Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/3sg6b927

ANTI-TUMOUR PROPERTIES OF ALOE VERA ETHANOLIC EXTRACT: EFFECTS ON DALTON'S ASCITIC LYMPHOMA IN SWISS ALBINO MICE

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Abstract
With cancer remaining a critical worldwide health concern and conventional treatments often leading to adverse effects, this research examined how ethanolic extract derived from Aloe vera impacts Dalton's Ascitic Lymphoma (DAL) in Swiss albino mice. The study involved intraperitoneal injection of DAL cells (1×10^6 cells/mouse) followed by Aloe vera extract administration at 100 mg/kg and 200 mg/kg doses over 14 days, using 5-fluorouracil (5-FU, 20 mg/kg) as a reference standard. The evaluation included measurements of tumour volume, weight changes, survival duration, blood parameters, and antioxidant activity. Results showed that Aloe vera's ethanolic extract significantly decreased tumour size and extended survival time proportional to dosage. Notably, the 200 mg/kg dose resulted in a 71.45% longer lifespan compared to untreated subjects. Blood parameters, including red blood cells, haemoglobin, and white blood cell counts, showed normalization in treated groups. The extract exhibited substantial antioxidant properties in both laboratory and living system tests, including DPPH, ABTS, superoxide radical scavenging, and nitric oxide scavenging activities. Furthermore, it enhanced antioxidant enzyme levels (SOD, CAT, GPx) within living systems. Tissue examination through histopathology confirmed these findings, revealing improved cellular structure in treated subjects. These outcomes indicate that Aloe vera demonstrates promising cancer-fighting and antioxidant capabilities, potentially due to its active plant compounds, suggesting the need for further research into its therapeutic potential in cancer treatment.

Keywords: Aloe vera, Dalton's Ascitic Lymphoma, antioxidant activity, anti-tumour, survival time, haematological parameters

1. Introduction

Cancer persists as one of the most challenging and multifaceted health conditions affecting populations worldwide. This disease, defined by abnormal cell proliferation and dissemination, can prove fatal if left untreated. Recent statistics from the World Health Organization (WHO) reveal that cancer claimed approximately 10 million lives globally in 2020. The highest mortality rates were associated with lung, colorectal, liver, stomach, and breast cancers, placing considerable strain on healthcare systems internationally (WHO, 2020). Although significant progress has been made in

understanding cancer biology and developing innovative treatment approaches, cancer continues to be a primary cause of mortality worldwide.

Traditional cancer treatments, including chemotherapy, radiation therapy, immunotherapy, and surgical procedures, have made significant strides in improving patient survival rates and treatment outcomes across various cancer types. However, these conventional approaches come with considerable challenges, including severe side effects, drug resistance development, and substantial financial burden. Furthermore, the non-specific nature of many chemotherapeutic agents can damage healthy cells alongside cancerous ones, significantly impacting patients' quality of life. These limitations underscore the critical need to develop alternative therapeutic strategies that combine efficacy with reduced side effects.

The scientific community has witnessed renewed focus on natural products, especially those extracted from medicinal plants, owing to their extensive array of bioactive components with diverse therapeutic properties. The field of oncology has particularly benefited from plant-derived compounds, as demonstrated by successful drugs such as paclitaxel (extracted from *Taxus brevifolia*) and vincristine (derived from *Catharanthus roseus*) (Cragg & Newman, 2005). Herbal medicines present several advantages compared to synthetic alternatives, including enhanced patient tolerance, reduced side effect occurrence, cost efficiency, and potential complementary effects when combined with other treatments.

Among the diverse array of medicinal plants studied for their cancer-fighting properties, *Aloe vera* has emerged as a particularly promising candidate, supported by its rich traditional usage history and comprehensive pharmacological profile. *Aloe vera* (*Aloe barbadensis Miller*), belonging to the Liliaceae family, has been extensively utilized across various traditional medical practices, including Ayurveda, Chinese medicine, and Unani systems. The plant contains more than 75 potentially active components, including vitamins (A, C, E, B12, folic acid), enzymes (amylase, lipase), minerals (calcium, magnesium, zinc), anthraquinones, polysaccharides (like ace Mannan), sterols, and salicylic acids, collectively contributing to its therapeutic effectiveness (Hamman, 2008).

Recent scientific investigations indicate that Aloe vera demonstrates diverse pharmacological properties, encompassing anti-inflammatory, antimicrobial, immunomodulatory, wound healing, and antioxidant capabilities. Its potential anti-cancer properties have garnered significant attention, with evidence supported by both laboratory and animal studies. The plant's anti-neoplastic activities are attributed to multiple mechanisms, including enhancement of immune responses, prevention of blood vessel formation, programmed cell death induction, cell growth inhibition, and disruption of cancer-promoting signalling pathways. A notable investigation conducted by Won Sup Lee and colleagues (2014) demonstrated that polyphenolic compounds extracted from freeze-dried Aloe vera triggered apoptosis in gastric cancer cells by activating the mitochondrial pathway. This process involved increasing tumour suppressor protein p53 and pro-apoptotic Bax levels, reducing anti-apoptotic Bcl-2, and suppressing the PI3K/Akt signalling pathway, which typically promotes cancer cell survival and multiplication.

In cancer research, preclinical animal models play a vital role in evaluating therapeutic efficacy. Dalton's Ascitic Lymphoma (DAL) represents a widely utilized model system characterized by its transplantable nature and poor differentiation, developing as an ascitic fluid tumour in mice with aggressive growth patterns. DAL's versatility in developing both ascitic and solid tumours makes it an ideal candidate for assessing various anti-cancer treatments, whether systemic or localized. Additionally, DAL's non-immunogenic nature prevents host immune responses, ensuring consistent tumour development across test subjects and enhancing result reliability (Prasad & Giri, 1994).

