Possible Protective Effect of Spanish Black Radish on Hematological Parameters aganist Benzene - Induced Leukemia in Rats

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Abstract

Background: The Spanish black radish, which is grown all over the world and is full of useful nutrients, helps in the therapy of many illnesses. **Objective:** The aim of this study was to evaluate the possible protective effect of Spanish black radish extracts against benzene-induced leukemia in rats. **Materials and Methods:** A total of 35 rats were purchased from the animal house at the College of Pharmacy, University of Karbala, Iraq. Benzene was used for the induction of leukemia in experimental animals. An extract of black radish was set after mixing up 50 g of the finely powdered plant material with two solvents (500 mL of 100% ethanol or water). After leukemia induction, rats were randomly divided into seven groups: first group, negative control group; second group, positive control group; third group, (lack radish aqueous extract 100 mg/kg; fourth group, black radish aqueous extract 200 mg/kg; fifth group, black radish ethanol extract 100 mg/kg; sixth group, black radish ethanol extract 200 mg/kg; and seventh group, cyclophosphamide 5 mg/kg. **Results:** Leukemia induction resulted in a severe increase in the total leukocyte count and a severe decrease in red blood cells, hemoglobin, and platelet levels of the experimental animals. However, there were no significant differences between the negatively treated group and the benzene-treated group regarding platelet count. Posttreatment with Spanish black radish extracts, especially the aqueous plant extract, shows an improvement in blood profiles compared to the benzene-treated group. **Conclusion:** Our study indicates that Spanish black radish might be a promising natural, antileukemic source.

Keywords: Benzene-induced leukemia, cyclophosphamide, platelet count, red blood cells, Spanish black radish, white blood cells

Background

Cancer is generally considered a lethal disease characterized by the uncontrolled growth and replication of cancer cell.[1] Cancer is considered as the second leading cause of death around the world, especially in developed countries, and the third leading cause of death in less-developed countries.[2,3] One of the most prevalent malignant diseases impacting people worldwide is leukemia. Leukemia was the fifteenth most often diagnosed cancer worldwide in 2018, with 437,033 cases and 309,006 fatalities, making it the eleventh cause of death from malignant illnesses.[4] A somatic mutation in the DNA causes leukemia, a malignancy of the blood or bone marrow, which is distinguished by an abnormal increase in blood cells, typically leucocytes. By turning on oncogenes or turning off tumor suppressor genes, certain mutations can cause leukemia by upsetting the balance between the control of cell death, differentiation, or division. These mutations are likely to be affected by genetic factors after expoure to radiation and carcinogens.[5]

Acute and chronic are the two categories of leukemia. Acute lymphoblastic leukemia is the most common type of diffuse bone marrow cancer among hematologic malignancies.[6] According to the reports, 6000 patients are diagnosed with this ailment each year, and approximately

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20-30% of all acute lymphoblastic leukemia(ALL) cases occur in patients older than 55-60 years.[7,8] Even though there are several treatments for this condition, acute lymphoblastic leukemia still has a relapse rate of between 15% and 20%.[9] Cyclophosphamide (CP) has demonstrated improved efficacy in individuals with acute lymphoblastic leukemia. According to Netíková *et al*.,[10] CP a heterocyclic amide nitrogen mustard. It is effective against a wide range of cancers, including ovarian, breast, lung, prostate, and leukemia.[11] Cytotoxic medication side effects are a significant barrier to effective treatment.

Our ability to fight cancer has been improved by using natural materials derived from a variety of sources, including plants, animals, and microbes. Around 60% of the current anticancer/antitumor medications were organically produced from these sources.[12] Plant products interact with cancer cells and the tumor microenvironment by affecting various biochemical and molecular cascades due to their complex nature.[13,14] Additionally, they are typically inexpensive and safe.

