^6$µl) | 5.14±0.187 | 2.82±0.093* | 4.60±0.160*# | 5.10±0.254# |
| Hb (gm/dl) | 13.75±0.263 | 7.74±0.304* | 12.99±0.366*# | 13.77±0.205# |
| HCT % | 44.33±0.943 | 27.37±0.585* | 40.77±0.746*# | 43.13±0.639# |
| Platelet ($x10^3$/µl) | 474.0±63.906 | 232.3±11.225* | 481.3±13.058# | 475.6±10.285# |

∗: Significance against Control group and #: significance against Leukemia group at $P < 0.05$.

*3.3 Histological observation*

Rats in the control group had typical splenic architecture, with spleen parenchyma made up of red and white pulp. White pulp, also known as lymphoid follicle, is made up of lymphoid aggregations arranged in the outer mantle zone, with a central arteriole surrounded by peri-arteriolar lymphoid sheath and an inner light-stained germinal center, separated from red pulp by a marginal zone of light stained lymphocytes (Fig. 2A & 3A). The leukemia group had a deformed splenic contour in the shape of an uneven white and red pulp contour. White pulp components were atrophied, and there was no marginal zone with nearby vacuoles. In series with dilated congested venous sinuses, red pulp developed with numerous foamy histiocytes and hemosiderin pigment deposits (Fig. 2B & 3B). The Leuk+ CSE treated group had nearly normal white pulp architecture with some degenerated cells and normal red pulp (Figs. 2C and 3C). The spleen architecture in the Leuk+CP group was practically normal. White pulp emerged with some deteriorated cells and congested red pulp (Figs. 2D and 3D). In H&E-stained sections, the mean diameters of the central arteriole along with the diameter of the splenic lymphoid follicle were considerably larger in the leukemia group than in the control group. In comparison to the leukemia group, the mean diameter of the central arteriole and the diameter of the splenic lymphoid follicle were considerably reduced following treatment with CSE and CP (table 3& Fig. 4).

**Table (3):** Morphometric analyses of spleen of the study groups.

| Parameters | Groups | | | |
|---|---|---|---|---|
| | Control | Leukemia | Leuk+CSE | Leuk+CP |
| Diameter of central artery (µm) | 19.54± 1.041 | 30.75± 1.720* | 21.26±1.320# | 22.20± 1.030# |
| Diameter of lymphoid follicle (µm) | 275.19±13.910 | 440.26±26.118* | 305.54±14.938# | 315.00±15.227# |
| Area% of collagen fibers | 14.32±1.433 | 34.28±2.078* | 15.07±1.210# | 15.68±1.886# |
| Area% of PAS positive Rx. | 4.67±0.623 | 16.81±1.414* | 6.55±0.546# | 6.43±0.724# |
| Area% of MPO positive Rx. | 0.13±0.007 | 21.84±1.308* | 3.90±0.477*# | 3.13±0.270*# |

Data are represented as Mean ± SEM, ∗: Significance against Control group and #: significance against Leukemia group at $P < 0.05$.

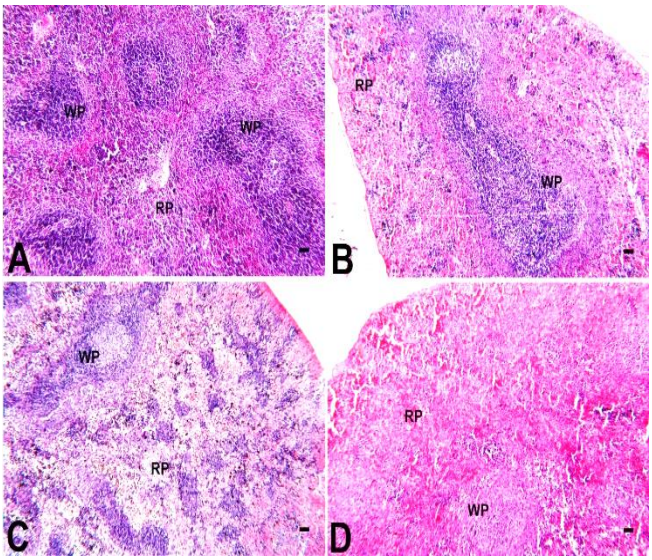



**Fig. (2):** photomicrograph of spleen sections of the study groups (H&E stain). **A** Control group showing normal architecture of spleen, spleen parenchyma consists of with red pulp (RP) and white pulp (WP). **B** Leukemia group showing distorted splenic contour in form of irregular contour of white pulp (WP), red pulp (RP). **C** Leuk+CSE treated group showing preserved architecture with clearly defined red pulp (RP) and white pulp (WP). **D** Leuk+CP treated group showing almost normal architecture of spleen

with congested red pulp (RP) and white pulp (WP). **Scale bar= 50µm**.

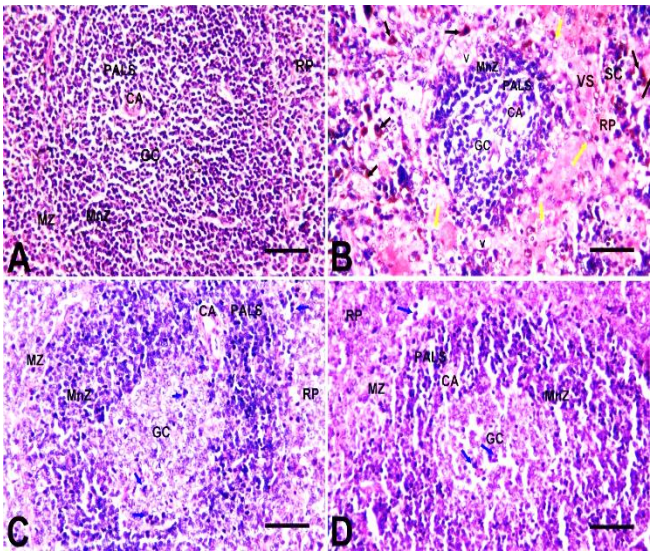
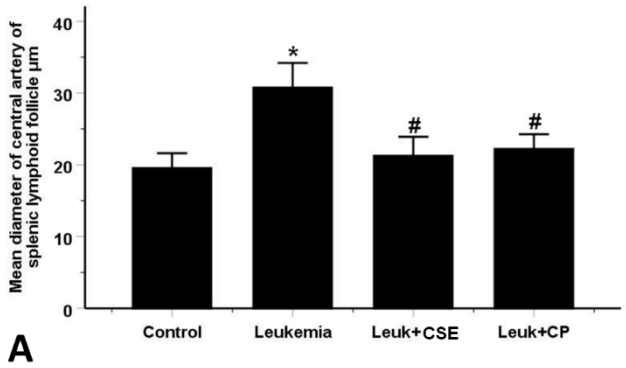



