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Insilico, Phytochemical and Evaluation of Anti-Inflammatory Activity from the Seed Extracts of *Erythrina Variegata* by Using Zebrafish Model

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ABSTRACT

The present study aimed to investigate the Phytochemical invstigation, Docking, Anti-inflammatory activity from the seed extracts of Erythrina variegata by using Zebra fish model and Insilico method. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, triterpenoids, saponins, and glycosides. GC–MS revealed stigmasterol, β-sitosterol, and prenylated flavonoids as major constituents. Acute toxicity studies established safety up to 100 mg/L (LD50 > 100 mg/L). Ethanolic extract (100 mg/L) inhibited carrageenan - induced edema by 48.6%, while chloroform extract reduced edema by 42.3%, compared with Indomethacin (10mg/kg). Histopathology showed only mild to moderate changes in extract-treated groups versus severe alterations in carrageenan controls. Docking studies revealed strong binding of stigmasterol and β-sitosterol with COX1 and COX-2. ADME analysis indicated high GI absorption, favourable log P values (3.5–4.2), TPSA < 100, and compliance with Lipinski's Rule. Protox3.0 categorized the major phytoconstituents under Toxicity Class 5 (LD50 ≈ 2000 mg/kg) with no hepatotoxic, mutagenic, or carcinogenic alerts. Erythrina variegata seed extracts demonstrated significant anti-inflammatory activity in zebra fish and exhibited favourable pharmacokinetic and safety profiles in Insilico method. These findings provide scientific validation for its traditional use and highlight its potential as a natural source of safe and effective Anti-inflammatory agents.

Keywords: Docking, Phytochemical investigation, GC-MS, Erythrina variegata seeds, Anti-inflammatory, zebra fish, ADME, Protox

INTRODUCTION

Pain and inflammation is a common complaint in most patients suffering from disease conditions. Inflammation is a host defense mechanism to combat or overcome the invading pathogen or the foreign particles. Non-steroidal anti-inflammatory drugs (NSAIDs) make up one of the largest groups of drugs used for pain and inflammation. Currently available anti-inflammatory agents are associated with unwanted side effects and have their own limitations[16]. Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, and extravasations of fluid, cell migration, tissue breakdown and repair. Inflammation is one among them, conventional drugs used to ameliorate this phenomenon are either too expensive or toxic and not commonly available to rural folks that constitute the major populace of the world[14].

Fish have long been considered useful indicators of environmental and ecological integrity. As a result, they are increasingly being employed as physiological and biomedical models in life science research. Amongst the most aquatic species, zebrafish are routinely being used as experimental models as environmental indicators and as substitutes for higher sentient laboratory animals for use in pharmacological, taxological, genetic and biomedical research and testing[18].

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The Indian coral tree belonging to the family Fabaceae, and the major role in Ayurveda, Unani, Siddha and Homeopathy[13].

The plant is safer and more dependable than costly synthetic drugs due to their adverse side effect. It is valuable as shade tree. In India it is found throughout Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs[12].

PLANT PROFILE [4,5]:

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Family : Fabaceae

Subfamily : Papilionoideae

Genus : *Erythrina* L – Coral tree

Species : *Erythrina variegata* L.

Scientific name : *Erythrina variegata var. orientalis*

Tamil name : Kalyana murungai

Origin : Oriental regions

Macroscopical characters of seed:

Shape - Seeds are kidney shaped or oblong oval.

Size - The seeds are approximately 1 to 2 cm in length and 0.5 to 1 cm in width.

Color - The outer surface is bright red or dark purple to red or orange red or sometimes scarlet. Some seeds may have a black spot.

Surface - The seed coat is smooth, glossy and hard.

Texture - The seeds are hard and firm to touch due the tough seed coat.

Taste - Bitter

Odour - odourless.

The seeds are known to be toxic if consumed raw due to the presence of alkaloids.

