ANTI-TUMOR POTENTIAL OF *CASSIA FISTULA LINN.*: A PHYTOCHEMICAL AND PRECLINICAL STUDY ON DALTON'S LYMPHOMA ASCITES

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ABSTRACT:

Cancer remains a major global health concern, with treatment effectiveness often limited by adverse effects and the development of drug resistance. Natural compounds, particularly those derived from plants, have gained attention as potential therapeutic agents. *Cassia fistula* Linn, recognized for its medicinal properties, was investigated for its anti-tumor and antioxidant activities against Dalton's Lymphoma Ascites (DLA) in a murine model. Methanolic extracts of *Cassia fistula* leaves were obtained and evaluated through both in vitro and in vivo studies. Swiss albino mice were categorized into different groups, receiving either the plant extract at doses of 250 mg/kg and 500 mg/kg or 5-fluorouracil (5-FU) as a reference drug. Tumor volume, mean survival time (MST), haematological parameters, and antioxidant potential were analyzed using DPPH, ABTS, and FRAP assays. Additionally, phytochemical screening was conducted to identify major bioactive constituents.

The extract demonstrated dose-dependent inhibition of tumor growth, with the 500 mg/kg dose exhibiting comparable efficacy to 5-FU. MST showed a 58.9% increase, while haemoglobin and RBC levels were restored to normal ranges. The antioxidant evaluation revealed strong free radical scavenging activity, with 80% DPPH inhibition at 500 µg/mL and an IC_{50} value of 71.29 µg/mL. Phytochemical analysis confirmed the presence of flavonoids, phenolics, and glycosides.

KEYWORDS:

Cassia fistula, anti-tumor activity, Dalton's Lymphoma Ascites, antioxidants, phytochemicals.

INTRODUCTION:

Cancer remains one of the most significant global health concerns, contributing substantially to both morbidity and mortality. It is characterized by the uncontrolled proliferation of cells, which can invade surrounding tissues and, in some instances, metastasize to distant organs. This abnormal growth arises due to genetic mutations, epigenetic modifications, and

disruptions in cellular regulatory pathways. The process of tumor development occurs in multiple stages, namely initiation, promotion, and progression [1-2]. During initiation, genetic mutations lead to the activation of oncogenes or the inactivation of tumor suppressor genes. The promotion phase supports the proliferation of these altered cells, while the progression stage results in the formation of malignant tumours capable of invading tissues and spreading throughout the body [3].

Cancer treatment typically involves a combination of surgery, chemotherapy, radiation therapy, and immunotherapy. However, these conventional methods are associated with significant limitations, including adverse side effects, toxicity to healthy cells, and the emergence of drug resistance. Consequently, there is a growing focus on identifying alternative treatment strategies that are both safer and more effective [4]. One promising area of research involves the use of natural products derived from plants, which have long been utilized in traditional medicine for their therapeutic properties. Many plants are rich in bioactive compounds, such as flavonoids, alkaloids, and polyphenols, which exhibit antioxidant, anti-inflammatory, and anti-cancer properties [5].

Cassia fistula Linn, commonly referred to as the golden shower tree or Indian laburnum, is widely acknowledged for its medicinal benefits [6]. Native to India and various regions of South Asia, this plant has been extensively used in Ayurvedic and other traditional medical systems. Different parts of Cassia fistula, including its leaves, bark, fruit, and seeds, have been utilized for the treatment of skin conditions, digestive issues, and infections [7].

Phytochemical investigations have identified numerous bioactive compounds within Cassia fistula, including flavonoids, anthraquinones, and glycosides. These compounds contribute to the plant’s pharmacological effects, which include antioxidant, antimicrobial, anti-inflammatory, and anti-cancer activities. Among them, flavonoids play a key role in scavenging free radicals and mitigating oxidative stress, a major factor in cancer progression [8].

The potential of Cassia fistula as an anti-cancer agent has drawn increasing scientific interest due to its ability to inhibit tumor growth and enhance the body's antioxidant defense mechanisms. Preliminary studies have indicated that extracts derived from the leaves of Cassia fistula can suppress cancer cell proliferation, induce apoptosis, and modulate immune responses. These findings highlight the plant’s potential as a natural source for developing innovative anti-tumor therapies with reduced side effects [9-11].

This study aims to provide a comprehensive evaluation of the anti-tumor efficacy of methanolic extracts from Cassia fistula Linn leaves in the context of Dalton’s Lymphoma

Ascites (DLA)-induced tumors in murine models. By emphasizing the relevance of plant-based therapies in cancer treatment, the research underscores the necessity of further exploring the medicinal properties of Cassia fistula. Understanding its phytochemical composition and therapeutic potential may contribute to the development of effective, safe, and affordable cancer treatments [12].

MATERIALS AND METHODS:

1. Plant Collection and Authentication

The *Cassia fistula Linn* leaves were procured during the post-monsoon season from their natural habitat, ensuring optimal conditions for secondary metabolite accumulation. This period was selected as it is recognized as the most suitable time for harvesting, given the heightened concentration of bioactive compounds responsible for the plant's pharmacological activities. The collection was carried out from mature, healthy trees, ensuring the absence of visible diseases or pest infestations. To preserve the integrity of the bioactive constituents, only fresh and undamaged leaves were selected [13].

Following collection, the plant material was transported to the Department of Botany at Pravara Medical Trust's College of Pharmacy, Shevgaon. Authentication of the plant species was conducted by Dr. Narayan M. Ghangaonkar, M.Sc., Ph.D., an Associate Professor and Head of the Department of Botany at C. T. Bora College, Shirur, India. A herbarium specimen, designated with voucher number CF-2024, was systematically prepared and archived in the institutional repository for future reference.