Black radish, primarily grown in Europe, is used in traditional medicine. Its roots, sprouts, seeds, and leaves have hepatoprotective, antioxidant, and anti-inflammatory properties.[15-17] These advantageous properties are frequently linked to its phenolic components, flavonoids, glucosinolates, and sulfur compounds.[18] Recently, there has been a rise in interest in diets enhanced with potentially bioactive natural substances that have anticancer characteristics. There is growing interest in diets containing bioactive natural substances with potential anticancer properties, specifically due to the presence of glucosinolates - secondary metabolites knowqn for their anti cancer effects.[19] Therefore, this study aimed to assess the anticancer activity of black radish extract against benzene-induced leukemia in male rats.

Materials and Methods

Black radish extract preparation

Black radish seeds were purchased from the Seed's valley center in Kerbala and then planted in the plant nursery between January 2022 and March 2022 [Figure 1]. Grown radish was then collected, transported to the lab, and rinsed in tap water to remove any surface contamination. The whole plant was dried and then grounded into a coarse powder. The extract of the plant under study was set after mixing up 50 g of the finely powdered plant material with two solvents (500 mL of 100% ethanol or water). An electric mixer was used to complete the mechanical crushing before the mixture was allowed to soak for 48 h at a temperature of 4°C. After filtering, the mixture was progressively dried in an air incubator at 37°C. Until usage, the extracts were kept in a firmly closed plastic container.

Animals and study procedure

A total of 35 male albino strain rats with an average weight of 199.8 g at the beginning of the study were purchased from the animal house of the College of Pharmacy, University of Karbala. The rats were kept in standard cages and subjected to the animal house conditions of the College of Veterinary Medicine, University of Karbala animal house with free access to food and water throughout the study. The rats were allowed to acclimatize for 7 days before the experiment.

By giving intravenous injections of diluted benzene solution every 2 days for 2 months, leukemia was induced in rats according to the procedure described in a previous study.[20] Regular hematological evaluation is a diagnostic technique for tracking the clinical state of blood-related diseases such as leukemia. Reduced red blood cells (RBCs), hemoglobin (Hg) contents, packed cell sizes, and platelet (PLT) counts are typical symptoms of the leukemogenic action of benzene in both human and animal models.[21]

After leukemia induction, the rats were randomly divided into seven groups (five rats per group):

1. Negative control group (untreated group), which received only distilled water for 30 days.
2. Positive control group [benzene (Bz)-treated group], which received only distilled water for 30 days.
3. Bz + Eq100, which received aqueous plant extract at a dose of 100 mg/kg orally for 30 days after benzene treatment.

Figure 1: Stages of growth-seed germination to a complete plant

4. Bz + Eq200, which received aqueous plant extract at a dose of 200 mg/kg orally for 30 days after benzene treatment.
5. Bz + Et100, which received ethanol plant extract at a dose of 100 mg/kg orally for 30 days after benzene treatment.
6. Bz + Et200, which received ethanol plant extract at a dose of 200 mg/kg orally for 30 days after benzene treatment.
7. Bz + CP, which received-cyclophosphamide at a dose of 5 mg/kg intraperitoneally every 48 h for 30 days after benzene treatment.

Hematological analyses

Hematological analysis of the blood samples was carried out using an automated hematology analyzer. The hematological parameters recorded were the total white blood cells (WBCs), RBCs, Hb concentration, hematocrit (HCT), and PLT count.

Statistical analysis

Statistical analysis was carried out using one-way analysis of variance test and post hoc Tukey test with a P value of <0.05 being the threshold for statistical significance among the group. The data are displayed as mean ± SE.

Table 1: Qualitative and quantitative disclosures for black radish aqueous extracts

		Measuring unit (mg/kg)
1	Alkaloids	20.611
2	Phenolics	15.118
3	Tannins	1.882
4	Flavonoids	10.107
5	Saponins	7.966

Ethical approval

The study was conducted in conformity with the moral guidelines that the Helsinki Declaration served as the foundation for. On December 16, 2021, a local ethics committee examined and approved the research protocol using the reference number 4347 to receive this permission.

