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## SCREENING OF ANTICANCER AND ANTI-OXIDANT ACTIVITY OF LEAVES OF *ANNONA RETICULATA* ON EAC INDUCED SOLID TUMOR

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### Keywords:

Ehrlich Ascites Carcinoma, Solid Tumor, 5-Fluorouracil, *Annona reticulata*

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**ABSTRACT: Objective:** The objective of the study was to evaluate the *in-vivo* anticancer and antioxidant activity of methanolic extract of leaves of *Annona reticulata* Linn. on EAC induced solid tumor. **Methodology:** Tumor cells ( $1 \times 10^6$  cells/mice/I.M.) were injected into the hind limb for solid tumor formation, and the treatment started from day 1 for 14 days. Group 1 served as a Normal control, Group 2 served as EAC control, whereas Group 3 served as a standard group (5-FU, 20 mg/kg/ip), & Group 4 & Group 5 served as test drug 100 & 200 mg/kg/po respectively. During the treatment, the different groups are checked with the parameters like Mean survival time, % increase in life span, Average body weight, Tumours size, on 15<sup>th</sup> day the blood was withdrawn for hematological parameters, SGOT, SGPT, ALP, and liver & tumor was isolated for histopathology studies, simultaneously antioxidant was carried out. **Results and Conclusion:** A significant increase in the life span and a decrease solid tumor volume were noted in the tumor-induced mice after treatment with the extract. The hematological parameters were also normalized by the extract in tumor-induced mice. These observations are suggestive of the protective effect of Methanolic Extract of leaves of *Annona reticulata* (MEAR) against Ehrlich Ascites Carcinoma induced solid tumor. Based on the present study, it could be accomplished that the potential anticancer activity of *Annona reticulata*, and it may be the promising anti-cancerous agent against solid tumor induced by EAC.

**INTRODUCTION:** Cancer is one of the most life-threatening diseases, which is more than 100 different types occurring due to changes that occur in the molecule within the cell<sup>1</sup>. It is estimated that about 12.5% of the world population dies due to cancer (WHO, 2004). This disease is widely prevalent, and in the west part of the world, almost a third of the population develops cancer during their life. Since the mortality due to cancer is very high, many treatments and mechanism of disease at the molecular level<sup>2</sup>.

Cancer is responsible for many deaths (1 in 8) worldwide. The cancer patients have been doubled between 1975 and 2000, and it is set to double again by 2020, and it will be nearly triple by 2030. Around 12 million new cancer deaths worldwide in the year 2008 and 20-26 million new cases and around 13-17 million deaths projected for 2030<sup>3</sup>. Cancer is the very serious public health problems in both developed and developing countries. Due to the limited success of clinical therapies including chemotherapy, radiation, surgery, and immunomodulation in treatment of cancer, which indicates that there is an imperative need for alternative strategies in the cancer management<sup>4</sup>.

Breast cancer is the most common form of cancer in women. The incidence of breast cancer is the highest in Pakistan among the south-central Asian countries.

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There is most frequently occurring malignancy in women and which accounts for 38.5% of all female cancers. Only about half (43.7%) of all breast cancers are detected in an advanced stage<sup>5</sup>. Due to this reason, the need for an effective treatment, management, and the cure of cancer is undoubtedly crucial. Since cancer is one of the leading causes of death in worldwide, so, the control of cancer is very important to form potential that resides in alternative therapies. Due to the conventional therapies which cause serious side effects and which also extend the patient's life span by a few years so, there is need of the present time to utilize alternative concepts or approaches to the prevention of cancer<sup>6</sup>.

For managing a cancer patient, should target the multiple biochemical and physiological pathways that support the tumor development and also minimizing normal tissue toxicity. Laboratory experiments and clinical trials both have demonstrated that when combined with chemotherapy, herbal medicines raise the efficacy level and lower the toxic reactions. Therefore, these facts raised the feasibility of the combination of herbal medicine and chemotherapy<sup>7</sup>.

In anaerobic organism, Reactive Oxygen species (ROS) are constantly generated under the normal condition, which is the part of metabolic processes. These generated species react with biological molecules to contribute to processes related to cellular signaling<sup>8, 9</sup>. However, during the abnormal metabolic conditions, there is an imbalance in the production of ROS and the biological systems inherent antioxidant defense mechanisms, and this phenomenon is known as oxidative stress (OS)<sup>10</sup>.

In the main cellular components which are affected by OS include the proteins, membrane lipids and nucleic acids<sup>11</sup> which lead to the development of pathologies such as cardiovascular, arteriosclerosis and neuro-degenerative diseases, rheumatism, diabetes and cancer<sup>12</sup>. Cancer can be attributed to the mutagenic effects of ROS on DNA, and this is one of the main causes of death in populations worldwide<sup>13</sup>. According to the WHO, different types of cancer with the greatest prevalence around the globe are Liver, Lungs, Colorectal, Mammary gland, and Esophageal cancer<sup>14</sup>.

There are many natural products which are discovered from various medicinal plants or secondary metabolites such as phenolic acids, terpenoids, tannins, ligans, flavonoids, coumarins, quinines, alkaloids which exhibit significant antioxidant and other activity, because of this they played an important role in the treatment of cancer<sup>15</sup>. Different studies have shown that many of the antioxidant compounds possess antitumor, anti-inflammatory, anti-carcinogenic, and anti-mutagenic activity<sup>16</sup>. Due to this, medicinal plants have thus become a focal point to improve the present and future health issues against cancer. Since the medicinal plants, secondary metabolites could maintain the health and also cure the various disease including cancer along with less harmful effects<sup>17</sup>.

*Annona reticulate* Linn. (Custard apple, Bullock's heart, Ramphal) is traditionally very important medicinal plant for the treatment of various diseases. This plant belongs to the family Annonaceae. The synonyms of the plant are *Annona excels* kunth, *A. custescens* and *A. Laevis*kunth<sup>18</sup>.

The different phytoconstituents have been identified from the different part of this plant. The bark of stem which contains an alkaloid, tannins, and phenolic compounds. Similarly, the leaves of this plant contain a wide range of chemicals like amino acids, carbohydrates, alkaloids, steroids, proteins, flavonoids, tannins, phenolics, and glycosides. Even the root has been identified to the content of alkaloid, flavonoids proteins, and tannins. The plant is rich in different types of minerals such as P, Ca, Na, Mg, S, Cl, Mn, Cu, Fe, Co, Se, Ni, and Cr<sup>19-21</sup>. One of the biologically important polyphenol constituents, (±)-catechin which was isolated from the fruit of *Annona reticulata* Linn. This isolated (±)-catechin was characterized by the IR, UV, H-NMR13, C-NMR, and it was found that it has antioxidant and anticancer activities<sup>22</sup>.

## MATERIALS AND METHODS:

**Collection of Plant Materials:** The leaves of *Annona reticulata* was collected from the forest, nearby Tirupati. The plant was identified by a Botanist, and voucher number (1017) was deposited in Rajiv Gandhi University of Health

Science, and a copy has been preserved for the future reference at the Karnataka College of Pharmacy, Department of Pharmacology. The collected plant material (leaves) was washed thoroughly with water to remove the adhering soil, mud, and debris. All old insect damage or fungus-infected leaves were removed. The plant material was dried in the shade at room temperature to a constant mass. The plant material was coarsely powdered using the blender. The powder was stored in an airtight container and protected from light.

