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Anticancer Potential Of Dillenia Bracteata: Evaluation Of Methanol Extract And Its Isolated Compound In Ehrlich Ascites Carcinoma-Induced Swiss Albino Mice

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ABSTRACT

Background: Cancer poses a significant health issue worldwide even though chemotherapy treatment creates major side effects in patients. Scientists find interest in the cancer prevention value of Dillenia bracteata Wight through herbal medicine research. This research analyzes the antitumor effects of Dillenia bracteata when given to Swiss albino mice with Ehrlich Ascites Carcinoma.

Objective: This research studied how MEDB and IF-D affect tumor growth in mice with EAC carcinoma and their effects on blood health and cell life along with liver function and inflammation markers.

Methods: Methanol extract was prepared from the dried leaves of Dillenia bracteata (MEDB) and active principle compound (IF-D) were isolated through column chromatography. Our research investigated MEDB and IF-D doses that were safe in the mice and then grouped them for investigation of invivo activity in EAC induced carcinoma. Researchers evaluated tumor size, weight, survival length, cell viability and essential blood chemistry results for all mice included in the study. Our research examined inflammatory markers like IL-6 and TNF along with hepatic condition tests and antioxidants SOD, GSH, CAT, MDA, plus total protein and enzyme measurements.

Results: Both MEDB and IF-D treatment brought about significant tumor size reduction and weight loss while making EAC-induced mice live longer. The isolated compound IF-D treated cancer cells better than MEDB and standard drug 5-fluorouracil (5-FU) activity. The treatment with IF-D therapy reduced tumor living cells while improving blood parameters and PCV. IF-D demonstrated great anti-inflammatory properties when it lowered IL-6 and TNF-α activity and strengthened natural defenses against oxidative damage. Hepatic parameters recovered completely because IF-D restored regular SGPT SGOT and ALP test results.

Conclusion: Results showed that the compound IF-D from Dillenia bracteata reduced cancer cells effectively while maintaining safe properties to study further its use in treating cancer patients. Research studies should test the plant extract IF-D because it shows great promise to control cancer development and protect liver health.

Keywords: *Dillenia, EAC induced carcinoma, 5-FU, antioxidant*

1. INTRODUCTION

Cancer continues to be an important worldwide disease challenge during present times (Tagne et al., 2014). The condition runs largely due to unchecked cell cycle evolution that triggers tissue cells to multiply in an unnatural way (Sumitra & Nagani, 2013). Cancer develops through modifications that affect important genetic pathways between oncogenes and tumour suppressor genes along with DNA repair genes. Multiple genetic disturbances in the population develop due to chemical exposures together with smoking, alcohol intake, ultraviolet rays, infectious agents and natural mutations (Mukherjee et al., 2007; Ganapathy et al., 2015). Standard treatment solutions like chemotherapy combined with radiotherapy and hormone therapy and surgical procedures lead to substantial side effects especially when patients receive chemotherapy according to (Krishnamoorthy & Ashwini, 2011). The insufficient treatment outcomes due to adverse effects have motivated researchers to find improved therapeutic alternatives. Plant-based therapies receive growing interest because of their

demonstrated potential as cancer treatments (Haghighi et al., 2017). Several pharmaceutical and research industries focus their increased dedication on developing and investigating herbal treatments for oncological use because of mounting global interest in herbal medicine. Effective and safe utilization of medicinal plants requires complete scientific evaluation (Chopra et al., 1956).

One species in Dilleniaceae family Dillenia bracteata Wight is known across India under its common name fish bone plant. The local communities use three different names to identify this plant: Chiruthaeku in Telugu and Bettadakanugalu in Kannada and Colikkay in Tamil. The species spreads from southern to southern India throughout its natural distribution range. The plant usually reaches 30–60 feet in height and features tomentose shoots combined with scarred branches as its distinctive characteristics. Each leaf of the plant appears simple and varies from ovate-elliptic to obovate-elliptic with dimensions between 16–46 cm in length and 5–19 cm in width. Each solitary as well as clustered flower of yellow color is followed by lanceolate or spathulate bracting structures. Within the flower Dillenia bracteata exhibits five ovate calyx sepals which are complemented by five obovate yellow petals of the corolla section. This plant contains many linear anthers supported by multiple curved styles along with linear stamens. The pseudocarp fruit takes the shape of subglobose while transforming orange when maturity occurs. The enclosed sepals protect the ovoid and black to dark brown inner carpels which measure about 5 mm across and have one or two seeds inside.

The medicinal use of Dillenia bracteata addresses both rheumatoid arthritis and dysentery and hepatitis and several inflammatory disorders and wounds and diabetes and gastrointestinal diseases. Scientific researchers have identified various properties of this plant including anti-inflammatory properties, antibacterial effects and antihemorrhagic behavior as well as immunomodulatory functions. Multiple modern research projects have established Dillenia bracteata exhibits both cancer-preventive features and healing capabilities for stomach ulcers (Shama et al., 2019). Thus this research was centered to isolate the anticancer constituents from the plant that work against EAC induced cancer in experimental animals.

2. MATERIALS AND METHODS

Chemicals and Reagents

All Chemicals solvents and reagents used in the study were of analytical grade and were procured from Sigma Aldrich India Ltd, India and Himedia Laboratories Ltd., Mumbai.