**Fig. (3):** photomicrograph of spleen sections of the study groups (H&E stain). **A** Control group showing normal architecture of spleen, spleen parenchyma consists of with red pulp (RP) and white pulp (WP). White pulp or lymphoid follicle consists of lymphoid aggregations arranged in outer mantle zone (MnZ) having a central artery (CA) surrounded by peri-arterial lymphoid sheath (PALS) and inner light-stained germinal center (GC), it is surrounded by marginal zone (MZ) of light stained lymphocytes separates white pulp from red pulp (RP). **B** Leukemia group showing degenerated WP with atrophied MnZ, PALS, CA, GC and absence of MZ with adjacent vacuoles (V). RP consists of splenic cords (SC) of lymphocytes with many foamy histiocytes (yellow arrow) and hemosiderin pigment deposits (black arrow) in series with dilated congested venous sinuses (VS). **C** Leuk+ CSE treated group showing almost normal architecture of WP with normal MnZ, MZ, PALS, CA, GC but with some degenerated cells (blue arrow) and normal RP. **D** Leuk+CP treated group almost normal architecture of WP with normal MnZ, MZ, PALS, CA, GC but with some degenerated cells (blue arrow) and normal RP. **Scale bar= 50µm.**



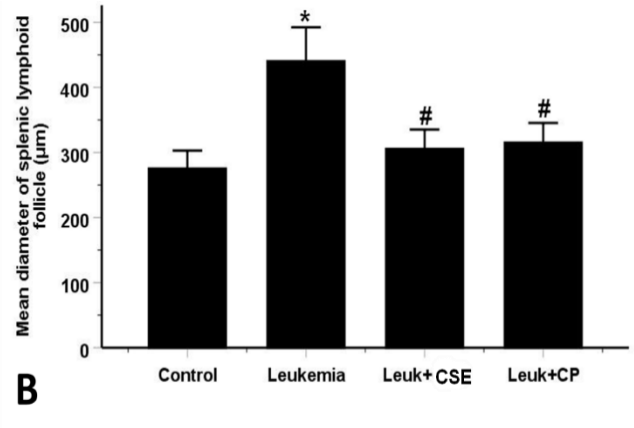




**Fig. (4):** Morphometric analysis (Mean± SEM) of H & E-stained spleen sections of; **A.** diameter of central arteriole of splenic lymphoid follicle. **B.** diameter of splenic lymphoid follicle.

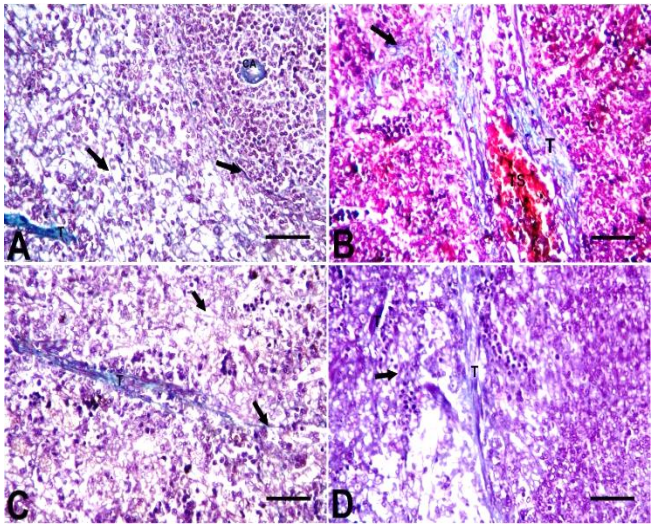



**Fig. (5):** photomicrograph of spleen sections of the study groups (Masson trichrome stain). A Control group showing thin blue-colored collagen fibers distributed in trabeculae (T), around central artery (CA) and in splenic stroma (black arrows). B Leukemia group showing thick aggregated collagen fibers in trabeculae (T) enclosing trabecular sinus (TS) and in splenic stroma (black arrow). C Leuk+ CSE treated group showing thin blue-colored collagen fibers distributed in trabeculae (T), and in splenic stroma (black arrows). D Leuk+CP treated group showing thin blue-colored collagen fibers distributed in trabeculae (T), and in splenic stroma (black arrows). Scale bar= 50µm.

In the control group, Masson trichrome stained spleen sections revealed thin blue-colored collagen fibers scattered in trabeculae, surrounding the major artery, and in the splenic stroma (Fig. 5A). Thick collagen fibers were seen in the trabeculae encircling the trabecular sinus and in the splenic stroma of the leukemia group (Fig. 5B). Few thin blue-

colored collagen fibers were seen in trabeculae and splenic stroma of the Leuk+ CSE and Leuk+CP treated groups (Figs. 5C and 5D). Morphometrically, the area % of collagen fibers in the leukemia group was substantially higher than in the control group. After CSE and CP therapy, it was much lower than in the leukemia group (table 3& Fig. 8A).

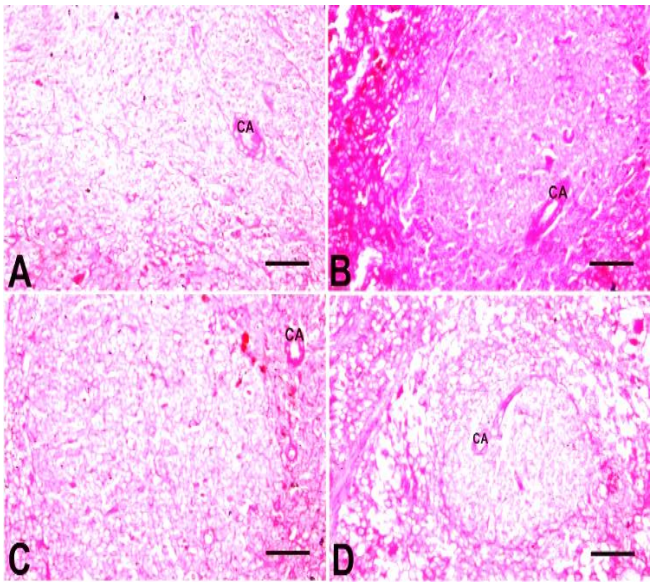



**Fig. (6):** photomicrograph of spleen sections of the study groups (Periodic acid Schiff reaction). **A** Control group showing mild to moderate PAS positive reaction of general carbohydrates content in splenic white pulp with central artery (CA). **B** Leukemia group showing strong PAS positive reaction of general carbohydrates content in splenic white pulp with central artery (CA). **C** Leuk+ CSE treated group showing moderate PAS positive reaction of general carbohydrates content in splenic white pulp with central artery (CA). **D** Leuk+CP treated group showing moderate PAS positive reaction of general carbohydrates content in splenic white pulp with central artery (CA). Scale bar= 50µm.