**Preparation of Extract:** Collected *Annona reticulata* Linn. Leaves were converted to small pieces and dried under the shade at room temperature. Powder of dried leaves (200 g) was prepared using a grinder. Then, the methanolic extract was obtained by Soxhlet extractor using 1 L methanol for 8 h at 64 °C, and the sample was concentrated with the help of rotary evaporator<sup>23</sup>.

**Experimental Animals:** Albino mice weighing between 20-25 g were used. They were maintained in standard laboratory conditions at room temperature (25 ± 2 °C) with 12 hrs light/dark cycle. The animals were given pellet chow, water *ad libitum*, except during experimentation. The study protocols were duly approved by the Institutional Animal Ethics Committee (KCP-IAEC/4/18-19/1/8.9.2018) at Karnataka College of Pharmacy, Bangalore. Studies were performed by the CPCSEA guidelines.

**Acute Toxicity Study:**<sup>24</sup> Acute toxicity study was conducted for the methanolic leaves extract of *Annona reticulata* as per OECD guidelines 425.

**Preparation of Dose:** A dose of 1/10<sup>th</sup> and 1/20<sup>th</sup> of 2000 mg/kg were considered to be a high dose and low dose prepared by dissolving in miliQ water. The doses were prepared as per the OECD guideline no. 425.

#### **In-vivo Models:**

**Ehrlich Ascites Carcinoma Model:**<sup>25</sup> Animals were injected with 1 × 10<sup>6</sup> cells (0.2 ml/mouse) EAC cells into the right hind limb (thigh) of all animals. Day of tumor implantation was assigned as day '0'. On day 1, the animals were randomized and divided into different groups. MEAR was used as the test compound and was administered orally

from day 1 to day 14 at doses of 100 mg & 200 mg/kg/day/po was given to group 4 & group 5. Control group received normal saline, while treatment with 5-FU (20 mg/kg/day) was given to a group of mice serving as the standard control group. The animals were weighed every five days for 15 days.

The mice were divided into five groups (each group of 6 mice), as follows:

**Group 1:** Normal control

**Group 2:** EAC control (mice were injected with EAC cells only)

**Group 3:** Standard control group (receives 5-Fluorouracil – 20 mg/kg/day I.P)

**Group 4:** Test group (receives MEAR extract low dose - 100 mg/kg/day orally)

**Group 5:** Test group (receives MEAR extract high dose - 200 mg/kg/day orally)

Drug treatments were given 24 h after the inoculation, once daily for 14 days. After 24 h of the last dose and then 18 h of fasting, animals of each group were sacrificed to measure tumour size, hematological parameters, and for histopathology studies.

**EAC Cells:** Cells were obtained through the courtesy of Amala Cancer Research Centre, Thrissur, Kerala, India. They were maintained weekly by intraperitoneal inoculation of 10<sup>6</sup> cells/mice.

The anticancer activity of *Annona reticulata* was screened concerning the following parameters:

#### **Parameters:**

**Effect on Survival Times:**<sup>26</sup> Animals were inoculated with EAC cells (1 × 10<sup>6</sup> cells/mouse) on the day '0' and the mean survival time (MST) of each group, consisting of 6 mice were noted.

$$\text{MST} = \text{Day of first death} \pm \text{day of last death} / 2$$

**Percentage Increase Life Span (% ILS):**<sup>26</sup> The effect of the drugs on tumor growth was monitored by recording the mortality daily for 6 weeks and percentage increase in life span (% ILS) was calculated.

$$\% \text{ ILS} = \frac{\text{Mean survival of treated group} - \text{Mean survival of EAC control group}}{\text{Mean survival of EAC control group}} \times 100$$

**Effect on Body Weight:** <sup>27</sup> Body weight of the experimental mice were recorded both at the beginning of the experiment (day 0) and sequentially on every 5<sup>th</sup> day during the treatment period.

Weight percentage was calculated using the following formula

% increase in body Weight = [(animal weight on the respective day / Animal weight on the 0<sup>th</sup> day) – 1] × 100

**Effect on Solid Tumor Volume:** <sup>28</sup> Tumor mass was measured from the 15<sup>th</sup> day of tumor induction. The measurement was carried out every 5<sup>th</sup> day for 30 days. The volume of tumor mass was calculated using the formula:

$V = 4/3 (\pi r^2)$ , where 'r' is the mean of 'r<sub>1</sub>' and 'r<sub>2</sub>' which are the two independent radii of the tumor mass.

**Effect on Haematological and Serum Biochemistry Parameters:**

**Collection of Blood Samples:** Blood was withdrawn on day 15<sup>th</sup> by cardiac puncture method from all animal groups in tubes containing EDTA (for hematological assays) and Clot Activator Tube (for serum biochemical analysis) in accordance with the method of Frankenberg <sup>29</sup> for the estimation of Haemoglobin (Hb%) content, Red Blood Cells count (RBC), White Blood Cells count (WBC), SGOT, SGPT, ALP, Triglyceride, Creatinine, total protein, Blood Urea Nitrogen (BUN), total bilirubin.

**Histopathology Studies:** <sup>30</sup> A portion of liver and tumor of animals in all groups were stored in a container for 12 h in 10% formalin (10 ml of formaldehyde in 90 ml of normal saline) solution and subjected to histopathological studies.

**Estimation of Liver Anti-oxidants:** The animals were sacrificed by chloroform entrapment, and the liver was isolated and quickly transferred to cold phosphate buffered saline (PBS pH-7.4). It was maintained free of blood and other tissue fluids and weighed. The liver was nicely chopped with a surgical blade into small slices. Then the pieces were placed in cold 0.25 M sucrose solution quickly placed on a filter paper. After that crushed and homogenized in cold tris HCl buffer of strength 10 mM of pH-7.4 to a concentration of 10% w/v. The obtained homogenate was centrifuged at 7000

rpm for 25 min under normal conditions. The clear supernatant fluid was used for different biochemical parameters estimation.

**Estimation of Superoxide dismutase (SOD):** <sup>31</sup> 0.8 ml of 0.1 M sodium carbonated buffer (pH 10.2), 0.1 ml of supernatant, 0.1 ml of epinephrine was added in a quartz cuvette. The absorbance at 295 nm by using spectrophotometer was measured.

**Estimation of Catalase:** <sup>32</sup> 1.95 ml phosphate buffer (50 MM, pH 7.0), 1.0 ml H<sub>2</sub>O<sub>2</sub> (0.17 MM), 0.05 ml homogenate (10%, w/v) in a total volume of 3.0 ml was added. The absorbance at 240 nm by using spectrophotometer was measured.

**Estimation of Lipid Peroxidation:** <sup>33</sup> To 1.0 ml of the sample, 2.0 ml of TCA- TBA-HCl reagent was added and mixed thoroughly. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1,000 rpm for 10 min. The absorbance was determined at 535 nm against a blank that contains all the reagents except the sample. The results were expressed as n moles of MDA formed/min/mg protein using an extinction coefficient of the chromophore  $1.56 \times 10^5$  Mm and expressed as n moles of MDA formed/min/mg protein

**Estimation of Glutathione Peroxidase:** <sup>34</sup> To 0.2 ml of tris buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide and 0.5 ml of tissue homogenate were added. To this mixture, 0.2 ml of glutathione and 0.1 ml of hydrogen peroxide were added. The contents were mixed well and incubated at 37 °C for 10 min along with a tube containing all the reagents except sample. After 10 min, the reaction was arrested with the addition of 0.5 ml of 10% TCA, centrifuged, and the supernatant was assayed for glutathione by Ellman's method. To 2.0 ml of the supernatant, 3.0 ml disodium hydrogen phosphate solution and 1.0 ml of DTNB reagent were added. The color developed was read at 412 nm. Standards in the range of 200-1000 µg were taken and treated similarly. The activity was expressed in term of glutathione consumed/min/mg protein.