Collection, Extraction and Isolation

The Leaves of the freshly collected Dillenia bracteata Wight was authenticated duly and dried under shade for about 5 days during March. The dried leaves were powdered and extracted using methanol using a soxhlet apparatus (Avinash et al., 2012). The obtained extract solution was filtered and the filtrate was evaporated to dryness using a rotary evaporator. The final methanol extract was named as MEDB (27.44%w/w) and stored in a dry place away form direct sun light and moisture until further use.

75g of methanol extract was weighed and mixed with silica gel and packed in a column. A mixture of dichloromethane and acetone were poured in different ratios (9:1 to 1:9) to isolate the constituents from the extract. All the fractions from the column were collected TLC was performed using various solvent combinations like Dichloromethane, acetone and ethanol (9:1:1; 6:2:2; 5:3:2; 3:3:4 and 1:4:5). The best combination which eluted all the constituents was used for re-column chromatogrpahy. Finally a combination of Methanol and Chloroform was used in combination (7:3) which yielded pure isolate compound (IF-D).

Experimental Animals

5 week old healthy Mice (Swiss albino) weighing 30-35g were procured and maintained in polypropylene cages with soft cushion and saw dust bedding which is changed regularly. Mice were allowed with free access to water and standard pellet diet. All the experiments were approved by IAEC and the procedures were conducted by following the guidelines of CPCSEA.

Acute Toxicity Studies

Acute toxicity studies were conducted following OECD guidelines with a starting dose of 2000mg/kg for extract and 500mg/kg for the isolated compound. When there are no changes noticed, then the the standard effective doses were fixed as 250mg/kg of MEDB and 50mg/kg of the IF-D (Schlede, 2002).

Anti-cancer activity of MEDB and IF-D on EAC induced carcinoma

Maintenance of EAC cell lines

The cell cultures of Ehrlich Ascites Carcinoma (EAC) strain were procured from the Department of Biochemistry and microbiology, Govt Degree College, Nellore. The procured culture was re-cultured to ensure purity and harvested (Alam et al., 2016). The cells were harvested about 2×10^6 cells/ml using ahemocytometer. an exclusion test with trypan blue dye (0.4%) was used to assess the viability of the tumor cells.

Grouping and Treatment

5 groups of 6 Swiss albino mice in each group was randomly divided and separated. EAC cells were intraperitoneally injected into other groups except group 1 (normal control). Day 1 was considered as the first day of implantation and the control group was injected with equal volume of normal saline solution. Group 2, 3, 4 and 5 served as EAC induced, Standard group (5-Fluorouracil-5FU-20mg/kg), methanol extract of Dillenea (MEDB-250mg/kg) and Isolated Fraction (IF-D-50mg/kg). The drug administration was performed 24hr after the EAC injection and 24hrs following that, animals were fasted for 18hrs. Mice were euthenized using ketamine and 0.5ml blood was collected from the retro-orbital plexus for evaluating the hematological, serum biochemical estimations (Parasuraman et al., 2011). Ascitic fluids and liver was carefully collected and the histopahtological examinations were conducted one part and other part was subjected for estimation of antioxidant enzymes.

Evaluation of markers

The extract and isolated compound were evaluated for the changes in tumor weight, volume, viable and non-viable cell counts using trypan blue staining method and respective percentages in the life span (Dolai et al., 2012). Also the MST and % ILS values were also calculated based on the standard formulae (Sur and Ganguly, 1994). The haemoglobin, RBC, WBC, and other blood based estimations were carried out from the blood extracted from the retro-orbital plexus (Mukherjee, 1990). Blood samples were coagulated and the serum was collected in the centrifuge tubes by subjecting to centrifuge at 8000rpm and the serum ws utlized for biochemical estimations like inflammatory markers (Mourtzikou et al., 2014), liver functions markers and antioxidant enzymes (Ellman 1959; Lowry et al., 1951; Paglia and Valentine, 1967). The excised liver tissue was preserved in 10% buffered formalin solution and dehydrated using ethanol and embedded in paraffin. Histological examinations were carried out by staining with hematoxylin and eosin.

Statistical analysis

All the values were taken for 6 animals in each group. Their means and standard error of means were calculated and ANOVA analysis was conducted to identify the significance of the difference among the groups indicated as ***P<0.001 using GraphPad Prism 5.0 (San Diego, CA, USA).

3. RESULTS

In the current research attempt was made to isolate the anticancer principle from the methanol extract of Dellenia bracteata (MEDB). The extract was subjected to column chromatography uing DCM and acetone which yielded 9 fractions out of which fraction 8 produced highest yield (19.88g). This fraciton was subjected to re-coloumn chromatography using DCM, acetone and ethanol which yielded in 5 sub fractions out of which the highest yielded fraction was subjected to re-coloum using Methanol and Chlorofrom. This yielded in two isolates our of which the highest yielded isolate was identified (IF-D) and further subjected for invivo analysis.

Acute oral toxicity

The administration of MEDB and IF-D at doses 2000 and 500mg/kg showed no toxicity as evident from absence of significant changes in the body weight of mice and also no behavioral or physiological changes noted. Thus it was confirmed that the administered drugs were safer at doses 250mg/kg and 50mg/kg respectively.