Results

The active ingredients in the aqueous extract of Spanish black radish were qualitatively assessed using colorimetric assays, and the presence of active metabolites was evidenced for alkaloids, phenols, tannins, flavonoids, and saponins [Table 1]. The aqueous extract of spanish black radish is a dependable source of phenols, flavonoids and saponin. however , tannins are found to be the least active ingredient present in this extract while alkaloids are abundant.

The outcomes of hematological parameters of different experimental groups are presented in Figures 2–6. Figures 2–6 show the statistical comparisons of various hematological parameters between the negative control group and the experimental groups.

Benzene has been demonstrated to be an effective way for inducing leukemia. The current data show that the benzene-induced leukemia group (positive control) had significantly decreased hematological parameters (RBC counts, Hb, and HCT) as compared to the negative control group. However, the total blood count revealed a significant increase ($P < 0.05$) in the total WBC counts when comparing the positive control group (9.080 ± 1.065) with the negative control group (4.440 ± 0.102) [Figures 2–6].

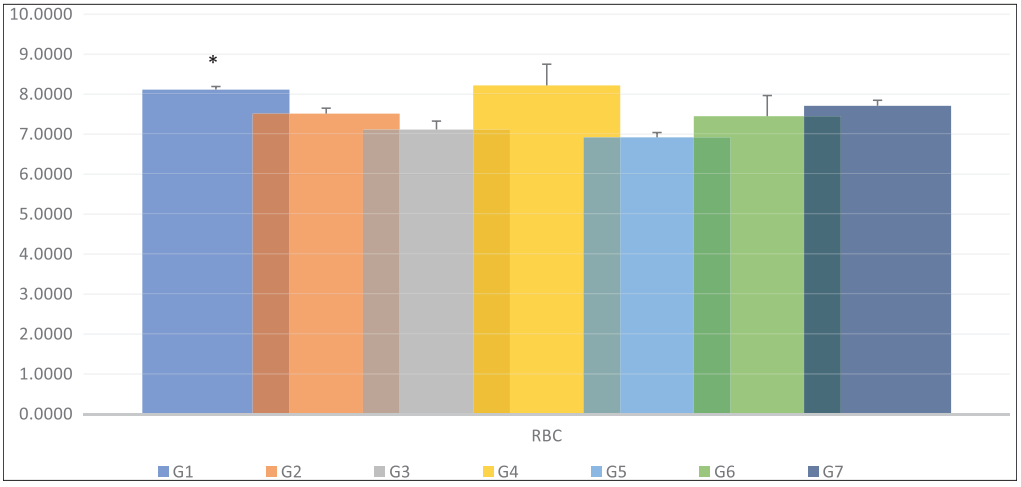

Figure 2: Comparison of the total red blood cell (RBC) count after treatment with black radish extracts between the positive control group (G2) and the other treatment groups: negative control group (G1), group treated with 100 mg/kg of aqueous extract (G3), group treated with 200 mg/kg of aqueous extract (G4), group treated with 100 mg/kg of ethanol extract (G5), group treated with 200 mg/kg of ethanol extract (G6), and cyclophosphamide-treated group (G7). Data are displayed as mean ± SE ($n = 5$). *$P < 0.05$ compared with benzene-induced leukemia rats