**Statistical Analysis:** Results have been reported as mean value ± SEM. The variation in a set of data has been estimated by performing one-way analysis of variance (ANOVA). P<0.05 indicate the

significance of the result. Individual comparisons of groups mean values were done using Dunnett's test.

## RESULTS:

**Results of Acute Oral Toxicity:** The LD<sub>50</sub> of the leaves extract of *Annona reticulata* was found to be 2000 mg/kg after performing the acute oral toxicity studies. 1/10<sup>th</sup> and 1/20<sup>th</sup> of the same dose were selected (200 mg/kg and 100 mg/kg respectively), and further study was carried out.

### In-vivo Data (EAC Model):

**Effect on Mean Survival Time (MST):** The effect of MEAR on the survival of tumour-bearing mice is shown in **Table 1**. The MST for the EAC control group was 16 ± 0.66 days; whereas it was 29 ± 0.94 days for 5-FU (20 mg/kg/day i.p); 25 ± 0.76 for MEAR (100 mg/kg/day p.o); and 30 ± 0.65 for MEAR (200 mg/kg/day p.o) treated groups.

**Effect on % ILS:** The increase in the life span of tumour-bearing mice treated with MEAR (100 and 200 mg/kg) and 5-FU was found to be 56.25%, 87.50%, and 81.25% respectively. The effect of MEAR on % ILS is shown in **Table 1**.

**TABLE 1: EFFECT OF MEAR ON MST, % ILS**

Treatment	Number of animals	% ILS	Mean survival time (days)
Group 1	6	100%	42 ± 0.00
Group 2	6	-	16 ± 0.66 <sup>###</sup>
Group 3	6	81.25%	29 ± 0.94 <sup>***</sup>
Group 4	6	56.25%	25 ± 0.76 <sup>**</sup>
Group 5	6	87.50%	30 ± 0.65 <sup>***</sup>

All values are expressed as mean ± SEM for 6 animals in each group. <sup>###</sup>p<0.05 statistically significant when compared to normal control. <sup>\*\*</sup>p<0.05, <sup>\*\*\*</sup>p<0.05 when compared with EAC control.

**TABLE 2: EFFECT OF MEAR ON BODY WEIGHTS**

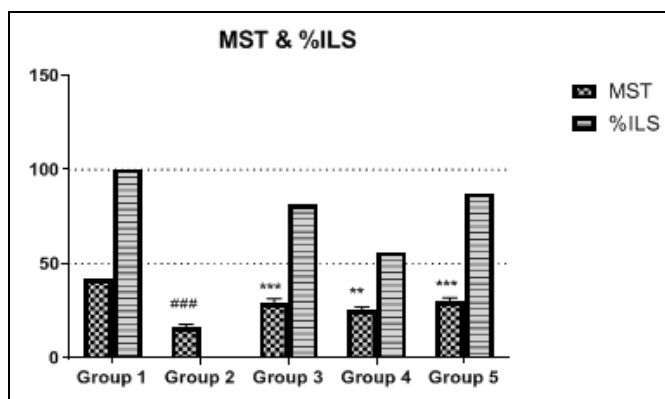
Treatment	0 <sup>th</sup> day		5 <sup>th</sup> day		10 <sup>th</sup> day		15 <sup>th</sup> day	
	Wt.	Wt. %	Wt.	Wt. %	Wt.	Wt. %	Wt.	Wt. %
Group 1	22.90 ± 0.78	23.51 ± 0.37	2.68%	24.77 ± 0.97	5.37%	25.04 ± 0.22	9.18%	
Group 2	33.12 ± 0.95 <sup>###</sup>	38.33 ± 0.64 <sup>###</sup>	15.75%	40.23 ± 0.74 <sup>###</sup>	21.49%	43.62 ± 0.23 <sup>###</sup>	31.71%	
Group 3	30.65 ± 0.96 <sup>***</sup>	31.80 ± 0.85 <sup>***</sup>	3.78%	32.52 ± 0.57 <sup>***</sup>	6.12%	33.33 ± 0.81 <sup>***</sup>	8.77%	
Group 4	26.24 ± 0.59 <sup>***</sup>	27.76 ± 0.19 <sup>***</sup>	5.80%	28.65 ± 0.67 <sup>***</sup>	9.21%	29.75 ± 0.78 <sup>***</sup>	13.41%	
Group 5	31.43 ± 0.55 <sup>***</sup>	32.75 ± 0.32 <sup>***</sup>	4.21%	33.79 ± 0.31 <sup>***</sup>	7.51%	34.74 ± 0.27 <sup>***</sup>	10.54%	

Values are expressed as Mean ± S.E.M. n=6; <sup>###</sup>p<0.05 statistically significant when compared with group 1; <sup>\*\*</sup>p<0.05 statistically significant when compared with group 2

**TABLE 3: EFFECT OF MEAR ON SOLID TUMOR VOLUME**

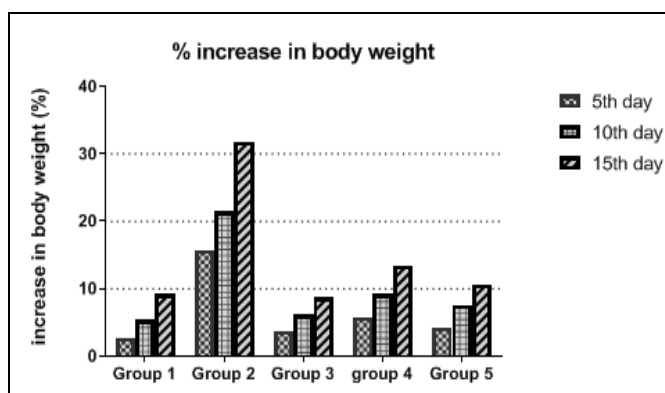
Treatment	15 <sup>th</sup> day	20 <sup>th</sup> day	25 <sup>th</sup> day	30 <sup>th</sup> day
Group 2 (EAC control)	3.54 ± 0.04	4.71 ± 0.08	5.32 ± 0.05	6.31 ± 0.05
Group 3 (EAC + 5-FU 20 mg/kg)	1.42 ± 0.04 <sup>***</sup>	1.68 ± 0.06 <sup>***</sup>	2.31 ± 0.07 <sup>***</sup>	2.65 ± 0.04 <sup>***</sup>
Group 4 (EAC + MEAR 100 mg/kg)	2.43 ± 0.07 <sup>**</sup>	3.39 ± 0.05 <sup>**</sup>	3.71 ± 0.05 <sup>**</sup>	4.36 ± 0.06 <sup>**</sup>
Group 5 (EAC + MEAR 200 mg/kg)	1.79 ± 0.04 <sup>***</sup>	2.38 ± 0.04 <sup>***</sup>	2.75 ± 0.05 <sup>***</sup>	3.29 ± 0.06 <sup>***</sup>

N = 6 animals in each group. Values are expressed as mean ± SEM. <sup>\*\*\*</sup>p<0.05 when compared with EAC control. <sup>\*\*</sup>p<0.05 when compared with EAC control.