Effect of MEDB and IF-D on the tumor paramters

The combined treatment with MEDB (25.77 ± 0.36) and IF-D (30.85 ± 0.35) elongated MST by significant duration compared to the EAC-induced group (15.17 ± 0.48). The anti-cancer ability of IF-D was evident as superior to the anti-cancer properties of MEDB because IF-D provided stronger and similar to 5-fluorouracil which led to a life span extension of 34.98 ± 0.59. The % ILS of MEDB (65.18 ± 0.20) and IF-D (91.83 ± 0.45) were significant in comaprison to the standard drug. The anti-cancer power of isolated compound (IF-D) (118.28 ± 0.72) is higher than methanol extract (MEDB) as it caused a significantly larger life span increase. Results showed MEDB-treated tumor volume was 5.06 ± 0.23 mL while IF-D-treated showed 4.24 ± 0.22 mL compared to EAC group of 10.03 ± 0.42 mL (***P < 0.001). The isolated compound (IF-D) demonstrated stronger tumor volume reduction than MEDB although both compounds showed significant anti-tumor activity.

Both MEDB (3.64 ± 0.22 g) and IF-D (2.49 ± 0.19 g) exhibited significant weight reduction of tumors which proved superior to tumors treated with EAC (5.39 ± 0.35 g, ***P < 0.001). The isolated fraction (IF-D) showed superior effectiveness compared to the methanol extract by yielding tumor weight results similar to 5-fluorouracil at 1.79 ± 0.31 g at P < 0.001 significance. PCV levels among the EAC-induced group reached 7.14 ± 0.20% yet all treatment groups achieved higher PCV levels. The highest PCV improvement was observed in 5-fluorouracil-treated (2.35 ± 0.21%) followed by MEDB (5.99 ± 0.33%) and IF-D (3.99 ± 0.21%) in their ability to combat anemia associated with cancer.

Effect of MEDB and IF-D on the cancer viability

The total counts of cells decreased to $4.57 \pm 0.19 \times 10^7$ cells in the MEDB group and to $3.57 \pm 0.21 \times 10^7$ cells in the IF-D

group with both reaching statistically significant levels (***P < 0.001). The examined groups receiving MEDB treatment (88.56 ± 1.65%, *P < 0.05) and IF-D treatment (76.17 ± 1.33%) showed higher viable cell percentages when compared to both control groups (77.79 ± 0.74%). The isolated fraction (IF-D) achieved superior cytotoxic effects compared to the methanol extract because it demonstrated lower viable cell percentage numbers as shown in table 1.

The EAC-induced group showed viable cell counts of $8.43\pm0.65\times10^7$ cells but both MEDB and IF-D decreased this value to $4.06 \pm 0.23 \times 10^7$ cells and $2.59 \pm 0.28 \times 10^7$ cells (***P < 0.001), respectively. The isolated compound reduced viable cell numbers at a higher level than the extract obtained from methanol. Tumor cell death became more efficient when the EAC-induced cells were treated with either MEDB ($0.50 \pm 0.05 \times 10^7$ cells) or IF-D ($0.99 \pm 0.12 \times 10^7$ cells) which resulted in significantly higher non-viable cell counts as compared to the EAC-induced group ($0.28 \pm 0.06 \times 10^7$ cells).

Table 1: Effect of Methanol Extract and Isolated Compound on Tumour Parameters

Parameter	EAC induced	5-Florouracil (20mg/kg)	Methanol Extract (MEDB-250mg/kg)	Isolated fraction (IF-D-50mg/kg)
MST	15.17 ± 0.48	34.98 ± 0.59***	25.77 ± 0.36***	30.85 ± 0.35***
% ILS	0.00	118.28 ± 0.72***	65.18 ± 0.20***	91.83 ± 0.45***
Tumor volume (ml)	10.03 ± 0.42	3.43 ± 0.24***	5.06 ± 0.23***	4.24 ± 0.22***
Tumor wt (gm)	5.39 ± 0.35	1.79 ± 0.31***	3.64 ± 0.22***	2.49 ± 0.19***
PCV	7.14 ± 0.20	2.35 ± 0.21***	5.99 ± 0.33***	3.99 ± 0.21***
Viable cells (10^7 cells)	8.43 ± 0.65	1.99 ± 0.27***	4.06 ± 0.23***	2.59 ± 0.28***
Non viable cells (10^7 cells)	0.28 ± 0.06	1.45 ± 0.18***	0.50 ± 0.05**	0.99 ± 0.12***
Total cells	9.70 ± 0.31	3.27 ± 0.28***	4.57 ± 0.19***	3.57 ± 0.21***
Viable %	95.79 ± 0.74	65.55 ± 3.57***	88.56 ± 1.65*	76.17 ± 1.33***
Non-viable %	3.34 ± 0.35	37.46 ± 3.06***	12.09 ± 1.57**	25.15 ± 1.41***

All the values were given as mean±SEM; ***indicates P<0.001 significance compared to EAC induced group