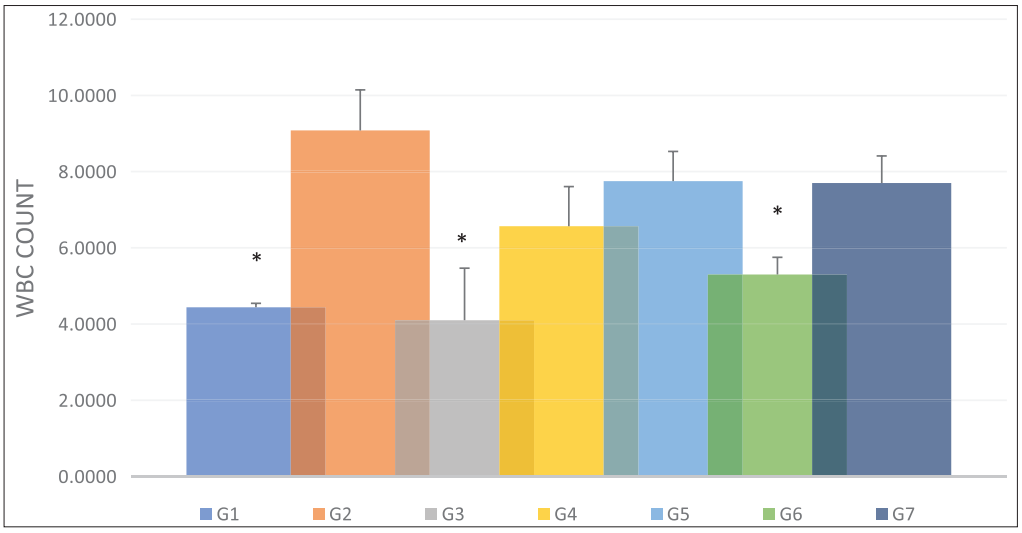

Figure 3: Comparison of the total white blood cell (WBC) count after treatment with black radish extracts between the positive control group (G2) and the other treatment groups: negative control group (G1), group treated with 100 mg/kg of aqueous extract (G3), group treated with 200 mg/kg of aqueous extract (G4), group treated with 100 mg/kg of ethanol extract (G5), group treated with 200 mg/kg of ethanol extract (G6), and cyclophosphamide-treated group (G7). Data are displayed as mean ± SE ($n = 5$). *$P < 0.05$ compared with benzene-induced leukemia rats

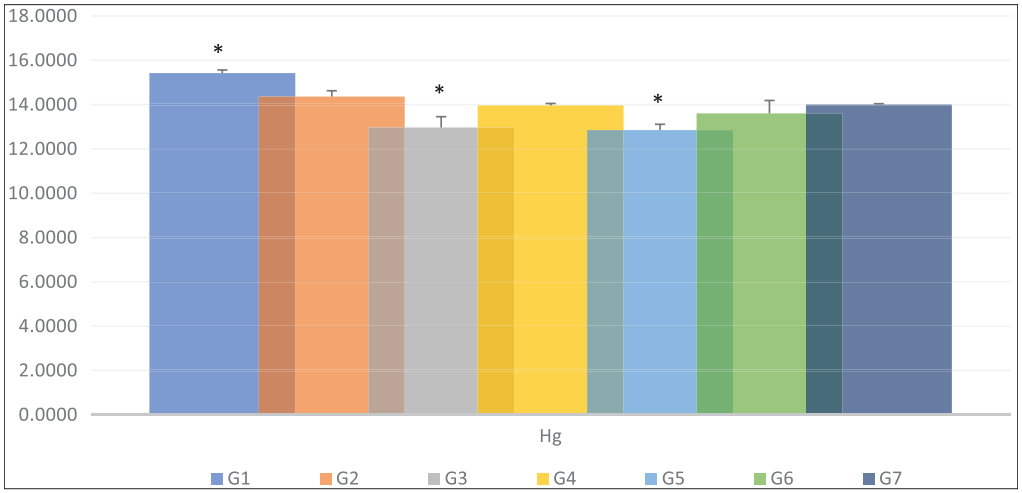

Figure 4: Comparison of the hemoglobin concentration (Hg) after treatment with black radish extracts between the positive control group (G2) and the other treatment groups: negative control group (G1), group treated with 100 mg/kg of aqueous extract (G3), group treated with 200 mg/kg of aqueous extract (G4), group treated with 100 mg/kg of ethanol extract (G5), group treated with 200 mg/kg of ethanol extract (G6), and cyclophosphamide-treated group (G7). Data are displayed as mean ± SE ($n = 5$). *$P < 0.05$ compared with benzene-induced leukemia rats

