

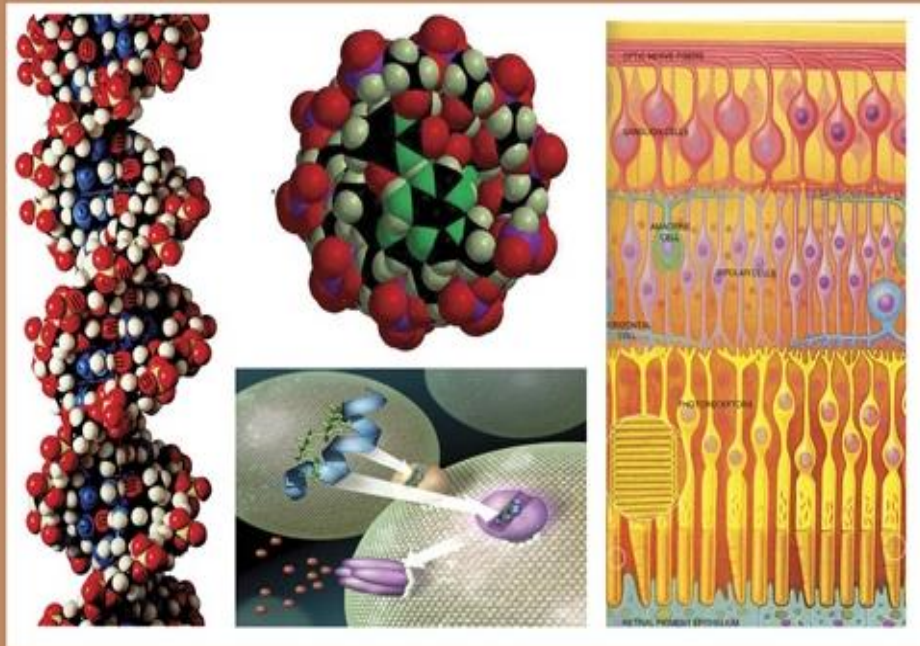


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## The Therapeutic and Synergistic Effect of *Saussurea costus* Extract against Induced Leukemia in Adult Male Rats.

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### ABSTRACT

Leukemia is a type of cancer affecting the blood and bone marrow and causing uncontrolled growth of abnormal white blood cells (WBCs). Treatment with antileukemic chemotherapy (cyclophosphamide, CP) results in several side effects including hepatotoxicity and nephrotoxicity. This study aimed to investigate the therapeutic and synergistic effect of *Saussurea costus* ethanolic extract (SEE) against induced leukemia in adult male rats. After induction of leukemia via benzene injection, both healthy and leukemic rats were divided into six groups (8 rats each) as follows: 1) healthy control group, 2) healthy rats ingested with SEE (300mg/Kg/day) for 4 weeks, 3) untreated leukemia-modeled rats, 4) leukemic rats treated orally with SEE for 4 weeks, 5) leukemic rats treated intraperitoneally with CP (7.5 mg/kg/48 hr.) for 4 weeks, and 6) leukemic rats treated with SEE in combination with CP for 4 weeks. The obtained results revealed that treatment of leukemic rats with SEE (either alone or combined with CP) efficiently succeeded to ameliorate the leukemic and CP-complication signs; this was evidenced by the significant reduction in WBCs count; also, SEE significantly improved the nephron-hepatic function, this was monitored from the marked decrease in serum ALAT, ASAT, urea, and creatinine values. In addition, hepatic MDA and NO levels were markedly reduced coupled with a valuable raise in hepatic GSH and SOD values. It could be concluded that SEE has antileukemic potential, succeeded to ameliorate the hazardous effects of CP, and enhanced the body tolerance ability against CP giving a promising application in the treatment of leukemia.

### INTRODUCTION

Leukemia is a type of cancer affecting the blood and bone marrow and causing uncontrolled growth of abnormal white blood cells (Chauhan, *et al.*, 2022). According to Bray (2018), leukemia is one of the most prevalent malignant disorders affecting the world population. The global prevalence of leukemia is reported to be 6.3 and 4.5 per 100,000 person-years in males and females, respectively (Kassahun *et al.*, 2020). Leukemia means “white blood” in the Latin language; it disrupts the body’s natural immunity and erythropoiesis. Leukemia induces the accumulation of cells outside the bone marrow, forming masses in vital organs of the body, such as the brain, lymph nodes, spleen, and liver (Varkesh *et al.*, 2013).

Also, leukemia was estimated to be the 15<sup>th</sup> and 11<sup>th</sup> most frequent cause of cancer incidence and cancer-related mortality worldwide, respectively (Sung *et al.*, 2021). Leukemia can result from chemically induced changes in the bone marrow from exposure to certain chemicals such as benzene, smoking and alcohol use, or exposure to radiation, viral infections and hereditary diseases (Balasubramaniam *et al.*, 2013; Bruzzoni-Giovanelli *et al.*, 2015).

Benzene is an aromatic hydrocarbon and a component of crude oil and gasoline (Schettgen *et al.*, 2010). And, it has been classified as a known human carcinogen by the International Agency for Research on Cancer (IARC) (Bahadar *et al.*, 2014). Chronic exposure to benzene is associated with a variety of hematological disorders, such as aplastic anemia, myelodysplasia, and leukemia, particularly acute myeloid leukemia (AML) (Arnold *et al.*, 2013; Wei *et al.*, 2017). The methods used for the treatment of cancer include chemotherapy, surgery, hormone therapy and radiation. Chemotherapy is one of the commonest and most frequent approaches used for the treatment of cancer; it delivers anticancer drugs systemically to patients for quenching the uncontrolled proliferation of cancerous cells (Jabir *et al.*, 2012).