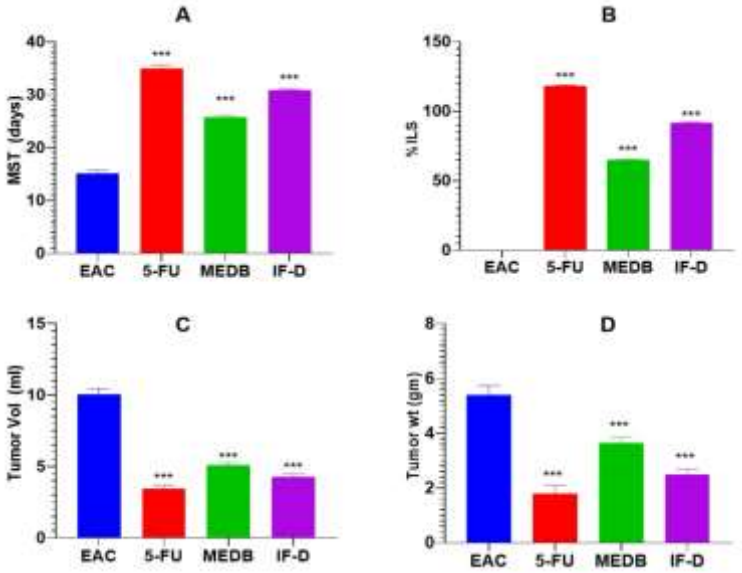

Figure 1: Effect of MEDB and IF-D on Tumor parameters

*****indicates P<0.001 significance compared to EAC induced group**

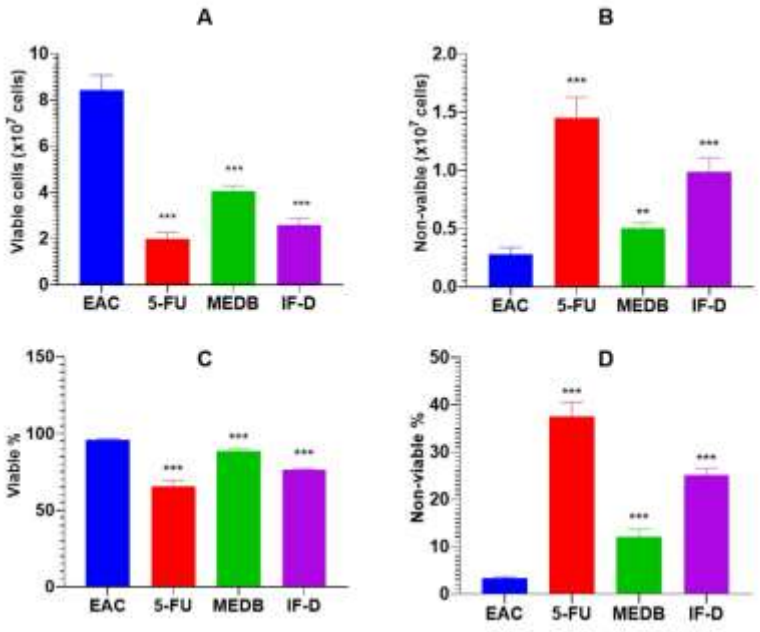

Figure 2: Effect of MEDB and IF-D on Cell viability

*****indicates P<0.001 significance compared to EAC induced group**

Effect of MEDB and IF-D on the hematological parameters

Studies investigating the blood parameter changes induced by MEDB extract as well as IF-D fraction and 5-fluorouracil in EAC-induced cancer were shown in Table 2. EAC-induced animals had significantly decreased levels of hemoglobin which accounted to 6.17 ± 0.48 g/dL when compared to normal control with 12.04 ± 0.37 g/dL. 5-fluorouracil treatment restored blood hemoglobin concentrations to 11.98 ± 0.59 g/dL. Blood tests showed significant enhancement in MEDB at 10.77 ± 0.36 g/dL and IF-D at 11.85 ± 0.35 g/dL alongside both reaching nearly normal control levels. EAC induction resulted in a substantial reduction of RBC count reaching 3.011 ± 0.384 × 10^6/µl compared to the normal control with 4.412 ± 0.327 × 10^6/µl. The use of 5-fluorouracil (4.283 ± 0.294 × 10^6/µl, ***P < 0.001) restored RBC count to a significant level together with MEDB (3.844 ± 0.241 × 10^6/µl, **P < 0.01). The isolated fraction (IF-D, 4.406 ± 0.332 × 10^6/µl) provided the most effective restoration of RBC count because it restored levels that were nearly similar to those of the normal control.

EAC-induced cancer resulted in an increase of WBC count to 10.03 ± 0.42 × 10^3/µl above the normal control of 5.63 ± 0.33 × 10^3/µl. Both 5-fluorouracil (6.43 ± 0.24 × 10^3/µl) and MEDB (8.06 ± 0.23 × 10^3/µl) decreased WBC counts and IF-D reduced white blood cell count to 6.24 ± 0.22 × 10^3/µl which matched results seen in 5-fluorouracil treatments. The 5-FU treatment successfully elevated platelet count back to normal (677.05 ± 29.31 × 10^3/µl, ***P < 0.001) as comapred to the EAC-induced group (509.32 ± 20.45 × 10^3/µl). Results showed a significant improvement in MEDB (602.98 ± 19.27 × 10^3/µl, **p < 0.01) and IF-D (688.65 ± 24.66 × 10^3/µl, ***p < 0.001) while both treatment groups displayed results closer to those of the normal control.