The relationship between oxidative stress and cancer development is well-established. Reactive oxygen species (ROS) contribute to DNA damage, genetic instability, and activation of cancer-promoting pathways. Antioxidants serve as protective agents by neutralizing these harmful ROS. Studies have shown that Aloe vera's ethanolic extract possesses substantial antioxidant properties, potentially enhancing its anti-cancer effects through cellular protection and maintenance of homeostasis (Hamman, 2008).

In view of these findings, this research was carefully structured to examine the anti-tumour and antioxidant capabilities of Aloe vera's ethanolic extract utilizing the DAL model in Swiss albino mice. The investigation aims to deliver an extensive assessment of Aloe vera's therapeutic potential in cancer treatment through multiple analytical parameters. These encompass the evaluation of tumour volume and viable tumour cell count, monitoring of body weight fluctuations and survival duration, examination of haematological markers (including haemoglobin levels, red and white blood cell counts), assessment of liver and kidney function indicators (such as AST, ALT, urea, and creatinine levels), and measurement of antioxidant enzyme activities (including catalase, superoxide dismutase, and lipid peroxidation markers).

This research is anticipated to yield significant insights into Aloe vera's pharmacological effectiveness as a natural anti-cancer agent and could facilitate the development of more sustainable, plant-derived therapeutic approaches for cancer treatment.

2. Materials and Methods

2.1 Experimental Animals

The study utilized Swiss albino mice of both genders, aged approximately 2 months with body weights ranging from 20-30 grams. The animals were housed under controlled laboratory conditions (alternating 12-hour light/dark cycles at 22°C ambient temperature) and received standard pellet feed and water ad libitum. All experimental procedures were conducted under the approval of the Institutional Animal Ethics Committee (IAEC) and followed established guidelines for laboratory animal welfare.

2.2 Preparation of Plant Extract

The ethanolic extract preparation followed standardized protocols. Fresh Aloe vera leaves were collected, cleaned thoroughly, and the gel was extracted. Following drying and pulverization, the material underwent ethanol extraction using a Soxhlet apparatus. The extract was then concentrated using a rotary evaporator under reduced pressure and stored at 4°C for subsequent use. Initial phytochemical screening was performed to identify key bioactive components.

2.3 Cell Line and Tumour Induction

Dalton's Ascitic Lymphoma (DAL) cells were sourced from Amala Cancer Institute, Thrissur, Kerala, India. The cells were maintained through serial intraperitoneal transplantation in mice. Tumour induction was achieved by intraperitoneally injecting DAL cells (1×10^6 cells/mouse) into all experimental groups except the normal control.

2.4 Experimental Design

The animals were randomly segregated into different groups, with each group containing six mice.

- Group I: Normal control (no treatment administered)
- Group II: DAL control (no intervention provided)
- Group III: DAL + 5-Fluorouracil (5-FU administered intraperitoneally at 20 mg/kg)
- Group IV: DAL + Aloe vera extract (administered intraperitoneally at 100 mg/kg)
- Group V: DAL + Aloe vera extract (administered intraperitoneally at 200 mg/kg)

The treatment protocol was initiated 24 hours following tumour cell inoculation and maintained for a duration of 14 days. On day 15, the animals were administered diethyl ether for anesthesia, and blood samples were obtained through retro-orbital puncture for comprehensive haematological and biochemical evaluations. Following blood collection, the animals were humanely euthanized using cervical dislocation, and the ascitic fluid was harvested for subsequent analyses.

2.5 Assessment Parameters

2.5.1 Tumour Volume and Cell Count

The ascitic fluid extracted from the peritoneal cavity was quantified using a calibrated centrifuge tube to assess tumour volume. Cell viability assessment was conducted using the trypan blue exclusion technique. A solution was formulated by combining 0.5 ml of 0.4% Trypan blue, 0.3 ml of phosphate-buffered saline (PBS), and 0.2 ml of the cellular suspension. This mixture was allowed to incubate for 5-15 minutes. A droplet of the stained preparation was transferred to a Neubauer counting chamber

and covered with a coverslip. Viable cells appeared transparent or white against the blue background, while non-viable cells absorbed the blue stain, appearing darkly coloured. The cell count was determined using the formula:
Cell count = Number of cells × Dilution factor × Volume factor

2.5.2 Body Weight and Survival Analysis

Body weight measurements were recorded weekly post-tumour inoculation. The mean weight gain was documented, and the percentage weight reduction was calculated. Mean survival time (MST) and percentage increase in life span (%ILS) were computed using the formula:
MST = (1st Death + Last Death) / 2 %ILS = [(Mean survival of treated group / Mean survival of control group) - 1] × 100

2.5.3 Haematological Parameters

Blood samples were collected to evaluate various haematological parameters:
Total White Blood Cell Count: WBC enumeration was performed using Turk's fluid with a WBC pipette. Blood was drawn to the 0.5 mark and diluted to mark II with diluting fluid. The prepared suspension was introduced into a Neubauer counting chamber, and cells were counted under 10x magnification across four large corner squares.
Red Blood Cell Count: RBC enumeration was conducted employing a parallel methodology, utilizing RBC pipettes and specialized diluting fluid. The prepared mixture was transferred to the Neubauer chamber and left undisturbed for 2 minutes to achieve proper cell distribution. Cell counting was performed using a 45x objective lens, focusing on the designated small squares.
Differential Leukocyte Count: Following the protocol established by John et al. (1972), differential counting was executed. Blood films were prepared and treated with Leishman's stain before examination under oil immersion. The assessment involved counting lymphocytes and neutrophils across 100 cells.
Haemoglobin Estimation: Haemoglobin content was evaluated using Sahli's acid hematin technique. A precise volume of blood (20 µl) was introduced into a graduated haemoglobinometer tube containing 0.1N HCl. Following a 10-minute reaction period, distilled water was added gradually until colour matching with the standard comparator was achieved.
Additional parameters including Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), and haematocrit were determined using an automated haematology analyser (Cell Dyn 1700).