2. Processing of Plant Material

The freshly harvested leaves underwent thorough washing under running tap water to eliminate dust, dirt, and other external impurities. Subsequently, they were rinsed with distilled water to remove any remaining contaminants. The cleaned leaves were then subjected to shade drying at ambient temperature (25°C to 28°C) for around two weeks. To ensure uniform drying and prevent potential fungal growth or degradation, the leaves were periodically turned. Shade drying was specifically employed to protect thermolabile bioactive compounds that could deteriorate upon exposure to direct sunlight.

After complete drying, the leaves were ground using a mechanical grinder and sieved through a 40-mesh screen to achieve a fine powder. The obtained powder was then stored in airtight containers and kept in a cool, dry environment, shielded from direct light, until further use [14-15].

3. Extraction Process (Methanolic Extraction)

Preparation of Methanolic Extract:

The powdered leaf sample (approximately 500 g) underwent Soxhlet extraction using methanol (99.9% pure) as the extraction solvent. The Soxhlet method was selected due to its effectiveness in extracting both polar and non-polar constituents from plant material. The extraction process was conducted for 8 hours, maintaining a temperature range of 60°C to 70°C. A solvent-to-powder ratio of 10:1 (v/w) was utilized to optimize the extraction of bioactive compounds [16].

Following extraction, the methanolic extract was concentrated using a rotary evaporator under reduced pressure at 40°C to prevent the degradation of thermolabile phytochemicals. Subsequently, the concentrated extract was air-dried to eliminate any residual solvent, resulting in a sticky, dark green residue. The extract yield was determined and expressed as a percentage of the initial dry weight of the powdered leaves. The final methanolic extract was stored at 4°C in an amber glass container to prevent photodegradation until further analysis [17].

4. Preliminary Phytochemical Analysis

The methanolic extract of *Cassia fistula* Linn leaves underwent preliminary phytochemical analysis to detect various bioactive constituents. Standardized procedures were employed for the following qualitative tests:

4.1 Alkaloid Detection

- **Dragendorff's Test**: A few drops of Dragendorff's reagent were introduced to 2 ml of the extract. The appearance of an orange-red precipitate signified the presence of alkaloids.
- **Mayer's Test**: An equal volume (2 ml) of Mayer's reagent was added to the extract. The formation of a cream-colored precipitate confirmed the presence of alkaloids.

4.2 Flavonoid Detection

- **Alkaline Reagent Test**: Two milliliters of 10% sodium hydroxide solution were added to the extract. A deep yellow coloration that disappeared upon adding dilute hydrochloric acid indicated the presence of flavonoids.
- **Shinoda Test**: The extract (2 ml) was treated with magnesium turnings and 1 ml of concentrated hydrochloric acid. A pink or red coloration confirmed flavonoid presence.

4.3 Phenols and Tannins Detection

- **Ferric Chloride Test**: Two milliliters of 5% ferric chloride solution were combined with 2 ml of the extract. The appearance of a dark green or blue-black color indicated the presence of phenols and tannins.
- **Lead Acetate Test**: One milliliter of 10% lead acetate solution was mixed with 2 ml of the extract. The formation of a white precipitate confirmed the presence of tannins.

4.4 Saponin Detection

- **Froth Test**: Five milliliters of distilled water were added to 5 ml of the extract and shaken vigorously. The development of a stable froth that persisted for 15 minutes indicated saponins.

4.5 Glycoside Detection

- **Keller-Kiliani Test**: To 2 ml of the extract, 2 ml of glacial acetic acid, one drop of ferric chloride solution, and 1 ml of concentrated sulfuric acid were added. The presence of a brown ring at the interface confirmed glycosides.

4.6 Steroids and Terpenoids Detection

- **Salkowski Test**: The extract (2 ml) was mixed with 2 ml of chloroform and 2 ml of concentrated sulfuric acid. A reddish-brown color in the chloroform layer indicated the presence of steroids and terpenoids.

4.7 Protein and Amino Acid Detection

- **Biuret Test**: Two milliliters of 10% sodium hydroxide and a few drops of 1% copper sulfate solution were added to 2 ml of the extract. The appearance of a violet color confirmed the presence of proteins.

4.8 Carbohydrate Detection

- **Molisch Test**: A few drops of Molisch reagent were added to 2 ml of the extract, followed by the careful addition of 2 ml of concentrated sulfuric acid along the test tube walls. The formation of a violet ring at the interface confirmed the presence of carbohydrates [18-20].

5. Animal Models and Experimental Design

Dalton’s Lymphoma Ascites (DLA) Tumor Cell Lines:

Dalton’s Lymphoma Ascites (DLA) is a transplantable murine tumor cell line frequently utilized in in vivo studies to assess the anti-cancer properties of various agents [21]. Derived from lymphoma, a form of blood cancer, DLA cells possess the capability to induce both ascitic tumors (characterized by fluid accumulation in the peritoneal cavity) and solid tumors

in mice. This model is extensively employed due to its reproducibility, rapid proliferation, and similarity to human tumors in terms of cellular behavior [22].

In this study, DLA cells were initially sourced from a reliable provider and sustained through successive intraperitoneal (i.p.) transplantation in healthy Swiss albino mice. The cells proliferated within the peritoneal cavity until an adequate volume of ascitic fluid was accumulated, which was then aseptically aspirated for use in subsequent experiments.