Seeds:

Seeds are kidney- shaped, dark purple to red and 1-1.5 cm in length. The aqueous extract of the seed of *Erythrina variegata* has an antineoplastic activity when tested against L- 1210 lymphoid leukemia in mouse and also it produced a sharp fall in b.p. but higher dose of the extract completely stopped the heart[3]. It also produced a contraction of isolated guinea pig ileum. In siddha system, the seeds are used for the treatment of stomatitis, dysentery, sterility, diabetes and eye disorders. The entire plant and seeds are reported to possess antihypertensive, antimicrobial, sedative, immunosuppressive and anti-inflammatory properties[4].

MATERIALS AND METHOD:

Collection of seeds:

The seeds were collected from month of December 2024. The collected seeds were cleaned to dust the adhering particle and dried under the shade. The dried seeds was coarsely powdered by means of mechanical grinder and the powder material was used for the extraction process.

PHYTOCHEMICAL SCREENING:

Organoleptic characters

Powder microscopy

Chemical test

POWDER MICROSCOPY:

Fine dried powdered seed sample was separately treated with different solutions i.e. aqueous saturated chloral hydrate, phloroglucinol in conc. HCl, 0.02N iodine reagent and picric acid mounted on slides with 50% glycerin following a standard protocol and observed under the binocular compound microscope at 10x and 40x magnifications.

PREPARATION OF EXTRACT:

Ethanol extract:

25g of *Erythrina variegata* seeds were soaked in 250ml of ethanol for 7 days. After they were filtered by using whatmann filter paper. The residue was evaporated under electrical waterbath. The obtained extract was stored in refrigerator[2].

Chloroform extract:

For chloroform extraction the same ethanol extraction procedure was followed.

PHYTOCHEMICAL SCREENING:

Phytochemical screening was carried out on seed extracts using different solventsto identify the major natural chemical groups such as tannins, saponins, flavonoids, alkaloids, glycosides, terpenoids.

GC-MS study of the plant extracts:

The mass spectrum of the GC-MS instrument was decoded using a library of more than 62000 designs from the NIST. The spectrum of the identified component kept in the NIST collection was contrasted with the spectrum of the unidentified molecule. To identify the components, the spectrum of the extracts was compared to the spectrum of the known compound from the NIST library.

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30m×0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1ml/min. The injector temperature was set at 260°C during the chromatographic run. The µL of extract sample injected into the instrument the oven temperature was 60°C (2 min); followed by 300°C at the rate 10°C min-1; and 300°C, where it was held for 6min. The mass detector conditions were: transfer line temperature 240°C; ion source temperature 240°C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

DOCKING:

Chemsketch is the drawing tool of choice to create 3D structures of proposed compounds in several formats such as pdb, mol, etc. In this study, anti-inflammatory protein is taken as a drug target. The 3D structure of this enzyme was retrieved from Protein Data Bank (PDB) (http://www.rcsb.org./pdb/). The PDB ID of this enzyme is trained by residues of amino-acids.

ACUTE ORAL TOXICITY OF ADULT ZEBRAFISH:

As per OECD test guidelines 203, the acute toxicity test were followed to obtain the maximum safety dose of the Ethanolic and Chloroform extracts of *Erythrina variegata* seeds. The fish will be exposed to the test substance preferably for a period of 96 hours. Mortalities were recorded at 24, 48, 72 and 96 hours, and the concentrations which kill 50 percent of the fish was recorded. The fish were inspected after 24, 48, 72 and 96 hours.

Concentrations of 25, 50, 75 and 100mg/L were selected as effective concentration for performing the main toxicity test of the plant extract of different concentration. The fish will be exposed to the sample based on a static exposure regime[10].

ANTI-INFLAMMATORY ACTIVITY-CARRAGEENAN INDUCED INFLAMMATION:

Adult zebra fish were randomly selected. weighed separately and inject intraperitoneally with carrageenan (300µg, 20µl) to induce abdominal edema in the adult zebra fish. The animals were anesthetized in water at temperature of 10±2°C and generally placed on a damp sponge. With the abdomen facing upwards, the needle was inserted parallel to the spine in to the midline of the abdomen, posterior to the pectoral fins. The animal did not remain out of the water for more than 10s. After injection, animals were placed in a separate tank with system water (25±2°c). The animals were individually weighed and their body weight will be measured every 1hr from 1 to 5 hr[1,8].