Cyclophosphamide (CP) is a cytotoxic alkylating agent used for the treatment of various diseases such as leukemia, lymphoma, breast, lung, prostate, ovarian cancers, and Hodgkin's disease (Al-Mousaw *et al.*, 2022). Several adverse events have been recorded following cyclophosphamide administration, including bone marrow suppression (Deng *et al.* 2018), hepatotoxicity (Ming *et al.* 2019), bladder toxicity (Mills *et al.* 2019), nephrotoxicity (Sheth *et al.* 2018), cardiovascular toxicity (Kurauchi *et al.* 2017), and hyponatremia (Clifton *et al.*, 2018). It is well-recognized that natural products have a variety of biological activities. Medicinal plants provide an inexhaustible source of anticancer drugs in terms of both variety and

mechanism of action. Accumulating reports have suggested that many naturally occurring substances exhibit cancer chemotherapeutic effects (Khan, *et al.*, 2013).

*Saussurea costus* (Falc.) Lipschitz, synonymous with *Saussurea lappa* C.B. Clarke (Parmar *et al.*, 2012) and is commonly known as costus (Gwari *et al.*, 2013), belongs to the family Asteraceae. Its roots are widely used in folk medicine (Amara *et al.*, 2017). It was used as a medicinal plant for the treatment of various ailments such as asthma, inflammatory diseases, ulcer, and stomach problems (Pandey *et al.*, 2007). Moreover, costus has been mentioned in the Holy Ahadith said by Prophet Muhammad (Peace be upon him) for the treatment of many diseases (AL-Kattan, 2013). *Saussurea costus* is one of the antioxidant-rich medicinal plants (Saleem *et al.*, 2013). It has various bioactive properties such as anticancer, anti-ulcer, anti-inflammatory, hepatoprotective, immunomodulator, hypoglycemic, spasmolytic, anticonvulsant, antidiarrheal and antiviral activities (Zahra *et al.*, 2014; Ghasham *et al.*, 2017). Because *Saussurea costus* roots are a rich source of various bioactive constituents such as alkaloids, cardiac glycosides, coumarins, flavonoids, phenols, quinones, resins, steroids, tannins, terpenoids, costunolide and dehydrocostus lactone are the major components in the essential oil of *Saussurea costus* roots. These phytochemical constituents are important for the use of health care (Abdallah *et al.*, 2017). The present study aimed to explore the therapeutic and CP-synergistic effect of *Saussurea costus* extract against induced leukemia in adult male rats.

## MATERIALS AND METHODS

### Plant Material:

Dried roots of *Saussurea costus* were obtained from Imtinan Company, Egypt. The dried roots were finely powdered using an electric grinder (KM-1500, London, UK), then kept in a dark container at 25C°.

### Extraction of *Saussurea costus*:

The ethanolic extract of dry powdered roots was carried out according to the modified method of Itarbone (1998). In brief, 500 g of the crude powder of *Saussurea costus* was soaked in 2.5 liters of ethanol (70%) with continuous stirring for 72 hours at room temperature, then the mixture was filtered using filter paper (Whatman number 1, England). The ethanolic filtrate was evaporated at 45°C under reduced pressure using a rotary evaporator; the aqueous residues were removed through

$$\text{Extract yield (g/100g crude herb)} = \frac{W_2 - W_1}{W_3} * 100$$

Where W1 is the weight of the clear empty dry quick fit flask (g), W2 is the weight of the flask containing the dry extract after lyophilization (g), and W3 is the weight of the crude powdered herb soaked in ethanol (g).

#### Determination of Radical Scavenging Activity (RSA):

The capacity of antioxidants of SEE to quench DPPH radicals was determined according to the method of Nogala-Kalucka *et al.* (2005); in this method, a certain amount from the crude

$$\text{RSA (\%)} = \left( \frac{A_{\text{control sample}} - A_{\text{sample extract}}}{A_{\text{control sample}}} \right) * 100$$

#### Determination of Total Phenolic Content (TPC):

The total phenolic content of the SEE was performed by dissolving 5 mg of the extract in a 10 ml mixture of acetone and water (6:4, v/v). Then, a sample of 0.2 ml was mixed with 1.0 ml of Folin-Ciocalteu reagent (10-fold diluted) and 0.8 ml of sodium carbonate solution (7.5%). After 30 min at room temperature, the absorbance was measured at 765 nm using Cary 100 UV-Vis spectrophotometer (Agilent Technologies, USA). Estimation of phenolic compounds as catechin equivalents was carried out using a standard curve of catechin (Jayaprakasha and Jaganmohan, 2000).

#### Experimental Animals and *in vivo* study:

Adult male albino rats (180-220g) were obtained from the Animal Farm of the Egyptian Holding Company for Biological

lyophilization process by freeze drier; finally, the stock extract was stored at -20 °C until further use. The dry extract was subjected to *in vitro* measurements before its application *in vivo* study.

#### *In vitro* Assessments:

##### Calculation of Total Yield:

The ethanolic extract filtrate was transferred to a quick-fit round bottom flask with a known weight (W1), then freeze-dried and weighed again (W2), and finally, the yield was calculated from the formula:

extract was dissolved in methanol to obtain a concentration of 200 ppm. A volume of 0.2 ml of this solution was completed to 4 ml by methanol; then 1 ml DPPH solution ( $6.09 \times 10^{-5}$  mol/L, dissolved in the same solvent) was added. The absorbance of the mixture and the blank (1 ml of DPPH solution and 4 ml methanol) was measured at 516 nm using a spectrophotometer (Cary 100 UV-Vis, USA) after 10 min standing; triplicate measurements were made, and the radical scavenging activity was calculated according to the following equation:

Products and Vaccines (VACSERA), Giza, Egypt and transferred to the animal house, faculty of science, Al-Azhar university where they were housed in plastic cages (5 rats each), and maintained under standard conditions (23±2°C, humidity 55±5, and 12hr light/dark cycle along the experimental period). The animals were kept on free access to food and water for a week before starting the experiment for acclimatization. All animals received human care in compliance with the standard institutional criteria for the care and use of experimental animals according to the ethical committee of the Faculty of Science, Al-Azhar University, Cairo, Egypt.