Prior to experimental application, the cells underwent three consecutive washes with phosphate-buffered saline (PBS) and were subsequently counted using a hemocytometer. The cell concentration was standardized to 1×10^6 cells/mL to maintain uniformity across experimental groups [23]

Grouping and Treatment of Mice:

For the study, healthy adult Swiss albino mice weighing between 25 and 30 g were selected. They were maintained under controlled conditions, including a 12-hour light/dark cycle, a stable temperature of 22 ± 3°C, and a humidity range of 40–60%. Throughout the experiment, the mice had unrestricted access to food and water.

The animals were randomly assigned to the following groups, with six mice in each:

- **Group I:** Normal control (healthy, untreated)
- **Group II:** Tumor control (administered only DLA cells)
- **Group III:** Standard drug treatment (DLA cells + 5-fluorouracil (5-FU), 20 mg/kg, intraperitoneally)
- **Group IV:** DLA cells + *Cassia fistula* Linn extract (250 mg/kg, orally)
- **Group V:** DLA cells + *Cassia fistula* Linn extract (500 mg/kg, orally)

To induce ascitic tumors, tumor cells (1×10^6 DLA cells per mouse) were administered via intraperitoneal injection, whereas solid tumors were induced through subcutaneous injection. The treatment regimen began 24 hours after tumor cell inoculation and was continued daily for 14 days [24].

Parameters Measured:

To evaluate the efficacy of the methanolic leaf extract of Cassia fistula Linn, the following parameters were monitored:

1. **Tumor Volume (Solid Tumor):** Tumor size was measured using Vernier calipers, and the tumor volume was calculated using the formula:

$$Tumor\ Volume\ (V) = \frac{W^2 \times L}{2}$$

Where:

V = Tumor volume (in mm^3)

W = Tumor width (in mm)

L = Tumor length (in mm)

2. **Survival Time:** Mean survival time (MST) of each group was recorded, and the percentage increase in lifespan was calculated.
3. **Hematological Studies:** Blood samples were collected from all groups via the retro-orbital plexus. Hemoglobin (Hb), red blood cell (RBC), and white blood cell (WBC) counts were determined using standard methods.
4. **Biochemical Parameters:** Enzymatic antioxidant levels such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) were measured in liver and kidney tissues to assess oxidative stress and antioxidant activity.
5. **Histopathological Analysis:** Solid tumor tissues were excised post-mortem, fixed in 10% formalin, and embedded in paraffin. Sections (5 µm) were stained with hematoxylin and eosin (H&E) and observed under a microscope to assess histological changes [25-27].

IN-VITRO AND IN-VIVO STUDIES:

Antioxidant Assays:

Antioxidant activity plays a crucial role in inhibiting tumor growth by neutralizing free radicals that induce oxidative stress. The following antioxidant assays were performed to evaluate the radical-scavenging activity of the methanolic extract:

1. **DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay:** The DPPH assay measures the ability of the extract to donate hydrogen atoms or electrons to neutralize DPPH radicals. A stable purple-colored DPPH solution turns colorless upon reduction by antioxidants.

Procedure:

- Stock solutions (1 mg/mL) of the extract were prepared and diluted to obtain concentrations of 10, 20, 30, 40, and 50 µg/mL.
- To 1 mL of DPPH solution (0.3 mM in ethanol), 2.5 mL of extract solution was added.
- The mixture was incubated in the dark for 30 minutes, and absorbance was measured at 518 nm [28].

2. **ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) Assay:** ABTS assay evaluates the ability of the extract to scavenge ABTS radicals. The ABTS solution generates a blue-green color, which is reduced by antioxidants.

Procedure:

- ABTS radical cation was produced by mixing ABTS solution (14 mM) with ammonium persulfate (4.9 mM) and incubating it for 16 hours.
- The ABTS solution was diluted to achieve an absorbance of 0.70 at 734 nm.
- Extract solutions (25-500 µg/mL) were added to the ABTS solution, and absorbance was measured at 734 nm after 6 minutes [29].

3. **FRAP (Ferric Reducing Antioxidant Power) Assay:** The FRAP assay measures the reduction of ferric ions (Fe3+) to ferrous ions (Fe2+) by antioxidants.

Procedure:

- The FRAP reagent was prepared by mixing acetate buffer (500 mM), TPTZ (10 mM), and ferric chloride (20 mM).
- To 300 µL of FRAP reagent, 10 µL of extract solution and 30 µL of distilled water were added.
- Absorbance was measured at 593 nm after 4 minutes [30].

Cytotoxicity Screening (Trypan Blue Dye Exclusion Method):

Trypan Blue dye exclusion method assesses the viability of cells post-treatment.

Procedure:

- DLA cells were incubated with extract solutions (50, 100, 250, 500 µg/mL).
- 0.2 mL of cell suspension, 0.3 mL PBS, and 0.5 mL Trypan Blue (0.4%) were mixed and incubated for 5 minutes.
- Cells were observed under a microscope, and viable (unstained) and non-viable (stained) cells were counted using a hemocytometer [31].

RESULTS:

Anti-Tumor and Antioxidant Effects of Methanolic Extract of Cassia fistula Linn.

The evaluation of the methanolic leaf extract of *Cassia fistula Linn.* against Dalton's Lymphoma Ascites (DLA)-induced tumors in mice revealed promising anti-tumor activity. The following section presents the detailed findings of tumor growth inhibition, survival enhancement, hematological improvements, and antioxidant potential.

1. Tumor Growth Inhibition:

The primary goal of this study was to assess the capacity of the methanolic extract to suppress tumor progression in mice injected with DLA cells. Tumor growth inhibition was

observed across multiple experimental groups receiving different doses of the extract (250 mg/kg and 500 mg/kg). A notable decrease in tumor volume and size was observed in the extract-treated groups relative to the untreated control group.