Histopathological analysis:

For the histopathological analysis of liver, intestine and kidney, the animals were fixed in bouins solution for 24hr and decalcified in EDTA solution for 48hrs. The samples were dehydrated in alcohol series (70, 80, 90 and 100%), diaphonized in xylene and embedded in paraffin. Sample was sectioned at 5µm using a microtome and the histopathological analysis was performed after tissue section using hematoxylin and eosin.

Assessment of histopathogical changes:

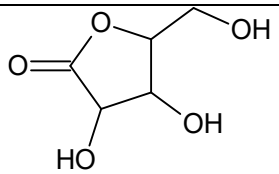
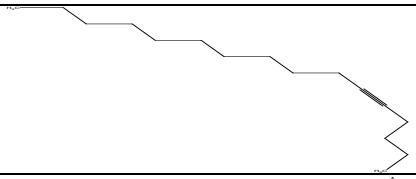
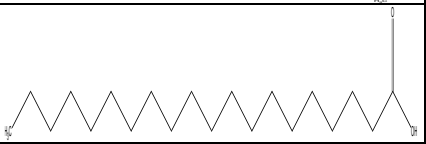
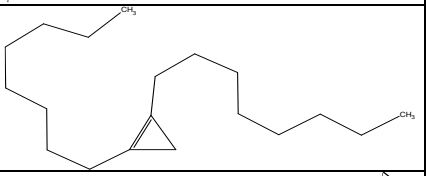
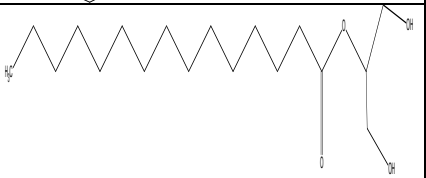
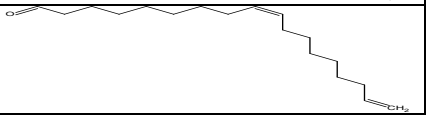
The IHC were calculated from the levels of tissue changes observed in the liver, intestine and kidney. The changes was classified into levels I ,II,III and the IHC value will be used to indicate whether the organ will be healthy (0 to 10), have mild to moderate changes (11 to20) or moderate to severe (21 to 50) damage, or will be irreversibly damage.

RESULTS AND DISCUSSION:

GC-MS study of the plant extracts:

By using GC-MS analysis, 10 components from ethanol extract and 5 components from chloroform extract of *Erythrina variegata* seeds could be identified and measured. The outcomes of the GC-MS analysis of the ethanol and chloroform extract from *Erythrina variegata* seeds were displayed in fig no 14-30. The major phytoconstituents included phytosterols such as β-sitosterol, stigmasterol, and campesterol, along with fatty acids and aldehydes including eicosanoic acid, 9,17-octadecadienal, and 7,11-hexadecadienal. Sterols are widely reported for their anti-inflammatory and lipid-regulating properties, while fatty acids and aldehydes exhibit antioxidant and membrane-stabilizing activities. The combination of these molecules suggests a synergistic pharmacological effect rather than a single-compound activity.

Table: 1 Summary of identified bioactive compounds from Ethanol extract of *Erythrina variegata* seeds

NAME OF THE COMPOUND	RT	MOL FORMULA	MOL STRUCTURE	MOL WEIGHT	AREA %
D-(+)-Ribonic acid. gamma.-lactone	16.554	$C_5H_8O_5$		148	25.920
5-Hexadecyne	18.215	$C_{16}H_{30}$		222	52.902
Eicosanoic acid	18.405	$C_{20}H_{40}O_2$		312	9.610
1,2-Dioctylcyclopropene	20.366	$C_{19}H_{36}$		264	0.962
Pentadecanoic acid, 2-Hydroxy-1-(hydroxymethyl)ethyl ester	21.126	$C_{18}H_{36}O_4$		316	1.824
9,17-Octadecadienal,(Z)-	22.276	$C_{18}H_{32}O$		264	1.052