#### Induction of Leukemia:

After the animals have been acclimatized to the experimental room conditions, a suitable number of the animals was subjected to induction of leukemia according to the method of Akanni *et al.*



(2014a). In brief, the rats were injected intravenously with 0.2 ml of diluted benzene chromasolv solution suspended in distilled water (1:10 ratio v/v) via the tail lateral veins (one injection every 48hr.) for 6 weeks. Post each two weeks, a blood sample was withdrawn from each animal (from retro-orbital venous plexus puncture using sterile heparinized glass capillary tubes) for white blood cell counting and testing of leukemia occurrence.

#### **Experimental Design:**

After induction of leukemia, both healthy and leukemic rats were divided into six groups (8 rats each) as follows: group (1) included healthy animals and served as control, group (2) included healthy animals orally administered with SEE (300mg/Kg/day) dissolved in distilled water for 4 weeks consecutively (Alnahdi *et al.*, 2016), group (3) included untreated induced-leukemic animals, group (4) included leukemic animals orally treated with SEE for 4 weeks, group (5) leukemic animals treated with an intraperitoneal injection of cyclophosphamide (7.5mg/kg/48hr) for 4 weeks (Akanni *et al.*, 2014a), and group (6) leukemic animals treated orally with SEE (300mg/kg/day), two-hour prior to intraperitoneal injection with cyclophosphamide (7.5 mg/kg/48 hr.) for 4 weeks.

#### **Blood and Tissue Sampling:**

At the end of the treatment period, rats were fasted overnight and then weighed; following anesthesia, blood samples were withdrawn using sterile glass capillaries; each blood sample was divided into two portions: the first portion was taken on EDTA for the determination of total leucocytes count; the second portion was left to clot, then cool-centrifuged at 3000 rpm for 15 minutes; the sera were separated, divided into aliquots, and stored at  $-80^{\circ}\text{C}$  until biochemical measurements were carried out. After blood collection, the animals were sacrificed soon with sudden decapitation, then the liver of each animal was dissected out, washed in saline, dried, rolled in a piece of aluminum foil, and stored at  $-80^{\circ}\text{C}$  for

determination of oxidative stress markers.

#### **Hepatic Tissue Homogenization:**

A specimen from the liver tissue was homogenized in ice-cold phosphate buffer (50 mM, pH 7.4) to give 10% homogenate (w/v) using an ultrasonic homogenizer (Sonics VCX-750 Vibra-Cell, USA); the homogenate was centrifuged in a cool-centrifuge (ROTANTA 460 R, Germany) at 3000 rpm for 20 min to remove the nuclear and mitochondrial fractions; the supernatant was separated, divided into aliquots and stored at  $-80^{\circ}\text{C}$  till the determination of the oxidative stress markers (Kunle *et al.*, 2019).

#### **Total Leucocytes Count:**

Blood Cell counter (Celltac MEK-6510K Nihon 53 kohden, Germany) was used for measuring of total leucocytes count.

#### **Biochemical Determinations:**

Using a spectrophotometer (Cary 100 UV-Vis, USA), serum alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) activities were determined according to the method of Breuer (1996) using reagent kits obtained from Egyptian Company for biotechnology spectrum, Egypt; serum urea and creatinine concentration was determined according to the method described by Tiffany *et al.* (1972) and Bowers and Wong (1980), using reagent kits obtained from Egyptian Company for biotechnology spectrum, Egypt.

#### **Hepatic Oxidative Stress Markers:**

Reduced glutathione (GSH) level was determined according to the method of Beutler *et al.* (1963), nitric oxide (NO) level was estimated according to the method of Montgomery *et al.* (1961), superoxide dismutase (SOD) activity was determined according to the procedure described by Nishikimi *et al.* (1972) using kits obtained from Bio-diagnostic Co., Dokki, Giza, Egypt. Malondialdehyde (MDA), indirect index for lipid peroxidation, level was determined chemically as described by Ruiz-Larrea *et al.* (1994); 0.5ml liver homogenate was added to 4.5 ml working reagent (0.8 g TBA was dissolved in 100 ml perchloric acid

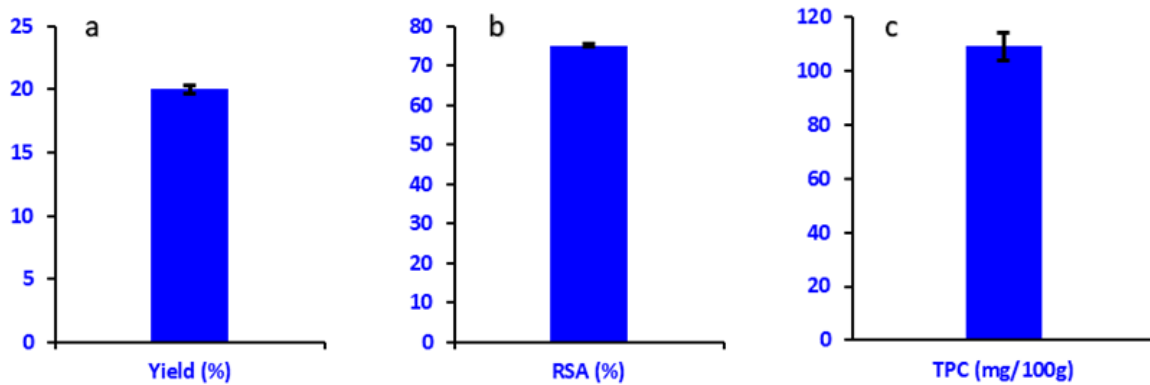
10%, and mixed with trichloroacetic acid (TCA) 20% in a volume ratio 1:3, respectively); then in a boiling and shaking water bath, the sample-reagent mixture was placed for 20 min, then carried to cool at room temperature and centrifuged for 5 min at 3000 rpm; finally, the absorbance of the clear pink supernatant was measured at 535 nm against reagent blank (0.5 ml distilled water + 4.5 ml working reagent) using spectrophotometer (Cary 100 UV-Vis, USA); MDA level (nmol/g tissue) was calculated according to the following formula:  $MDA \text{ (nmol/g tissue)} = \left[ \frac{A_{535} \times 10^9}{(1.56 \times 10^5) \times 10^3} \times AD \right] \times 10$ . Where  $1.56 \times 10^5 \text{ M}^{-1}\text{L}^{-1}\text{cm}^{-1}$  is MDA extinction coefficient and AD is assay dilution (10).