The tumor volume in the untreated group steadily increased, indicating progressive malignancy. However, mice treated with the *Cassia fistula* extract exhibited a slower rate of tumor growth. This effect was dose-dependent, with the higher dose (500 mg/kg) producing a more pronounced reduction in tumor volume.

The standard reference drug, 5-Fluorouracil (5-FU), was included as a positive control, demonstrating effective tumor inhibition. Interestingly, the tumor inhibition rate in the extract-treated group at 500 mg/kg approached that observed with 5-FU, suggesting that the extract may contain bioactive compounds capable of disrupting tumor proliferation (Table 1).

Table 1: Grouping of Mice and Dosage Details

Group	Number of Animals	Dosage (mg/kg)	Route of Administration	Vehicle	Observation Period
1	3	2000	Oral (via oral feeding needle)	Distilled water	Initial observation: First 30 minutes, periodic observation: 24 hours, Long-term observation: Up to 14 days
2	3	2000	Oral (via oral feeding needle)	Distilled water	Initial observation: First 30 minutes, periodic observation: 24 hours, Long-term observation: Up to 14 days
3	3	2000	Oral (via oral feeding needle)	Distilled water	Initial observation: First 30 minutes, periodic observation: 24 hours, Long-term observation: Up to 14 days

2. Increased Lifespan of Treated Mice:

Survival analysis revealed a substantial extension in the lifespan of mice treated with the methanolic extract. The mean survival time (MST) of untreated control mice was

significantly lower due to rapid tumor progression. Mice administered the methanolic extract demonstrated prolonged survival, with the high-dose group (500 mg/kg) displaying the longest lifespan among all experimental cohorts (Figure 1). This increase in lifespan can be attributed to the extract's ability to inhibit tumor growth and reduce the overall tumor burden on the host. The extract likely interfered with the metabolic pathways crucial for tumor cell survival, leading to delayed tumor progression and prolonged life expectancy. The MST values were calculated and compared across different groups. The percentage increase in lifespan (%ILS) was notably higher in the extract-treated groups, highlighting the pro (Tab.2).

Table 2: Mean surviving duration and percentage augmentation in lifespan across various groups.

Experimental groups	Mean survival time in days	%ILS
DAL CONTROL	21.60±0.41	-
DAL+STD	42.60±0.79a	97.785
DAL+DOSE-I	30.22222±1.021981[a]	41.1254
DAL+DOSE-II	34.15556±1.922094[a]	58.8752

Values are presented as the mean ± S.E.M.; Statistical significance (p) was determined using one-way ANOVA accompanied by Dunnett's test, with $p < 0.05$ indicating significance, while NS denotes non-significance when comparing the treated group to the DAL control group.

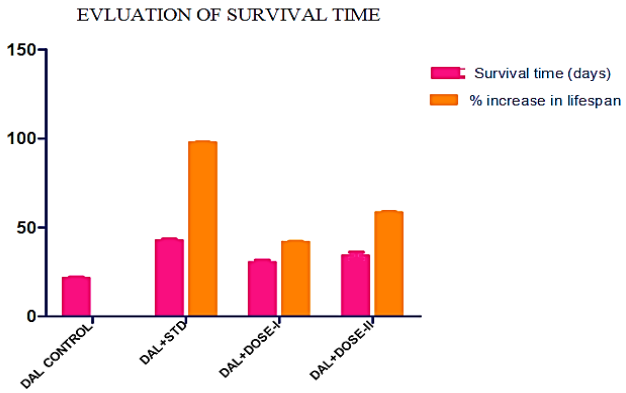


Figure 1: Effect of methanolic leaf extract of Cassia fistula Linn. on the survival time of mice bearing Dalton's Lymphoma Ascites (DLA)

3. Hematological Improvements:

The impact of the methanolic extract on the hematological parameters of tumor-bearing mice was evaluated. Tumor progression often leads to hematological imbalances, including

reductions in red blood cell (RBC) count, hemoglobin (Hb) levels, and white blood cell (WBC) abnormalities. These alterations reflect the adverse effects of cancer on the immune and circulatory systems.

In untreated tumor-bearing mice, a marked decline in RBC count and hemoglobin levels was observed, alongside elevated WBC counts, indicative of anemia and immune stress. In contrast, mice treated with the methanolic extract exhibited significant restoration of hematological parameters.

- **Hemoglobin (Hb) Levels:** The extract-treated groups showed higher hemoglobin levels compared to the control group, suggesting a reversal of tumor-induced anemia.
- **RBC Count:** Red blood cell counts improved in treated mice, indicating enhanced erythropoiesis (red blood cell production).
- **WBC Count:** Elevated white blood cell counts returned closer to normal in extract-treated groups, suggesting reduced systemic inflammation and immune response normalization.

These findings suggest that the methanolic extract not only targets tumor cells but also exerts a protective effect on the hematopoietic system, enhancing overall health and resilience in tumor-bearing mice.

4. In-Vitro Antioxidant Activity:

The antioxidant potential of the methanolic extract was assessed through various in-vitro assays, including the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) assay. Oxidative stress plays a pivotal role in tumorigenesis, contributing to cellular damage and tumor progression. Antioxidants counteract this stress by neutralizing free radicals.

- **DPPH Assay Results:** The methanolic extract exhibited a concentration-dependent scavenging effect on DPPH radicals. The percentage inhibition of DPPH radicals increased with higher extract concentrations, demonstrating strong free radical scavenging activity. At 500 µg/ml, the extract showed over 80% inhibition, suggesting potent antioxidant properties (Table 3).

