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IN-VITRO AND *IN-VIVO* ACTIVITY OF NOVEL CHALCONES DERIVATIVES TARGETING CANCER

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ABSTRACT

We synthesized 10 different chalcone derivatives by the Claisen–Schmidt condensation methods. Here, acetonapthone was treated with various benzaldehyde derivatives to obtain the derivatives of the chalcones. The reaction takes place in an ice bath with continuous stirring for 4 to 6 hrs. The reaction medium is ethanol and uses sodium hydroxide as a catalyst to boost the reaction and the derivatives are formed as AR1 to AR10. Their characterization and identification were done by physical and chemical methods like TLC, Melting point, FT-IR, NMR, and Mass spectroscopy. Out of them AR1 to AR3 are showing higher activity in case of breast cancer cell line i.e. MCF7 and MDA MB (453) whereas some shows high actions on lung cancer cell line i.e. A431 cell line. The IC_{50} value of the compounds AR1, AR2, and AR3 for MCF7 was found to be 24 µg, 59 µg, and 45 µg; for MDA MB (453) was found to be 50 µg, 52 µg, and 49 µg; for A431 was found to be 53 µg, 60 µg, and 46 µg respectively. *In-vitro* studies have been conducted on 6 different types of cell lines like MCF-7, MDA MB (453), A431, HCT-116, HL-60, and HeLa cell lines. But the compound AR1 to AR3 is showing superior activity than standard drugs in the case of MCF-7, MDA MB (453), A431 cell lines. In the case of other cell lines, they are showing less activity than the standard drug. Their *in-vivo* activities were also studied by comparing their body weight, RBC count, WBC count, mean survival time, and several anti-oxidants parameters as well. All the results are expressed in p-value.

KEYWORDS: Claisen–Schmidt condensation, cytotoxicity, Docking, EAC model, Spectroscopy, cancer cells, and anti-oxidants.

1. INTRODUCTION

Cancer is normally a disease in which immortal and genetically altered cells begin to grow without natural apoptosis and invade the normal healthy cells; the latter process is called metastasizing. Metastasis constitutes a major cause of cancer death. Cancer is the world's second-largest cause of death, with an estimated 9.6 million deaths in 2018.[1] The chalcones are α, β- unsaturated ketones with keto ethylene reactive group -COCH = CH-, the presence of α, β- unsaturated carbonyl frame in chalcone allows it biologically active. Some substituted chalcones and their derivatives have been reported to exhibit a wide range of biological properties such as anthelmintic[2], anti-microbial[3], antimycobacterial[4], antifungal[5], anti-cancer[6-9], antioxidant[10], and anti-inflammatory activity[11], etc. Cancer that induces inflammation, such as hepatitis and human papilloma infection (HPV), accounts for up to 25 percent of malignant development in low-paying nations and centers of pay.[12] The monetary effect of the disease is enormous and is gradually increasing. In 2010 the gross average monetary cost of malignant development was measured at almost US$ 1.16 trillion.[13] Prostate, lung, colorectal, skin cancer, breast, stomach, etc. are the most prevalent cancers. Lung, colorectal, prostate, liver, and breast cancer are among the most common cancer that causes death. Cancer is the type of disease that may affect every section of the body under which the cells expand unregulated and can or may not attack the surrounding cells by design. Because of the lack of early diagnosis and also the inefficiency of the target-oriented therapy, it is very difficult to cure cancer until now but in some cases, multiple therapies along with surgery are done. Cancer can be caused by several pathways and its medication is done by blocking its particular pathways.[14]

2. CHEMISTRY

2.1. Synthesis

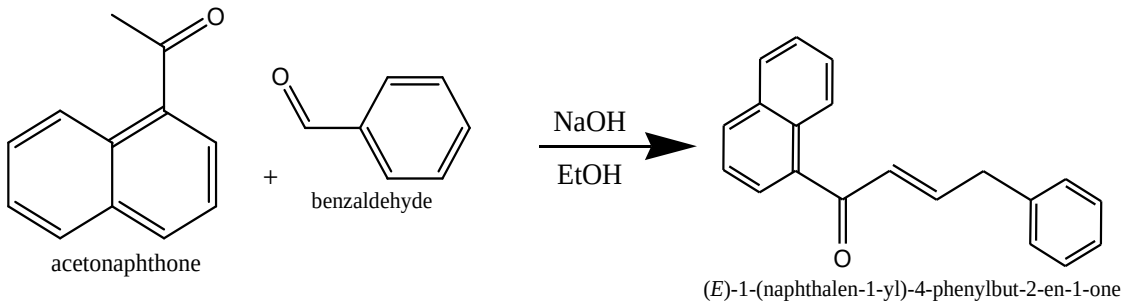


Fig: 1: Synthesis of Chalcone derivative.

Derivatives are ethoxy, methoxy and nitro compounds

2.2. Identification and characterization

The compound synthesized were identified and characterized by following methods such as:

- **Melting point determination**

An open capillary tube approach was used to determine the melting point of organic material. Melting point identification is the most important and simplest way to distinguish the physical constant from one compound from another.

- **Thin-layer chromatography**

The pre-coated TLC plate made of F254 silica gel was used for calculating the Rf size. TLC is an important tool used in synthetic chemistry to infer compound formation based on the Rf meaning. It also assists in verifying reaction development. Pet. ether: ethyl acetate (1:1) used as the solvent.[15]

- **Infra-red spectroscopy**

In tensor 27 spectrophotometer, Brucker optic (Germany) used the ATR technique, the infrared spectra were recorded using the FT-IR technique. IR is one of the most important instruments for assessing the specific functional groups and the future chemical structure. The significant benefit of IR over the other technique is that it gives simple fingerprints (1300-650) cm-1 providing details on the composition of molecules (functional group, bonding with each other). Any two substances have an area of the same fingerprint. This method is based on the compound's molecular vibration, such that every bond will vibrate at different frequencies and when this vibration frequency corresponds to the IR frequency, spectra will be formed for every bond.[16,17]

- **Nuclear magnetic resonance spectroscopy**

Proton NMR is one of the most effective methods for determining the various protons present in the system. The relationship between compound and electromagnetic force can be detected by subjecting a material to two magnetic forces simultaneously, each of which differs at some radiofrequency. Energy is absorbed by the sample at a specific combination of field, and absorption can be detected as a signal shift produced by the radio frequency detector and amplifier. This energy of absorption can be related to a magnetic dipolar nature of the spinning nuclei. This method is known as Magnetic Resonance to the Scientific. This method is useful in determining molecular structure.[16-18]

➢ **Mass spectroscopy**

Mass spectroscopy is a technique by which characterization of molecules is conducted according to the way they disperse when bombarded with high energy electrons. In addition to elucidating or understanding the composition of molecules, mass spectra are useful in determining molecular weight; the theory behind mass spectrometry is that a charged particle moves through the magnetic field in a distance that is proportional to the mass to charge ratio (m / e) is deflected in a circular trajectory. A high-energy electron beam is used in an electron impact mass spectrometer to displace an electron from the organic molecule, creating a radical cation known as the molecular ion. If the molecular ion becomes too weak then the presence of other small ions will fragment. The ion collection is then centered on a beam and guided into the magnetic field and deflected according to the masses of the ions into radial paths. By changing the magnetic field, the ion will be centered on the detector and recordable.[16-18]

3. MATERIALS AND METHOD

3.1. Materials

Sodium carbonate (Karnataka Fine Chem, India), Potassium sodium tartrate (Karnataka Fine Chem, India), Dipotassium hydrogen orthophosphate (Karnataka Fine Chem.), Potassium hydrogen phosphate (Karnataka Fine Chem, India.), Ferric chloride hydrous (Karnataka Fine Chem, India), Diphenylamine (MERCK.), Sodium nitroprusside dehydrate (MERCK), Phosphoric acid (ROLEX.), DMSO (Himedia), Triss-buffer (Karnataka Fine Chem, India.), Tetra-sodium pyrophosphate (Priya Research Labs.), Hydrogen peroxide (Karnataka Fine Chem, India), MTT (Thermo fisher scientific.), etc. and all other chemicals used in this project were of analytical grade.

3.2. Cell lines

The assays were performed using HL-60, Hela, MCF-7, HCT-116, A431, MDA MB (453) cell line (National Centre for Cell Research, Pune, India).

3.3. MTT assay

A cell culture: cancer cell line was preserved in the RPMI-1640 medium logarithmic growth process, augmented by heat-inactivated 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin incubated in a CO_2 incubator (5% CO2 / 95% humidified air).

Principle

MTT assay is a standard quantitative colorimetric assay designed to quantify mammalian cell growth, cell survival, and cell proliferation based on live-cell capacity. It can also be used to determine the cytotoxicity of possible therapeutic agents and other toxic materials. In this process, MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (pale yellow) enters the cell and passes into viable cell mitochondria where mitochondrial dehydrogenase enzymatically reduces MTT to formazan (dark blue) crystals by cleaving the tetrazolium chain. Under aqueous solutions, the formazan crystals thus formed are insoluble. The cells are then treated with an organic solvent, acid-isopropanol (0.04 N HCl in isopropanol) to remove in a colored solution the insoluble violet formazan. The absorbance of this colored solution can be quantified by using a single well scanning spectrophotometer (ELISA reader) to measure wavelengths from (490 and 600 nm). Because the reduction of MTT can occur only in metabolically active cells, the activity level is a measure of the cells' viability.[19-21]

Percentage cell viability was calculated using the equation

$$\% \text{ Cell viability} = \frac{\text{The absorbance of cells (treated)}}{\text{The absorbance of cells (untreated)}} \times 100$$

3.4. *IN VIVO* STUDIES: EAC model

Ehrlich Ascites Carcinoma (EAC) cancer cells were obtained from Amala Cancer Research Centre, Thrissur, Kerala 680 555.

- **Drug solution preparation & Dose selection**

The synthesized derivatives chalcone molecules were measured at mice body weight doses of (200 mg/kg). The molecules have been dissolved properly in water with a pinch of acacia, and the drug will be freshly formulated and each mouse has been given drug suspension for test animals according to their body weight.