### Statistical Analysis:

All numerical data were statistically analyzed using one-way analysis of variance (ANOVA) followed by post hoc (LSD) test at  $p \leq 0.05$  using the Statistical Package for Social Sciences (SPSS/PC) computer program (version 26).

### RESULTS

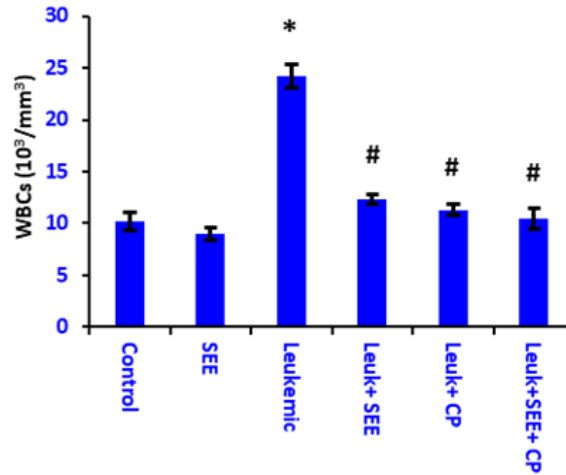
The *in vitro* assessment indicated that *Saussurea costus* gave a considerable yield (20%) when extracted with ethanol. Interestingly, this extract performed a high percentage of radical scavenging activity (75.2 %) and recorded a high content (109mg%) of total phenolic compounds (Fig. 1).



**Fig.1:** In vitro data of SEE; a) the yield, b) radical scavenging activity (RSA), and c) and total phenolic content (TPC). Data are expressed as the mean of three replicates

With respect to the leukemic marker, this study showed a significant increase in the count of white blood cells (WBCs) in the induced-leukemic animals' group as compared with the normal control group. Favorably, treatment of leukemic animals

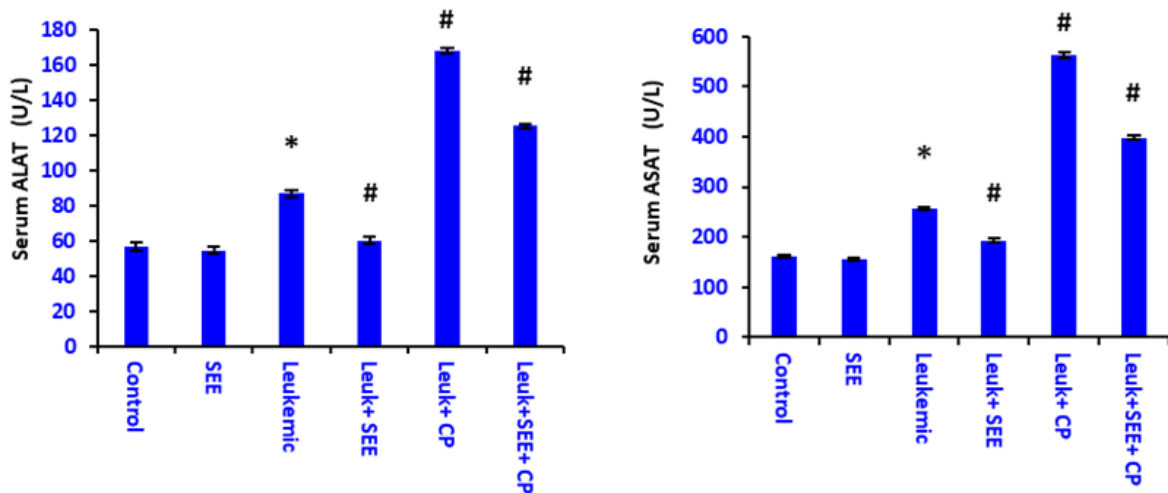
with either SEE or CP, alone or in combination, resulted in a significant reduction in WBCs count; however, the combined SEE-CP exhibited the highest degree of improvement (Fig. 2).



**Fig. 2.** White blood cells count of the treated leukemic- animals' groups in compared to the control one. Data are presented as mean  $\pm$  standard error and were subjected to one-way ANOVA followed by post hoc (LSD) test at  $p \leq 0.05$ . \* is significantly different from the control group, # is significantly different from leukemic group, SEE is *Saussurea costus* ethanolic extract, and Leuk is leukemic, and CP is Cyclophosphamide.