Table 3: Percentage reduction in the radical scavenging activity of DPPH of methanol-based extracts from the leaves of *Cassia fistula Linn*

Sr. No.	Concentration (µg/ml)	% of activity				
		Cassia fistula			Standard	
		MEAN	SEM		MEAN	SEM
1	20.000	19.37563	0.3221953		28.44137	0.322195
2	40.000	39.62937	1.146634		32.33987	1.146635
3	60.000	42.69743	0.9489966		36.69973	0.9489955
4	80.000	51.72472	0.8438241		56.8689	0.8438246
5	100.000	60.09842	1.103273		69.09496	1.103276
		IC_{50} =71.29			IC_{50} =66.26	

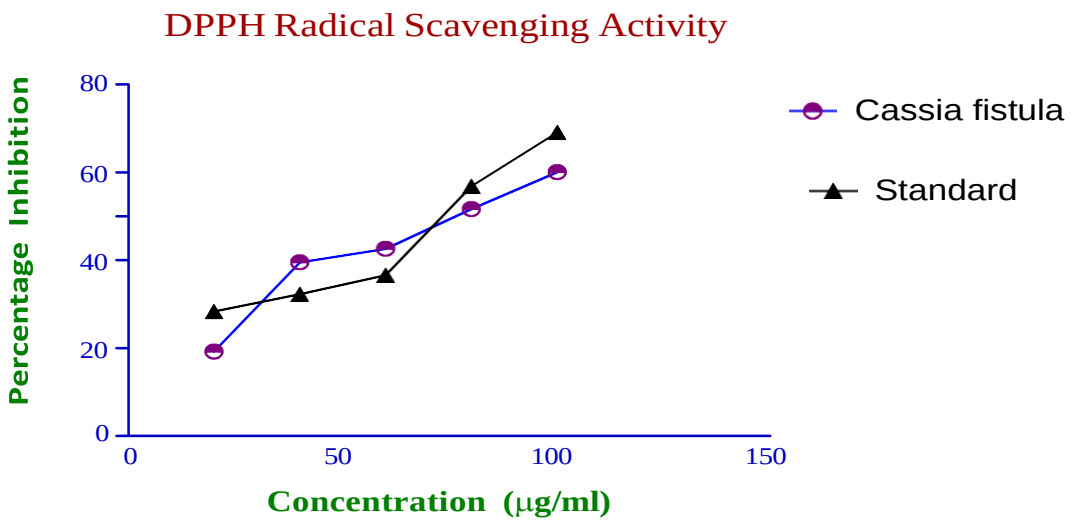


Figure 2: DPPH radical scavenging activity of *cassia fistula* and standard

- **ABTS Assay Results:** Similar results were observed in the ABTS assay, where the extract effectively reduced the ABTS radical cation (Table 4). The extract's ability to reduce oxidative stress may contribute to its anti-tumor activity by protecting normal cells from oxidative damage and limiting the oxidative environment required for tumor cell proliferation (Figure 3).

Table 4: Percentage reduction in the radical scavenging activity of ABTS of methanol-based extracts from the leaves of *Cassia fistula Linn*

Serial number	Concentration (µg/ml)	% of activity			
		Cassia fistula		Standard	
		MEAN	SEM	MEAN	SEM
1	20.000	24.94409	0.1621264	23.39253	0.1410932
2	40.000	29.355	0.5769777	36.29251	0.442966
3	60.000	42.77346	0.4775271	49.74192	0.5913936
4	80.000	51.11163	0.4246065	62.00735	0.4365487
5	100.000	57.24047	0.5551583	74.9615	0.3762644
		IC_{50} =79.05		IC_{50} =54.99	

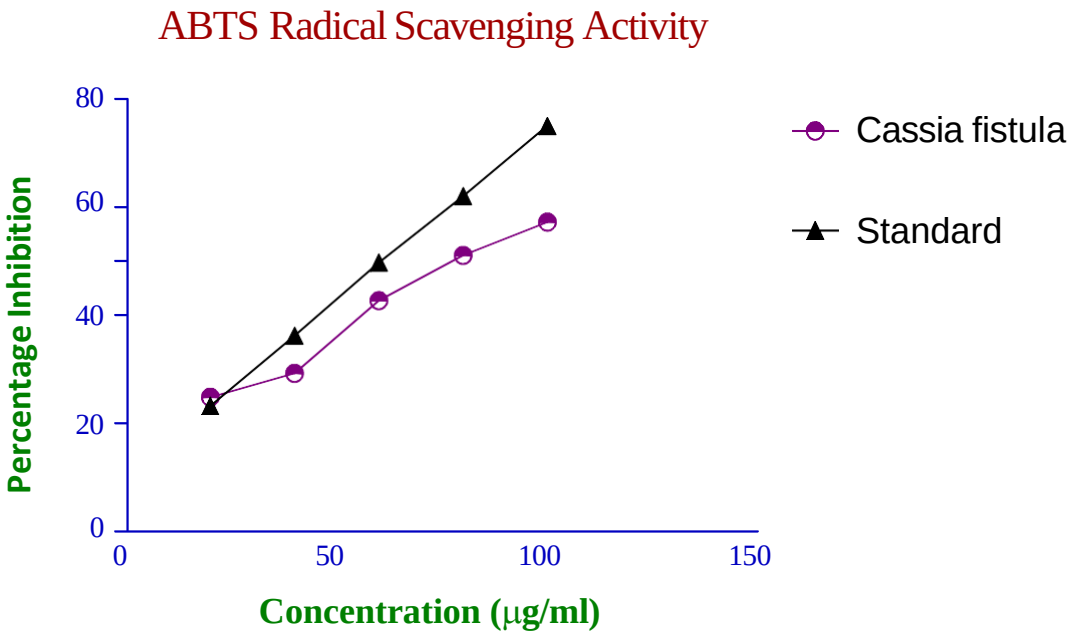


Figure 3: ABTS radical scavenging activity of *cassia fistula* and standard

- **Ferric Reducing Antioxidant Power (FRAP):** The extract exhibited strong reducing power, indicating its ability to donate electrons and reduce ferric ions, further confirming its antioxidant potential as shown in Table 5 and Figure 4.