In the control group, PAS-stained spleen sections revealed a modest to moderate PAS positive reactivity of general carbohydrates abundance in splenic white pulp with central artery (Fig. 6A). The PAS positive reaction of overall carbs content in splenic white pulp with central artery was highest in the leukemia group (Fig. 6B). The PAS positive reaction of general carbs content in splenic white pulp with central artery was modest in the Leuk+ CSE and Leuk+CP treated groups (Figs. 6C and 6D). Morphometrically, the area % of PAS positive response in the leukemia group was substantially higher than in the control group. After CSE and CP therapy, it was much lower than in the leukemia group (table 3& Fig. 8B).

The immunoreaction of immature myeloid cells in spleen sections to myeloperoxidase (MPO) was negative in the control group (Fig. 7A). The leukemia group demonstrated a high positive immunoreaction to MPO in immature myeloid cells in the spleen (Fig. 7B). Mild to moderate MPO positive immunoreactivity of immature myeloid cells in the spleen was seen in the Leuk+ CSE and Leuk+CP treated groups (Figs. 7C and 7D). Morphometrically, the area % of MPO positive immunoreaction in the leukemia group was substantially higher than in the control group. After CSE and CP therapy, it was much lower than in the leukemia group (table 3& Fig. 8C).

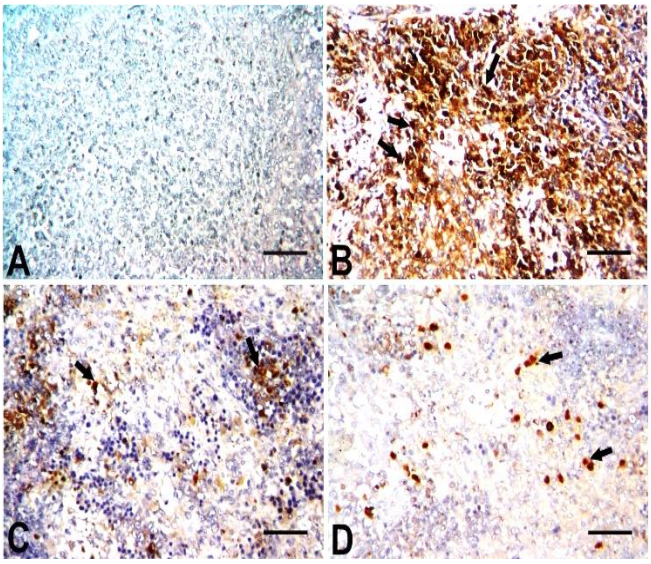
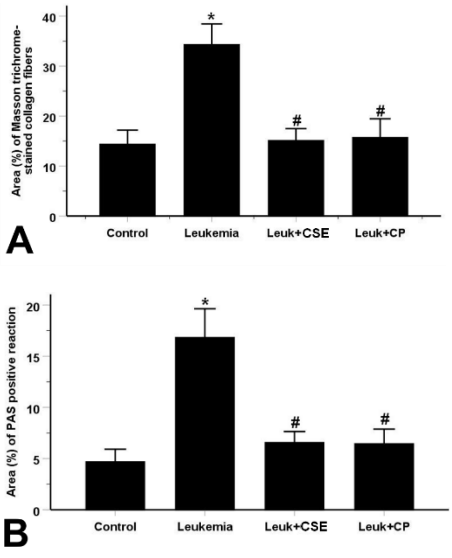



**Fig. (7):** photomicrograph of spleen sections of the study groups (Myeloperoxidase MPO immunoreaction). **A** Control group showing negative MPO immunoreaction of immature myeloid cells in spleen. **B** Leukemia group showing strong MPO positive immunoreaction of immature myeloid cells in spleen (black arrows). **C** Leuk+CSE treated group showing mild to moderate MPO positive immunoreaction of immature myeloid cells in spleen (black arrows). **D** Leuk+CP treated group showing mild to moderate MPO positive immunoreaction of immature myeloid cells in spleen (black arrows). Scale bar= 50µm.



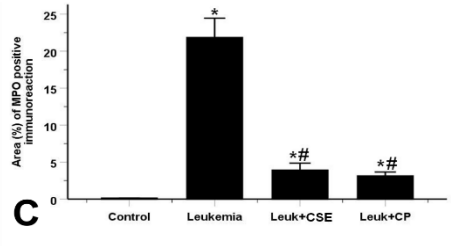



**Fig. (8):** Morphometric analysis (Mean± SEM) of H & E-stained spleen sections of; **A.** area % of Masson trichome-stained collagen fibers. **B.** area % of PAS positive reaction **C.** area % of MPO positive immunoreaction

## 4. Discussion

Leukemia remains a kind of cancer that impacts the blood and bone marrow, resulting in the uncontrollable multiplication of aberrant white blood cells [1]. Chemically, it is caused by chronic benzene exposure in the bone marrow; chronic benzene exposure has been associated to a range of hematological disorders, including aplastic anemia and acute myeloid leukemia [6, 12].

When the leukemia group was compared to the control group, the total blood count showed a significant increase (P 0.05) in WBC counts (marked leukocytosis) and a significant decrease (P 0.05) in RBC counts, Hb content, HCT%, and platelet count. These findings show that intravenous injections of benzene Chromasolv (0.2 mL twice a week) successfully induce leukemia in albino rats.

In the current study, the benzene induce-leukemic animals group demonstrated a significant increase in WBC count (leukocytosis) and demonstrated successful induction of leukemia in rats. This discovery is consistent with prior research [41, 42], which linked it to important molecular and cellular mechanisms that may lead to the development of myeloid leukemia. Following the event of exposure, benzene is mainly metabolized in the liver through cytochrome P450 2E1 and 2F1; however, its reactive metabolites like catechol, hydroquinone, benzoquinone, and others consider how benzene exerts its toxicity; benzene and its metabolites spread and accumulate in bone marrow tissue, where they produce their selective damage to hematopoietic stem cells or progenitor cells [43]. Various stages of hematopoietic cell differentiation are affected by benzoene metabolites. These interactions result in genomic instability, chromosomal, or epigenetic abnormalities, and altered hematopoietic stem cell proliferation and differentiation. Ultimately, this leads to the formation of mutated hematopoietic cells, which in turn cause clonal evolution to leukemia [27, 44].