- **Dose selection**

Acute toxicity analysis for synthesized pyrimidine derivative compounds was performed by modifying Committee up & down procedure for the purpose of animal laboratory monitoring and oversight (CPCSEA), OECD guidelines 425. The study was performed using AOT425Statpgm (Version: 1.0), and the research findings and guidelines were focused on the predictive system for acute oral toxicity (OECD Guideline 425). For the study were used the Swiss female albino mice weighing between 20-25 g. To order to monitor differences to

autonomic or behavioral activity, individuals were consistently monitored for 12 h and persisted for 24 h. Mortality was detected for 48 h. The research, as per the key check, was started at 175 mg/kg, followed by 550 mg/kg and completed at 2000 mg/kg p.o with a maximum dose of 2000 mg/kg. The subject was examined for 14 days for any evidence of toxicity.[22]

3.5. Methods

Healthy Swiss albino mice 25 ± 3 g of body weight was procured from Central Animal Facilities, Acharya & BM Reddy Pharmacy School, Bengaluru. Animals were kept in polypropylene containers and kept under normal conditions. They were supplemented with water and normal food. The animals were treated in compliance with the standards for the treatment and use of laboratory animals established by CPCSEA (Committee for the Purpose of Regulation and Supervision of Experimental Animals). Institutional Animal Ethics Committee (IAEC), Acharya & BM Reddy College of Pharmacy, Karnataka Bengaluru, has approved the research protocol number IAEC / ABMRCP/2018–2019/20.

- **CANCER CELL COUNT AND INDUCTION**

Intraperitoneally 1 ml of regular saline (0.9 percent) was injected into the donor mouse. When saline was applied, 1 ml of ascites fluid was immediately obtained from the peritoneal cavity and diluted up to 10 ml with normal saline. From this, 10 µl ascites were taken and inserted in the chamber of neubauer and the number of cells existed in the chamber was determined and the concentration of 1×10^6 cells was injected intraperitoneally into each mouse.

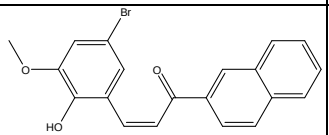
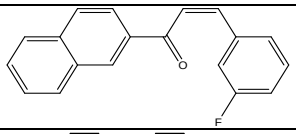
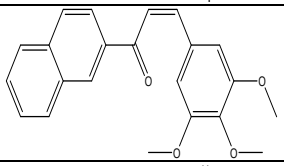
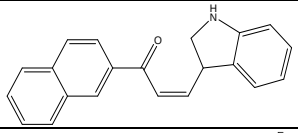
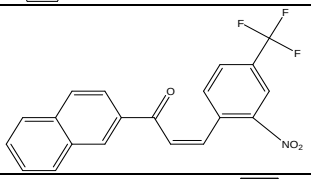
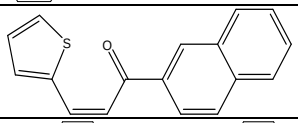
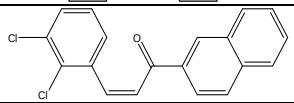
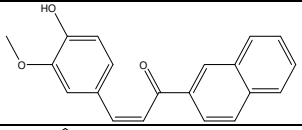
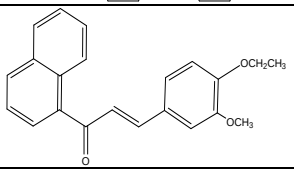
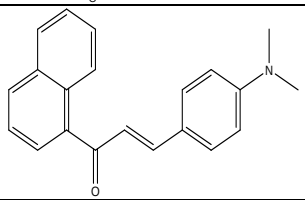
4. RESULT

4.1. Synthesis of Chalcone derivatives

The Claisen – Schmidt condensation methods have performed the synthesis of the chalcone derivatives. Here, acetonapthone was processed with various benzaldehyde derivatives to obtain the derivatives of the chalcones. The reaction takes place in an ice bath with constant stirring for 4 to 6 hours. The reaction medium is ethanol, which uses sodium hydroxide as a catalyst to improve the reaction.

4.2. Physical properties

Table 1: Physical properties of the derivatives.

S. No	Compound Code	IUPAC Name	Structure	Melting Point (°C)	Rf value
1	AR 1	(Z)-3-(5-bromo-2-hydroxy-3-methoxyphenyl)-1-(nahthalen-7-yl) prope-2-en-1-one		>300	0.55
2	AR 2	(Z)-3-(3-fluorophenyl)-1-(nahthalen-7-yl) prope-2-en-1-one		52-54	0.8
3	AR 3	(Z)-3-(3,4,5-trimethoxyphenyl)-1-(naphthalene-7-yl) prope-2-en-1-one		78-80	0.521
4	AR 4	(Z)-3-(indolin-3-yl)-1-(naphthalene-7-yl) prope-2-en-1-one		79-81	0.79
5	AR 5	(Z)-3-(4-(trifluoromethyl)-2-nitrophenyl))-1-(naphthalene-7-yl) prope-2-en-1-one		60-62	0.73
6	AR 6	(Z)-1-(naphthalene-7yl)-3-(thiophen-2-yl) prope-2-en-1-one		>300	0.890
7	AR 7	(Z)-3-(2,3-dichlorophenyl)-1-(naphthalene-7-yl) prope-2-en-1-one		58-60	0.76
8	AR 8	(Z)-3-(4-hydroxy-3-methoxyphenyl)-1-(naphthalene-7-yl) prope-2-en-1-one		50-52	0.875
9	AR 9	(E)-3-(4-ethoxy-3-methoxyphenyl)-1-(naphthalene-1-yl) prope-2-en-1-one		68-70	0.81
10	AR 10	(E)-3-(4-(dimethylamino)phenyl)-1-(naphthalene-1-yl) prope-2-en-1-one		72-74	0.88

4.3. Spectral Characterization

a. FT-IR Spectra

i. AR 1

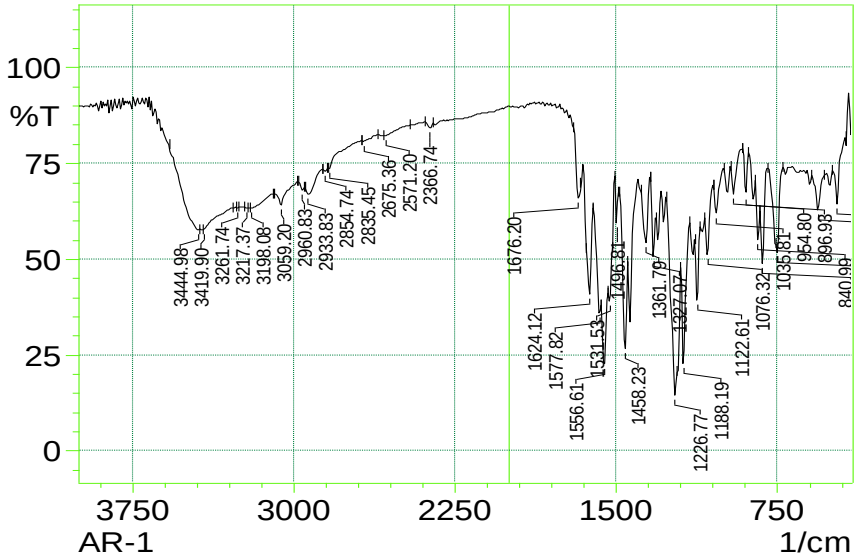


Fig: 2: FT-IR Spectra of AR1.

FT-IR (cm-1)

C-H Ar	3059.20 Streching
C-H Al	2933.83 Str
C=O	1676.20 Str
C=C	1361.79 str
C-Br	954.80 bending
O-H	3444.98 str

ii. AR 2

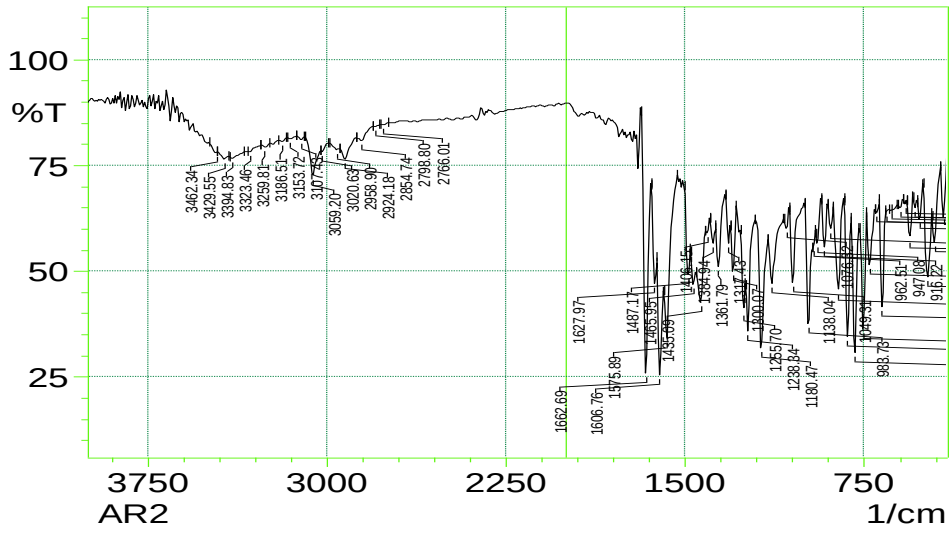


Fig: 3: FT-IR Spectra of AR2.