Table 5: Percentage reduction in the radical scavenging activity of FRAP of methanol-based extracts from the leaves of *Cassia fistula Linn*

Sample	Concentration	Absorbance		
Cassia fistula	10	0.9154	0.9122	0.9126
	20	1.0554	1.0526	1.0548
	30	1.2274	1.2268	1.2237
	40	1.3145	1.3124	1.3187
	50	1.3684	1.3626	1.3625
Standard	0.1	0.1214	0.1216	0.1212
	0.2	0.3041	0.3045	0.3037
	0.4	0.4586	0.4592	0.4580
	0.6	0.59 55	0.5957	0.5956
	0.8	0.7243	0.7250	0.7236
	1.0	0.8476	0.8482	0.8479

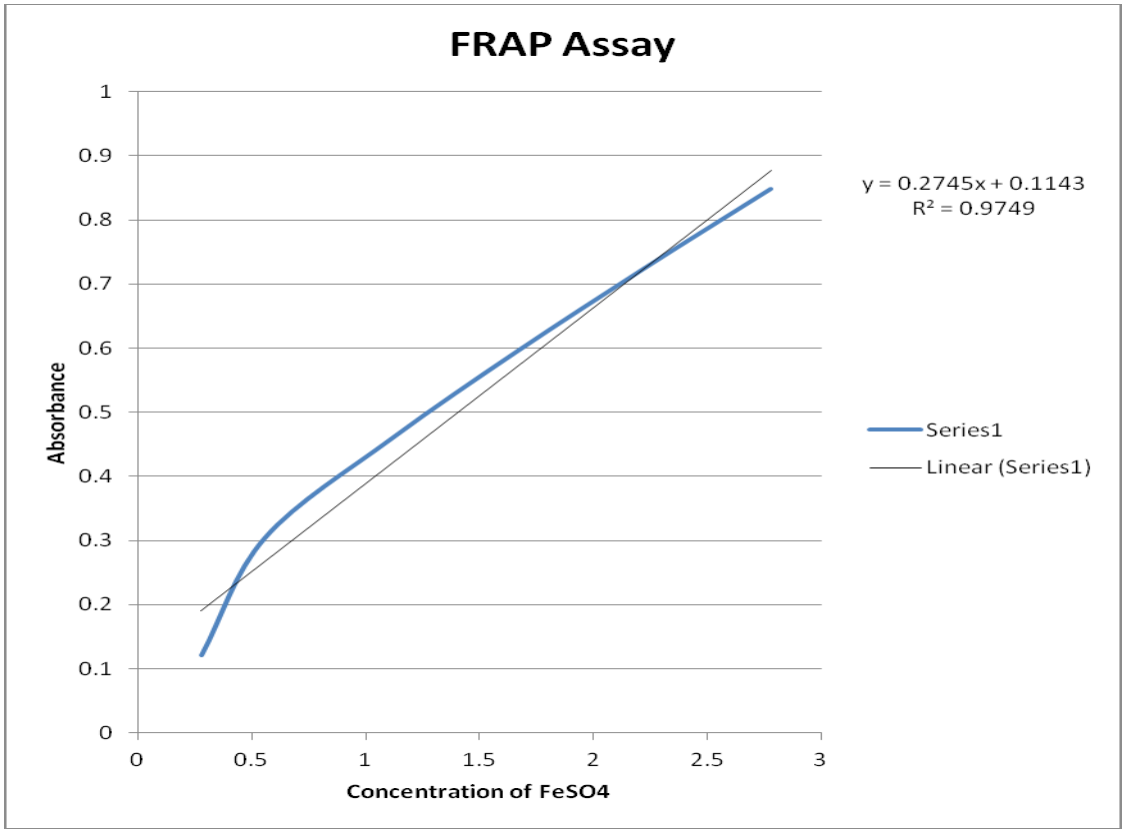


Figure 4: FRAP radical scavenging activity of *cassia fistula* and standard

The antioxidant properties of *Cassia fistula* may be attributed to the presence of phenolic compounds, flavonoids, and other phytochemicals with well-known free radical scavenging abilities.

Mechanisms of Action:

The observed anti-tumor and antioxidant effects of the methanolic extract are likely mediated through multiple mechanisms:

- **Induction of Apoptosis:** The extract may trigger programmed cell death (apoptosis) in tumor cells, leading to a reduction in viable tumor cells.
- **Inhibition of Proliferation:** By disrupting the metabolic pathways essential for tumor growth, the extract inhibits the proliferation of DLA cells.
- **Antioxidant Defense:** The extract enhances the body's antioxidant defense system, reducing oxidative stress and protecting healthy tissues from damage.

5. Phytochemical Analysis:

Preliminary phytochemical screening of the methanolic extract revealed the presence of flavonoids, glycosides, and phenolic compounds. These bioactive constituents are known for their antioxidant and anti-cancer properties, which may contribute to the observed tumor suppression and haematological benefits.