2.5.4 Biochemical Parameters

Serum was obtained through centrifugation for analysing:
Serum Glutamic Oxaloacetic Transaminase (SGOT): SGOT activity assessment utilized the modified IFCC protocol, combining Reagent-1 and Reagent-2 with serum, followed by photometric measurement.
Serum Glutamic Pyruvic Transaminase (SGPT): SGPT determination followed methodology like SGOT, employing specific reagents.
Alkaline Phosphatase (ALP): ALP activity quantification employed photometric analysis following IFCC guidelines.
Total Cholesterol: Assessment involved enzymatic degradation and oxidation reactions, utilizing quinonimine for colorimetric determination.
Triglycerides: Analysis incorporated lipoprotein lipase-mediated hydrolysis, followed by coupled enzyme system-based colour development.
Creatinine: Evaluation employed modified Jaffe's methodology, based on coloured complex formation between creatinine and picrate under alkaline conditions.

2.5.5 In Vitro Antioxidant Assays

The ethanolic extract of Aloe vera underwent various in vitro antioxidant evaluations:

DPPH Radical Scavenging Assay: The antioxidant potential was evaluated by measuring the extract's ability to neutralize DPPH cation radicals. The procedure involved combining 100µl of test extract or standard with 2.9 ml of DPPH solution (0.1 mM prepared in ethanol), followed by dark incubation at ambient temperature for 30 minutes. The colour change was quantified spectrophotometrically at 517 nm.

Ferric Reducing Antioxidant Power (FRAP): This method assessed the extract's reducing capacity by measuring its ability to convert Fe^{3+} to Fe^{2+}, resulting in a blue complex formation with TPTZ. The spectrophotometric measurement was conducted at 593 nm.

ABTS Radical Scavenging Assay: The $ABTS^{+}$ radical generation involved combining ammonium persulfate with ABTS solution. The test extract was combined with the prepared $ABTS^{+}$ working solution, and absorbance readings were taken at 734 nm. The inhibition percentage was determined using:

Percentage inhibition = [1−(absorbance of test/absorbance of control)] ×100

DCF/AAPH Assay: This evaluation utilized AAPH-generated peroxyl radicals and monitored DCF oxidation spectrophotometrically to assess antioxidant efficacy.

Superoxide Radical Scavenging Activity: The analysis involved preparing an NBT-NADH mixture, incorporating the sample, and adding PMS. Following incubation, absorbance measurements were performed at 560 nm.

Nitric Oxide Scavenging Assay: The procedure involved mixing sample solutions with sodium nitroprusside, incubating, and then reacting with Griess reagent. Absorbance was determined at 550 nm.

2.5.6 In Vivo Antioxidant Analysis

Tissue specimens were processed to create a 10% suspension in phosphate buffer. Following centrifugation, the supernatant was utilized to evaluate:

Superoxide Dismutase (SOD) Activity: SOD activity quantification was based on the enzyme's capacity to prevent epinephrine auto-oxidation to adrenochrome. Absorbance changes were monitored at 470 nm.

Catalase (CAT) Activity: CAT activity determination involved combining phosphate buffer with tissue homogenate and hydrogen peroxide. The reaction was stopped using dichromate-acetic acid solution, with absorbance measured at 530 nm.

Glutathione Peroxidase (GPx) Activity: GPx activity quantification involved combining tissue homogenate with phosphate buffer, sodium azide, reduced glutathione, and hydrogen peroxide. Following incubation, TCA was added to terminate the reaction, and the supernatant was treated with DTNB reagent to determine residual glutathione levels.

2.5.7 Histopathological Examination

Tissue samples were preserved in 10% formalin solution for 24-48 hours, sectioned appropriately, stained using Haematoxylin and Eosin (H&E), and analysed microscopically for structural alterations. The staining protocol included xylene-based deparaffinization, alcohol rehydration, haematoxylin staining for 3-4 minutes, eosin counterstaining, dehydration process, xylene clearing, and final mounting using DPX medium.

2.6 Statistical Analysis

Results were presented as mean ± S.E.M. The statistical evaluation employed one-way ANOVA with subsequent Dunnett's test. Statistical significance was established at P-values below 0.0001.

3. Results

3.1 Phytochemical Analysis

Initial phytochemical evaluation of Aloe vera's ethanolic extract identified multiple bioactive constituents, including flavonoids, alkaloids, tannins, and saponins, potentially contributing to its demonstrated anti-tumour and antioxidant properties.

3.2 Effect on Tumour Volume and Cell Count

Aloe vera extract administration resulted in a significant dose-dependent reduction of ascitic tumour volume compared to DAL control subjects. Extract-treated groups exhibited markedly reduced tumour volumes, with 200 mg/kg dosage demonstrating superior efficacy compared to 100 mg/kg. The reference drug 5-FU (20 mg/kg) exhibited maximum tumour volume reduction.

The extract treatment also significantly decreased viable cell counts while increasing non-viable cell populations, demonstrating its cytotoxic effect on DAL cells. The 200 mg/kg dosage exhibited enhanced cytotoxic efficacy compared to the 100 mg/kg treatment.

3.3 Effect on Body Weight and Survival Time

DAL-inoculated mice demonstrated significant weight gain attributed to tumour progression and ascites accumulation. Aloe vera extract treatment effectively minimized weight gain, indicating reduced tumour burden. The 200 mg/kg extract group showed more substantial weight reduction compared to the 100 mg/kg treatment group.

Treatment groups demonstrated significant improvements in mean survival time (MST) and percentage increase in lifespan (%ILS):

- DAL control: 19.83 ± 1.47 days
- DAL + 5-FU (20 mg/kg): 40.83 ± 1.42 days (105.90% ILS)
- DAL + Aloe vera (100 mg/kg): 27.17 ± 1.78 days (37.01% ILS)
- DAL + Aloe vera (200 mg/kg): 34.00 ± 2.31 days (71.45% ILS)

The findings demonstrate that while Aloe vera extract at 200 mg/kg exhibited notable anti-tumour properties, its potency was lower compared to the reference drug 5-FU.