FT-IR (cm-1)

C-H Ar	3059.20 str
C-H Al	2924.18 str
C=O	1662.69 str
C=C	1361.79 str
C-F	983.73 bend

iii. AR 3

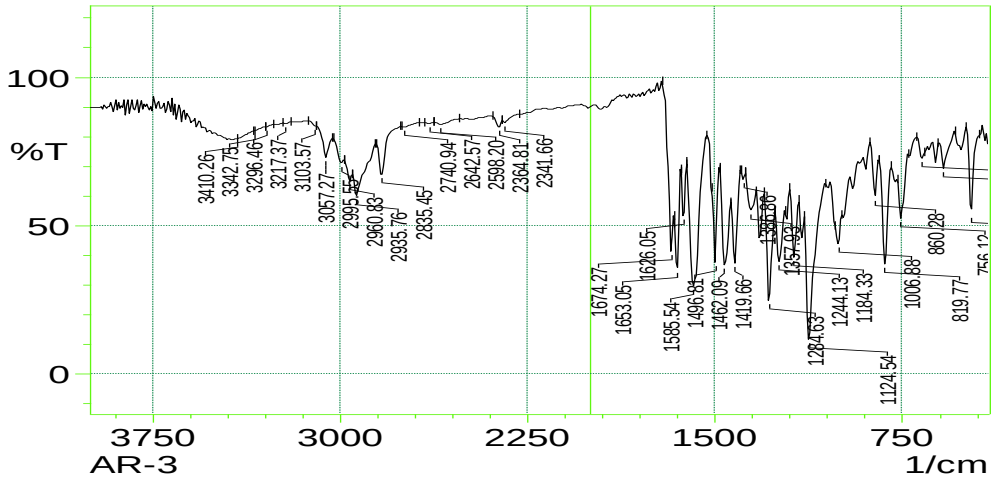


Fig: 4: FT-IR Spectra of AR3.

FT-IR (cm-1)

C-H Ar	3057.27 str
C-H Al	2935.76 str
C=O	1674.27 str
C=C	1357.93 str
C-O	1006.88 str

b. NMR Spectra

i. AR 1

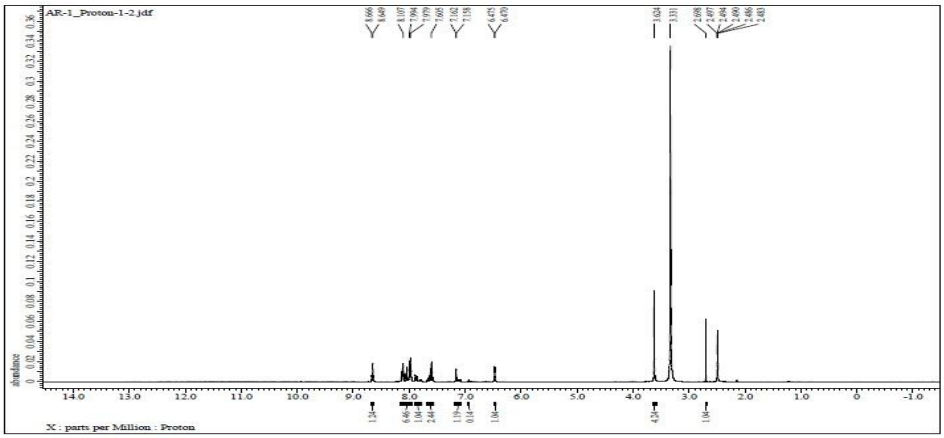


Fig: 5: NMR Spectra of AR1.

δ ppm

7.085-7.116	d, 1H, ethylene
7.782-7.814	d, 1H, ethylene
7.576-7.632	m, 2H, benzene
7.858-8.666	m, 7H, naphthalene
3.331	s, 3H, OCH_3
3.624	s, 1H, OH

ii. AR 2

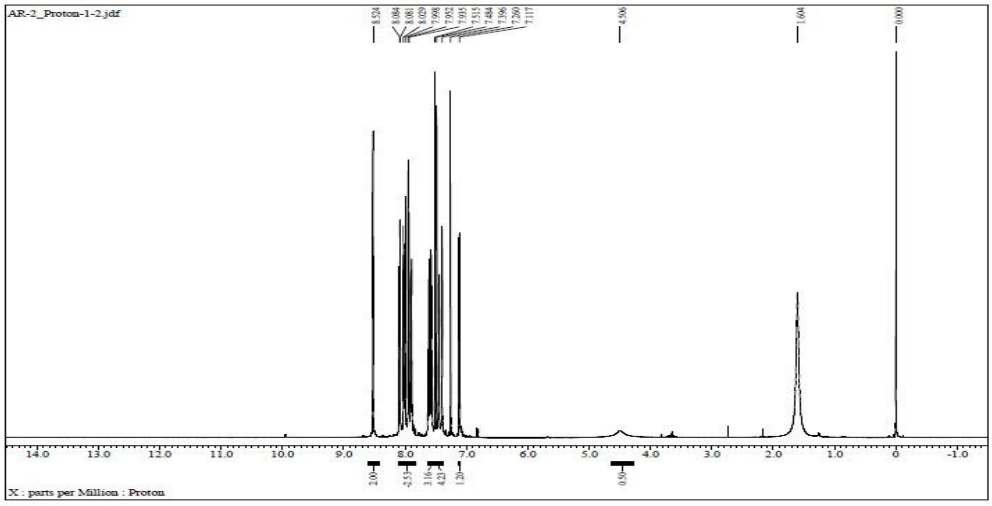


Fig: 6: NMR Spectra of AR2.

δ ppm

7.396-8.524	m, 7H, naphthalene
7.570-7.617	m, 4H, benzene
7.114-7.117	d, 1H, ethylene
8.098-8.101	d, 1H, ethylene

iii. AR 3

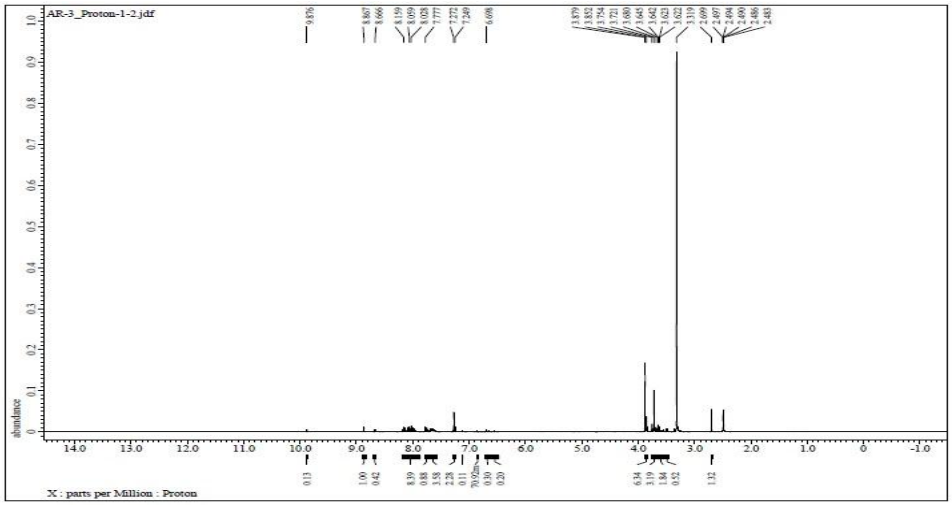


Fig: 7: NMR Spectra of AR3.

δ ppm

3.721	s, 3H, OCH_3
3.879	s, 3H, OCH_3
3.852	s, 3H, OCH_3
7.644-7.687	m, 2H, benzene
8.138-8.159	d, 1H, ethylene
7.746-7.777	d, 1H, ethylene
8.867-8.001	m, 7H, naphthalene

c. Mass Spectra

i. AR 1

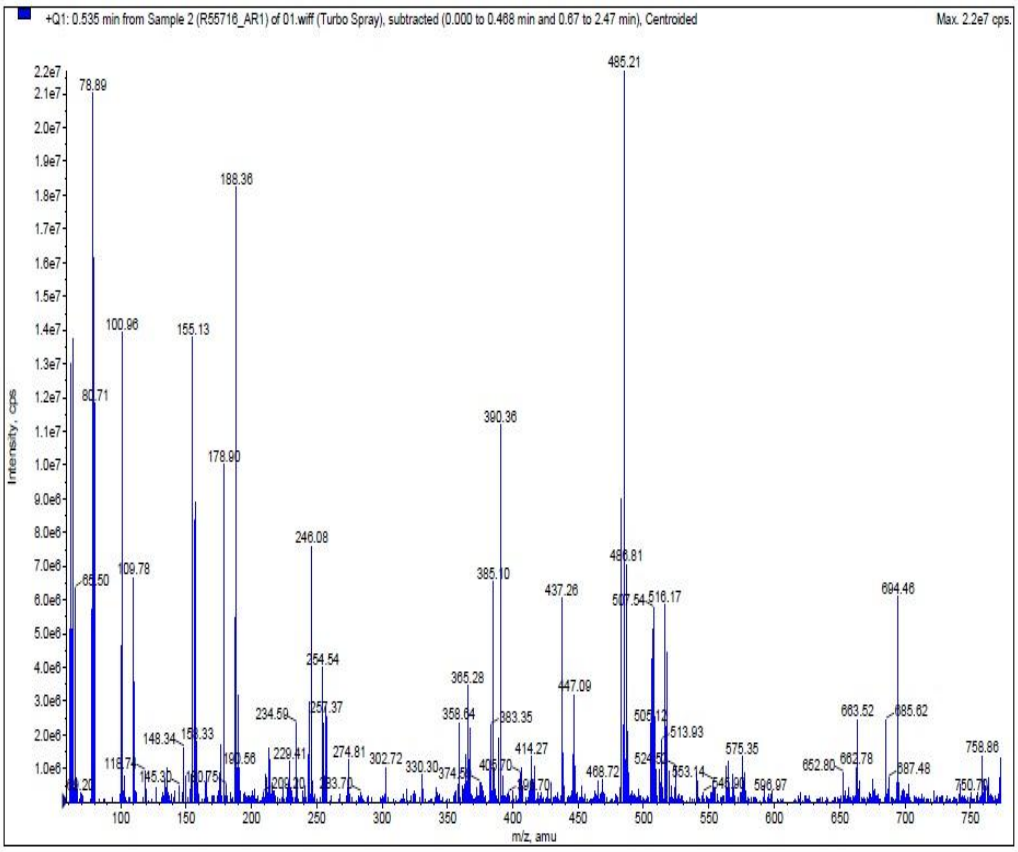


Fig: 8: Mass Spectra of AR1.