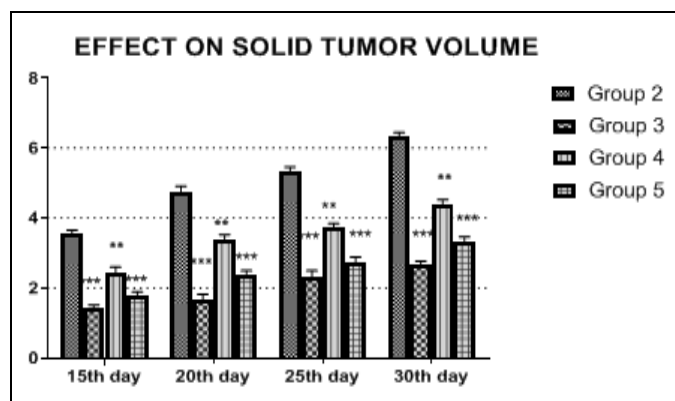


**GRAPH 1: MST & % ILS ON VARIOUS TREATED GROUPS**

**Effect on Body Weights:** The effect of MEAR and 5-Fluorouracil on the body weight & % increase in weights of tumour-bearing mice is shown in **Table 2**. The body weight of the group 2 (EAC control group) was 43.62 ± 0.23 g, whereas 33.33 ± 0.81 g for group 3 (5-FU) and 29.75 ± 0.78 g for group 4 (low dose -100 mg/kg) and 34.74 ± 0.27 g for group 5 (high dose - 200 mg/kg) on 15<sup>th</sup> day.



**GRAPH 2: INCREASE IN BODY WEIGHT (%) ON VARIOUS TREATED GROUPS**



GRAPH 3: SOLID TUMOR VOLUME ON VARIOUS TREATED GROUPS

**Effect on Solid Tumour Volume:** There was a significant reduction in the tumour volume of mice treated with MEAR (100 & 200 mg/Kg). Tumour volume of control animals was  $6.31 \pm 0.05$  mL

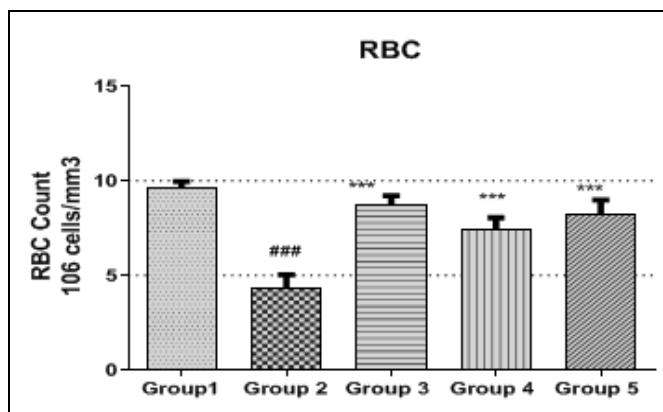
whereas it was  $2.65 \pm 0.04$  mL for 5-FU and  $4.36 \pm 0.06$  &  $3.29 \pm 0.06$  mL for the groups treated with MEAR (100 and 200 mg/Kg) respectively **Table 3**.

**Effect on Haematological & Serum Biochemistry Parameters:** Haematological parameters of EAC control group day 15 showed significant changes when compared to the vehicle control mice as shown in **Table 4**. In EAC control, the total WBC count was found to increase with a reduction in Haemoglobin content and the RBC count. On the other hand, the MEAR treated group changed these altered parameters to nearly normal values in a dose-dependent manner, and the highest dose of MEAR has produced a superior effect which is comparable to 5-Fluorouracil.

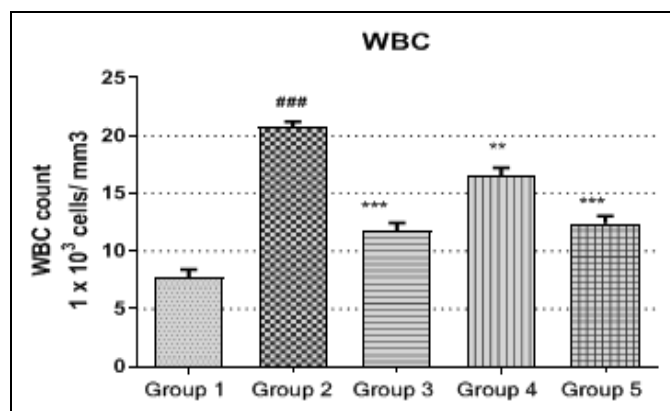
TABLE 4: EFFECT ON HAEMATOLOGICAL PARAMETERS

Treatment	RBC ( $10^6/\text{mm}^3$ )	WBC ( $1 \times 10^3/\text{mm}^3$ )	HB% (g %)
Group 1	$9.63 \pm 0.14$	$7.74 \pm 0.28$	$12.640 \pm 0.35$
Group 2	$4.35 \pm 0.26^{###}$	$20.66 \pm 0.19^{###}$	$8.60 \pm 0.29^{###}$
Group 3	$8.77 \pm 0.17^{***}$	$11.73 \pm 0.26^{***}$	$12.29 \pm 0.30^{***}$
Group 4	$7.38 \pm 0.26^{***}$	$16.32 \pm 0.29^{**}$	$10.74 \pm 0.26^{**}$
Group 5	$8.27 \pm 0.28^{***}$	$12.33 \pm 0.25^{***}$	$11.74 \pm 0.27^{***}$

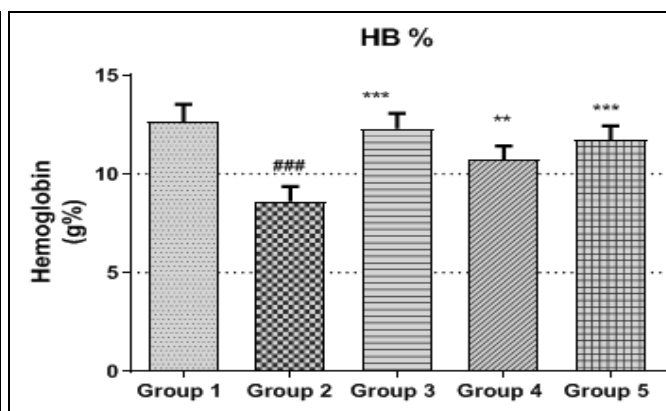
Values are Mean  $\pm$  S.E.M. n=6;  $^{###}$ p<0.05 statistically significant when compared with Normal control;  $^{***}$ p<0.05 statistically significant when compared to EAC control group;  $^{**}$ p<0.05 statistically significant when compared to EAC control group



GRAPH 4: EFFECT OF MEAR ON RBC COUNT



GRAPH 5: EFFECT OF MEAR ON WBC COUNT



GRAPH 6: EFFECT OF MEAR ON HAEMOGLOBIN COUNT

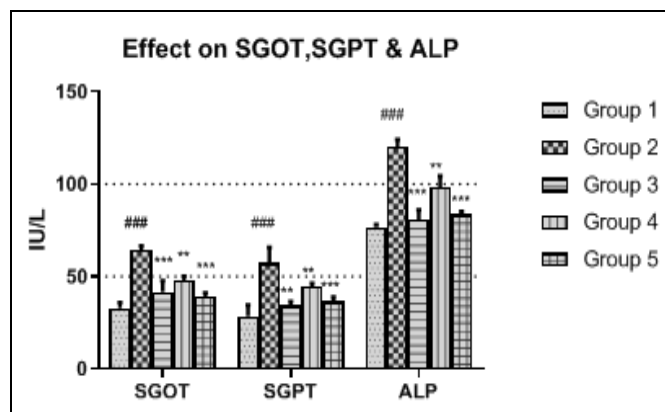
The effects of MEAR on hematological profiles of experimental animals were represented in **Table 4** and represented graphically in **Graph 4, 5, and 6**. Effect of MEAR on the level of various serum biochemical parameters was studied and tabulated. The level of SGOT, SGPT, ALP, TG, Creatinine,

etc. on tumour-bearing experimental animals after oral administration of various doses levels of MEAR for 14 days were shown in **Table 5** and **6** and represented graphically in **Graph 7, 8, 9, 10, 11 and 12**.