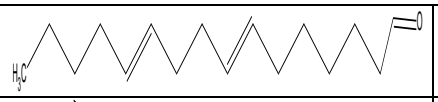
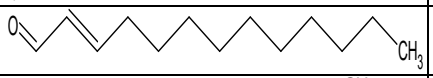
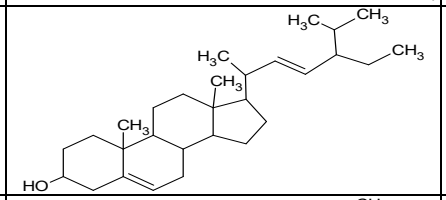
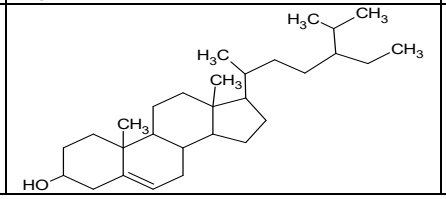
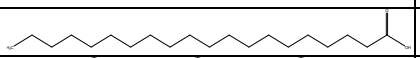
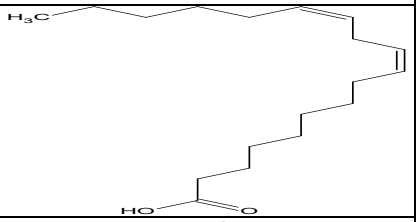
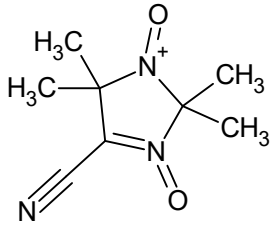
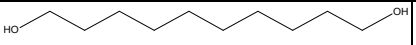
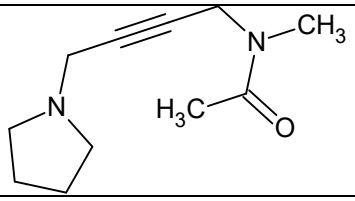
7,11-Hexadecadienal	22.471	$C_{16}H_{28}O$		236	4.705
2-Tridecenal,(E)-	22.712	$C_{13}H_{24}O$		196	0.799
Stigmasterol	26.253	$C_{29}H_{48}O$		412	1.017
Beta-sitosterol	26.678	$C_{29}H_{50}O$		414	1.209

Table 2: Summary of identified bioactive compounds from Chloroform extract of *Erythrina variegata* seeds

NAME OF THE COMPOUND	RT	MOL FORMULA	MOL STRUCTURE	MOL. WEIGHT g/mol	AREA %
Eicosanoic acid	16.859	$C_{20}H_{40}O_2$		312	18.742
9,12-Octadecadienoic acid(Z,Z)	18.675	$C_{18}H_{32}O_2$		280	73.037
4-Cyano-2,2,5,5-tetramethyl-3-imadazoline-3-oxide-1-oxyl	18.805	$C_8H_{12}O_2N_3$		182	5.085
1,10-Decanediol	22.556	$C_{10}H_{22}O_2$		174	1.533
Acetamide,N-methyl-N-(4-(1-pyrrolidinyl)-2-butynyl)-	26.753	$C_{11}H_{18}ON_2$		194	0.788

BINDING ENERGY OF PyRx

Table 3: Binding energy of PyRx

SI.NO	COMPOUND NAME	6y3c	6COX
1.	Indomethacin	-7.2	-8.5
2.	D-(+)-Ribonic acid. Gamma-lactone	-5.4	-5
3.	5-Hexadecyne	-5.6	-6
4.	Eicasanoic acid	-6.7	-6.4
5.	1,2- Dioctylcyclopropene	-5.8	-5.5