MS (m/z): 383.35 (C20H15 BrO3, M+)

ii. AR 2

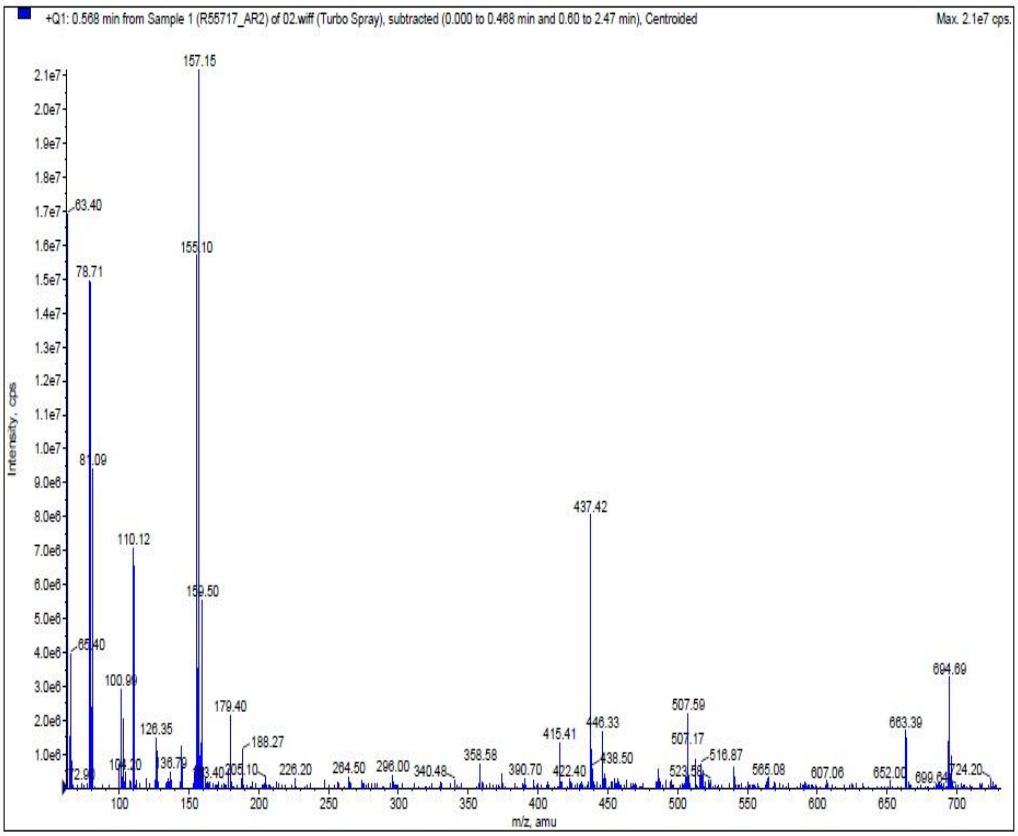


Fig: 9: Mass Spectra of AR2.

MS (m/z): 278.15 (C19H13 FO, M++2)

iii. AR 3

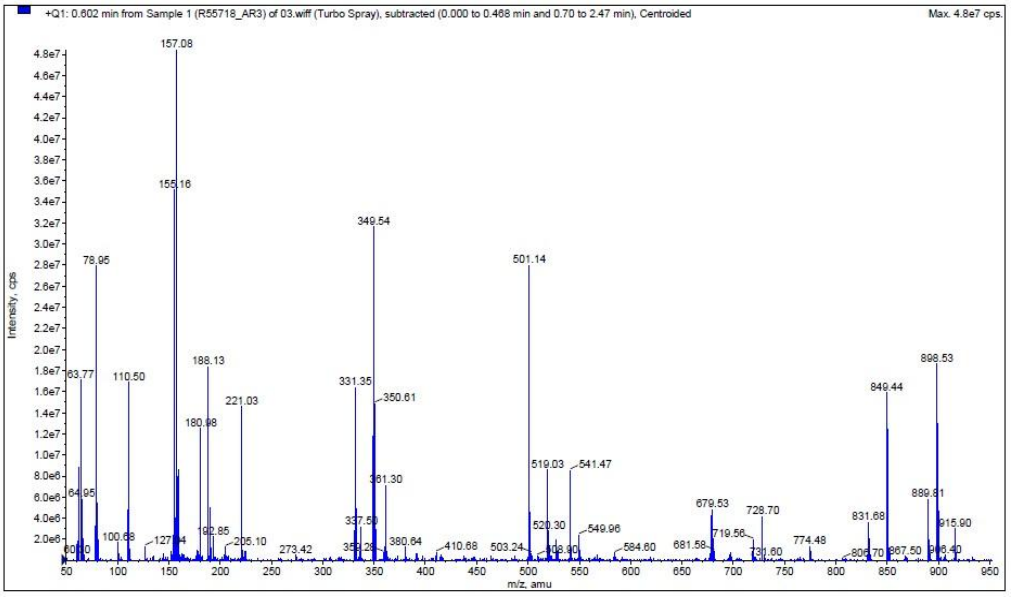


Fig: 10: Mass Spectra of AR3.

MS (m/z): 349.54 (C22H20 O4, M++1)

4.4. *In vitro* studies

4.4.1. Cytotoxicity activity

- The action of the drugs on the MCF-7 cell line

Table: 2: Cytotoxicity activity on MCF-7 cell line

Compounds	25 µg	50 µg	75 µg	100 µg
AR 1	49.8±0.622	62.2±0.176	72.3±0.471	81.1±0.937
AR 2	28.6±0.461	51.2±0.288	64±0.110	70±0.730
AR 3	35.1±0.536	53.5±0.512	69.4±0.401	83±0.712
AR 4	-46.9±0.875	5.46±0.450	17.3±0.440	29±0.585
AR 5	-23.4±0.713	18.9±0.146	32.5±0.316	53±0.423
AR 6	-3.33±0.278	29.1±0.356	48±0.630	60.7±0.196
AR 7	2.52±0.357	17.8±0.339	28.8±0.413	53.5±0.170
AR 8	23.4±0.656	41.8±0.212	57.4±0.344	68.1±0.186
AR 9	-56.2±0.435	9.31±0.419	32.5±0.381	48.7±0.456
AR 10	23.7±0.429	36.3±0.312	50.7±0.381	63.7±0.456
STD	31.1±0.403	-	-	-

Note: Cells are treated with drugs in triplicate.

Here, the different derivatives of chalcone have been used in the MCF-7 cell line and the following response is obtained. Among the 10 different derivatives AR 1, AR 2 and AR 3 show potent activity. While comparing them AR 1and AR 3 are showing great action in less concentration of the dose. If we compare the inhibition action in comparison to standard drug AR1 is showing the best action.

- The action of the drugs on the MDA MB (453) cell line.

Table: 3: Cytotoxicity activity on MDA MB (453) cell line.

Compounds	50 µg	75 µg	100 µg
AR 1	49.4±0.376	65.2±0.259	72.4±0.373
AR 2	50.3±0.177	55.4±0.409	76.2±0.459
AR 3	50.9±0.245	69.7±0.542	81.6±0.332
AR 4	22.2±0.0572	42.6±0.485	60.5±0.407
AR 5	35.5±0.427	47.8±0.995	68.2±0.194
AR 6	-56.6±0.715	3.43±0.284	20.6±0.463
AR 7	37.6±00408	49.4±0.505	69.3±0.503
AR 8	54.9±0.168	63.5±0.341	75.5±0.464
AR 9	41.6±0.435	55.4±0.595	70.5±0.104
AR 10	15±0.624	27.8±0.877	62.7±0.405
STD	47.7±0.181	-	-

Note: Cells are treated with drugs in triplicate.

Here, if we compare the activity of the 10 different chalcone derivatives we can see that AR 1, AR2 AR 3, and AR 8 are showing higher activity when we compare their minimum dose activity with standard drugs. While increasing the dose of the drugs we can see that the action is also increasing. But at 100 µg AR 3 is showing the best activity.

- The action of the drug in the A431 cell line

Table: 4: Cytotoxicity activity on A431 cell line.

Compounds	50 µg	75 µg	100 µg
AR 1	47.7±0.713	69.7±0.266	81.6±0.365
AR 2	44.5±0.275	58±0.663	69±0.363
AR 3	51.5±0.267	70.6±0.417	83.5±0.275
AR 4	17.8±0.196	27.5±0.407	49.5±0.253
AR 5	31.3±0.212	50.6±0.495	68.8±0.619
AR 6	26.4±0.325	46.6±0.287	61.5±0.260
AR 7	28.3±291	54.6±0.228	65.5±0.488
AR 8	40.6±0.319	56.3±0.459	66.5±0.374
AR 9	32.3±0.204	55.5±0.303	74.3±0.211
AR 10	37.7±0.207	46.5±0.339	58.8±0.274
STD	43.4±0.383	-	-

Note: Cells are treated with drugs in triplicate.

Here, in the case of lung cancer cell line which is A431 cells among the 10 different derivatives of chalcone AR1, AR 2 and AR 3 are showing the higher action. We can also clearly see that on increasing the dose of the derivatives the action also increases simultaneously. So we can say that they are dose-dependent. Also by comparing their minimum concentration activity with the standard, these are showing better results than standard drugs. Among the best 3 compounds, the AR 3 is showing the potent activity.

- The action of the drug in HCT-116 cell line.

Table 5: Cytotoxicity activity on HCT-116 cell line.

Compounds	50 µg	75 µg	100 µg
AR 1	1.68	21	53.5
AR 2	48.5	58.7	71.2
AR 3	47.7	58.2	74
AR 4	53.4	61.3	72.6
AR 5	50.9	61.3	72.6
AR 6	48.6	65.5	83.3
AR 7	52.2	61.5	77.9
AR 8	13.6	22.8	58.6
AR 9	42.6	55.3	70.4
AR 10	-258	32.4	7.11
STD	64.1	-	-

Note: Cells are treated with drugs in triplicate.