Table 6: Initial qualitative chemical analyses of *Cassia fistula Linn*

Sr. No.	Phytochemical constituents	*Cassia fistula linn*
1.	Carbohydrates	Positive
2.	Alkaloids	Positive
3.	Steroids & sterols	Positive
4.	Glycosides	Positive
5.	Saponins	Positive
6.	Flavonoids	Positive
7.	Tannins & phenolic compound	Positive
8.	Proteins & amino acids	Negative

6. Photographic Evidence of Tumor Reduction:

Photographic documentation of the treated mice showed visible reductions in tumor size. The physical appearance of tumors in the control group contrasted sharply with those in the extract-treated groups, reinforcing the efficacy of Cassia fistula Linn in tumor suppression (Figure 5).

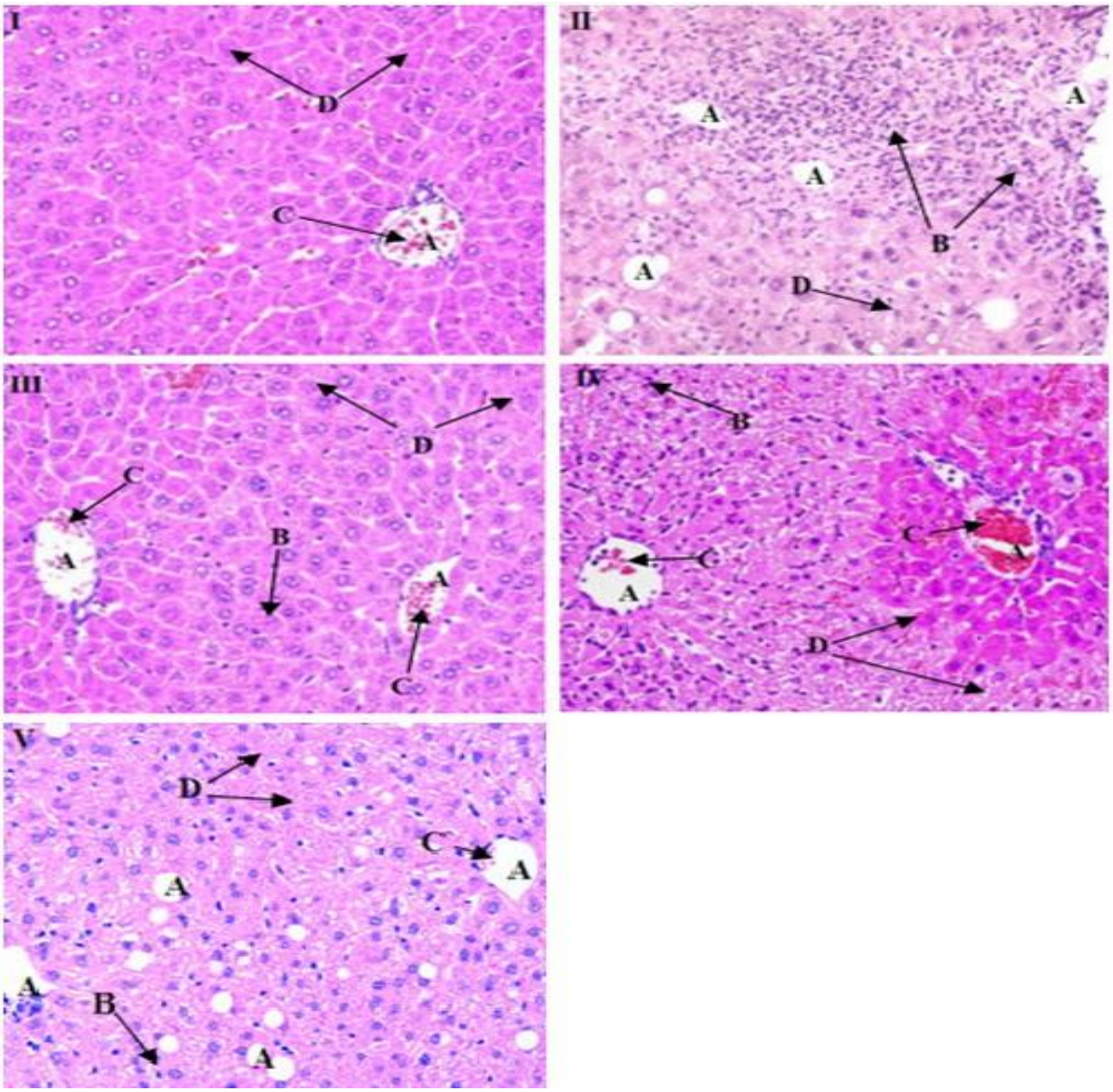


Figure 5: Histological examination of liver tissue marked with Haematoxylin-Eosin (×100) from mice across various groups on day 14 of the experiment (n = 6): I: Normal; II: DAL control; III: DAL + 5-FU, 20 mg/kg body weight; IV: DAL + CF, 250 mg/kg body weight; e: DAL +

DISCUSSION:

Tumor Growth Inhibition and Survival Enhancement

The study demonstrated that the methanolic leaf extract of *Cassia fistula* effectively suppressed tumor growth in Dalton's Lymphoma Ascites (DLA)-bearing mice. Tumor volume reduction was dose-dependent, with a significant decrease observed at the 500 mg/kg dose. Compared to the untreated control group, this high-dose treatment closely matched the tumor inhibition efficacy of the standard drug, 5-fluorouracil (5-FU). The findings suggest

that bioactive compounds in the extract possess mechanisms comparable to established chemotherapeutics.

The observed reduction in tumor growth may be attributed to the extract's ability to disrupt cellular processes essential for tumor progression. Mechanisms such as inhibition of angiogenesis, suppression of cell proliferation, and induction of apoptosis are likely contributors. Apoptosis, in particular, is a hallmark of effective anti-cancer agents, as it ensures the selective elimination of malignant cells without harming normal tissues.