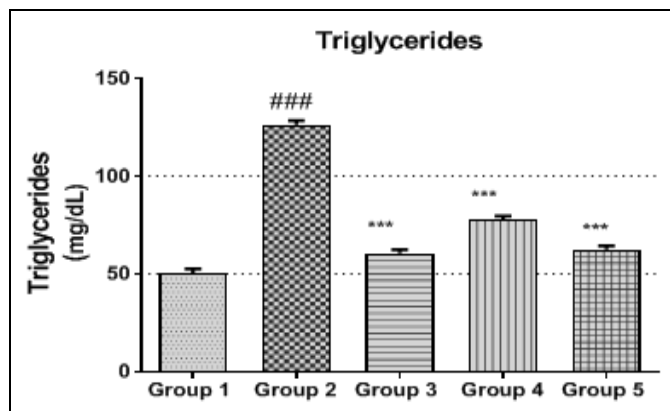
**TABLE 5: EFFECT ON SERUM BIOCHEMISTRY PARAMETERS**

Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Triglycerides (mg/dL)	Creatinine (ml/dL)
Group 1	32.68 ± 1.23	28.47 ± 2.34	76.51 ± 0.57	50.50 ± 0.76	0.41 ± 0.01
Group 2	64.19 ± 0.89 <sup>###</sup>	57.32 ± 3.31 <sup>###</sup>	120.09 ± 1.53 <sup>###</sup>	126.50 ± 0.76 <sup>###</sup>	0.93 ± 0.07 <sup>###</sup>
Group 3	41.64 ± 2.34 <sup>***</sup>	34.26 ± 0.79 <sup>***</sup>	80.54 ± 2.12 <sup>***</sup>	60.51 ± 0.76 <sup>***</sup>	0.61 ± 0.02 <sup>***</sup>
Group 4	47.78 ± 0.91 <sup>**</sup>	44.48 ± 0.69 <sup>**</sup>	98.27 ± 2.34 <sup>**</sup>	78.00 ± 0.58 <sup>***</sup>	0.53 ± 0.01 <sup>***</sup>
Group 5	39.18 ± 0.72 <sup>***</sup>	36.58 ± 0.81 <sup>***</sup>	83.57 ± 0.58 <sup>***</sup>	62.50 ± 0.76 <sup>***</sup>	0.62 ± 0.02 <sup>***</sup>

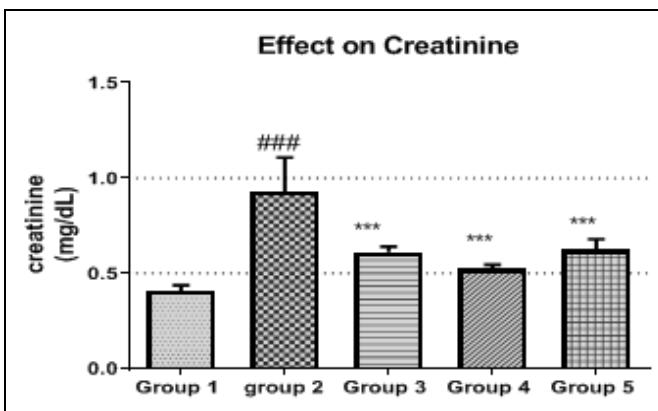
All values are expressed as mean ± SEM for 6 animals in each group; <sup>###</sup>p<0.05 statistically significant when compared with Normal control; <sup>\*\*\*</sup>p<0.05 statistically significant when compared to EAC control group; <sup>\*\*</sup>p<0.05 statistically significant when compared to EAC control group.



**GRAPH 7: EFFECT OF MEAR ON SGOT, SGPT & ALP**



**GRAPH 8: EFFECT OF MEAR ON TRIGLYCERIDES**

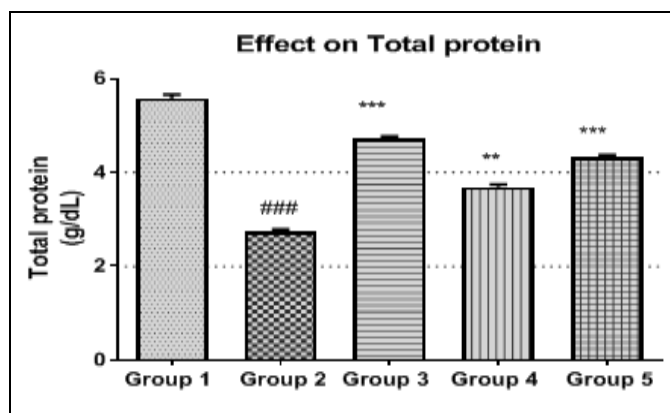


**GRAPH 9: EFFECT OF MEAR ON CREATININE**

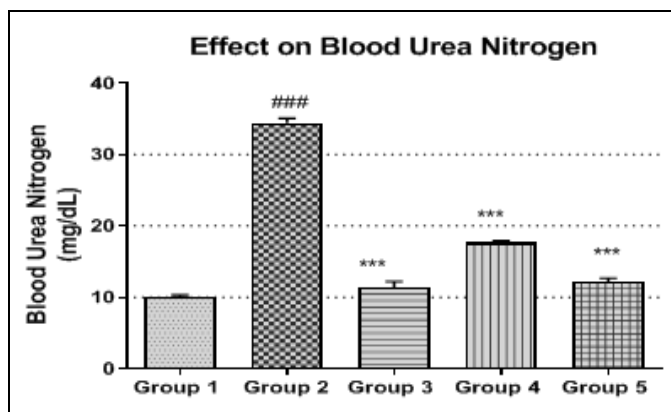
**TABLE 6: EFFECT ON SERUM BIOCHEMISTRY PARAMETERS**

Treatment	Total Protein (g/dL)	Blood Urea Nitrogen (mg/dL)	Total Bilirubin (mg/dL)
Group 1	5.58 ± 0.08	10.50 ± 0.10	0.40 ± 0.01
Group 2	2.72 ± 0.05 <sup>###</sup>	34.41 ± 0.24 <sup>###</sup>	1.46 ± 0.08 <sup>###</sup>
Group 3	4.71 ± 0.06 <sup>***</sup>	11.42 ± 0.31 <sup>***</sup>	0.42 ± 0.02 <sup>***</sup>
Group 4	3.69 ± 0.05 <sup>**</sup>	17.67 ± 0.11 <sup>**</sup>	0.64 ± 0.03 <sup>***</sup>
Group 5	4.33 ± 0.04 <sup>***</sup>	12.20 ± 0.18 <sup>***</sup>	0.52 ± 0.01 <sup>***</sup>