Here, the chalcone derivatives are not showing the noticeable type of action in the case of colon cancer cell lines in the minimum dose of 50 µg as compared to the standard drug. But among the derivatives, AR 2, AR 3 AR 4, AR 5, AR 6, and AR 7 are showing some action than other derivatives. Among the derivatives, AR 4 is showing some action that is near to the standard drug.

- The action of the drug in the HL-60 cell line.

Table: 6: Cytotoxicity activity on HL-60 cell line.

Compounds	25 µg	50 µg	75 µg
AR 1	6.41	59.1	75.5
AR 2	-13.9	20.6	60.6
AR 3	8.91	32.8	65.2
AR 4	-142	42.7	67.6
AR 5	-41.5	27.4	46.3
AR 6	10.7	45.6	69.2
AR 7	-18.3	16.5	28.7
AR 8	-214	41.3	66.6
AR 9	-6.03	26.5	44.7
AR 10	28.3	45.2	61.5
STD	52.5	-	-

Note: Cells are treated with drugs in triplicate.

Here, the action of the chalcone derivatives has been compared with the standard drug in HL 60 cell line which is a human leukaemia cell line. By seeing the results we can say that these derivatives are not working well as compared to the standard drug. The potent drug among the chalcone derivatives is AR 10 which is showing half of the action when compared to the standard in the concentration of 25 µg. But when the dose concentration is increased then the activity also got increased in a dose-dependent manner and shoving the action superior to standard of 25 µg in the concentration of 75 µg.

- The action of the drug on HELA cell line.

Table: 7: Cytotoxicity activity on HELA cell line.

Compounds	25 µg	50 µg	75 µg
AR 1	52.8	62.2	70.8
AR 2	36.9	47.6	68.6
AR 3	52.5	64.3	76.4
AR 4	7.81	27.3	42.5
AR 5	34.6	48.3	63.7
AR 6	10.9	27.5	42.5
AR 7	13.7	26.5	51.5
AR 8	29.8	41.6	64.7
AR 9	6.39	18.5	38.6
AR 10	0.572	10.5	18.7
STD	55.3	-	-

Note: Cells are treated with drugs in triplicate.

Here, the actions of the chalcone derivatives are studied on the human cervical cancer cell line which is HeLa cells. Among the other derivative AR 1, AR 2, and AR 3 are showing a response. Specifically, if we say that AR 1 and AR 3 are showing somewhat similar response to that of standard in the concentration of 25 µg. These are also showing the dose-dependent activity where on increasing the activity the response is also increased.

4.4.2. IC_{50} value

Table 8: IC50 value of Selected compounds.

S. No	Compound	MCF-7	MDA MB (453)	A431	HCT-116	HL-60	HeLa
1	AR 1	24 µg	50 µg	53 µg	98 µg	52 µg	18 µg
2	AR 2	59 µg	52 µg	60 µg	54 µg	65 µg	54 µg
3	AR 3	48 µg	49 µg	46 µg	58 µg	64 µg	17 µg

Note: Cells are treated with drugs in triplicate.

Comparing the activity of the chalcone derivatives on 6 different types of cancer cell lines AR 1, AR 2, and AR 3 are showing better results than others. Here the IC50 values of the selected compound are given. These derivatives are showing good action and their IC50 value has been calculated by taking the 50% inhibition in the particular dose of the compounds.

5.1. *In vivo* studies

- **Bodyweight**

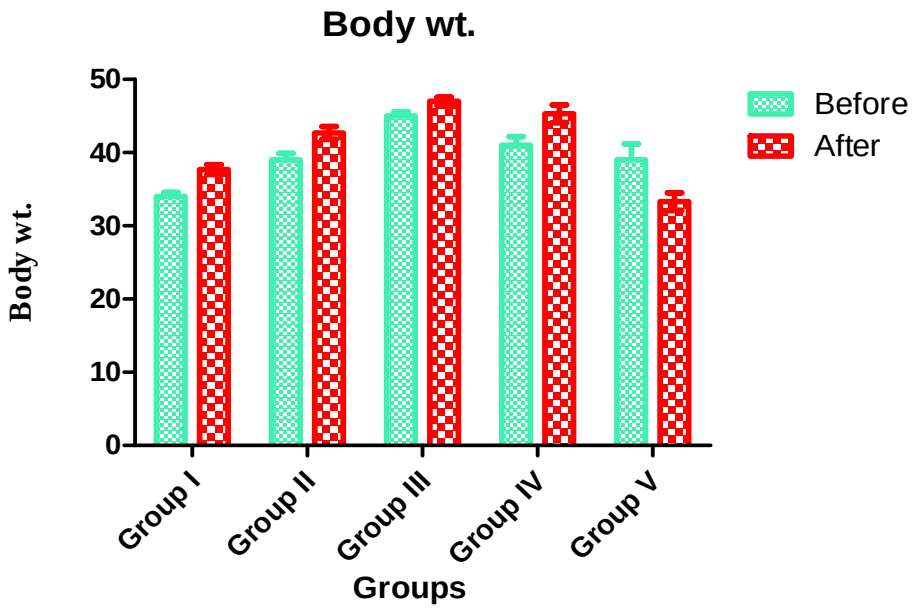


Fig 11: Change in body weight before and after the dosing.

Here, we can see that in the Group I or controlled group there is a drastic change in the body weight after the induction of cancer but in the standard group or Group II there is a slight gain in the bodyweight but in the treatment group like Group III, Group IV, and Group V there are no such changes in the body weight. Among the treatment groups, there is the least difference in body weight before and after the induction is Group V. Whereas in Group IV and Group V there is small change only.

Table 9: Change in the mean of body weight before and after the dosing.

S. No	Group	Before	After
1	Group I	34±0.58	37.7±0.667
2	Group II	39±0.88 *	42.7±0.882*
3	Group III	45±0.58 ***	47±0.577***
4	Group IV	41±1.20 **	45.3±1.20***
5	Group V	39±2.20 *	33.3±1.20*

The number of samples; N=3 and values were measured as Mean ± SEM. Results were tested by one-way ANOVA followed by Dunnett's multiple t-tests. Where $P < 0.05$ (*), $P<0.01$ (**), $P<0.001$ (***), have been taken as significant.

- **Haemoglobin**

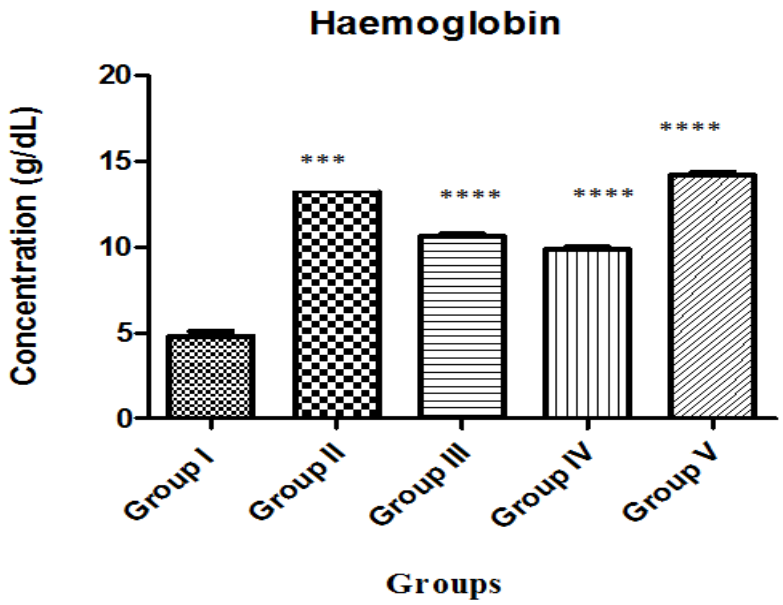


Fig: 12: Change in haemoglobin in different groups.

Here, we can see that there is a decrease in number of haemoglobin in Group I which is an untreated group but there is increasement in the treated group that may be standard treated or maybe test drug-treated. Group II (standard drug) is showing more number of haemoglobin then Group III and Group IV but less than Group V.

Table 10: Change in the mean of haemoglobin in different groups.

S. No	Group	Haemoglobin
1	Group I	4.80±0.289
2	Group II	13.3±0.0667***
3	Group III	10.6±0.145***
4	Group IV	9.87±0.145***
5	Group V	14.2±0.173***

The number of samples; N=3 and values were measured as Mean ± SEM. Results were tested by one-way ANOVA followed by Dunnett's multiple t-tests. Where P < 0.05 (*), P<0.01 (**), P<0.001 (***), have been taken as significant.

- **RBC**

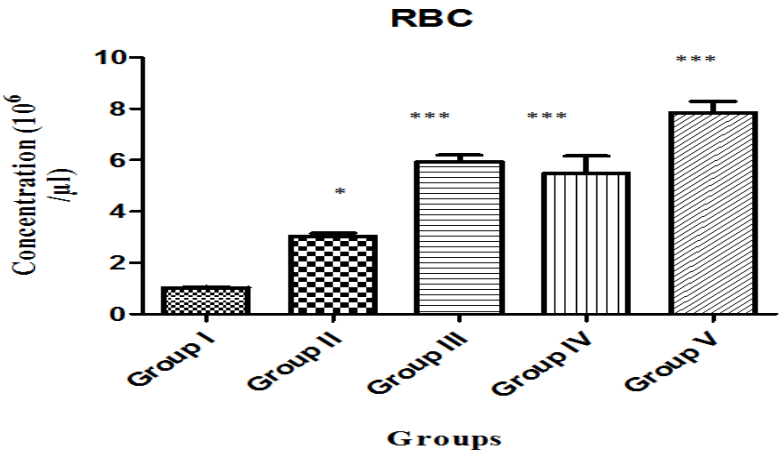


Fig: 13: Change in RBC in different groups.

Here**,** we can see that after the treatment the number of RBCs increases gradually. As per the result, the maximum number of increasement of RBC's is seen in Group V then Group II.

Table 11: Change in the mean of RBC in different groups.