Furthermore, occupational exposition to benzene has been demonstrated to produce oxidative stress in gas station staff. Furthermore, metabolic activation of benzene to metabolites that include hydroquinone and 1, 2, and 4-benzenetriol produces ROS, degrades the antioxidant defense system, and hence increases oxidative stress [45]. Benzene intoxication has been connected to a variety of disorders, many of which have pathologically substantial levels of hematological indices [46]. In the current study, benzene injection drastically affected hematological indicators. The rats in the control group had significantly lower levels of PCV. A low PCV level is frequently used to diagnose anemia. Benzene's hematotoxicity and chronic toxicity are the outcome of a series of biotransformation processes that begin with the creation of reactive intermediates.

In an animal model, benzene exposure decreased packed cell volume, which is a significant predictor of the amount of circulating erythrocytes and the degree of anemia or polycythemia, and produced leukocytosis [47]. Acute myelogenous leukemia patients often appear with nonspecific symptoms that develop gradually or abruptly as a result of anemia, leukocytosis, leucopoenia or leukocyte malfunction, or thrombocytopenia [48]. Benzene exposure has been demonstrated to reduce the number of WBC, RBC, PLT, and HGB concentration, which is compatible with findings Sun *et al*. [49]. Furthermore, earlier research has shown that giving mice 130, 260, 390, and 520 mg/kg benzene promotes aplastic anemia, a greater exposure dosage, and severe pancytopenia [49].

WBC counts fell in rats administered (350 mg/kg) CSE and (7.5 mg/kg/48 h) CP, whereas RBC counts, Hb content, hematocrit, and platelet count increased. Blood parameters enhanced in both treatment groups, showing that this natural material may have an anti-leukemic effect equivalent to anti-leukemic medications (CP). The current study's significant decrease in WBCs coincides with the findings of Salem *et al*. [50] and Alazzouni *et al*. [38], which attributed that antitumor impact to CP's likely ability to promote the immune-response by inducing the mobilization of hematopoietic stem cells and dendritic cells from bone marrow. Furthermore, phosphor-amide mustard (a highly effective cyclophosphamide metabolite that kills cancer cells) has been shown to alkalize cellular nucleic acids and decrease cancer cell growth and division [51].

Tea has become one of the most popular beverages in the world, especially green tea, which contains phenolic compounds including epigallocatechin gallate, catechins, and epicatechin gallate. The antioxidant activity of green tea polyphenols has been hypothesized as a potential mechanism for cancer prevention [52, 53]. Furthermore, the anti-leukemic effect of CSE might be explained by the fact that epigallocatechin-3-gallate (EGCG; the most potent catechin in green tea extract) can activate caspases and induce leukemic cell death [54]. EGCG produces oxidative stress in the AML cell line using the MPO/H2O2/halide pathway [55].

Kuo et al. [56] observed that drinking large amounts of green tea with more than 550 units of catechins reduces the risk of leukemia. EGCG induced necrosis-like cell death in chronic myeloid leukemia cells, outperforming apoptosis resistance [57]. The control group in the new study exhibits normal splenic architecture, with spleen parenchyma constituted of red and white pulp. The leukemia group's splenic outlines were changed, with irregular white and

crimson pulp contours. There was no marginal zone with neighboring vacuoles, and the white pulp components had atrophied. Red pulp with many foamy histiocytes and hemosiderin pigment deposits formed in tandem with dilated congested venous sinuses. Furthermore, the mean diameters of the central arteriole as well as the diameter of the splenic lymphoid follicle were significantly greater in the leukemia group than in the control group.

Acute inflammatory immune responses are a result of the overall pathological response to a toxicant, as discovered by Sureshkumar *et al*. [58]. The authors asserted that the irritative effect of benzene, the attraction of inflammatory cells, and the generation of several inflammatory mediators, reactive oxygen species (ROS), or free radicals were most likely the causes of these changes.

In this investigation, benzene chlorosolv was the source of the histopathological damage to the spleen. These findings are in line with those of Akanni *et al*. [59], who found that groups exposed to the carcinogen benzene displayed coronary congestion, severe vacuolar degeneration, necrosis of hepatocytes with cellular infiltration by mononuclear cells, and a range of heart lesions (from mild to marked). Hetal [60] also found that exposure to benzene damages liver tissues significantly, resulting in necrosis and sinusoidal dilatation. The agent's ability to damage DNA is connected to the many splenic lesions found in this study [61].

According to certain studies, benzene causes apoptosis, which is a potentially harmful effect of benzene that leads to proliferation, cytotoxicity, and diseases [62, 63 & 64]. Benzene has been demonstrated to stimulate the generation of reactive oxygen species (ROS), which destroys cell ultra-structures and induces cell death [65].

The presence of necrotic patches in a white pulp with foamy macrophages might suggest spleen toxicity, which would likely impede erythrocyte evacuation efficiency [66]. Because the spleen controls peripheral erythrocyte and platelet levels by eliminating old or damaged cells, these macrophages may include phagocytized faulty RBCs and platelets [67].

Benzene exposure affects the number of immune cells in the bone marrow, thymus, spleen, and liver [68] implying that benzene exposure is linked to immunosuppression [69]. The spleen, which serves as an immune cell reserve, is critical to the adaptive immune system [70]. The mechanism of benzene-induced spleen destruction contributes to an improved comprehension of benzene immune toxicity. Benzene can affect the spleen by promoting transgene mutation and lowering lymphocyte count [46, 68]. Gene mutations [46], increased oxidative stress [71], and chronic inflammation [69] are all linked to benzene-induced spleen dysfunction.

Surprisingly, CSE treatment of leukemic rats produced a significant anti-leukemic effect, as evidenced by a significant decline in WBCs that approached the normal control animal group. CSE's antioxidant and scavenging capabilities may contribute to CSE therapy's ameliorative activity on the toxic effects of Benzene Chromasolv on spleen structure. Antioxidants offer protection or repair by scavenging ROS that damage DNA and cause diseases such as cancer [72].

According to the results of Emara and El-Bahrawy's [73] study, drinking green tea during benzene exposure can reduce (or completely reverse/prevent) a variety of toxicities linked to benzene-induced oxidative stress in pump workers. As a consequence, green tea may be a prospective therapeutic treatment for reducing particular features of benzene-induced toxicity as a dietary supplement. The CP-induced oxidative stress in rats triggered many intracellular signaling pathways, resulting in an increase in pro-inflammatory cytokine production. The release of proteolytic enzymes and free radicals induced the accumulation of inflammatory neutrophils, macrophages, and lymphocytes [16, 74]. According to Abdelaziz et al. [75], CP promotes oxidative damage by decreasing antioxidant enzyme activity and increasing lipid peroxidation. Oxidative damage can cause apoptosis, or programmed cell death (Wu et al., 2019).