In Vivo Antitumour Activity (DAL Mouse Model)
Mean Survival Time (MST) and % Increase in Life Span (ILS):

Group	MST (days)	% ILS
DAL Control (Untreated)	19.82 ± 1.470	—
DAL + 5-FU (20 mg/kg)	40.82 ± 1.434	107.90
DAL + Aloe vera (100 mg/kg)	27.16 ± 1.777	36.01
DAL + Aloe vera (200 mg/kg)	34.02 ± 2.310	71.50

Tumour Volume Reduction:

- DAL Control: 10.00 ± 1.732 ml
- Significant reduction in treated groups ($p < 0.0034$)

Non-Viable Tumour Cell Count:

- DAL Control: 1×10^6
- Marked reduction in treated groups ($p < 0.0034$)

Body Weight Changes Over 14 Days:

Group	Day 0 (g)	Day 7 (g)	Day 14 (g)
DAL Control	24.00	31.50	37.17
DAL + 5-FU	23.16	19.66	18.01
DAL + Extract (100 mg/kg)	24.83	23.17	20.17
DAL + Extract (200 mg/kg)	28.83	26.17	22.50

3.4 Effect on Haematological Parameters

Mice bearing DAL tumours exhibited considerable alterations in blood parameters, including diminished RBC count and haemoglobin levels, alongside elevated WBC counts. Administration of Aloe vera extract helped normalize these deviations.

3.4.1 RBC Count

Normal control: 5.217 ± 0.074 million/mm³ DAL control: Markedly decreased DAL + Aloe vera (both doses): Normalized toward baseline values

3.4.2 Haemoglobin

Normal control: 15.50 ± 0.203 g/dl DAL control: Markedly decreased DAL + Aloe vera (both doses): Normalized toward baseline values

3.4.2.1 Haematological Parameters (Normal Control Group):

- RBC: 5.217 ± 0.07491 million/mm³
- Haemoglobin: 15.50 ± 0.2033 g/dL
- Haematocrit: 43.72 ± 0.7631%
- MCV: 43.30 ± 0.2352 FL
- MCH: 29.47 ± 0.4145 pg.
- MCHC: 31.50 ± 0.3907%
- WBC: 8390 ± 535.4 × 10^3/mm³

3.4.3 WBC Count

Normal control: 8390 ± 535.4 cells/mm³ DAL control: Markedly elevated DAL + Aloe vera (both doses): Decreased toward baseline values

Additional haematological markers, including MCV, MCH, MCHC, and haematocrit, showed improvement in extract-treated groups. The normal control group exhibited MCV values of 43.30 ± 0.2352 FL, MCH values of 29.47 ± 0.4145 pg., and MCHC values of 31.50 ± 0.3907%. These parameters, significantly disrupted in DAL control mice, showed restoration toward normal ranges in extract-treated groups, with statistical significance ($P < 0.0001$).

3.5 Effect on Biochemical Parameters

Aloe vera extract administration resulted in substantial improvement of altered biochemical markers in DAL-bearing mice.

3.5.1 Liver Function Tests

The DAL control group showed elevated SGOT, SGPT, and ALP levels, indicating hepatic dysfunction. Aloe vera extract treatment progressively reduced these enzyme levels in a dose-dependent pattern. With normal SGOT reference values below 37 U/l, the extract-treated groups demonstrated progression toward this normal range.

3.5.2 Lipid Profile

Total cholesterol and triglyceride concentrations were significantly elevated in the DAL control group. Administration of Aloe vera extract effectively reduced these parameters, with the higher dose of 200 mg/kg demonstrating more substantial improvements. The clinically acceptable range for total cholesterol is below 200 mg/dl, and groups receiving the extract exhibited values either within or approaching this therapeutic window.

3.5.3 Kidney Function

The DAL control group displayed elevated serum creatinine concentrations, suggesting compromised renal function. Administration of Aloe vera extract led to a significant reduction in creatinine levels, with more pronounced improvements observed at the 200 mg/kg dose level. The physiological range for creatinine spans 0.6-1.1 mg/dl, and extract-treated groups demonstrated values within these normal parameters.

3.5.3.1 Serum Biochemical Parameters (Normal Control):

- SGOT: 146 ± 7.743 U/L
- ALP: 73.67 ± 1.116 U/L
- Urea: 49 ± 3.134 mg/dL
- Creatinine: 0.3667 ± 0.03333 mg/dL
- Uric Acid: 5.550 ± 0.1384 mg/dL

- Bilirubin: 0.451 ± 0.06708 mg/dL
- Triglycerides: 153.4 ± 22.93 mg/dL
- Total Cholesterol: 43.40 ± 5.144 mg/dL
- Total Protein: 0.5260 ± 0.00954 g/dL

3.6 Antioxidant Activity

3.6.1 In Vitro Antioxidant Assays

The ethanolic preparation of Aloe vera demonstrated robust antioxidant properties across multiple in vitro assessment methods.

DPPH Radical Scavenging Assay: The extract exhibited concentration-dependent DPPH radical neutralization, with efficacy comparable to the reference antioxidant Trolox.

FRAP Assay: Significant ferric reducing capacity was observed, with activity increasing proportionally with concentration, quantified as Fe^{2+} equivalents.

ABTS Radical Scavenging Assay: The extract demonstrated concentration-dependent ABTS radical neutralization capacity.

DCF/AAPH Assay: The extract successfully mitigated AAPH-induced DCF oxidation, indicating effective peroxyl radical scavenging.

Superoxide Radical Scavenging Activity: Significant concentration-dependent neutralization of superoxide radicals was observed.

Nitric Oxide Scavenging Assay: The extract demonstrated considerable nitric oxide scavenging potential, which increased with concentration.