S. No	Group	RBC
1	Group I	1.03±0.0306
2	Group II	3.03±0.122*
3	Group III	5.95±0.249***
4	Group IV	5.48±0.686***
5	Group V	7.84±0.452***

The number of samples; N=3 and values were measured as Mean ± SEM. Results were tested by one-way ANOVA followed by Dunnett's multiple t-tests. Where P < 0.05 (*), P<0.01 (**), P<0.001 (***), have been taken as significant.

- **WBC**

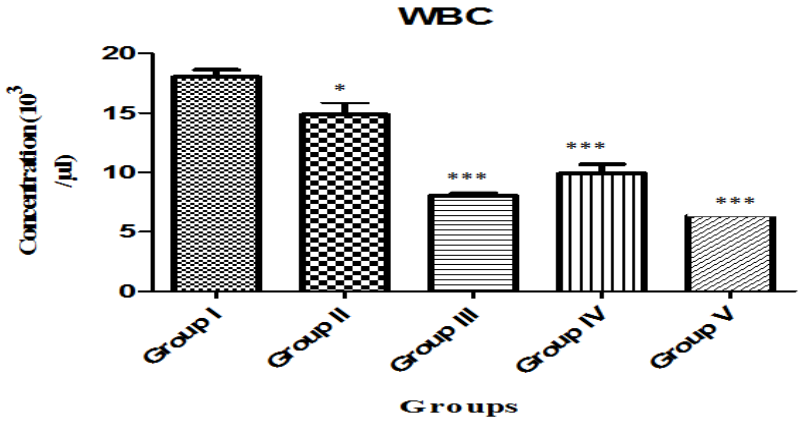


Fig: 14: Change in WBC in different groups.

Here, we can see that there is an increased level of WBC in cancer-induced mice in case of the absence of treatment. But when they are treated the numbers of WBC get reduced in the number. According to the result, it is clear that the Group V is showing good activity than Group II.

Table: 12: Change in a mean of WBC in different groups.

S. No	Group	WBC
1	Group I	18.0±0.590
2	Group II	14.9±1.03*
3	Group III	8.07±0.219***
4	Group IV	9.91±0.787***
5	Group V	6.37±0.353***

The number of samples; N=3 and values were measured as Mean ± SEM. Results were tested by one-way ANOVA followed by Dunnett's multiple t-tests. Where P < 0.05 (*), P<0.01 (**), P<0.001 (***), have been taken as significant.

- **Mean Survival time**

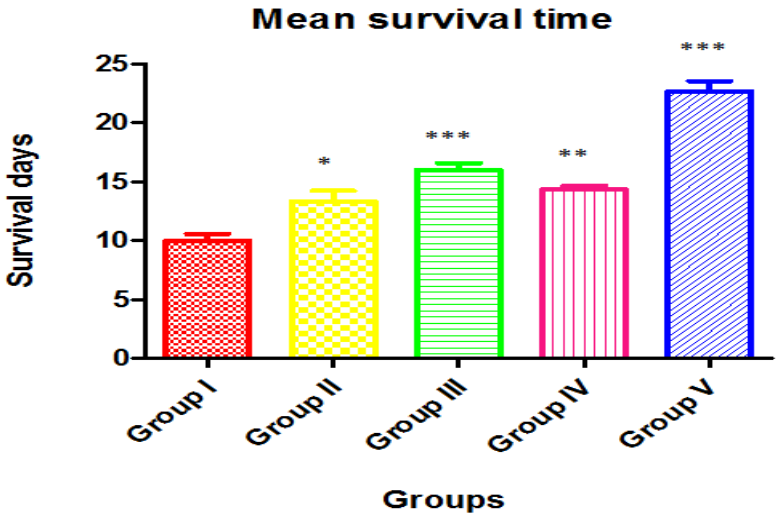


Fig 15: Change in mean survival time in different groups.

From the given graph we can see that the mean survival time of the mice got increased after the treatment than without treatment. Group I is the control group whereas Group II is the standard drug-treated group and the remaining groups are treated with test drugs. Here, Group V is showing the best activity.

Table 13: Change in the mean of Mean survival time in different groups.

S. No	Group	Mean survival time
1	Group I	10.0±0.577
2	Group II	13.3±0.882*
3	Group III	16.0±0.577***
4	Group IV	14.3±0.333**
5	Group V	22.7±0.882***

The number of samples; N=3 and values were measured as Mean ± SEM. Results were tested by one-way ANOVA followed by Dunnett's multiple t-tests. Where P < 0.05 (*), P<0.01 (**), P<0.001 (***), have been taken as significant.

- **SOD**

✓ **Liver**

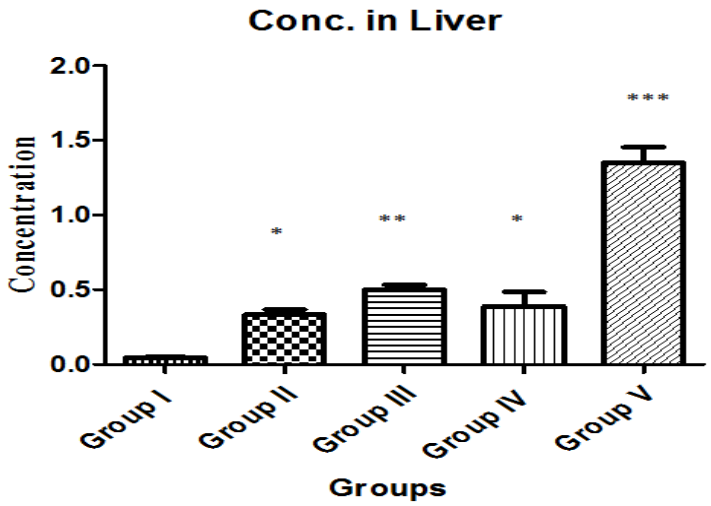


Fig 16: Change in SOD in the liver in different groups.

From the graph, we can see that there is a decrease in the concentration of anti-oxidant in the liver of mice after the induction of cancer. But when they are treated the concentration of the anti-oxidant SOD gets increased. Here Group II is the standard drug-treated group and Group III to Group V are test drug-treated groups. Among the Group V shows the best action.

Table 14: Change in mean SOD in the liver in different groups.

S. No	Group	SOD conc.
1	Group I	0.0447±0.0044
2	Group II	0.334±0.0333*
3	Group III	0.501±0.0335**
4	Group IV	0.389±0.0975*
5	Group V	1.35±0.106***

The number of samples; N=3 and values were measured as Mean ± SEM. Results were tested by one-way ANOVA followed by Dunnett's multiple t-tests. Where P < 0.05 (*), P<0.01 (**), P<0.001 (***), have been taken as significant.

✓ **Kidney**

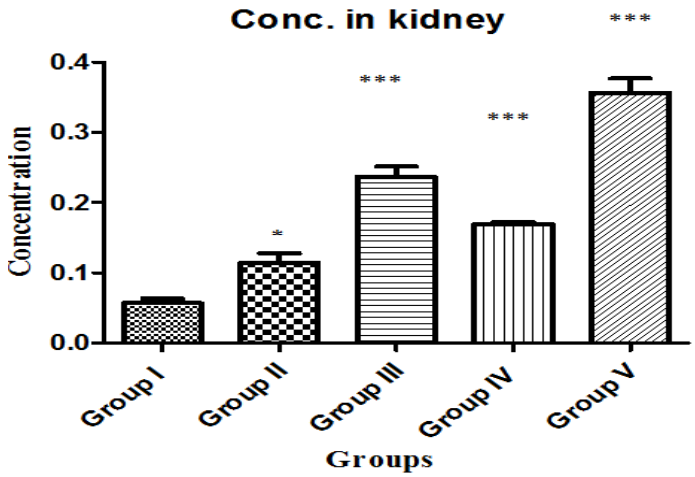


Fig: 17: Change in SOD in the kidney in different groups.

Here, we can see that in the kidney of the mice also there is decreased in the number of anti-oxidant in mice. Group I is the controlled group after the induction of cancer where the concentration of SOD is less but when they have treated the concentration of the SOD gets increased.

Table: 15: Change in mean SOD in the kidney in different groups.

S. No	Group	SOD conc.
1	Group I	0.0568±0.00672
2	Group II	0.114±0.0132*
3	Group III	0.236±0.0150***
4	Group IV	0.169±0.00348***
5	Group V	0.356±0.0203***

The number of samples; N=3 and values were measured as Mean ± SEM. Results were tested by one-way ANOVA followed by Dunnett's multiple t-tests. Where P < 0.05 (*), P<0.01 (**), P<0.001 (***), have been taken as significant.

- **GSH**
- ✓ **Liver**

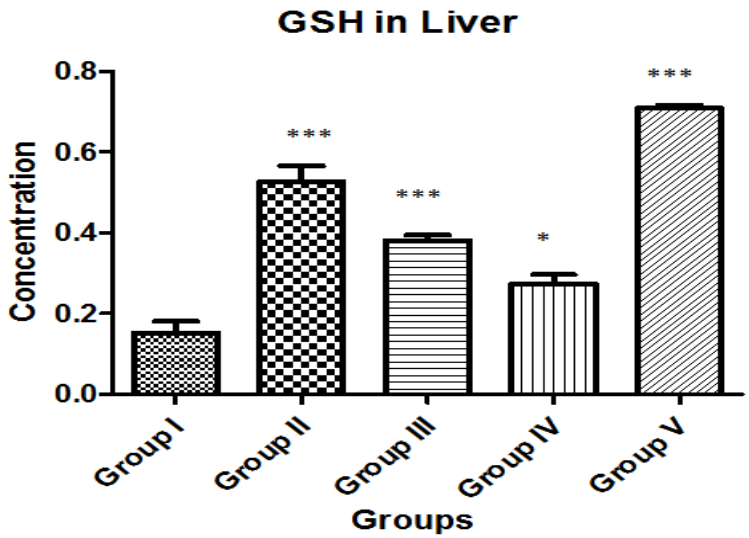


Fig 18: Change in GSH in the liver in different groups.