The obtained data showed a significant increase in serum ALAT and ASAT activities of the induced-leukemic animals' group when compared with the normal control group. Interestingly, treatment of leukemic animals with SEE resulted in a significant decline in serum ALAT and ASAT activities, while CP-treated leukemic animals showed a

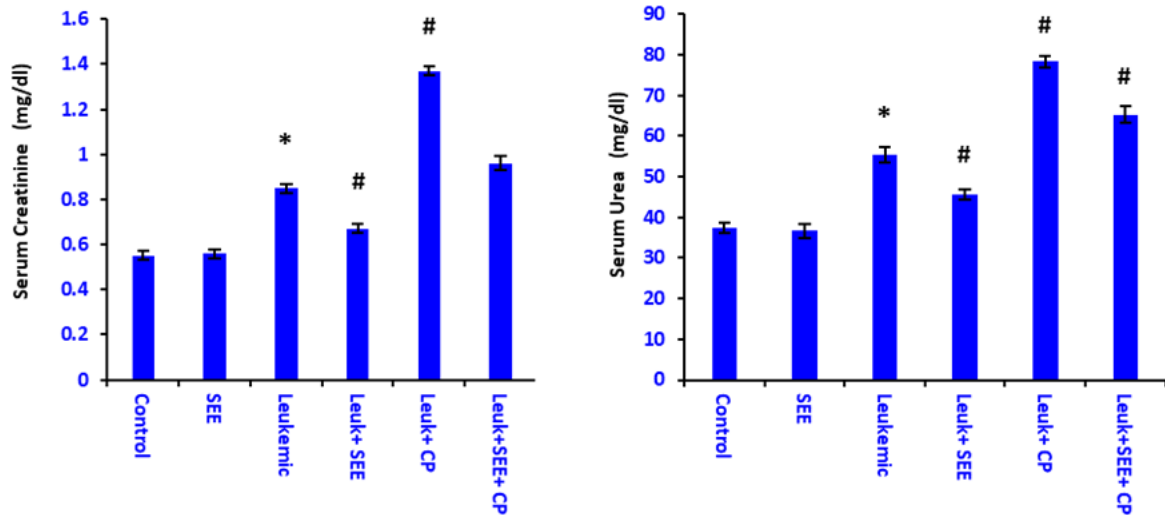
significant severe raise in serum ALAT and ASAT activities. Moreover, SEE-CP-treated leukemic animals pointed to a significant increase in serum ALAT and ASAT activities (but less than in the case of treatment with CP alone), when these groups were compared to untreated leukemic animals' group (Fig. 3).



**Fig. 3.** Serum ALAT and ASAT activities of the treated leukemic- animals' groups in comparison to the control one. Data are presented as mean  $\pm$  standard error and were subjected to one-way ANOVA followed by a post hoc (LSD) test at  $p \leq 0.05$ . \* is significantly different from the control group, # is significantly different from leukemic group, SEE is *Saussurea costus* ethanolic extract, and Leuk is leukemic, and CP is Cyclophosphamide.

Similarly, Figure 4 shows a significant elevation in serum creatinine and urea level in leukemic animals' group when compared with the control group. Compared with the leukemic animals' group, the SEE-treated leukemic animals' group showed a significant decrease in serum creatinine and urea level, while treatment of leukemic

animals with CP alone or combined with SEE performed a significant increase in creatinine and urea level. However, co-treatment of leukemic animals with SEE and CP together resulted in a remarkable reduction in creatinine and urea level in comparison with leukemic animals treated with CP alone.