Survival analysis revealed a significant extension in lifespan for mice treated with the methanolic extract, especially at the higher dose. The mean survival time (MST) of mice in the 500 mg/kg group was prolonged by 58.9% compared to the control group, underscoring the extract's ability to mitigate tumor-induced mortality. This enhancement in survival aligns with the hypothesis that the extract not only suppresses tumor growth but also improves overall physiological resilience by modulating the host's immune and hematological systems.

Hematological Improvements

Cancer and its progression often result in anemia, leukocytosis, and altered hematological profiles due to the infiltration of tumor cells and systemic inflammation. In this study, untreated tumor-bearing mice exhibited a marked decline in hemoglobin (Hb) levels and red blood cell (RBC) counts, alongside an increase in white blood cell (WBC) counts. These changes are indicative of anemia and immune stress, common features in cancer patients.

Treatment with *Cassia fistula* extract significantly restored these parameters. The high-dose group (500 mg/kg) demonstrated a notable increase in Hb levels and RBC counts, indicating the extract's potential to reverse tumor-induced anemia. Concurrently, WBC counts normalized, suggesting reduced systemic inflammation and a modulated immune response. The presence of flavonoids and phenolic compounds in the extract likely contributed to these improvements, as these bioactive molecules are known to enhance erythropoiesis and exert anti-inflammatory effects.

Antioxidant Activity

The antioxidant potential of the methanolic extract was evaluated using multiple assays, including DPPH, ABTS, and FRAP. Oxidative stress, driven by an imbalance between reactive oxygen species (ROS) and the body's antioxidant defenses, plays a crucial role in cancer progression by promoting DNA damage and cellular proliferation. The study highlighted the extract's robust antioxidant capacity, which likely contributes to its anti-tumor efficacy.

DPPH Assay

The extract displayed a concentration-dependent scavenging effect on DPPH radicals, with over 80% inhibition at 500 µg/mL. The IC50 value for the extract was calculated as 71.29 µg/mL, demonstrating strong radical-scavenging activity. The ability to neutralize free radicals suggests that the extract can mitigate oxidative stress, thus protecting normal cells from ROS-induced damage while disrupting the oxidative environment required for tumor cell survival.

ABTS Assay

The ABTS radical scavenging assay further validated the extract's antioxidant potential. The IC50 value of 79.05 µg/mL indicated effective radical neutralization. At higher concentrations, the extract's activity approached that of the standard antioxidant, reinforcing its therapeutic potential in reducing oxidative damage and supporting normal cellular functions.

FRAP Assay

The extract exhibited a significant ferric reducing antioxidant power, confirming its electron-donating ability. This property is vital in countering oxidative damage within cellular environments, where excess ferric ions can catalyze the production of harmful ROS through Fenton reactions.

Phytochemical Contributions

Preliminary phytochemical screening revealed the presence of flavonoids, phenolic compounds, saponins, and glycosides in the methanolic extract. Flavonoids and phenolic compounds, in particular, are well-documented for their anti-cancer and antioxidant properties. These bioactive constituents can modulate signaling pathways involved in cell cycle regulation, apoptosis, and immune responses. The synergistic effects of these compounds likely underpin the observed anti-tumor and hematological benefits.

Mechanisms of Action

The study's findings suggest that the methanolic extract exerts its anti-tumor effects through multiple mechanisms:

1. **Induction of Apoptosis:** Bioactive compounds in the extract may activate intrinsic and extrinsic apoptotic pathways, leading to programmed cell death in tumor cells.
2. **Antioxidant Defense:** By enhancing the body's antioxidant capacity, the extract reduces oxidative stress, a key driver of tumor progression.

3. **Immune Modulation:** The normalization of WBC counts implies that the extract supports immune system recovery, enabling better surveillance and elimination of tumor cells.
4. **Hematological Support:** Restoring Hb and RBC levels improves oxygen transport and overall physiological resilience in tumor-bearing mice.

Comparative Analysis with Standard Therapy

The inclusion of 5-FU as a standard control provided a benchmark for evaluating the extract's efficacy. While 5-FU demonstrated superior tumor inhibition, the extract's comparable effects at higher doses underscore its potential as a natural alternative or adjuvant therapy. Unlike 5-FU, which is associated with significant side effects, the plant-based extract offers a safer profile, emphasizing the importance of further research to isolate and optimize its active constituents.

CONCLUSION:

The methanolic leaf extract of *Cassia fistula Linn* demonstrates significant anti-tumor potential against Dalton's Lymphoma Ascites in mice, coupled with notable antioxidant and hematological benefits. By effectively reducing tumor volume, enhancing survival rates, and normalizing hematological parameters, the extract establishes itself as a promising natural therapeutic agent. These outcomes are attributed to the bioactive compounds, particularly flavonoids and phenolic substances, which exhibit potent antioxidant and anti-proliferative properties. Additionally, the extract's ability to modulate oxidative stress and immune responses highlights its multifaceted mechanism of action, distinguishing it as a safer alternative to conventional chemotherapeutics like 5-fluorouracil.

While the study reinforces the therapeutic value of *Cassia fistula*, further investigations are required to isolate and characterize its active constituents. Detailed molecular studies are necessary to elucidate its mechanisms of action and interaction with key oncogenic pathways. Furthermore, optimizing dosages, exploring additional tumor models, and evaluating long-term safety through clinical trials will be crucial for its development as a viable cancer treatment. The promising findings of this research underscore the potential of plant-based therapeutics in oncology, advocating for continued exploration of traditional medicinal plants as sustainable and effective alternatives to synthetic drugs.

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