Here, we can see that there is a decrease in the GSH anti-oxidant after the induction of cancer. But when they are treated the concentration gets increased. Group II is the standard treated group that is showing in the increase in the GSH concentration then Group III and Group IV but less in concentration then Group V. And also Group V is showing the best action among others.

Table: 16: Change in mean GSH liver in different groups.

S. No	Group	GSH
1	Group I	0.15±0.028
2	Group II	0.53±0.040***
3	Group III	0.38±0.014***
4	Group IV	0.27±0.022*
5	Group V	0.71±0.0085***

The number of samples; N=3 and values were measured as Mean ± SEM. Results were tested by one-way ANOVA followed by Dunnett's multiple t-tests. Where P < 0.05 (*), P<0.01 (**), P<0.001 (***), have been taken as significant.

✓ **Kidney**

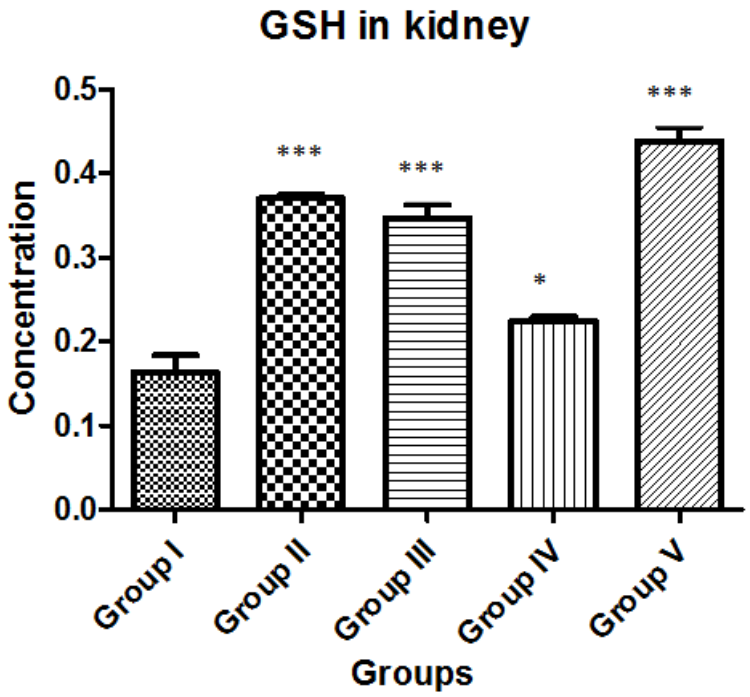


Fig 19: Change in GSH in the kidney in different groups.

Similarly, as stated in the liver the concentration of GSH will decrease in the kidney after the induction of cancer, and when they have treated the concentration of GSH get increased. Here Group II is showing more activity then Group III and Group IV but less than Group V.

Table: 17: Change in mean GSH in kidney different groups.

S. No	Group	GSH
1	Group I	0.163±0.0202
2	Group II	0.370±0.00414***
3	Group III	0.346±0.0163***
4	Group IV	0.224±0.00480*
5	Group V	0.438±0.0170***

The number of samples; N=3 and values were measured as Mean ± SEM. Results were tested by one-way ANOVA followed by Dunnett's multiple t-tests. Where P < 0.05 (*), P<0.01 (**), P<0.001 (***), have been taken as significant.

- **Catalase**

✓ **Liver**

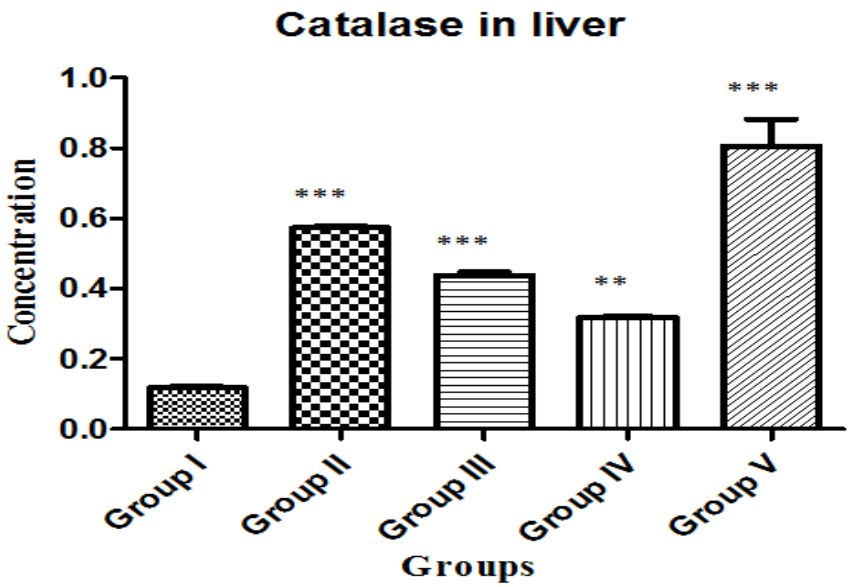


Fig 20: Change in Catalase in the liver in different groups.

After the induction of cancer the concentration of the catalase gets reduced in the liver but when they are treated the level of catalase anti-oxidant gets increases. Here also in Group II, the concentration of catalase is higher than Group III and Group IV but lesser than Group V.

Table 18: Change in mean catalase in liver different groups.

S. No	Group	Catalase
1	Group I	0.118±0.00455
2	Group II	0.573±0.00679***
3	Group III	0.439±0.00857***
4	Group IV	0.320±0.000127**
5	Group V	0.806±0.0760***

The number of samples; N=3 and values were measured as Mean ± SEM. Results were tested by one-way ANOVA followed by Dunnett's multiple t-tests. Where P < 0.05 (*), P<0.01 (**), P<0.001 (***), have been taken as significant.

✓ **Kidney**

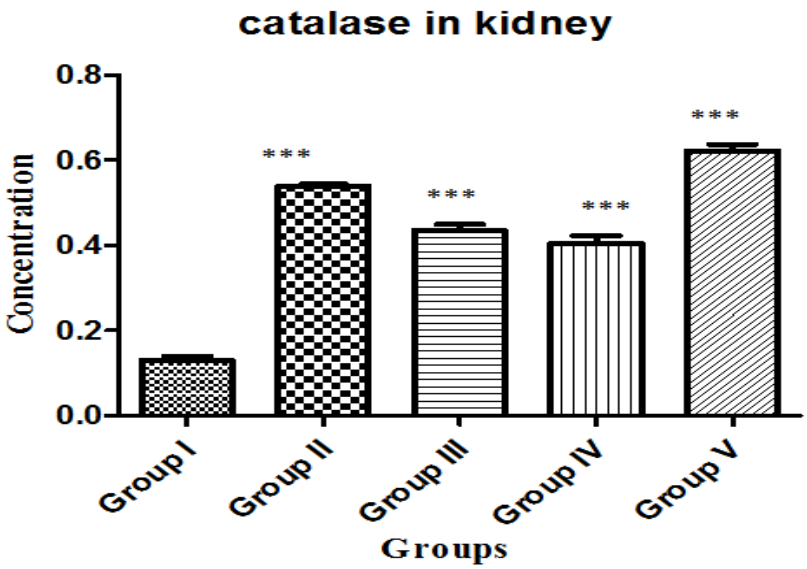


Fig: 21: Change in Catalase in the kidney in different groups.

Likewise in the liver, the concentration of catalase also decreases in the kidney of the mice after the induction of cancer. But increases when they are treated either with Group III to Group IV. Here Group V shows the best response than others.

Table 19: Change in mean catalase in kidney different groups.

S. No	Group	Catalase
1	Group I	0.130±0.00957
2	Group II	0.540±0.00373
3	Group III	0.435±0.0143
4	Group IV	0.404±0.0187
5	Group V	0.623±0.0147

The number of samples; N=3 and values were measured as Mean ± SEM. Results were tested by one-way ANOVA followed by Dunnett's multiple t-tests. Where P < 0.05 (*), P<0.01 (**), P<0.001 (***), have been taken as significant.

- **Nitric Oxide**
- ✓ **Liver**

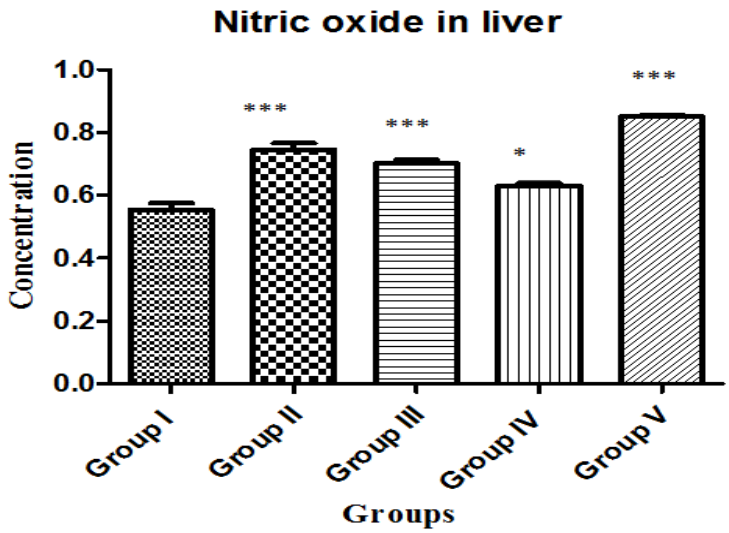


Fig: 22: Change in NO in the liver in different groups.

Here, we can see that after the induction of cancer there is a reduction in the concentration of nitric oxide (NO). When they have treated the concentration of NO in the liver gets increased. Group II is showing slightly greater concentration then Group III and Group IV but slightly lesser than Group V.

Table: 20: Change in mean NO in liver different groups.

S. No	Group	Nitric oxide
1	Group I	0.553±0.0216
2	Group II	0.744±0.0212***
3	Group III	0.701±0.0107***
4	Group IV	0.630±0.0101*
5	Group V	0.851±0.00456***

The number of samples; N=3 and values were measured as Mean ± SEM. Results were tested by one-way ANOVA followed by Dunnett's multiple t-tests. Where P < 0.05 (*), P<0.01 (**), P<0.001 (***), have been taken as significant.