Table 2: Effect of Methanol Extract and Isolated Compound on Blood Parameters

Parameter	Hemoglobin (g/dL)	RBC (×106/µl)	WBC (×103/µl)	Platelets (x103/l)
Normal control	12.04±0.37	4.412±0.327	5.63±0.33	694.42±23.94
EAC induced	6.17 ± 0.48	3.011± 0.384	10.03 ± 0.42	509.32±20.45
5-Florouracil (20mg/kg)	11.98 ± 0.59***	4.283±0.294***	6.43 ± 0.24***	677.05±29.31***
Methanol Extract (MEDB-250mg/kg)	10.77 ± 0.36***	3.844±0.241**	8.06 ± 0.23***	602.98±19.27**

Isolated fraction (IF-D-50mg/kg)	11.85 ± 0.35***	4.406±0.332***	6.24 ± 0.22***	688.65±24.66***

All the values were given as mean±SEM; ***indicates P<0.001 significance compared to EAC induced group

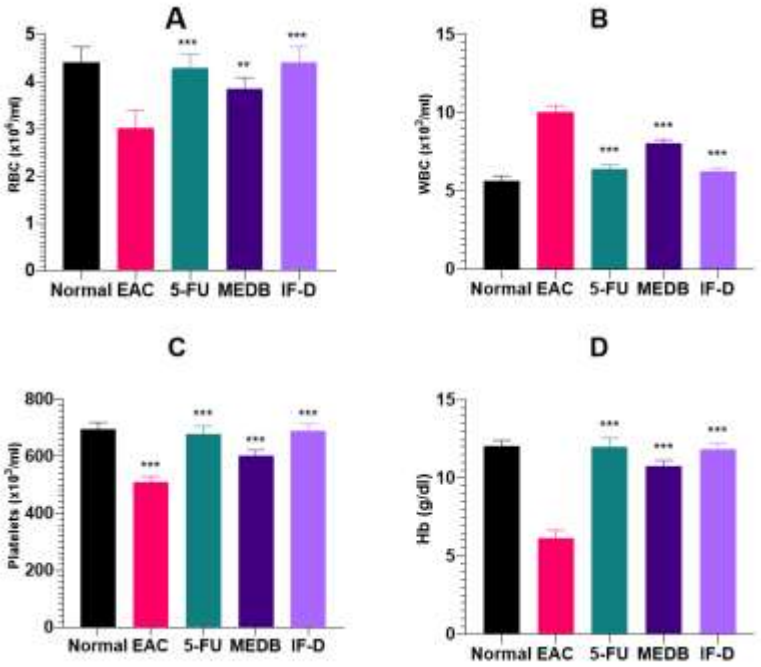

Figure 3: Effect of MEDB and IF-D on hemotological parameters

*****indicates P<0.001 significance compared to EAC induced group**

Effect of MEDB and IF-D on the inflammatory markers

The EAC-induced group showed high levels of IL-6 values at 7.11 ± 0.65 pg/ml while normal controls showed 2.32 ± 0.27 pg/ml. The IL-6 levels displayed a major reduction of 4.43 ± 0.31 pg/ml in the MEDB treatment group while IF-D treatment resulted in levels of 2.92 ± 0.25 pg/ml (*P < 0.001). Both treatments produced effects similar to the normal control values as shown in table 3. The exposure to EAC resulted in TNF-α levels reaching 18.99 ± 1.52 pg/ml, 5-Fluorouracil treatment combined with TNF-α levels decreased to 2.94 ± 0.22 pg/ml while MEDB and IF-D also decreased to 10.29 ± 1.03 pg/ml and 2.64 ± 0.13 pg/ml respectively although IF-D showed the most pronounced reduction. TNF-α levels in the group treated with EAC reached 25.03 ± 2.27 pg/ml. The experimental drugs MEDB and IF-D significantly lowered TNF-α levels to comparable levels as 5-fluorouracil through significant reductions of 17.42 ± 1.27 pg/ml (***P < 0.001) and 7.72 ± 0.86 pg/ml (***P < 0.001) respectively.

Table 3: Effect of Methanol Extract and Isolated Compound on Inflammatory Markers

Parameter	IL-6 (pg/ml)	TNF-α (pg/ml)	TNF-α (pg/ml)
Normal control	2.32±0.27	2.76±0.39	7.28±0.46
EAC induced	7.11±0.65	18.99±1.52	25.03±2.27
5-Florouracil (20mg/kg)	2.21±0.19***	2.94±0.22***	7.88±0.38***
Methanol Extract (MEDB-250mg/kg)	4.43±0.31***	10.29±1.03***	17.42±1.27***
Isolated fraction (IF-D-50mg/kg)	2.92±0.25***	2.64±0.13***	7.72±0.86***

All the values were given as mean±SEM; ***indicates P<0.001 significance compared to EAC induced group