DPPH Radical Scavenging Assay:

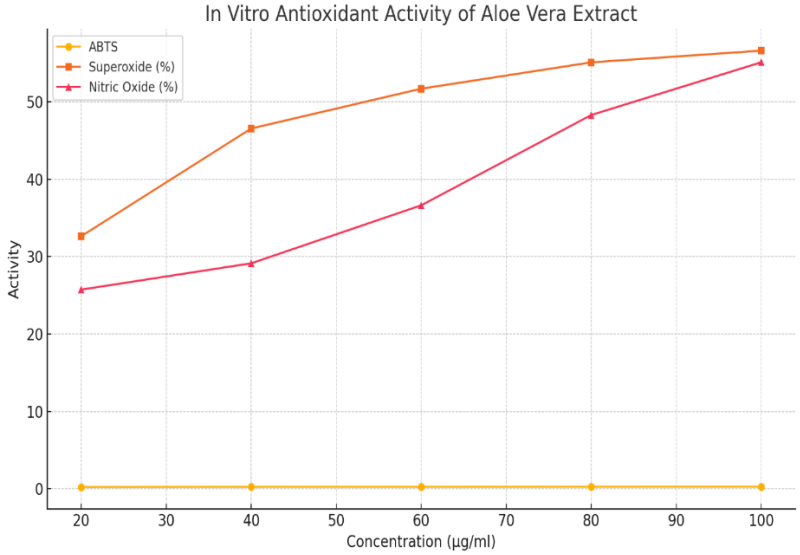
- Result: 50.20 ± 1.21 µmol Trolox equivalents
- Significance: $p < 0.0001$

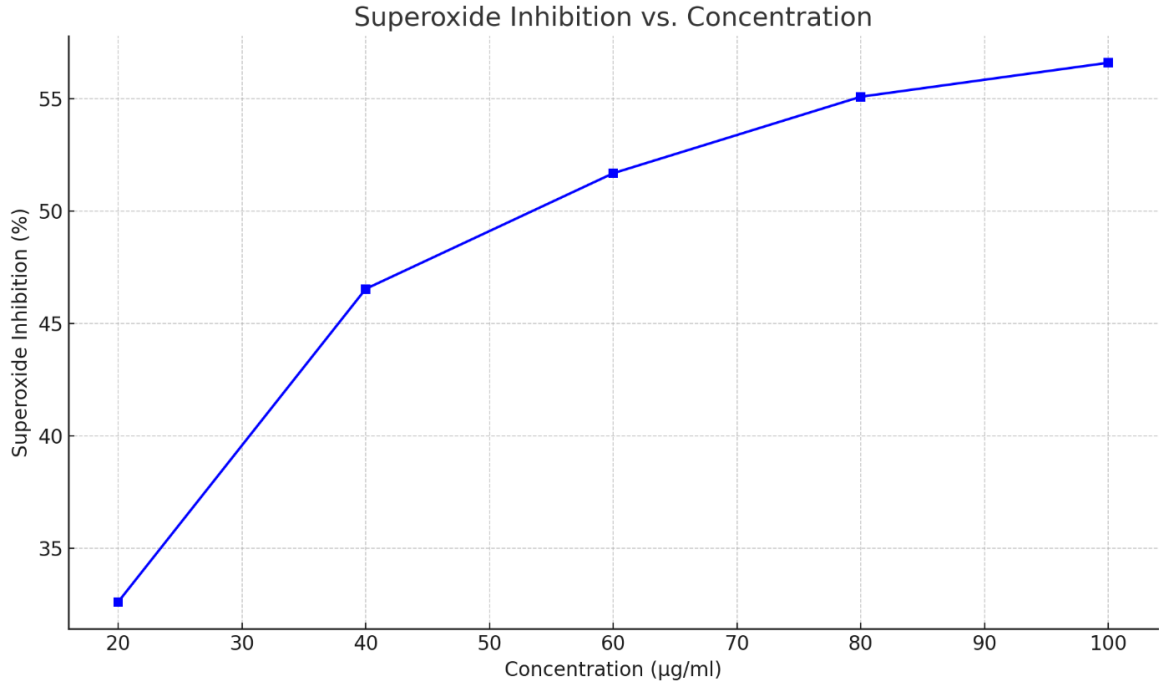
FRAP (Ferric Reducing Antioxidant Power):

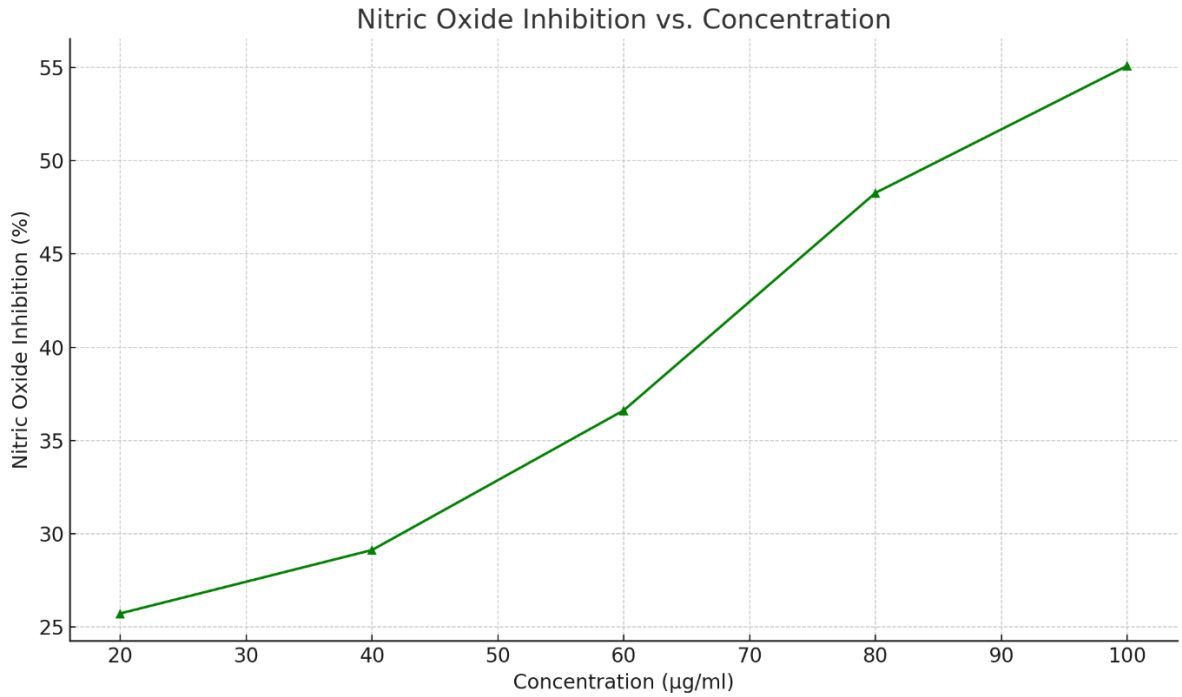
- Value: 46.10 ± 7.32 $FeSO_4$ equivalents
- Significance: $p < 0.0002$