✓ **Kidney**

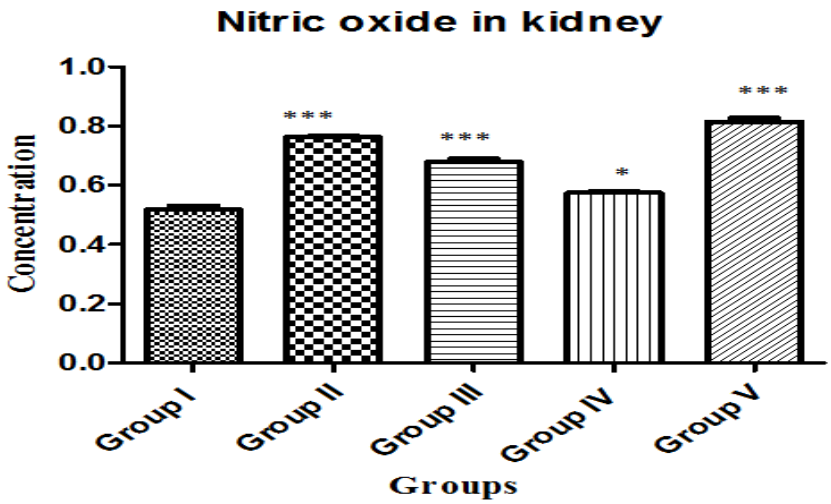


Fig: 23: Change in NO in the kidney in different groups.

Similarly, to the liver, the concentration of NO also decrease in the kidney after the induction of cancer. Group II is showing the significant concentration of NO which is greater than Group III and Group IV but lesser than Group V.

Table: 21: Change in mean NO in the kidney of different groups.

S. No	Group	Nitric oxide
1	Group I	0.518±0.0127
2	Group II	0.762±0.00491***
3	Group III	0.678±0.0121***
4	Group IV	0.574±0.00674*
5	Group V	0.814±0.0142***

The number of samples; N=3 and values were measured as Mean ± SEM. Results were tested by one-way ANOVA followed by Dunnett's multiple t-tests. Where P < 0.05 (*), P<0.01 (**), P<0.001 (***), have been taken as significant.

➢ **Total Protein**

✓ **Liver**

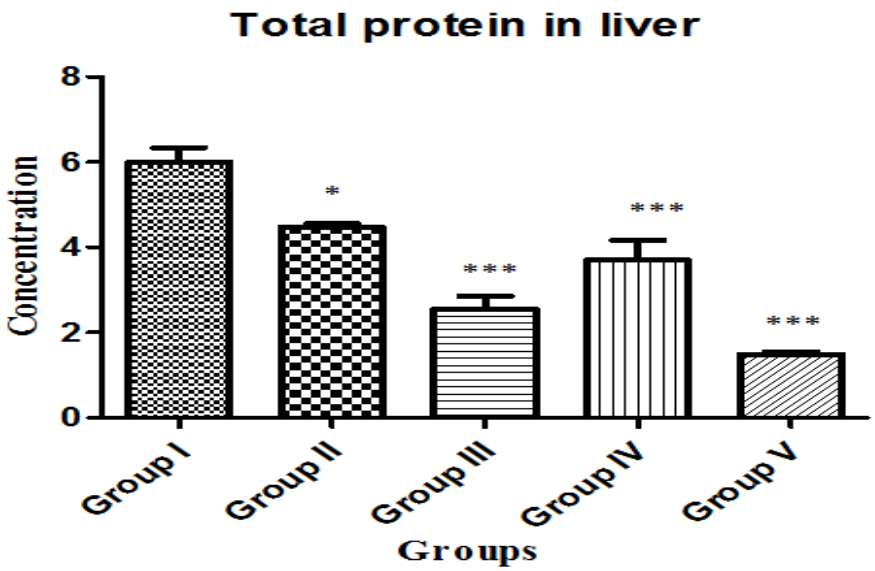


Fig: 24: Change in total protein concentration in the liver in different groups.

Here, we can see the excess amount of total protein in the liver in cancer-induced mice because of the denaturation of the cells. When they are treated the concentration of the total protein gets reduced. An increase in the concentration of total protein indicates the increase in cell damage. Group III is showing good activity then group II and Group IV but lesser inactivity then Group V.

Table: 22: Change in the mean concentration of total protein in liver different groups.

S. No	Group	Total Protein
1	Group I	6.0±0.346
2	Group II	4.47±0.0882*
3	Group III	2.57±0.285***
4	Group IV	3.70±0.473***
5	Group V	1.50±0.0577***

The number of samples; N=3 and values were measured as Mean ± SEM. Results were tested by one-way ANOVA followed by Dunnett's multiple t-tests. Where P < 0.05 (*), P<0.01 (**), P<0.001 (***), have been taken as significant.

- **Kidney**

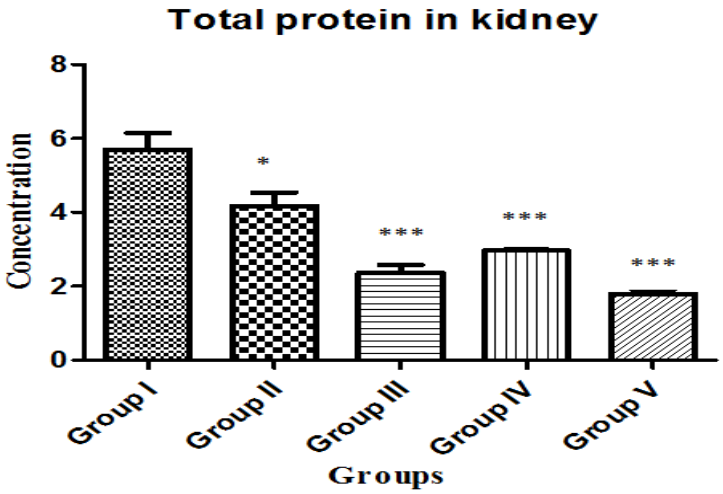


Fig: 25: Change in total protein concentration in the kidney in different groups.

Same as in the liver concentration of the total protein in more in the kidney in mice which indicates the induction of cancer. After the treatment, the concentration of total protein decreases. Lesser the number of total protein more the activity in mice. Group III is greater than Group II and Group IV but less than Group V.

Table 23: Change in the mean concentration of total protein in kidney different groups.

S. No	Group	Total Protein
1	Group I	5.70±0.451
2	Group II	4.17±0.371
3	Group III	2.37±0.219
4	Group IV	2.97±0.0333
5	Group V	1.80±0.115

The number of samples; N=3 and values were measured as Mean ± SEM. Results were tested by one-way ANOVA followed by Dunnett's multiple t-tests. Where $P < 0.05$ (*), $P<0.01$ (**), $P<0.001$ (***), have been taken as significant.

5. DISCUSSION

Molecular docking is a bioinformatics technique by which the receptor protein interaction of two or more molecules is analyzed. Via this strategy, we will classify the orientation of drug molecules with its receptor. We prepared 10 novel derivatives of the chalcone, i.e. AR1 to AR 10 is analyzed with the help of the chem-draw program and its association with the receptor tyrosine kinase epidermal growth factor which is 1M17. The stability of the molecules is dependent on the C-docker interaction and C-docker energy in which c-docker

interaction in the molecule's binding energy in comparison to its receptor protein and c-docker energy is. C-docker energy exchange and c-docker stability of the strongest 3 compounds i.e. AR1, AR2, and AR3 are found to be 29.445, 36.3456, 37.9818, and 12.738, 11.5037, 12.97173.

The study of cytotoxicity is the study of the compound or drug toxic effects in cells. It is one of the methods of biological appraisal and screening. This helps one to track the activation and viability of cells after the compounds/drugs have touched. The presence of our compounds on various cell lines such as breast cancer cell lines (MCF-7, MDA MB (453)), lung cancer (A431), colon cancer (HCT-116), cervical cancer (HeLa) and leukemia cells (HL-60) was verified here. The inhibitor concentration resulting in 50 percent inhibition (IC50) was calculated by plotting the inhibitor percentage and concentration. There the drugs demonstrate greater activity on the cell lines of breast cancer rather than on other types of cancers and in other types of cell line drugs they display equal or less activity relative to normal medications. Their operation was reported on the table described above.

Study *in vivo* is the study of drug/compound activity in the human body. Here the results and outcomes of both the procedure and the regular drug are analyzed and measured. Through this, the animals are infected with diseases and are then treated with normal medicines and examination. Here we measured all parameters of the body such as body weight, haemoglobin, RBC, WBC, mean survival time, etc., as well as other measures of enzymes such as SOD, GSH, catalase, nitric oxide, and total protein. During the tests review, we find that the concentration of the enzymes increases in the body following treatment with medications as well as test compounds but the concentration of total protein decreases. Among the 10 novel derivatives AR1, AR2 and AR3 show positive results in the living organism. When comparing drug activity AR3 shows stronger action than AR1 and AR2.

6. CONCLUSION

Here, with the aid of TLC, Melting point, FT-IR, NMR, and mass spectroscopy, we synthesized 10 novel chalcone derivatives that characterized them. The synthesis is carried out with the aid of a Claisen Schmidt reaction involving the acetonapthone and different forms of benzaldehyde, which is a single-step solution. After reviewing all of the findings including docking, experiments in vitro as well as in vivo indicated that various compounds exhibit specific activity on different cell lines among the 10 novel chalcone derivatives. For this, we have used the EAC model that is used for this AR1, AR2, and AR3 in the study of

breast cancer because of all other compounds they display excellent inhibition efficacy. Yet in the case of other cell lines, they do not display as much of an active activity relative to other compounds. Even, AR1, AR2, and AR3 demonstrate strong activity on cell line A431.

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8. CONFLICT OF INTEREST

There aren't any conflicts of interest related to this publication.

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