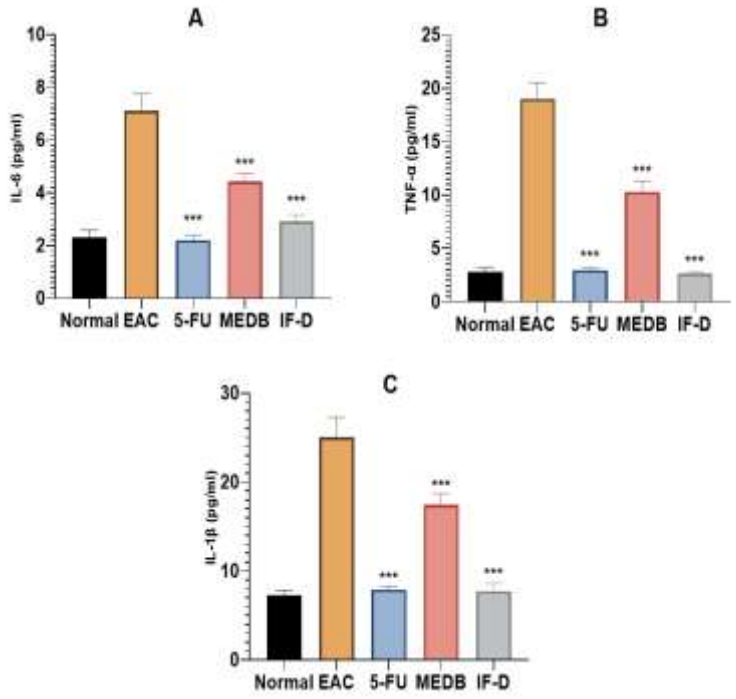

Figure 4: Effect of MEDB and IF-D on Inflammatory markers

*****indicates P<0.001 significance compared to EAC induced group**

Effect of MEDB and IF-D on the Hepatic function markers

SGPT levels reached a significantly higher level (58.95 ± 0.29 IU/L) within EAC-induced group when comparing to normal control (27.12 ± 1.04 IU/L). SGPT levels decreased markedly when mice received treatment with 5-FU reaching 24.46 ± 1.12 IU/L (***P < 0.001), together with MEDB (34.98 ± 1.59 IU/L, ***P < 0.001) and IF-D (25.63 ± 3.47 IU/L, ***P < 0.001). The cells treated with EAC developed significantly lower SGOT values amounting to 36.07 ± 2.13 IU/L compared to normal controls whose values, however both MEDB-treated cells (41.77 ± 1.40 IU/L, ***P < 0.001) and IF-D-treated cells (53.16 ± 3.10 IU/L, ***P < 0.001) showed significant better activity. The levels of ALP in EAC-induced subjects reached 80.79 ± 3.25 U/L while (IF-D) showed a lower ALP level of 98.55 ± 3.24 U/L (***P < 0.001) demonstrating superior anti-cancer properties than that of MEDB (88.45 ± 3.63 U/L, **P < 0.01).

EAC induction elevated the LDH levels by 251.21 ± 10.46 IU/L beyond the normal control range of 125.43 ± 8.74 IU/L. All treatments with 5-fluorouracil and MEDB and IF-D successfully lowered LDH levels (146 ± 1.74 IU/L, 185 ± 2.72 IU/L, 144 ± 4.11 IU/L) yet IF-D caused the most significant reduction similar to 5-fluorouracil levels. When EAC was induced in Mice the total protein level dropped to 3.22 ± 0.53 g/dL compared to normal controls at 8.43 ± 0.63 g/dL. The total protein content reached significant improvements in both the MEDB (5.38 ± 0.33 g/dL, **P < 0.01) and IF-D (8.06 ± 0.65 g/dL, ***P < 0.001) groups when compared to the control. However, IF-D showed protein values that closely matched those of the normal control group as shown in table 4.

Table 4: Effect of Methanol Extract and Isolated Compound on Hepatic Function Markers

Parameter	SGPT (IU/L)	SGOT (IU/L)	ALP (U/L)	LDH (IU/L)	Total Protein
Normal control	27.12±1.04	121.88±3.29	45.69±1.69	125.43±8.74	8.43±0.63
EAC induced	58.95 ±0.29	36.07 ± 2.13	80.79 ± 3.25	251.21±10.46	3.22±0.53
5-Florouracil	24.46±1.12***	72.17±1.16***	124.41±5.29***	146±1.74***	8.41±0.75***

(20mg/kg)					
Methanol Extract (MEDB-250mg/kg)	34.98±1.59***	41.77 ± 1.40***	88.45 ± 3.63**	185±2.72***	5.38±0.33**
Isolated fraction (IF-D-50mg/kg)	25.63 ± 3.47***	53.16 ± 3.10***	98.55 ± 3.24***	144±4.11***	8.06±0.65***

All the values were given as mean±SEM; ***indicates P<0.001 significance compared to EAC induced group