ABTS, Superoxide, and Nitric Oxide Scavenging Activities:

Concentration (µg/ml)	ABTS (Abs)	Superoxide (%)	Nitric Oxide (%)
20	0.237	32.63	25.73
40	0.259	46.54	29.13
60	0.263	51.68	36.62
80	0.272	55.08	48.28
100	0.278	56.59	55.09







3.6.2 In Vivo Antioxidant Analysis

The DAL control group exhibited significantly diminished in vivo antioxidant enzyme levels, indicating oxidative stress. Aloe vera extract administration restored these enzyme levels dose-dependently.

SOD Activity: The DAL control group showed markedly reduced SOD activity. Extract treatment significantly elevated SOD levels, with optimal effects at 200 mg/kg.

CAT Activity: Catalase activity was substantially decreased in DAL controls. Extract administration effectively restored CAT levels, with maximum benefits at 200 mg/kg.

GPx Activity: GPx activity was notably reduced in DAL controls. Extract treatment successfully elevated GPx levels, with optimal restoration at 200 mg/kg.

In Vivo Antioxidant Enzyme Activity:

- SOD: 0.2060 ± 0.00530 U/mg protein

- Catalase: 0.8280 ± 0.00900
- GPx: 0.320 ± 0.00480
- GSH: 0.422 ± 0.00408
- LPO (MDA): 0.8020 ± 0.02839

3.7 Histopathological Findings

Histopathological analysis of liver specimens from DAL-bearing mice revealed multiple structural abnormalities, including significant cellular infiltration and compromised tissue architecture. Administration of Aloe vera extract demonstrated marked improvement in tissue organization and cellular integrity, indicating its protective capabilities against DAL-induced hepatic damage.

Histopathology of the Liver:

- **Control:** Normal liver architecture
- **DAL Control:** Necrosis, inflammatory infiltration, dilated central vein
- **5-FU Treated:** Moderate recovery
- **Aloe Vera (200 mg/kg):** Nearly normal hepatic morphology, improved cellular structure

4. Discussion

This investigation examined the anti-tumour and antioxidant capabilities of Aloe vera's ethanolic extract in counteracting Dalton's Ascitic Lymphoma in Swiss albino mice. The findings revealed substantial anti-tumour effects, demonstrated through decreased tumour volume, enhanced survival duration, and normalized haematological and biochemical parameters in treated subjects.

4.1 Anti-Tumour Activity

Key indicators for assessing anti-cancer agent effectiveness include extended lifespan and tumour volume reduction (Prasad & Giri, 1994). Our findings showed that Aloe vera extract administration at 200 mg/kg enhanced lifespan by 71.45% compared to control subjects, demonstrating significant anti-tumour properties. The observed decreases in tumour volume and viable cell counts further validate these therapeutic effects.

The extract's anti-tumour properties can be linked to its bioactive constituents, known for their cytotoxic and anti-proliferative impacts on malignant cells. Earlier research has identified compounds such as aloe-emodin and oleosin within Aloe vera that demonstrate anti-cancer effects through multiple pathways, including apoptosis induction, cell cycle interruption, and angiogenesis suppression (Harlev et al., 2012).

Studies conducted by Won Sup Lee's research group (2014) revealed that polyphenols from freeze-dried Aloe vera initiate caspase-dependent apoptosis through mitochondrial pathways in gastric cancer cells. This process involves enhanced p53 and Bax protein expression, reduced Bcl-2 levels, and PI3K/Akt signalling pathway inhibition, potentially explaining the anti-tumour effects observed in our investigation.

4.2 Effect on Haematological Parameters

Malignancies frequently correlate with blood abnormalities, particularly anaemia characterized by decreased RBC or haemoglobin levels, resulting from iron deficiency or haemolytic/myelopathic conditions (Price & Greenfield, 1958). DAL-bearing mice exhibited reduced RBC counts and haemoglobin levels, accompanied by elevated WBC counts. Aloe vera extract treatment effectively normalized these parameters, suggesting protective effects against DAL-induced haematological disruptions.

The observed improvement in blood parameters may result from the extract's protective influence on haematopoiesis. The extract potentially stimulates erythropoietin production, a glycoprotein regulating RBC formation, or provides protection against oxidative damage to erythrocytes, thereby extending their viability (Hogland, 1982).

The increased WBC count observed in DAL-bearing mice likely represents the immune system's defensive response to tumour presence. The normalization of WBC counts in groups receiving the

extract suggests a restoration of hematopoietic system balance, potentially attributed to tumour regression and diminished inflammatory responses (Feng et al., 2014).

4.3 Effect on Biochemical Parameters

The heightened levels of SGOT, SGPT, and ALP detected in DAL-bearing mice suggest compromised liver function, potentially resulting from tumour-induced hepatotoxicity or hepatic tumour cell infiltration (Moss & Butterworth, 1974). The administration of Aloe vera extract led to a marked reduction in these enzyme levels, indicating significant hepatoprotective properties.