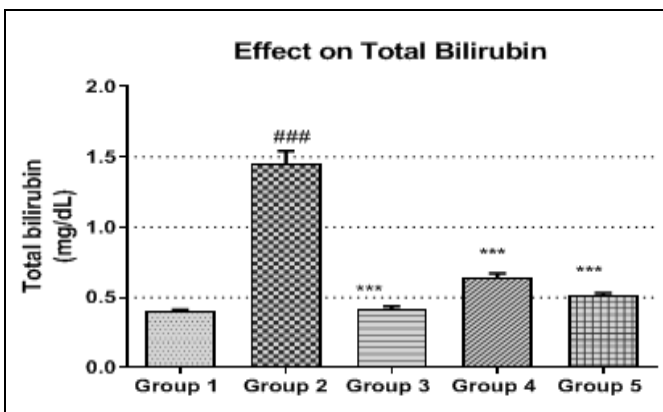
All values are expressed as mean ± SEM for 6 animals in each group. <sup>###</sup>p<0.05 statistically significant when compared with Normal control; <sup>\*\*\*</sup>p<0.05 statistically significant when compared to EAC control group; <sup>\*\*</sup>p<0.05 statistically significant when compared to EAC control group.



GRAPH 10: EFFECT OF MEAR ON TOTAL PROTEIN



GRAPH 11: EFFECT OF MEAR ON BUN



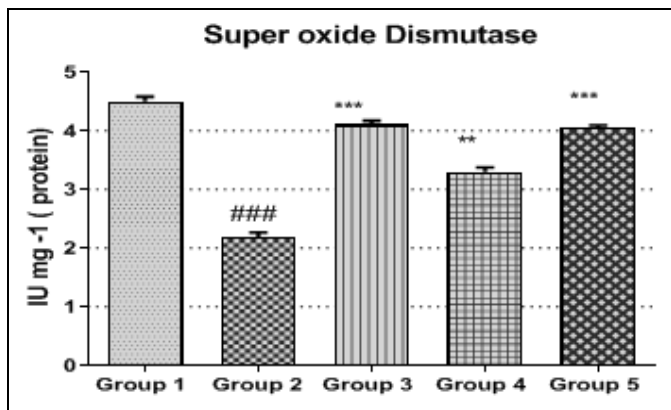
GRAPH 12: EFFECT OF MEAR ON TOTAL BILIRUBIN

**Estimation of Liver Anti-Oxidants:**

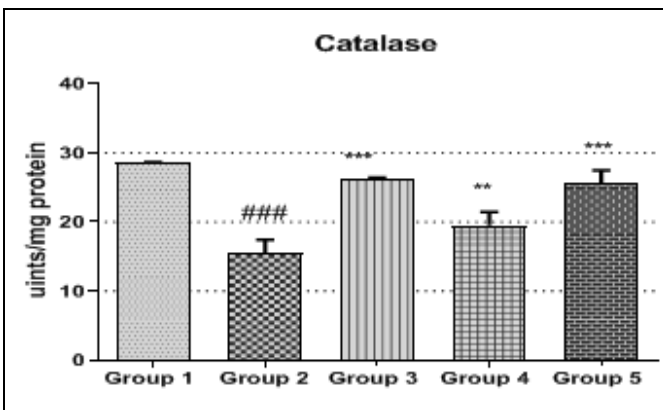
**TABLE 7: EFFECT OF THE EAC CELLS, STANDARD & TEST DRUG-ON SUPEROXIDE DISMUTASE, CATALASE, LIPID PEROXIDATION, AND GLUTATHIONE PEROXIDASE**

S. no.	Treatment	SOD (units/mg protein)	Catalase (units/mg protein)	Lipid peroxidation (units/mg protein)	Glutathione peroxidase mmol/mg protein/min
1	Group 1	4.50 ± 0.07	28.50 ± 0.06	0.75 ± 0.01	3.39 ± 0.58
2	Group 2	2.18 ± 0.09 <sup>###</sup>	15.50 ± 0.76 <sup>###</sup>	1.37 ± 0.06 <sup>###</sup>	1.40 ± 0.55 <sup>###</sup>
3	Group 3	4.11 ± 0.06 <sup>**</sup>	26.10 ± 0.12 <sup>**</sup>	0.61 ± 0.02 <sup>**</sup>	3.16 ± 0.60 <sup>**</sup>
4	Group 4	3.31 ± 0.07 <sup>**</sup>	19.50 ± 0.76 <sup>**</sup>	0.92 ± 0.03 <sup>**</sup>	2.33 ± 0.49 <sup>**</sup>
5	Group 5	4.05 ± 0.04 <sup>**</sup>	25.50 ± 0.75 <sup>**</sup>	0.75 ± 0.01 <sup>**</sup>	2.73 ± 0.52 <sup>**</sup>

The data are expressed as Mean ± S.E.M (n=6) mice in each group; <sup>###</sup>P<0.05 when compared with Normal Control; <sup>\*\*</sup>P<0.05 when compared with EAC cells group; <sup>\*</sup>P<0.05 when compared with EAC cells group