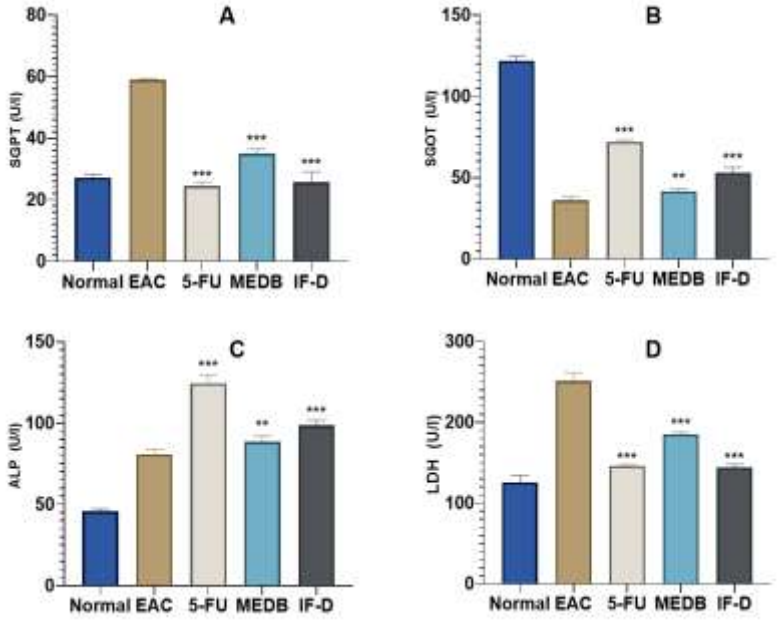

Figure 5: Effect of MEDB and IF-D on Hepatic function markers

*****indicates P<0.001 significance compared to EAC induced group**

Effect of MEDB and IF-D on the Antioxidant markers

The effect o MEDB and IF-D on the antioxidant markers was investigated and the results were tabulated in table 5. SOD levels from the EAC-induced group reached levels at 1.84 ± 0.14 U/ml while 5-fluorouracil increased to 4.12 ± 0.10 U/ml above normal levels (**P < 0.001). IF-D (4.57 ± 0.14 U/ml) and MEDB (3.4 ± 0.14 U/ml) increased SOD levels significantly yet IF-D had a stronger impact which brought levels closer to those of the normal control. EAC induction led to a substantial drop of GSH levels to 28.74 ± 2.44 U/ml which was lower than the normal control value of 55.44 ± 1.82 U/ml. The tested agents MEDB (41.16 ± 3.56 U/ml, ***P < 0.001) and IF-D (61.49 ± 0.12 U/ml, ***P < 0.001) produced statistically significant increases in GSH levels.

The EAC-induced group exhibited reduced levels of CAT at 39.85 ± 2.64 U/ml and both MEDB treatment and IF-D treatment achieved comparable results of 56.46 ± 5.21 U/ml and 72.88 ± 4.16 U/ml respectively. IF-D treatment demonstrated an effect comparable to 5-fluorouracil (72.65 ± 5.52 U/ml). IF-D-treated group (4.61 ± 0.38 nmol/mg, ***P < 0.001) resulted in significant MDA level reductions that matched the 5-fluorouracil group resutls (4.44 ± 0.22 nmol/mg) which were significant compared to the EAC induced group (9.39 ± 0.52 nmol/mg).

Table 5: Effect of Methanol Extract and Isolated Compound on Antioxidant Enzymes

Parameter	SOD (U/ml)	GSH (U/ml)	CAT (U/ml)	MDA (nmol/mg)
Normal	3.99± 0.34	55.44 ±1.82	76.05± 4.64	4.69 ± 1.25

EAC induced	1.84 ± 0.14	28.74± 2.44	39.85±2.64	9.39 ± 0.52
5-Florouracil (20mg/kg)	4.12 ±0.10***	59.63 ± 5.07***	72.65 ±5.52***	4.44 ±0.22***
Methanol Extract (MEDB-250mg/kg)	3.4 ±0.14*	41.16 ±3.56***	56.46 ± 5.21***	6.52 ± 0.36***
Isolated fraction (IF-D-50mg/kg)	4.57 ± 0.14***	61.49± 0.12***	72.88 ± 4.16***	4.61 ± 0.38***

All the values were given as mean±SEM; ***indicates P<0.001 significance compared to EAC induced group

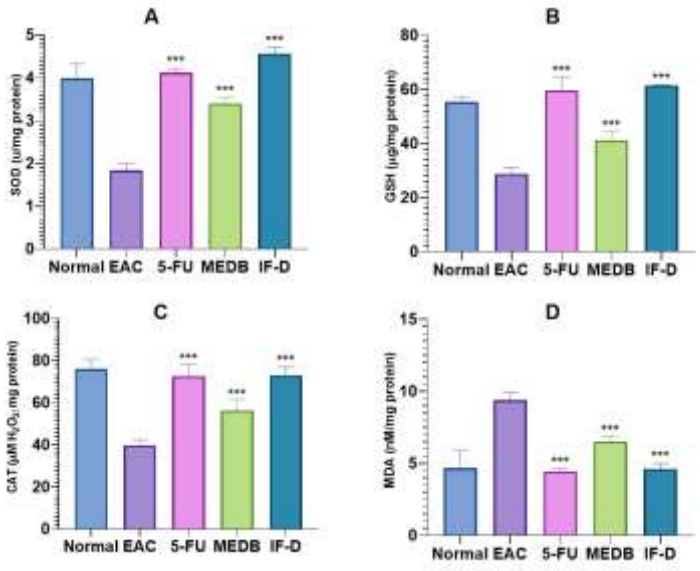

Figure 6: Effect of MEDB and IF-D on antioxidant enzymes

*****indicates P<0.001 significance compared to EAC induced group**

4. DISCUSSION

The rate of spread of cancer worldwide leads to deaths while meagre medical treatments that produce extra side effects make cancer treatment increasingly challenging (Mamun et al., 2016). Scientists searches for alternative treatment options which herbal medicines offer as dependable and safer measures for addressing cancer. The research investigated Dillenia bracteata regarding its invivo anticancer and antioxidant properties on EAC induced cancer in the swiss albino mice. Previous research has shown the medicinal properties of Dillenia bracteata when used to treat diabetes through various methods and inflammation as well as wounds (Shama et al., 2019).