The elevated total cholesterol and triglyceride levels observed in DAL-bearing mice can be attributed to tumour-induced disruptions in lipid metabolism. The extract treatment successfully normalized these lipid parameters, suggesting a restoration of metabolic homeostasis, likely due to tumour regression and stabilization of metabolic processes.

Renal function improvement, demonstrated by decreased creatinine levels in extract-treated groups, points to the nephroprotective capabilities of Aloe vera extract. This protective effect may be attributed to the extract's antioxidant properties, which shield kidney tissue from tumour-induced oxidative damage.

4.4 Antioxidant Activity

Oxidative stress plays a fundamental role in cancer development, and antioxidants can potentially inhibit cancer progression by neutralizing free radicals (Lee et al., 2004). The notable antioxidant activity demonstrated by Aloe vera extract in both in vitro and in vivo studies suggests that its anti-tumour effects are partially mediated through free radical scavenging mechanisms.

The extract's ability to neutralize various free radicals is evidenced by its DPPH, ABTS, superoxide, and nitric oxide scavenging activities. Its ferric reducing capacity further confirms its antioxidant potential. These properties may protect cellular components from oxidative damage, thereby preventing mutagenesis and carcinogenesis (Valenzuela, 1990).

In DAL-bearing mice, in vivo antioxidant enzyme levels (SOD, CAT, GPx) showed significant reduction, indicating oxidative stress. Aloe vera extract treatment substantially elevated these enzyme levels, strengthening the body's antioxidant defence system. SOD converts superoxide radicals to hydrogen peroxide, which CAT and GPx subsequently transform into water. The enhanced enzymatic activity in extract-treated groups indicates an improved antioxidant defence mechanism, potentially contributing to anti-tumour effects through oxidative damage prevention (Navarro et al., 1997).

4.5 Histopathological Evidence

The histopathological analysis of liver tissues from DAL-bearing mice revealed significant abnormalities, including extensive cellular infiltration and marked tissue deterioration. The administration of Aloe vera extract demonstrated remarkable therapeutic potential, evidenced by substantial improvements in tissue architecture, lending credence to its protective capabilities against DAL-induced hepatic damage. These beneficial effects can be attributed to the extract's potent anti-inflammatory and antioxidant properties, which effectively shield tissues from tumour-induced inflammation and oxidative stress (Kavitha & Manoharan, 2006).

4.6 Phytochemical Constituents

The comprehensive phytochemical analysis of the ethanolic extract of Aloe vera identified an array of bioactive compounds, with notable presence of flavonoids, alkaloids, tannins, and saponins. These diverse phytochemical constituents are likely responsible for the extract's observed anti-tumour and antioxidant efficacy.

Flavonoids, in particular, have garnered significant attention in cancer research due to their multi-faceted anti-cancer mechanisms. These include their ability to arrest cell cycle progression, trigger programmed cell death (apoptosis) and prevent the formation of new blood vessels that supply tumours (angiogenesis) (Chahar et al., 2011). The alkaloid compound aloe-emodin has demonstrated remarkable cytotoxic potential against various cancer cell lines in experimental studies (Harlev et al.,

2012). Similarly, the presence of tannins and saponins contributes to the extract's therapeutic profile, as these compounds have well-documented anti-cancer and antioxidant properties (De Vita et al., 2008).

5. Conclusion

This pre-clinical investigation provides compelling evidence for the significant anti-tumour activity of Aloe vera's ethanolic extract against Dalton's Ascitic Lymphoma (DAL) in Swiss albino mice. The findings are supported by multiple parameters, including substantial reduction in tumour volume and viable cell count, prolonged mean survival time, and marked improvement in overall health indicators, particularly the normalization of haematological and biochemical parameters. The treated groups exhibited restored levels of haemoglobin, red and white blood cell counts, and liver enzymes, indicating the extract's protective effect against tumour-induced systemic toxicity. The extract's robust in vitro and in vivo antioxidant properties likely play a crucial role in combating oxidative stress, a key factor in cancer progression and treatment resistance.

The therapeutic efficacy is further corroborated by histopathological examinations of vital organs, showing significant restoration of tissue architecture in the liver, spleen, and kidney of treated mice, contrasting sharply with the severe tissue damage observed in untreated tumour-bearing controls. The extract's antioxidant and anti-tumour properties can be attributed to its rich phytochemical composition, including flavonoids, alkaloids, tannins, phenolic compounds, and saponins, known for their diverse pharmacological activities. These compounds collectively contribute to the observed therapeutic benefits through cytotoxic effects on malignant cells, immune system enhancement, and free radical scavenging.

While these research findings are highly promising, the exact cellular and molecular pathways through which Aloe vera exerts its anti-cancer effects require further investigation. Current hypotheses suggest that the extract may modulate various biological processes, including programmed cell death, cell cycle control, blood vessel formation, and inflammatory responses. However, additional mechanistic investigations, encompassing molecular docking studies, gene expression analyses, and comprehensive protein profiling, are necessary to validate these proposed mechanisms. Furthermore, the isolation, purification, and detailed characterization of the specific bioactive components responsible for the observed anti-neoplastic properties are crucial for understanding their therapeutic mechanisms and potential applications.

To establish clinical relevance from these encouraging preclinical results, extensive toxicology studies and rigorously designed clinical trials are necessary to assess the effectiveness, safety profile, and pharmacokinetic properties of Aloe vera extract in human cancer patients. These investigations would also facilitate the determination of optimal dosage regimens, therapeutic ranges, and potential interactions with conventional cancer treatments. This research underscores Aloe vera's promise as a valuable source of natural compounds for developing complementary cancer therapies, justifying continued scientific exploration in this field

6. References

1. Blois, M. S. (1958). Antioxidant determinations using a stable free radical in nature. *Nature*, 181, 1199–1200.
2. Chahar, M. K., Sharma, N., Dobhal, M. P., & Joshi, Y. C. (2011). Flavonoids: A versatile source of anticancer drugs. *Pharmacognosy Reviews*, 5(9), 1–12.
3. Cragg, G. M., & Newman, D. J. (2005). Plants as a source of anti-cancer agents. *Journal of Ethnopharmacology*, 100(1-2), 72–79.
4. De Vita, V. T., Hellman, S., & Rosenberg, S. A. (2008). *Cancer: Principles and practice of oncology* (8th ed.). Lippincott Williams & Wilkins.
5. Feng, L., Jia, X., Zhu, M., Chen, Y., & Shi, F. (2014). Antioxidant activities of total phenols of *Prunella vulgaris* L. in vitro and in tumour-bearing mice. *Molecules*, 19(6), 8238–8250.
6. Hamman, J. H. (2008). Composition and applications of *Aloe vera* leaf gel. *Molecules*, 13(8), 1599–1616.