WBC count significantly decreased ($P < 0.05$) in rats receiving 100 mg/kg aqueous extract from Spanish black radish (4.100 ± 0.102) when compared with the positive control group (9.080 ± 1.065) and rats receiving CP (7.700 ± 0.711), whereas RBC count (8.220 ± 0.531) Hb (13.966 ± 0.084), HCT (37.400 ± 1.063), and PLT count (1162.333 ± 125.953) increased in rats receiving 200 mg/kg aqueous extract.

Rats receiving CP therapy (antileukemic medications) induced a decrease in the total WBC count compared with the benzene-treated group (positive control) although did not reach the level of significance.

Discussion

Compared to the negative control group, benzene-induced leukemia was significantly related with higher WBCs and severe anemia. By producing reactive oxygen species (ROS), benzene induces leukemia. It can also bond to biological macromolecules. In addition to increasing lipid peroxidation, ROS functions as a signaling molecule that controls the regulation of cell death, cell proliferation, and gene expression.[22] An overall proposal for benzene-induced leukemia is previously proposed, which suggested that benzene may play a role in or have an impact on a few important

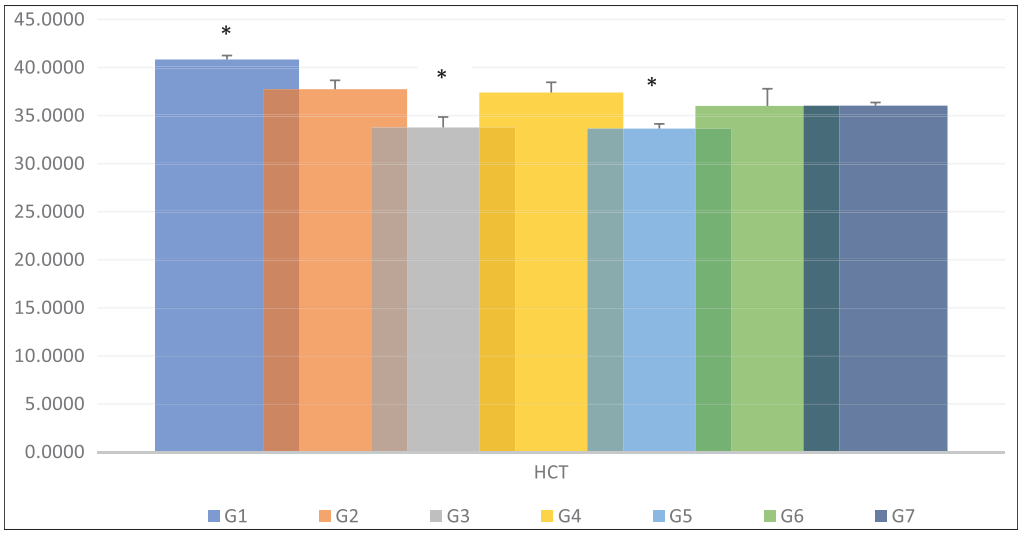

Figure 5: Comparison of the hematocrit (HCT) after treatment with black radish extracts between the positive control group (G2) and the other treatment groups: negative control group (G1), group treated with 100 mg/kg of aqueous extract (G3), group treated with 200 mg/kg of aqueous extract (G4), group treated with 100 mg/kg of ethanol extract (G5), group treated with 200 mg/kg of ethanol extract (G6), and cyclophosphamide-treated group (G7). Data are displayed as mean ± SE ($n = 5$). *$P < 0.05$ compared with benzene-induced leukemia rats