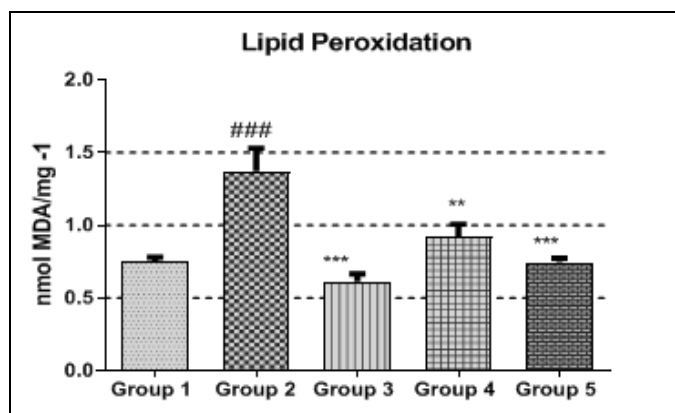


GRAPH 13: EFFECT OF MEAR ON SUPER OXIDE DISMUTASE LEVELS

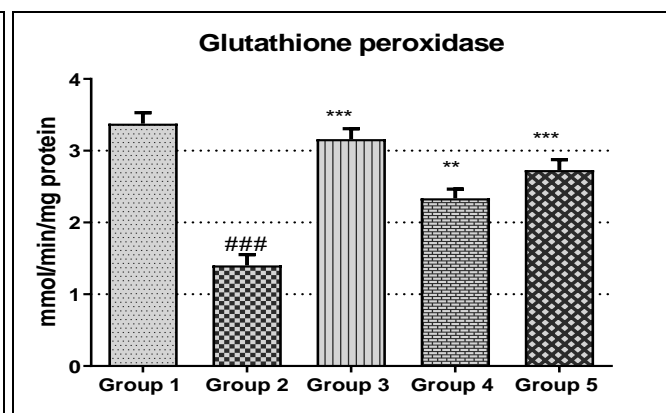


GRAPH 14: EFFECT OF MEAR ON CATALASE LEVELS





GRAPH 15: EFFECT OF MEAR ON LIPID PEROXIDATION LEVELS



GRAPH 16: EFFECT OF MEAR ON GLUTATHIONE PEROXIDASE LEVELS

**Histopathology Analysis:**

**Effect on Liver and Tumour of Normal Control:**

**Liver:** Normal histology with hepatic cords of hepatocytes, central vein and portal triad, etc.

**Tumour:** Normal nucleoli in nuclear, structure and function, regular in size and shape with normal apoptosis.

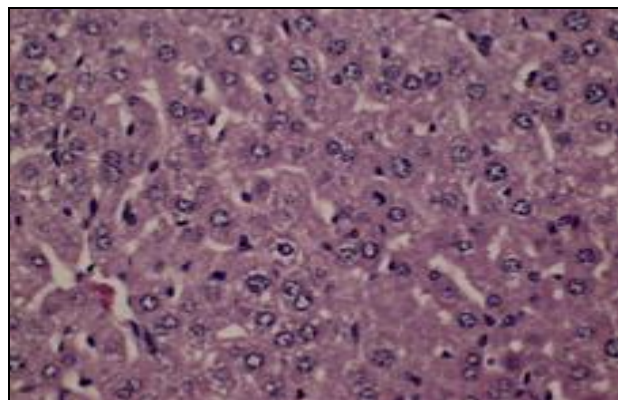


FIG. 1: LIVER-NORMAL CONTROL

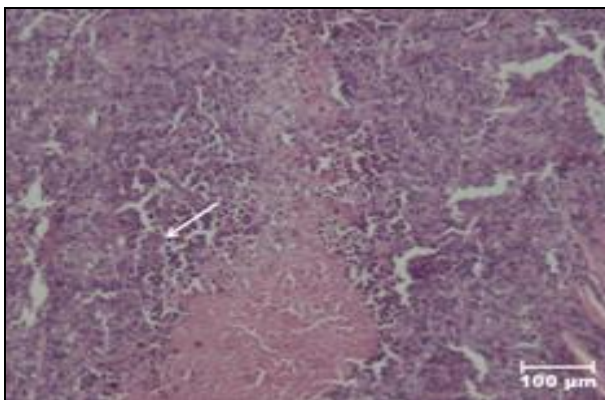


FIG. 2: TUMOR-NORMAL CONTROL

**Effect on Liver and Tumor of EAC Control Group:**

**Liver:** Infiltration of neutrophils- 4, cellular vocalization -3, loss of cellular integrity-3, and single cell necrosis-2, nuclear pleomorphism-3.

variation in size and shape of nucleus-3, Apoptosis-4.

**Tumour:** Prominent nucleoli in the nucleus-4, Nuclear structure, and function of nucleus-4,

Grading Pattern	Score
Minimal	1
Mild	2
Moderate	3
Severe	4

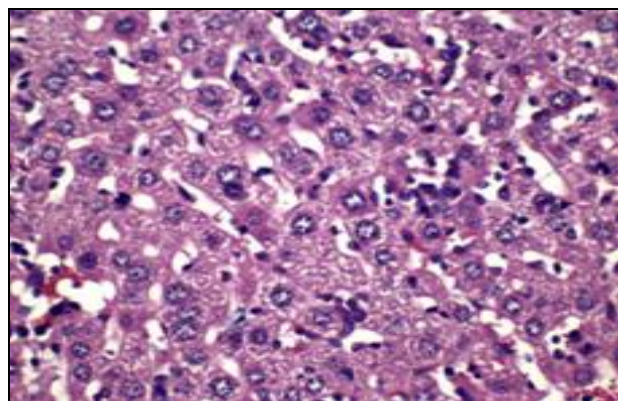


FIG. 3: LIVER-EAC CONTROL

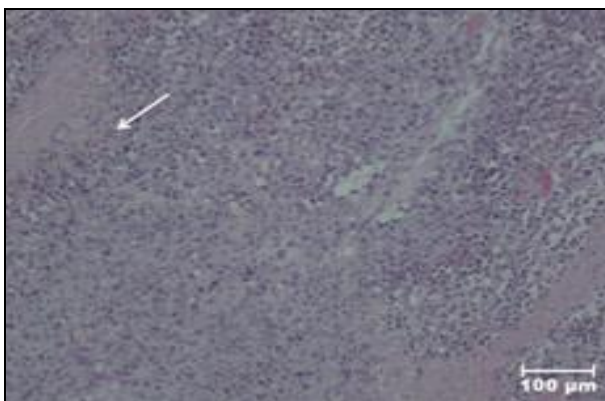


FIG. 4: TUMOR-EAC CONTROL

**Effect on Liver and Tumour of 5-FU Control Group:**

**Liver:** Infiltration of neutrophils-1, cellular vocalization -1, loss of cellular integrity-1, and single cell necrosis-1, nuclear pleomorphism-1.

**Tumour:** Prominent nucleoli in the nucleus-1, Nuclear structure, and function of nucleus-1, variation in size and shape of nucleus-1, Apoptosis-1.

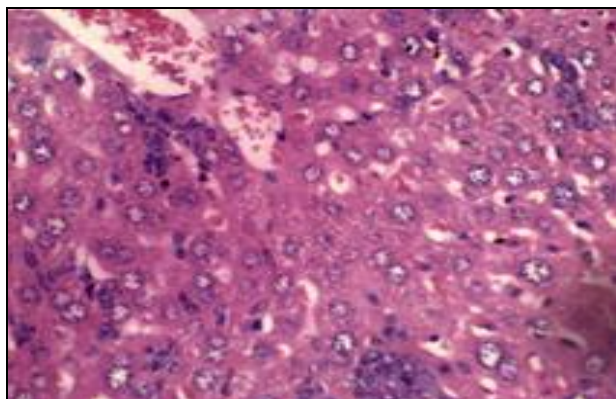


FIG. 5: LIVER-5 FU GROUP

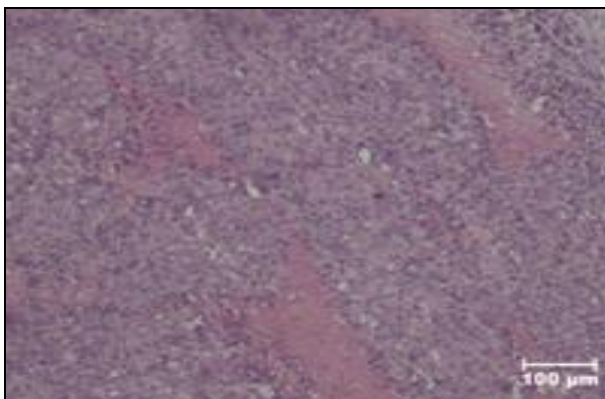


FIG. 6: TUMOR-5 FU GROUP

**Effect on Liver and Tumor of Low Dose Group:**

**Liver:** Infiltration of neutrophils-2, cellular vocalization-1, loss of cellular integrity-2, and single cell necrosis-2, nuclear pleomorphism-1

**Tumour:** Prominent nucleoli in the nucleus-2, Nuclear structure, and function of nucleus-3, variation in size and shape of nucleus-2, Apoptosis-1.