The leukemia group showed thick collagen fibers in the trabeculae surrounding the trabecular sinus and in the splenic stroma, with an elevated mean area percent, according to continuing study. CSE and CP treatment groups indicated few thin blue-colored collagen fibers dispersed in trabeculae and splenic stroma with a lower mean area percent. Organ failure and dysfunction are caused by an increase in extracellular matrix proteins in fibrotic disorders such pulmonary silicosis. The responsible cells for the development of fibrotic processes have been identified as myofibroblasts derived from different sources, such as quiescent tissue fibroblasts and epithelial or endothelial cells. These cells up-regulate the expression of α-SMA and increase the synthesis of extracellular matrix proteins, such as different types of collagen [76].

According to continuing studies, the leukemia group reacted strongly (PAS positive) and had a higher mean area percent to the total amount of carbohydrates in splenic white pulp. With a lower mean area percent, the CSE and CP treated groups' total carbohydrate content in splenic white pulp exhibited a mild PAS positive reaction.

The leukemia group had a higher mean area% and a substantial MPO positive immunoreaction of immature myeloid cells in the spleen in the current study. MPO positive immunoreactions of immature myeloid cells in the spleen were mild to moderate in the CSE and CP therapy groups, and the mean area percent was decreased. The presence of extramedullary hematopoiesis (EMH) cells and many apoptotic hematopoietic cells in the spleens of benzene-treated rats was the most noticeable histological abnormality in Elazab et al.'s (2022) investigation. Furthermore, benzene changed the form and organization of the spleen, resulting in the accumulation of megakaryocytes. Because the spleen was unable to function as an alternate hematopoietic organ with platelets, liberation was hampered, and megakaryocytes accumulated in the spleen [77].

Previous studies by Johns and Christopher [78] and Chiu *et al*. [79] revealed that pathophysiological alterations in hematopoietic stem/progenitor cells, as well as the ectopic emergence of their niche in these tissues—a process known as EMH—cause hematopoiesis to occur outside the BM, including the liver and spleen.

It has been discovered that the antioxidant effect of green tea (GT) is attributed to its bioactive phytochemicals [80]. Additionally, green tea polyphenols, or catechins, were shown by Shimizu *et al*. [81] to have anti-inflammatory and anti-oxidant qualities.

Because polyphenols, particularly EGCG, can block enzymes involved in DNA synthesis and cell division, they have chemopreventive properties [82]. Additionally, EGCG increased leukemia cell line apoptosis while blocking DNA replication and vascular endothelial growth factor (VEGF) [54, 83].

## Conclusions

Because *Camellia sinensis* extract (CSE) greatly reduces the percentage of blasts entering the spleen and restores normal total WBC, RBC, and platelet counts in peripheral blood, it may be used to treat rats with artificially created leukemia. Moreover, much of the splenic histology linked to leukemia was improved by CSE. The antioxidant qualities of the phytochemical components in CSE may be responsible for its anti-leukemic effects.

## Acknowledgment

The authors would like to express their gratitude to the Zoology Department's authorities at Helwan University in Cairo, Egypt's Faculty of Science.

## Ethical approval

Every relevant institutional, national, and/or international guideline pertaining to the use and care of animals was adhered to. None of the authors' studies involving human subjects are included in this article. The Institutional Animal Care and Use Committee for Laboratory Animals gave its approval to the experiment. The Science Faculty's Zoology Department of Helwan University has received permission (HU-IACUC/Z/SR2910-37).

## References

1. Chauhan, S., Rai, S., and Huddar, V.G. A review on clinical and experimental studies on Ayurveda and leukemia. Medical Journal of Dr. DY Patil Vidyapeeth, 2022,15(2), 158.

2. Javed, F., Utreja, A., Correa, F. O. B., Al-Askar, M., Hudieb, M., Qayyum, F., Al-Rasheed, A., Almas, K. and AlHezaimi, K. Oral health status in children with acute lymphoblastic leukemia. Critical reviews in oncology/hematology, 2012, 83, 303-309.

3. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., and Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians, 2018, 68(6), 394-424.

4. Kassahun, W., Tesfaye, G., Bimerew, L. G., Fufa, D., Adissu, W., and Yemane, T. Prevalence of leukemia and associated factors among patients with abnormal hematological parameters in Jimma Medical Center, Southwest Ethiopia: A cross-sectional study. Advances in Hematology, 2020, 1-7.

5. Varkesh, H., Mokhtari, N., Moeini, M., Baser, R. S., Masoomi, Y., and Moeini, M. The dentist's role in improving the life's quality of children with leukemia. American Journal of Research Communication, 2013, 1, 66-7.

6. Bruzzoni-Giovanelli, H., Gonzalez, J. R., Sigaux, F., Villoutreix, B. O., Cayuela, J. M., Guilhot, J., and Rousselot, P. Genetic polymorphisms associated with increased risk of developing chronic myelogenous leukemia. Oncotarget, 2015, 6(34), 36269-36277.

7. Balasubramaniam, G., Saoba, S. L., Sarhade, M. N., and Kolekar, S. A. Lifestyle factors including diet and leukemia development: a casecontrol study from Mumbai, India. Asian Pacific Journal of Cancer Prevention, 2013, 14(10), 5657-5661.

8. Sonoda, T., Nagata, Y., Mori, M., Ishida, T., Imai, K. Meta-analysis of multiple myeloma and benzene exposure. J Epidemiol., 2001, 11(6), 249-54.

9. Schettgen, T., Ochsmann, E., Alt, A., and Kraus, T. A biomarker approach to estimate the daily intake of benzene in non-smoking and smoking individuals in Germany. Journal of Exposure Science & Environmental Epidemiology, 2010, 20(5), 427-433.

10. Bahadar, H., Mostafalou, S., and Abdollahi, M. Current understandings and perspectives on non-cancer health effects of benzene: a global concern. Toxicology and applied pharmacology, 2014, 276(2), 83-94.

11. Arnold, S.M., Angerer, J., Boogaard, P. J., Hughes, M. F., O'Lone, R. B., Robison, S. H., and Robert Schnatter, A. The use of biomonitoring data in exposure and human health risk assessment: benzene case study. Critical Reviews in Toxicology, 2013, 43(2), 119-153.