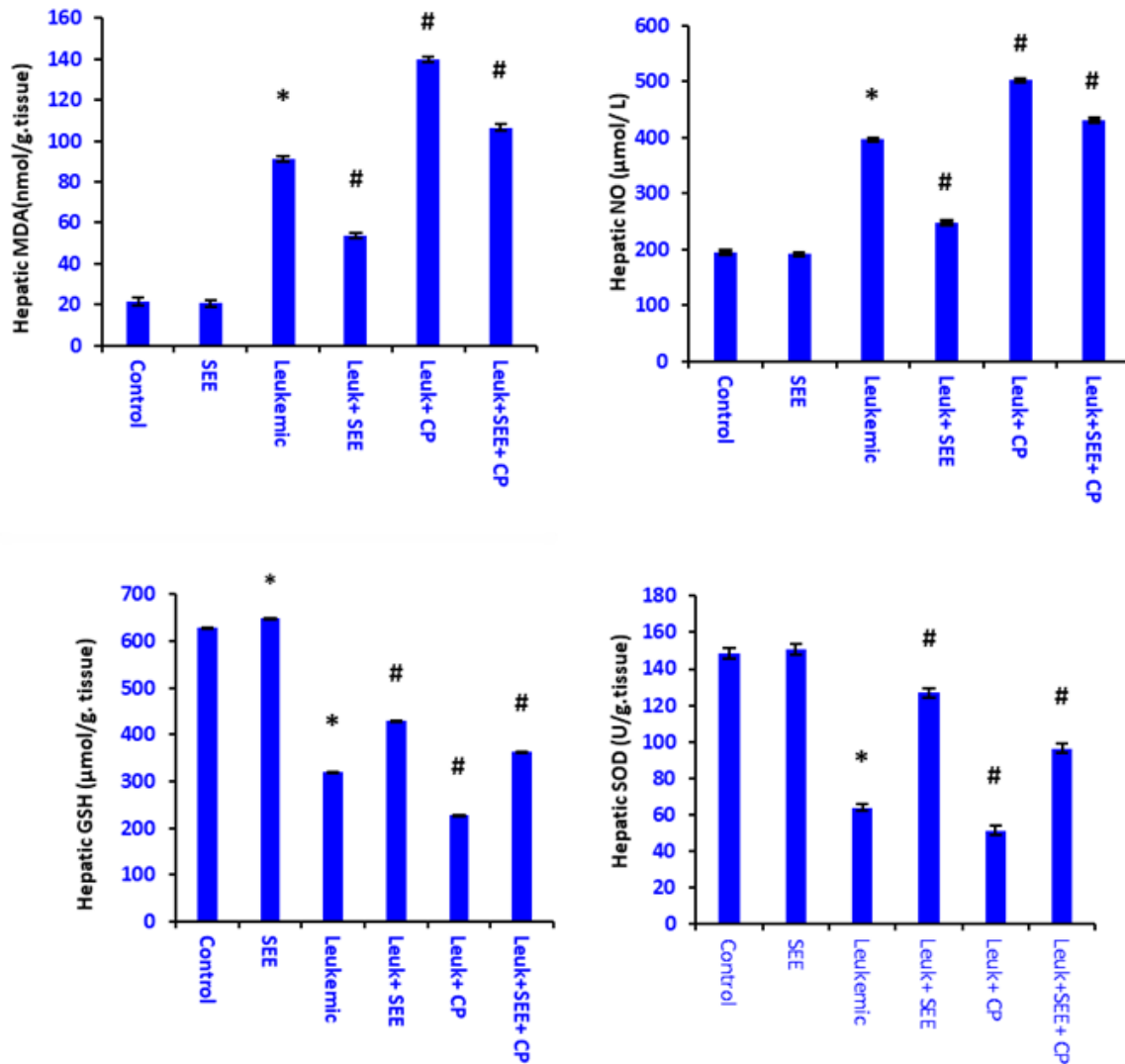


**Fig. 4.** Serum creatinine and urea levels of the treated leukemic- animals' groups in compared to the control one. Data are presented as mean  $\pm$  standard error and were subjected to one-way ANOVA followed by post hoc (LSD) test at  $p \leq 0.05$ . \* is significantly different from the control group, # is significantly different from leukemic group, SEE is *Saussurea costus* ethanolic extract, and Leuk is leukemic, and CP is Cyclophosphamide.

The current study revealed that the induced-leukemic animals' group displayed a significant increase in the hepatic MDA and NO levels accompanied by a significant decline in hepatic GSH and SOD when compared with the control group. Favorably, SEE treatment of leukemic animals resulted in a significant drop in both MDA and NO levels associated with a marked increase in GSH and SOD values; while treatment of leukemic animals with CP alone performed a more significant elevation in hepatic MDA

and NO levels coupled with a more significant decline in hepatic GSH and SOD values when the two animals' groups compared with the untreated leukemic group. However, co-treatment of leukemic animals with SEE besides CP resulted in a remarkable reduction in MDA and NO levels associated with notable raise in GSH and SOD values in comparison with leukemic animals treated with CP alone, but still significantly deteriorated in comparison with the untreated leukemic group (Fig. 5).





**Fig 5.** Serum hepatic level of oxidative (MDA and NO) and antioxidant (GSH and SOD) markers of the treated leukemic- animals' groups in compared to control one. Data are presented as mean  $\pm$  standard error and were subjected to one-way ANOVA followed by post hoc (LSD) test at  $p \leq 0.05$ . \* is significantly different from the control group, # is significantly different from leukemic group, SEE is *Saussurea costus* ethanolic extract, and Leuk is leukemic, and CP is Cyclophosphamide.

## DISCUSSION

Leukemia is a type of cancer affecting the blood and bone marrow, causing uncontrolled growth of abnormal white blood cells (Chauhan, *et al.*, 2022). It results, chemically, post-exposure of the bone marrow to certain chemicals such as benzene; chronic exposure to benzene is associated with a variety of hematological disorders, such as aplastic anemia and acute myeloid leukemia (Bruzzoni-Giovanelli *et al.*, 2015; Wei *et al.*, 2017). Cyclophosphamide is a cytotoxic alkylating

chemotherapy used for the treatment of various diseases including leukemia, but it has wide adverse side effects like bone marrow suppression (Deng *et al.* 2018), hepatotoxicity (Ming *et al.* 2019), nephrotoxicity (Sheth *et al.* 2018), cardiovascular toxicity (Kurauchi *et al.*, 2017). Several reports have suggested that many naturally occurring substances exhibit cancer chemotherapeutic effects, one of these natural substances is *Saussurea costus* which has been used as a medicinal plant for the treatment of various ailments (Khan *et*

*al.*, 2013; Ghasham *et al.*, 2017); therefore, the present study aimed to explore the therapeutic and CP-protective-synergistic efficiency of *Saussurea costus* ethanolic extract against induced leukemia in adult male albino rats.

In the present study, the benzene induce-leukemic animals' group showed a significant increase in WBCs count (leukocytosis) and demonstrated the successful induction of leukemia in rats. This finding runs in line with previous studies (Olufemi *et al.*, 2014; Li *et al.*, 2019) which attributed this finding to the significant molecular and cellular events that may contribute to the development of myeloid leukemia. Following exposure, benzene is mostly metabolized in the liver by cytochrome P450 2E1 and 2F1; however, its reactive metabolites such as catechol, hydroquinone, benzoquinone, and others interpret how benzene exerts its toxicity; benzene and its metabolites redistribute and accumulate in bone marrow tissue, where they exert their selective toxicity to hematopoietic stem cells or progenitor cells (Khalade *et al.*, 2010). In the bone marrow, benzene metabolites interact with hematopoietic cells at various stages of differentiation, causing genetic, chromosomal, or epigenetic abnormalities, genomic instability, and altered hematopoietic stem cell proliferation and differentiation, which results in the formation of mutated hematopoietic cells and subsequent clonal evolution to leukemia (Roy *et al.*, 2014; Musial *et al.*, 2020).