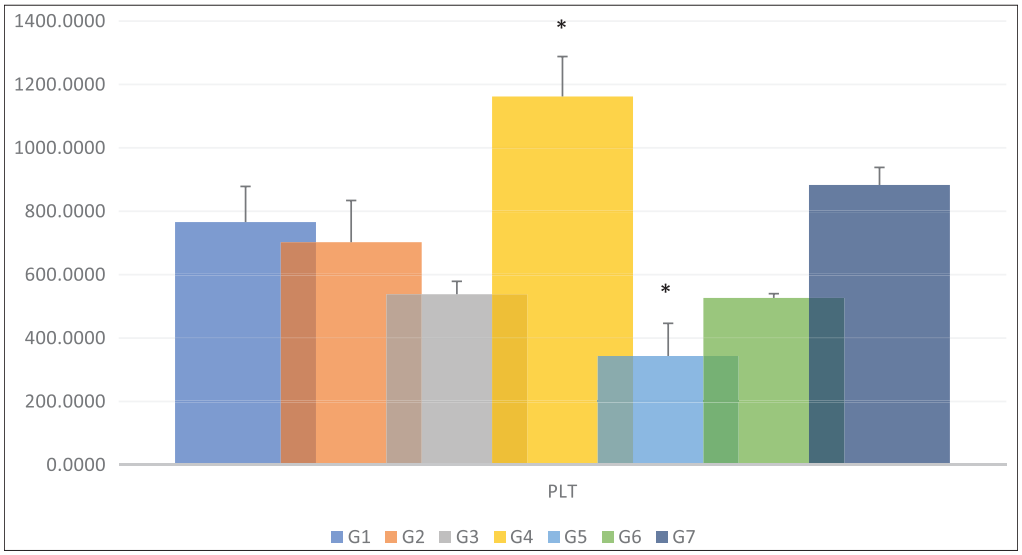

Figure 6: Comparison of the platelet (PLT) count after treatment with black radish extracts between the positive control group (G2) and the other treatment groups: negative control group (G1), group treated with 100 mg/kg of aqueous extract (G3), group treated with 200 mg/kg of aqueous extract (G4), group treated with 100 mg/kg of ethanol extract (G5), group treated with 200 mg/kg of ethanol extract (G6), and cyclophosphamide-treated group (G7). Data are displayed as mean ± SE ($n = 5$). *$P < 0.05$ compared with benzene-induced leukemia rats

molecular and cellular variables during the development of myeloid leukemogenesis.[23]

Previous research has explained the antileukemic activity of black radish in their results using various cancer cells.[19] It is believed that phytochemicals are responsible for these positive impacts. For example, alkaloids are significant chemical substances that provide a huge resource for the development of new medicines. Several alkaloids that have been extracted from natural herbs have been shown to have both *in vitro* and *in vivo* antiproliferative and antimetastatic effects on different forms of cancer. Alkaloids such as vinblastine have already been effectively transformed into anticancer medications.[24,25] Flavonoids are antioxidant, anti-cardioprotective and anti-cancer , in addition to their potential benefits to human health, they are supported by in vitro epidemiological research and protect against chronic non-communicable diseases.[26]

This observation suggests that Spanish black radish has beneficial effects on anemia in animals with leukemia. The results of this study are supported by the previous studies on the hematological effects of *Kigelia africana*

and *Moringa oleifera* leaves on benzene-induced leukemia in rats.[20,27]

Both types of black radish extract demonstrated some ability to reduce the total WBCs of the animals. The decrease in WBCs at the concentration of 100 mg/kg for aqueous extract and the increase in the total RBC and PLT counts in rats receiving 200 mg/kg of the aqueous extract demonstrate that a dose-dependency exists.

CP may possibly enhance immune responses depending on the dosage and timing of the course of treatment.[28] The decrease in the total number of leukocytes is in line with the previous studies on the effect of CP in mice.[29,30] To sum up, the data reveal improvements in blood parameters across aqueous extract–treated groups, which raises the possibility that these natural compounds could serve as antileukemic agents like antileukemic medications (CP).

Conclusion

Our study indicates that Spanish black radish might be a promising natural, antileukemic source.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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