12. Wei, C., Chen, M., You, H., Qiu, F., Wen, H., Yuan, J., and Yang, X. Formaldehyde and co-exposure with benzene induce compensation of bone marrow and hematopoietic stem/progenitor cells in BALB/c mice during post-exposure period. Toxicology and Applied Pharmacology, 2017, 324, 36-44.

13. Jabir, N. R., Tabrez, S., Ashraf, G. M., Shakil, S., Damanhouri, G. A., & Kamal, M. A. Nanotechnology-based approaches in anticancer research. International journal of nanomedicine, 2012, 7, 4391–4408.

14. Al-Mousaw, M., Bustani, G. S., Barqaawee, M. J. A., and AL-Shamma, Y. M. Evaluation of histology and sperm

parameters of testes treated by in cyclophosphamide - induced cystitis of the lycopene against cyclophosphamide that induced testicular toxicity in Male rats. AIP Conference Proceedings, 2022, 2386(1), 020040.

15. Alhowail, A., Sajid, S., Almogbel, Y., Rabbani, S., and Alsharidah, M.J.J.o. P.R.I. Effect of Cyclophosphamide and Its Combination with Metformin on the Survival Rate in Mice. Journal of Pharmaceutical Research International, 2019, 30(2), 1-6.

16. Abdel-Hafez, S.M.N., Rifaai, R.A., and Abdelzaher, W.Y. Possible protective effect of royal jelly against cyclophosphamide induced prostatic damage in male albino rats; a biochemical, histological and immuno-histochemical study. Biomed. Pharma-cother. 2017, 90, 15-23.

17. Deng, J., Zhong, Y.F., Wu, Y.P., Luo, Z., Sun, Y.M., Wang, G.E., and He, R.R. Carnosine attenuates cyclophosphamide-induced bone marrow suppression by reducing oxidative DNA damage. Redox biology, 2018, 14, 1-6.

18. Ming, Z., Yongqiang, Z., Zijin, Z., Yan, X., Di, C., and Xiaoxin, T. Severe and prolonged cyclophosphamide-induced hepatotoxicity in a breast cancer patient carrying a CYP2B6* 7 variant. Pharmacogenomics, 2019, 20(16), 1119-1124.

19. Mills, K. A., Chess-Williams, R., and McDermott, C. Novel insights into the mechanism of cyclophosphamide-induced bladder toxicity: chloroacetaldehyde's contribution to urothelial dysfunction in vitro. Archives of Toxicology, 2019, 93(11), 3291-3303.

20. Sheth, V.G., Navik, U., Maremanda, K.P., and Jena, G. Effect of diethyldithiocarbamate in cyclophosphamide-induced nephrotoxicity: Immunohistochemical study of superoxide dismutase 1 in rat. Indian Journal of Pharmacology, 2018, 50(1), 4.

21. Kurauchi, K., Nishikawa, T., Miyahara, E., Okamoto, Y., and Kawano, Y. Role of metabolites of cyclophosphamide in cardiotoxicity. BMC research notes, 2017, 10(1), 1-10.

22. Clifton, K., Barnett, C., Martinez, A., Karuturi, M., Saleem, S., & Litton, J. Severe hyponatremia following cyclophosphamide infusion in breast cancer patients. The breast journal, 2018, 24(4), 691.

23. Khan, M.K., Ansari, I.A., and Khan, M.S. Dietary phytochemicals as potent chemotherapeutic agents against breast cancer: Inhibition of NF-κB pathway via molecular interactions in rel homology domain of its precursor protein p105. Pharmacognosy magazine, 2013, 9(33), 51-57.

24. Balentine, D. A., Wiseman, S. A., and Bouwens, L. C. The chemistry of tea flavonoids. Critical reviews in food science and nutrition, 1997, 37(8), 693-704.

25. Fujiki, H., Sueoka, E., and Watanabe, T. Synergistic enhancement of anticancer effects on numerous human cancer cell lines treated with the combination of EGCG, other green tea catechins, and anticancer compounds. Journal of Cancer Research and Clinical Oncology, 2015, 141(9), 1511-1522.

26. Del Rio D., Stewart A.J., Mullen W., Burns J., Lean M.E.J., Brighenti F. and Crozier A. HPLC-MSnAnalysis of Phenolic Compounds and Purine Alkaloids in Green and Black Tea. J. Agric. Food Chem., 2004, 52, 2807-2815.

27. Musial, C., Kuban-Jankowska, A., and Gorska-Ponikowska, M. Beneficial properties of green tea catechins. International journal of molecular sciences, 2020, 21(5), 1744.

28. Maiti, S., Nazmeen, A., Medda, N., Patra, R., & Ghosh, T. K. Flavonoids green tea against oxidant stress and inflammation with related human diseases. Clinical Nutrition Experimental, 2019, 24, 1–14. https://doi.org/10.1016/j.yclnex.2018.12.004

29. Mokra, D., Joskova, M., & Mokry, J. Therapeutic Effects of Green Tea Polyphenol (–)-Epigallocatechin-3-Gallate (EGCG) in Relation to Molecular Pathways Controlling Inflammation, Oxidative Stress, and Apoptosis. International journal of molecular sciences, 2022, 24(1), 340.

30. Almatroodi, S.A.; Almatroudi, A.; Khan, A.A.; Alhumaydhi, F.A.; Alsahli, M.A.; Rahmani, A.H. Potential Therapeutic Targets of Epigallocatechin Gallate (EGCG), the Most Abundant Catechin in Green Tea, and Its Role in the Therapy of Various Types of Cancer. Molecules, 2020, 25, 3146.

31. Romano, A. and Martel, F. The Role of EGCG in Breast Cancer Prevention and Therapy. Mini Rev. Med. Chem., 2021, 21, 883-898.

32. Voloshin, V.N., Koveshnikov, V.G., and Voloshina, I.S. Morphology of the spleen in adult albino rats after whole-body exposure to low-level of toluene. Int. J. Anat. Res., 2014, 2(2), 421-430.

33. Cesta, M.F. Normal structure, function, and histology of the spleen. Toxicologic Pathol.,2006, 34, 455-465.

34. Omman, R.A., Kini, R.A. Acute leukemias. In: Keohane, E.M., Otto, C.N., Walenga, J.M. (Eds.), Rodak's Hematology (6th Ed.), Elsevier, 2020, pp. 540-54.

35. Kim, Y., Yoon, S., Kim, S.J., Kim, J.S., Cheong, J.W., and Min, Y.H. Myeloperoxidase expression in acute myeloid leukemia helps identifying patients to benefit from transplant. Yonsei medical journal, 2021, 53(3), 530-536.