The research followed two objectives to evaluate MEDB derived from Dillenia bracteata and IF-D for their anticancer properties against Ehrlich Ascites Carcinoma (EAC) induced cancer cells in mice. Analysis of the study revealed that tumor growth reduction together with enhanced survival outcomes occurred when exposed to MEDB and IF-D treatments although IF-D showed better efficiency in several biomarker tests.

As the plant is rich in flavonoids and this compound was expected to a flavonoid that shows its rising value for therapeutic applications. Various scientific investigations confirm that flavonoid compounds and their analogs display powerful cancer-fighting properties through three key molecular processes which include apoptosis initiation and cell cycle blocking while controlling tumor spread (Gao et al., 2018, Dang et al., 2015). Research findings showed that flavonoids applied successfully reduced tumor development through enhanced mean survival time (MST) and inhibited tumor volume expansion while decreasing tumor weights in EAC-induced Mice. Results showed that the separated compound IF-D displayed stronger

cancer inhibition abilities than both the medication MEDB and the widely used chemotherapy drug 5-FU. Testing indicated that the anti-tumor effects of IF-D produced both an extended survival duration and decreased tumor dimensions and weights.

The cellular death process of apoptosis becomes more effective when cells contain flavonoids because their ability to activate caspases through the mitochondrial pathway and guides Bcl-2 family proteins (Chen et al., 2023). The anticancer activity of compounds functions similarly to other mechanisms by prompting death of cancer cells in EAC tumors. Various cancer cells undergo G2/M phase halting when exposed to flavonoids which subsequently prevents malignant cell proliferation (Choi et al., 2018). Treatment with IF-D in combination with MEDB decreased viable tumor cell numbers substantially suggesting both anti-apoptotic and anti-proliferative effects on tumor cells. IF-D demonstrates better anticancer effects against cell proliferation and apoptotic cell death than MEDB and 5-FU because it simultaneously triggers both cell cycle arrest and programmed cell death.

The development of cancer tumors and tumor metastasis depends on inflammation because TNF-α and IL-6 function as pro-inflammatory cytokines which help tumors form and spread (Dang et al., 2015). The study demonstrated that both MEDB and IF-D treatment successfully decreased the production of inflammatory cytokines. The anti-inflammatory results from IF-D depend on its ability to block NF-κB pathways that direct inflammatory cytokine production. Previous research confirms how flavonoids decreases NF-κB activation in cancer cells to limit both inflammation and tumor development (Kaur et al., 2024). The findings of this study demonstrating reduced IL-6 and TNF-α levels explain how IF-D serves as an anti-inflammatory therapeutic agent in cancer management.

The key factor in cancer development is oxidative stress therefore cancer prevention becomes possible through the modulation of antioxidant enzymes. The investigation revealed IF-D boosted SOD, GSH and CAT antioxidant enzyme performance and lowered MDA lipid oxidation rates in addition to its activity. IF-D achieves the dual purpose of defending regular cells against free radical attacks and leading to cancer cell demise through its ability to reduce oxidative damage. Research proves that flavonoids demonstrates powerful antioxidant capabilities because this property aids cancer treatment due to its ability to decrease oxidative stress while shifting the redox system toward tumor suppression (Gao et al., 2018).

Chemotherapy treatment as well as cancer causes considerable harm to the liver functions. Both IF-D and MEDB test solutions helped normalize hepatic markers SGPT SGOT and ALP levels in addition to positive effects on liver function parameters in our research. The hepatoprotective properties of IF-D can be explained through its ability to protect the liver from oxidative damage because flavonoids demonstrate known antioxidant capacity (Saleh et al., 2022). Analysis indicates IF-D functions as both a strong anticancer agent and a protective substance against damaging effects chemical therapy or cancer treatment inflicts on the liver.

The cancer treatment agent 5-FU remains popular despite facing two main issues with its adverse effects and patients developing resistance to the medication. The anticancer effects of 5-FU appeared substantial in this study since it produced changes in tumor volume, survival period and tumor mass measurement. The anticancer activity of IF-D showed similar as well as superior performance by enhancing both liver function and hematological parameter values. The results indicate that IF-D presents potential as a medical option or supplementary treatment to conventional chemotherapeutic agents that causes reduced adverse effects.

5. CONCLUSION

The study demonstrates strong evidence about isolated compound IF-D as an anticancer agent extracted from Dillenia bracteata in mice models of EAC-induced cancer. The anticancer properties of this compound result from its dual capability to regulate apoptosis while controlling inflammatory and liver functional and oxidative stress processes. Research on isolated compound as a new therapeutic option for cancer treatment should advance through pre-clinical and clinical trials due to its encouraging effectiveness with low toxicity levels. Also structural elucidation of the compound could be of significant advantage for elucidating the mechanisms tandem with molecular modelling and docking.

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