Interestingly, treatment of leukemic animals with SEE resulted in a significant anti-leukemic effect, this was established by the marked decrease in WBCs near its level in the normal control animals' group. This anti-leukemic effect of SEE may be due to its phytochemical compounds like alkaloids, saponins, steroids, terpenes, polyphenols, flavonoids, sterols, tannins, and glycosides; these agents are well known as hemopoietic factors and have a direct influence on the production of blood, and endogenous antioxidant system, and serve on

inhibition of free radical and ameliorate blood components (Pandey, 2012). This result agrees with Alnahdi, (2017). who reported that administration of costus extract to rats intoxicated with Deltamethrin insecticide, markedly decreased WBCs count. Moreover, the remarkable reduction of WBCs post-treatment of leukemic rats with CP, in the current study, is concomitant with the finding of Salem *et al.* (2010) and Alazzouni *et al.* (2021). who attributed this antitumor effect to the probable ability of CP to activate the immuno-response via inducing the mobilization of the hematopoietic stem cells and dendritic cells from bone marrow. Also, it was reported that phosphor-amide mustard (an effective metabolite of cyclophosphamide that kills cancer cells) can alkalize cellular nucleic acids and inhibit the proliferation and division of cancer cells (Jiang *et al.*, 2020). Also, treatment of leukemic animals with SEE in combination with CP revealed a more significantly decreased in the count of WBCs than occurred in CP-treated leukemic animals; this result could be attributed to the antileukemic synergistic effect of both as cyclophosphamide has antileukemic activity (Al-Mousaw *et al.*, 2022) and SEE possesses antitumor effect (Li *et al.*, 2020) that resulted in a marked improvement in the WBCs count.

The current study showed that serum ALAT and ASAT activities (liver function markers) were significantly increased in the induced-leukemic animals' group as compared with the control group; this result agrees with the report of Ola and Sofolahan (2021), and Owagboriaye *et al.* (2021) who concluded that activities of serum aminotransferases were significantly increased in benzene-induced leukocytosis rats. It was suggested that the elevation in the activities of these aminotransferases might be probably because of the toxic impact on the cell membranes of hepatocytes where petroleum hydrocarbons were oxidized into free radicals or reactive intermediates (Nwanjo and Ojiako, 2007). Interestingly, treatment of the leukemic rats

with SEE exhibits an ameliorative effect against the benzene resultant-hepatotoxicity, this effect was achieved from the marked lowering potential of these aminotransferases activities; This result concomitant with some previous studies (Deabes *et al.* 2021; Ashry *et al.* 2022 ), and may be due to the antioxidant potential of SEE-included phytochemical compounds, such as flavonoids and chlorogenic acid, which act as antioxidant agents and led to stabilization of the lipid peroxidation resultant free radicals, consequently preventing benzene-induced hepato-toxicity (Shabnam *et al.*, 2018). Otherwise, a significant extra increase in the activity of serum ALAT and ASAT in the CP-treated leukemic animals' group was observed compared with the induced-leukemic animals' group; these findings are consistent with Abdel-Wahhab *et al.*, (2021) who found that CP-injection induced significant elevations in serum ASAT and ALAT activities; the excessive elevation of ASAT and ALAT activity (occurred post CP-treatment of leukemic animals) could be attributed to the excessive oxidative stress that results in inflammation and hepatocellular damage leading to excessive disturbance in the cellular integrity, selective permeability of the hepatocyte membrane, and leakage of these enzymes to the bloodstream (Dang *et al.*, 2008). Co-treatment of the leukemic rats with SEE besides CP led to a significant reduction in the serum ALAT and ASAT activities compared to either the untreated or CP-treated leukemic rats; this result reflects the protective effect of SEE against CP which was monitored from the marked reduction in the serum ALAT and ASAT activities post-treatment of leukemic rats with SEE together with CP; this preferable protective efficiency of SEE could be attributed to the antioxidant behavior of SEE-phytochemical constituents that enhanced the body tolerance against the CP-complications and improved the hepatic oxidative status (Al-Duais and Al-Awthan, 2017).

The present results displayed that serum urea and creatinine concentrations

were significantly elevated in the induced-leukemic animals' group; this finding goes in line with Alshareef and Ibrahim (2020) who attributed the rise in serum urea and creatinine to benzene exposure causes dilated proximal tubules with degenerated lining, degenerated distal tubules with atrophy, and exudate of the renal corpuscle. Serum creatinine and urea are good biomarkers of renal damage by environmental pollutants as serum creatinine, (breakdown product of creatine phosphate) reflects the rate of renal (glomerular) filtration; however, a rise in serum urea and creatinine level has been recognized as a good diagnostic marker of renal dysfunction (Tizhe *et al.*, 2014). Thus, the increased levels of serum urea and creatinine observed in the modeled leukemic animals' group indicate a reduced glomerular filtration process and dysfunction of the kidney tubules. The present study pointed out that SEE-treated leukemic animals performed a marked reduction in levels of creatinine and urea as compared to the untreated leukemic ones reflecting the nephroprotective capacity of SEE against benzene-resultant nephrotoxicity; this might be due to the high concentration of flavonoids and alkaloids that exhibiting high antioxidant activity (Giri *et al.*, 2019). Our result is inconsistent with Kadhem (2019) who reported that the ethanolic extract of *Saussurea lappa* roots acts as an antioxidant agent and has a hepato-nephroprotective effect against toxicity induced by paracetamol in male rabbits. Unfortunately, our study performed a marked elevation in serum creatinine and urea levels post-treatment of leukemic animals with either CP alone or combined with SEE in comparison with the untreated leukemic group; this result may be attributed to resultant excessive nephrotoxicity because of CP-metabolite, acrolein (Sheth *et al.*, 2018). This finding runs in line with Tian *et al.* (2022). In a favorable manner, treatment of leukemic animals with SEE in combination with CP causes a marked improvement in serum creatinine and urea levels compared

with the CP-treated leukemic group. CP treatment of leukemia exhibits side effects, including nephrotoxicity, and SEE possesses antioxidant activity; therefore, co-administration of SEE along with CP in leukemic rats succeeded to ameliorate and regenerate kidney cell damage; consequently reducing urea and creatinine levels. It was found that SEE is rich in bioactive molecules, such as dehydrocostus lactone, costunolide, cynaropicrin, monoterpenes, sesquiterpenoids, flavonoids, lignans, triterpenes, steroids and glycosides (Abdallah *et al.*, 2017); and the successive therapeutic activities of SEE (including the ability to reduce oxidative stress) are very familiar with these compounds (Abd Eldaim *et al.*, 2019). The reason for the inhibition of CP-induced alterations could be the reduction of oxidative stress; SEE could be a cytoprotective agent as it showed high free radical scavenging activity (*in vitro* results) that causes extensive damage to cell components (Bolkiny *et al.*, 2019).