36. Pakkirisamy, M., Kalakandan, S.K. and Ravichandran, K. Phytochemical screening, GC-MS, FT-IR analysis of methanolic extract of curcuma caesia Roxb (Black turmeric). Pharmacog. J.,2017, 9(6), 952-956.

37. Akanni, E. O., Adedeji, A. L., Adedosu, O.T., Olaniran, O. I., and Oloke, J. K. Chemopreventive and antileukemic effects of ethanol extracts of Moringa oleifera leaves on wistar rats bearing benzene induced leukemia. Current pharmaceutical biotechnology, 2014a, 15(6), 563-568.

38. Alazzouni, A., Al-Brakati, A., Rabie, S., Gabry, M., and Hassan, B. Effects of Moringa oleifera, Curcumin, and Green Tea Extracts on Histopathological Changes in Mesenteric

Lymph Nodes and Spleen of Albino Rats with Benzene-Induced Leukemia. Pakistan Journal of Zoology, 2021, 54(4), 1791-1802.

39. Suvarna, S.K., Layton, C., Bancroft, J.D., Eds. Bancroft's theory and practice of histological techniques. 2018, 8th Ed. Amsterdam: Elsevier.

40. Layton, C., Bancroft, J.D., Suvarna, S.K. 4-Fixation of tissues. In: Suvarna, S.K., Layton, C., Bancroft, J.D. (Eds.) Bancroft's theory and practice of histological techniques, 2019, (8th Ed.), Elsevier, pp. 40–63.

41. Olufemi, A. E., Folarin, O. R., and Igbeneghu, C. Tumour suppressive and organ protective effects of aqueous andrographis paniculata leaves extract on benzene induced leukaemia bearing rats. Annual Research & Review in Biology,2014, 1070-1079.

42. Li, L., Cui, Y., Shen, J., Dobson, H., and Sun, G. Evidence for activated Lck protein tyrosine kinase as the driver of proliferation in acute myeloid leukemia cell, CTV-1. Leukemia research, 2019, 78, 1220.

43. Khalade, A., Jaakkola, M. S., Pukkala, E., and Jaakkola, J.J. Exposure to benzene at work and the risk of leukemia: a systematic review and meta-analysis. Environmental Health, 2010, 9(1), 1-8.

44. Roy, S., Deb, N., Basu, S., and Besra, S. E. Apoptotic activity of ethanolic extract of Moringa oleifera root bark on human myeloid leukemia cells via activation of caspase cascade. World Journal of Pharmaceutical Science, 2014, 3(10), 1138-1156.

45. Uzma, N., Kumar, B.S., and Hazari, M.A.H. Exposure to benzene induces oxidative stress, alters the immune response and expression of p53 in gasoline filling workers. American journal of industrial medicine, 2010, 53(12), 1264-1270.

46. Spatari G., Allegra A., Carrieri M., Pioggia G., and Gangemi S. Epigenetic Effects of Benzene in Hematologic Neoplasms: The Altered Gene Expression. Cancers (Basel), 2021, 13(10), 2392.

47. Akanni, E.O., Adedeji, A.L., Akanni, R.A. and Oloke, J.K., Up-regulation of TNF-α by ethanol extract of Moringa oleifera in benzene-induced leukemic wister rat: A possible mechanism of anticancer property. Cancer Res., 2014b, 74, 3792.

48. Sharma, M. and Porte, S.M. Role of Ayurveda in management of leukemia (Raktarbuda). IJPSR, 2016 7(2), 520-530.

49. Sun, R., Xu, K., Ji, S., Pu, Y., Man, Z., Ji, J., Chen, M., Yin, L., Zhang, J., and Pu, Y. Benzene exposure induces gut microbiota dysbiosis and metabolic disorder in mice. The Science of the total environment, 2020, 705, 135879.

50. Salem, M.L., Al-Khami, A. A., El-Naggar, S.A., Diaz-Montero, C.M., Chen, Y., and Cole, D.J. Cyclophosphamide induces dynamic alterations in the host microenvironments resulting in a Flt3 ligand-dependent expansion of dendritic cells. The journal of immunology, 2010, 184(4), 1737-1747.

51. Jiang, X., Ren, Z., Zhao, B., Zhou, S., Ying, X., and Tang, Y. Ameliorating effect of pentadecapeptide derived from cyclina sinensis on cyclophosphamide- induced nephrotoxicity. Marine drugs, 2020, 18(9), 462.

52. Hou, Z., Sang, S., You, H., Lee, M. J., Hong, J., Chin, K. V., and Yang, C. S. Mechanism of action of (-)-epigallocatechin-3-gallate: auto-oxidation-dependent inactivation of epidermal growth factor receptor and direct effects on growth inhibition in human esophageal cancer KYSE 150 cells. Cancer research, 2005, 65(17), 8049-8056.

53. Butt, M.S. and Sultan, M. T. Green tea: nature's defense against malignancies. Critical reviews in food science and nutrition, 2009, 49(5), 463-473.

54. Lee, Y. K., Bone, N. D., Strege, A. K., Shanafelt, T. D., Jelinek, D. F., & Kay, N. E. VEGF receptor phosphorylation status and apoptosis is modulated by a green tea component, epigallocatechin-3-gallate (EGCG), in B-cell chronic lymphocytic leukemia. Blood, 2004, 104(3), 788–794.

55. Nakazato, T., Ito, K., Miyakawa, Y., Kinjo, K., Yamada, T., Hozumi, N., Ikeda, Y., & Kizaki, M. Catechin, a green tea component, rapidly induces apoptosis of myeloid leukemic cells via modulation of reactive oxygen species production in vitro and inhibits tumor growth in vivo. Haematologica, 2005, 90(3), 317–325.

56. Kuo, Y.C., Yu, C.L., Liu, C.Y., Wang, S.F., Pan, P.C., Wu, M.T., Ho, C.K., Lo, Y.S., Li, Y. and Christiani, D.C., (). A population-based, case–control study of green tea consumption and leukemia risk in southwestern Taiwan. Cancer Causes Contr., 2009, 20, 57-65.

57. Yao, S., Zhong, L., Chen, M., Zhao, Y., Li, L., Liu, L., Xu, T., Xiao, C., Gan, L. and Shan, Z.,. Epigallocatechin-3-gallate promotes alltransretinoic acid-induced maturation of acute promyelocytic leukemia cells via PTEN. Int. J. Oncol., 2017, 51, 899-906.

58. Sureshkumar, V., Paul, B., Uthirappan, M., Pandey, R., Sahu, A.P., Lal, K., Prasad, A.K., Srivastava, S., Saxena, A. and Mathur, N. Proinflammatory and anti-inflammatory cytokine balance in gasoline exhaust induced pulmonary injury in mice. Inhalation Toxicol., 2005, 17, 161-168.