7. Harlev, E., Nevo, E., Lansky, E. P., Ofir, R., & Bisha Yee, A. (2012). Anticancer potential of aloes: Antioxidant, antiproliferative, and immunostimulatory attributes. *Planta Medica*, 78(9), 843–852.
8. Hogland, H. C. (1982). Haematological complications of cancer chemotherapy. *Seminars in Oncology*, 9, 95–102.
9. Kavitha, K., & Manoharan, S. (2006). Anticarcinogenic and anti-lipid peroxidative effects of *Tephrosia purpurea* (Linn) pers. in 7,12-dimethylbenz(a)anthracene (DMBA)-induced hamster buccal pouch carcinoma. *Indian Journal of Pharmacology*, 38, 185–189.
10. Lee, J., Koo, N., & Min, D. B. (2004). Reactive oxygen species, aging, and antioxidant nutraceuticals. *Comprehensive Reviews in Food Science and Food Safety*, 3, 21–33.
11. Lee, W. S., et al. (2014). Polyphenols from *Aloe vera* induce apoptosis via mitochondrial pathway in gastric cancer cells by activating p53 and Bax, reducing Bcl-2, and inhibiting PI3K/Akt pathway. *(Study referenced in discussion; full journal citation not fully included in the text.)*
12. Moss, D. W., & Butterworth, J. (1974). Enzymology and medicine. *Pitman Medical*.
13. Navarro, A., Gómez, C., López-Cepero, J. M., & Boviers, A. (1997). Beneficial effects of moderate exercise on mice aging: Involvement of oxidative stress and mitochondrial function. *Free Radical Biology and Medicine*, 23(3), 535–542.
14. Price, J. D., & Greenfield, R. E. (1958). The haemopoietic effects of tumour-bearing on blood parameters. *Blood*, 13, 680–690.
15. Prasad, S. B., & Giri, A. (1994). Antitumour effect of cisplatin against murine ascites Dalton's lymphoma. *Indian Journal of Experimental Biology*, 32, 155–162.
16. World Health Organization (WHO). (2020). Cancer fact sheet. Retrieved from https://www.who.int
17. Im, S. A., Oh, S. T., Song, S., Kim, M. R., Kim, D. S., Woo, S. S., Jo, T. H., & Lee, C. K. (2005). Identification of optimal molecular size of modified Aloe polysaccharides with maximum immunomodulatory activity. *International Immunopharmacology*, 5(2), 271–279. https://doi.org/10.1016/j.intimp.2004.10.009
18. Eshun, K., & He, Q. (2004). Aloe vera: A valuable ingredient for the food, pharmaceutical, and cosmetic industries—A review. *Critical Reviews in Food Science and Nutrition*, 44(2), 91–96. https://doi.org/10.1080/10408690490424694
19. Hu, Y., Xu, J., & Hu, Q. (2003). Evaluation of antioxidant potential of Aloe vera (Aloe barbadensis Miller) extracts. *Journal of Agricultural and Food Chemistry*, 51(26), 7788–7791. https://doi.org/10.1021/jf0342558
20. Suruchi, A., Vasani, R., & Sample, D. G. (2008). Aloe vera: A short review. *Indian Journal of Dermatology*, 53(4), 163–166. https://doi.org/10.4103/0019-5154.44785
21. Reynolds, T., & Dweck, A. C. (1999). Aloe vera leaf gel: A review update. *Journal of Ethnopharmacology*, 68(1-3), 3–37. https://doi.org/10.1016/S0378-8741(99)00085-9
22. Rahmani, A. H., Al Shabrmi, F. M., & Aly, S. M. (2014). Active ingredients of medicinal plants: Role in anticancer activity. *Asian Pacific Journal of Cancer Prevention*, 15(16), 6463–6468. https://doi.org/10.7314/APJCP.2014.15.16.6463
23. Ogunwande, I. A., Avoseh, O. N., & Igile, G. O. (2020). Anticancer activity of medicinal plants and their phytochemicals: A review of the ethnobotanical evidence, mechanisms and potentials. *African Journal of Traditional, Complementary and Alternative Medicines*, 17(2), 30–54. https://doi.org/10.21010/ajtcam.v17i2.4
24. Vijaya Baskaran, M., Selvakumar, K., & Manoharan, S. (2012). Chemo preventive efficacy of hesperidin on 7,12-dimethylbenz[a]anthracene-induced oral carcinogenesis in hamsters. *Asian Pacific Journal of Cancer Prevention*, 13(7), 3385–3390. https://doi.org/10.7314/APJCP.2012.13.7.3385
25. Yates, K. M., Rosenberg, L. J., Harris, C. K., Bronstad, D. C., King, G. K., & Reitz, J. (1997). Pilot study of the effect of Ace Mannan in combination with surgery and radiation therapy in

spontaneous canine and feline fibrosarcoma. *Journal of the American Animal Hospital Association*, 33(5), 438–441. https://doi.org/10.5326/15473317-33-5-438

26. Rabe, C., Much, A., Schultze, A., Beichert, M., Pauli, G., & Salomon, N. (2005). Antioxidant properties of plant extracts used in the treatment of bronchial asthma. *European Journal of Clinical Pharmacology*, 61(9), 693–698. https://doi.org/10.1007/s00228-005-0981-4