In the current study, untreated leukemic animals showed a significant increase in hepatic oxidative voltage (MDA and NO levels) accompanied with a significant decline in hepatic antioxidant battery (GSH and SOD values). These findings flow in consistent with Owagboriaye *et al.* (2021) who found that gasoline fumes exposure has induced redox imbalance and oxidative stress in the liver of the exposed rats, which was evidenced by the marked increase in hepatic MDA and ROS levels accompanied by a reduction in SOD and GSH values. Excessive production of ROS overwhelmed the protective roles of the antioxidant molecules in the liver of the modeled leukemic rats, and this resulted in peroxidation of the hepatic membrane polyunsaturated fatty acids. Moreover, occupational exposure to benzene has been documented to induce oxidative stress among workers in gasoline filling stations. In addition, metabolic activation of benzene to metabolites, such as hydroquinone and 1, 2, and 4-benzenetriol has been reported to generate ROS, impair the antioxidant

defense system, and consequently induce oxidative stress (Uzma *et al.*, 2010). Thus, the presence of benzene, to a great extent, may be responsible for hepatic ultra-oxidative stress (Uboh *et al.*, 2022).

The treatment of leukemic animals with SEE resulted in a significant decrease in hepatic MDA and NO levels matched with a significant elevation in hepatic GSH and SOD values. These findings are in accordance with Abdel-Rahman *et al.* (2020) who interpreted the oxidative status ameliorative potential of costus through its potentiation of the antioxidant defense system that is represented by the elevated antioxidant markers and decreased oxidative markers. In addition, many previous studies demonstrated the ability of costus to donate electrons to reactive radicals, converting them into more stable and unreactive species (Elemike *et al.*, 2017; Shediwah *et al.*, 2019; Benedetto *et al.*, 2019). The decreased MDA concentrations in the SEE-treated leukemic group may be due to the protective effect of costus from the deleterious effect of ROS-mediated lipid peroxidation of tissue macromolecules (Anyasor *et al.* 2014). Dehydro-costus lactone isolated from *Saussurea costus* inhibited the production of nitric oxide by suppressing inducible nitric oxide synthase (iNOS) enzyme expression (Pandey *et al.* 2007). So, the suppression of NO production may be due to the inhibitory action on the inducible nitric oxide synthase gene expression, and the increase in the level of GSH may be due to the enhancement of its synthesis.

Treatment of leukemic animals with CP led to a marker extra raise in hepatic MDA and NO levels associated with a marked over reduction in hepatic GSH and SOD values. These findings agree with Sun *et al.* (2021). CP-mediated hepatic injury has also been associated with disturbances in redox homeostasis; the generation of free radicals post-treatment with CP leads to an attack on the membrane of hepatocytes and triggers lipid peroxidation, which reflects the induction of oxidative insults and cellular damage (Mahmoud *et al.*, 2017).

Additionally, acrolein, the active metabolite of CP, can activate xanthine oxidase and enhance ROS production, resulting in the induction of oxidative responses, including the peroxidation of the lipid membrane (Stojiljkovic *et al.*, 2019). The significant increase in NO production may be due to the upregulation of *Nos2* expression in the liver tissue following treatment with CP. The increased levels of NO production were associated with hepatic injury after exposure to CP (Andersson *et al.*, 2008), which may be due to the cytotoxic peroxynitrite radicals that are produced following the interaction of NO with superoxide radicals (Radi, 2018). The observed depletion of GSH in the liver of CP-treated leukemic rats could be a result of the direct conjugation of CP-metabolites with GSH (Yousefipour *et al.*, 2005). GSH plays a significant role against oxidative stress by neutralizing the hydroxyl radicals (Circu and Aw, 2011); the conjugation of GSH with CP, or its active metabolites, may explain the reduction in the hepatic GSH level. Indeed, the increased MDA and nitric oxide production, along with the depletion of glutathione, following CP treatment, have been suggested to increase oxidative stress and consequently hepatic damage (Singh *et al.* 2018). The current study showed that co-treatment of leukemic animals with CP besides SEE resulted in a marked decrease in hepatic MDA and NO levels coupled with a notable increase in GSH and SOD values in comparison with CP-treated leukemic animals; this reflects the protective effect of SEE against the oxidative stress deteriorations induced by either benzene and/or CP. This protective efficacy of SEE could be a result of the actions of its phytochemical constituents that prevent the actions of the toxic metabolites of either CP and or benzene, consequently, stabilizing the cell membranes of the intracellular biomolecules (Parker *et al.*, 2017). Additionally, it was stated that costus ingredients prevented the generation of ROS, inhibited the formation of MDA, and regulated the activities of CAT, GPx, and SOD (Chen *et al.* 2018).

## CONCLUSION

The present study has demonstrated that *Saussurea costus* ethanolic extract (SEE) exhibits pronounced antioxidative activity making it promise as an anti-leukemic and protective agent against induced leukemia and cyclophosphamide (CP) complications, respectively. Also, SEE performed a synergistic anti-leukemic potential with CP, as it enhanced the therapeutic effect of CP against leukemia. The dual effect of SEE (antileukemic and protective effect against CP-induced side effects) strengthened the antileukemic therapeutic efficacy; this improved the recovery possibility. These activities of SEE could be attributed to the antioxidant behavior of its phytochemical constituents that performed an anti-leukemic role and enhanced the body's tolerance against the CP-complications.

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