59. Akanni, E.O., Folarin, O.R. and Igbeneghu, C. Tumour suppressive and organ protective effects of aqueous andrographis paniculata leaves extract on benzene induced leukaemia bearing rats. Annual Research & Review in Biology, 2014c, 4(7), 1070-1079.

60. Hetal, R. Evaluation of benzene induced histopathological alterations in the rat. Int. J. Adv. Res., 2016, 4, 563-567.

61. Nakajima, T., Vares, G., Ninomiya, Y., Wang, B., Katsube, T., Tanaka, T., Maruyama, K. and Nenoi, M., Diallyl disulfide mitigates DNA damage and spleen tissue effects after irradiation. Int. med. J. exp. clin. Res., 2019, 25, 8920-8927.

62. Chen, Y., Sun, P., Guo, X., & Gao, A. MiR-34a, a promising novel biomarker for benzene toxicity, is involved

in cell apoptosis triggered by 1,4-benzoquinone through targeting Bcl-2. Environmental pollution (Barking, Essex: 1987), 2017, 221, 256–265.

63. Chen, Y., Sun, P., Bai, W., & Gao, A. MiR-133a regarded as a potential biomarker for benzene toxicity through targeting Caspase-9 to inhibit apoptosis induced by benzene metabolite (1,4-Benzoquinone). The Science of the total environment, 2016, 571, 883–891.

64. Qian, S., Han, Y., Shi, Y., Xu, W., Zhu, Y., Jiang, S., Chen, Y., Yu, Z., Zhang, S., Yang, Y., Yu, K., & Zhang, S. Benzene induces haematotoxicity by promoting deacetylation and autophagy. Journal of cellular and molecular medicine, 2019, 23(2), 1022–1033.

65. Chen, Y., Zhang, W., Guo, X., Ren, J., & Gao, A. The crosstalk between autophagy and apoptosis was mediated by phosphorylation of Bcl-2 and beclin1 in benzene-induced hematotoxicity. Cell death & disease, 2019, 10(10), 772.

67. Tavasoli, M. Structure and functions of the spleen. In: Williams, J.W., Beutler, E., 66. Erslev, A.J., Litchman, M.A., eds. Hematology. 4th Ed., 1991, Ney York, NY: McGraw-Hill. p. 54–62.

68. Karaulov, A.V., Mikhaylova, I.V., Smolyagin, A.I., Boev, V.M., Kalogeraki, A., Tsatsakis, A. M., and Engin, A. B. The immunotoxicological pattern of subchronic and chronic benzene exposure in rats. Toxicology letters, 2017, 275, 1-5.

69. Guo, H., Ahn, S., and Zhang, L. Benzene-associated immunosuppression and chronic inflammation in humans: a systematic review. Occupational and environmental medicine, 2020, oemed-2020-106517.

70. Lewis Steven, M., Williams, A. and Eisenbarth Stephanie, C., Structure and function of the immune system in the spleen. Sci. Immunol., 2019, 4 (33), eaau6085.

71. Amin, M.M., Rafiei, N., Poursafa, P., Ebrahimpour, K., Mozafarian, N., Shoshtari-Yeganeh, B., Hashemi, M., & Kelishadi, R. Association of benzene exposure with insulin resistance, SOD, and MDA as markers of oxidative stress in children and adolescents. Environ. Sci. Pollut. Res. Int., 2018, 25 (34), 34046–34052.

72. Aziz, M.A., Diab, A.S. and Mohammed, A.A., Antioxidant categories and mode of action. In: Antioxidants. InTech Open. 2019, doi: 10.5772/intechopen.83544.

73. Emara, A. M., and El-Bahrawy, H. Green tea attenuates benzene-induced oxidative stress in pump workers. Journal of immunotoxicology, 2008, 5(1), 69-80.

74. Hamsa, T.P., and Kuttan, G. Ipomoea obscura ameliorates cyclophosphamide-induced toxicity by modulating the immune system and levels of proinflammatory cytokine and GSH. Can. J. Physiol. Pharmacol., 2010, 88, 1042-1053.

75. Abdelaziz, T.E., Borai, E.-B.N., Kamal, K.H., Hanem, E.-G.J.G.B., and Sciences, P. Contribution of garlic for improving the cytoprotective effect of mesna against cyclophosphamide toxicity in rats. GSC Biological and Pharmaceutical Sciences,2019, 07(03), 008–020.

76. Kendall, R.T. and Feghali-Bostwick, C.A. (). Fibroblasts in fibrosis: Novel roles and mediators. Front. Pharmacol., 2014, 5, 123.

77. Boll, I.T., Domeyer, C., Bührer, C. Human megakaryoblastic proliferation and differentiation events observed by phase-contrast cinematography. Acta Haematol., 1997, 97, 144-52.

78. Johns, J. and Christopher, M.M. Extramedullary hematopoiesis: a new look at the underlying stem cell niche, theories of development, and occurrence in animals. Vet. Pathol., 2012, 49, 508-23.

79. Chiu, S.C., Liu, H. H., Chen, C.L., Chen, P.R., Liu, M.C., Lin, S.Z. and Chang, K.T. Extramedullary hematopoiesis (EMH) in laboratory animals: offering an insight into stem cell research. Cell transplantation, 2015, 24(3), 349-366.

80. Serafini, M., Del Rio, D., Yao, D.N., Bettuzzi, S, and Peluso, I. Chapter 12. Health benefits of tea. In: Benzie, I.F.F., Wachtel-Galor, S. (Eds). Herbal Medicine: Biomolecular and Clinical Aspects, 2011, 2nd Ed., CRC Press\Taylor & Francis: Boca Raton (FL).

81. Shimizu, M., Shirakami, Y., Sakai, H., Kubota, M., Kochi, T., Ideta, T., Miyazaki, T., and Moriwaki, H. Chemopreventive potential of green tea catechins in hepatocellular carcinoma. International journal of molecular sciences, 2015, 16(3), 6124-6139.

82. Fujiki, H., Watanabe, T., Sueoka, E., Rawangkan, A. and Suganuma, M. Cancer Prevention with Green Tea and Its Principal Constituent, EGCG: From Early Investigations to Current Focus on Human Cancer Stem Cells. Mol Cells, 2018, 41, 73-82.

83. Smith, D.M. and Dou, Q.P. Green Tea Polyphenol Epigallo catechin Inhibits DNA Replication and Consequently Induces Leukemia Cell Apoptosis. Int. J. Mol. Med., 2001, 7: 645-652.

.