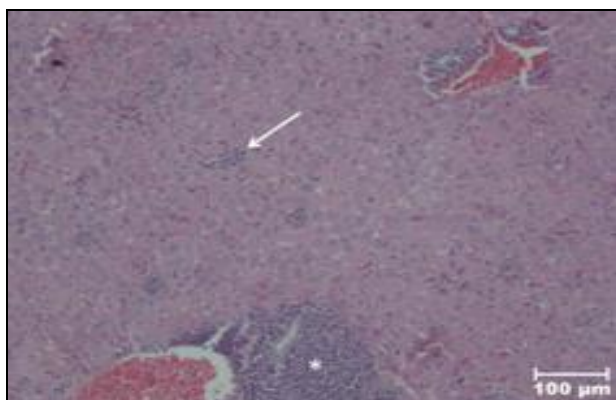


FIG. 7: LIVER-MEAR (100mg)

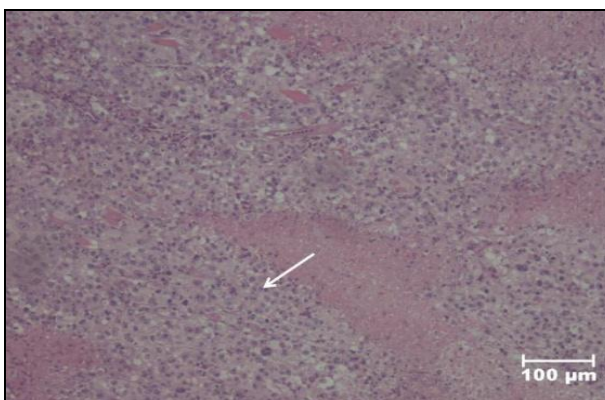


FIG. 8: TUMOR-MEAR (100mg)

**Effect on Liver High Dose Group:**

**Liver:** Infiltration of neutrophils -1, cellular vocalization -1, loss of cellular integrity-2 and single cell necrosis-1, nuclear pleomorphism-1

**Tumour:** Prominent nucleoli in the nucleus-1, Nuclear structure, and function of nucleus-2, variation in size and shape of nucleus-1, Apoptosis-1.

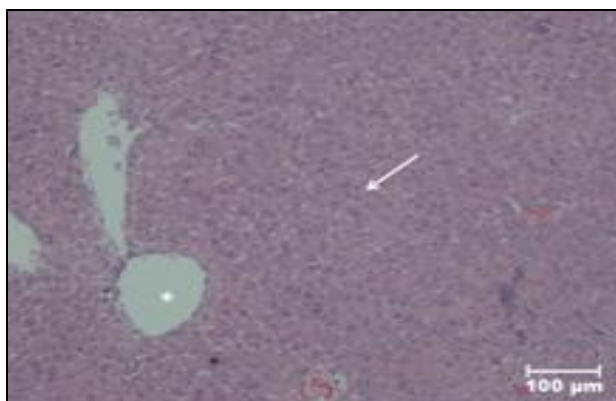


FIG. 9: LIVER-MEAR (200mg)



FIG. 10: TUMOR-MEAR (200mg)

**DISCUSSION:** The present study was carried out to evaluate the anticancer & anti-oxidant activity of leaves of *Annona reticulata* on EAC induced solid tumor. The evaluation of different parameters was carried out. The chemotherapeutic agent has been shown that synthetic cancer drug causes nonspecific killing of cells, whereas natural plant products offer the protective and therapeutic actions to all cells with low cytotoxicity<sup>35</sup>.

The study revealed that methanolic extract of *Annona reticulata* leaves showed significant antitumor activity against the solid tumor. Both dose levels significantly increased the life span, inhibit the body weight, the tumor volume of the mice when compared to the EAC control. The steadfast criteria for judging the potency of any anticancer drug are a prolongation of life span and decrease in WBC<sup>36</sup>. The methanolic extract delayed the cell division, thereby suggesting the reduction in EAC volume and increased survival time in mice.

Methanolic leaves extract (100 and 200 mg/kg b.w.) significantly improved the MST in tumor-bearing mice. No visible sign of toxicity and changes in vital functions were observed in any of the treated animals. The prolongation of life span is a reliable criterion for judging the efficacy of anticancer drugs<sup>37</sup> and the extract of this plant were able to meet this criterion. Myelosuppression and anemia (reduced Haemoglobin) have been frequently observed in ascites carcinoma. Anaemia encountered in ascites carcinoma mainly due to iron deficiency, either by hemolytic or myelopathic conditions which finally lead to reduced RBC number<sup>38</sup>.

In this study, elevated WBC count, reduced Haemoglobin and RBC count was observed in EAC control mice, and the oral administration of *Annona reticulata* restored Haemoglobin content and maintained normal values of RBC and WBC, thus supporting its hematopoietic protecting activity without inducing myelotoxicity, the most common side effects of cancer chemotherapy.

The phytochemical study indicated the presence of flavonoids, alkaloids, and terpenoids in Methanol extract. Flavonoids have been shown to possess antimutagenic and antimalignant effects<sup>39,40</sup>.

Furthermore, flavonoids have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation<sup>41</sup> and angiogenesis<sup>42</sup>. Terpenoids, steroids, and phenolic compounds have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation, and angiogenesis has been reported<sup>43</sup>. Thus, the anticancer effect produced by the methanolic extract of leaves of *Annona reticulata* may be due to the presence of flavonoids, phenolic compounds, and there antioxidant potential<sup>44</sup>.

Hepatotoxicity may occur due to the cytotoxic agent itself or due to its toxic metabolites. In some cases, they can be carcinogenic, *i.e.* they may themselves cause cancer. If there is rapid cell destruction with extensive purine catabolism, urates may precipitate in the renal tubules and cause kidney damage<sup>45, 46</sup>. From the result, it can be concluded that in EAC inoculated increase the level of a liver enzyme like SGOT, SGPT, ALP and decrease the level of total protein and albumin. These levels are markedly reversed by the administration of MEAR.

Increase lipid peroxidation's causes degeneration of tissues and lipid peroxide formed in the primary site could be transferred through circulation and cause the damage by propagating the process of lipid peroxidation<sup>47</sup>. Glutathione, which is a potent inhibitor of a neoplastic process that plays an important role as an endogenous antioxidant system that is found particularly in high concentration in liver and is known to have a crucial function in the protective process<sup>48</sup>. MEAR reduced the elevated levels of lipid peroxidation and increased the glutathione content in EAC bearing mice.

CAT is present in all oxygen metabolizing cells, and their function is to provide a defense against the potentially damaging reactivity of hydrogen peroxide and superoxide<sup>49</sup>. In the present study, administration of MEAR at different dose increase the CAT levels in a dose-dependent manner, which indicate the antioxidant and free scavenging property of MEAR. However, further studies are needed to identify, isolate, characterize, and screen organ protective activity.

**CONCLUSION:** The methanolic extract of leaves of *Annona reticulata* significantly inhibit solid

tumor in EAC induced cancer in Swiss albino mice when compared with the standard drug. The activity was confirmed by significant enhancement of MST, % ILS, decrement of gain in average body weight, reduction in tumor volume, increase in the RBC and hemoglobin (Hb%), a decrease of WBC count were observed. On the other isolated compound, *Annona reticulata* can also be used as adjuvant therapy in combination with the existing anticancer drug like 5-Fluorouracil or methotrexate.

Based on the present study, it can accomplish that the potential anticancer activity of *Annona reticulata*, and it may be the promising anti-cancerous agent against a solid tumor induced by Ehrlich Ascites Carcinoma.

Further studies are needed to characterize the anticancer activity of the isolated extract to find out the exact mechanism involved so that it can be formulated and may be tried clinically.

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**CONFLICT OF INTEREST:** The authors declare that there is no conflict of interest.

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