6.	Pentadecanoic acid, 2-hydroxy-1-(Hydroxymethyl)ethyl ester	-4.2	-5.3
7.	9,17-Octadecadienal,(Z)	-5	-5.6
8.	7,11-Hexadecadienal	-4.9	-4.9
9.	2-Tridecenal,(E)-	-4.7	-4.5
10.	Stigmasterol	-5.1	-8.7
11.	Beta-sitosterol	-7.5	-9.6
12.	9,12-Octadecadienoic acid(z,z)-	-5.2	-5
13.	4-cyano-2,2,5,5-tetramethyl-3-imidazoline-3-oxide-1-oxyl	-4.5	-5.4
14.	1,10-Decanediol	-5.1	-4.5
15.	Piperazine,1,2,4-Trimethyl	-9	-4.7
16.	2,2-dimethyl-N-Tert-Butylaziridine-1-Carboxamide	-5.3	-5.9
17.	Acetamide, N-methyl-N-(4-(1-Pyrrolidinyl)-2-Butynyl)-	-5.5	-6.2
18.	Campesterol	-8.8	-7.9
19.	1-methylcyclooctene	-6.1	-6.1
20.	1,9-nonanediol	-4.9	-4.8
21.	4-Tridecene(z)-	-5.9	-5.5
22.	1 Pyrrolidineethamine	-4.7	-4.6

BINDING ENERGY OF ARGUS

Table 4: Binding energy of Argus lab

SI.NO	COMPOUND NAME	BINDING ENERGY (Kcal/Mol) 6y3c	BINDING ENERGY (Kcal/Mol) 6COX
1.	Indomethacin	-7.05	-9.24
2.	D-(+)-Ribonic acid. Gamma-lactone	-7.97	-7.38
3.	5-Hexadecyne	-13.15	-11.93
4.	Eicasanoic acid	-8.7	-8.5
5.	1,2- Dioctylcyclopropene	-6.6	-6.2
6.	Pentadecanoic acid, 2-hydroxy-1-(Hydroxymethyl)ethyl ester	-7.1	-7.7
7.	9,17-Octadecadienal,(Z)	-7.3	-8.8
8.	7,11-Hexadecadienal	-10.2	-9.9
9.	2-Tridecenal,(E)-	-11.43	-11.25
10.	Stigmasterol	-14.47	-14.44
11.	Beta-sitosterol	-15.48	-16.00
12.	9,12-Octadecadienoic acid(z,z)-	-7.5	-8.5
13.	4-cyano-2,2,5,5-tetramethyl-3-imidazoline-3-oxide-1-oxyl	-7.49	-7.71
14.	1,10-Decanediol	-8.91	-8.85
15.	Piperazine,1,2,4-Trimethyl	-5.68	-5.64
16.	2,2-dimethyl-N-Tert-Butylaziridine-1-Carboxamide	-6.84	-6.67
17.	Acetamide, N-methyl-N-(4-(1-Pyrrolidinyl)-2-Butynyl)-	-7.42	-7.59

18.	Campesterol	-15.17	-15.42
19.	1-methylcyclooctene	-10.80	-10.53
20.	1,9-nonanediol	-8.09	-8.28
21.	4-Tridecene(z)-	-12.22	-11.83
22.	1 Pyrrolidineethamine	-6.75	-7.05

Molecular docking studies further confirmed the anti-inflammatory potential of the identified compounds. Docking against COX-2 (6COX) and 6y3c proteins revealed that sterols such as β-sitosterol (–9.6 kcal/mol), campesterol (–8.8 kcal/mol), and stigmasterol (–8.7 kcal/mol) showed the strongest binding affinities, in some cases superior to the standard anti-inflammatory drug indomethacin (–8.5 kcal/mol). Argus docking provided even stronger binding energies, with β-sitosterol (–16.0 kcal/mol), campesterol (–15.42 kcal/mol), and stigmasterol (–14.44 kcal/mol) demonstrating remarkable stability within the protein active sites. Fatty acids and aldehyde derivatives also showed moderate docking scores, contributing to the overall pharmacological effect.

EVALUATION OF PHARMACOLOGICAL PROPERTIES:

Swiss ADME predictions provided insights into the pharmacokinetic behavior of the compounds. Fatty acid derivatives and aldehydes exhibited high gastrointestinal absorption and, in some cases, blood–brain barrier permeability, suggesting good bioavailability and potential central nervous system activity. In contrast, sterols displayed low GI absorption and poor BBB penetration, which may limit oral bioavailability. However, all three major sterols complied with Lipinski's Rule of Five and showed minimal CYP450 inhibition, reducing the likelihood of metabolic drug–drug interaction.

EVALUATION OF PHARMACOLOGICAL PROPERTIES BY USING SWISS ADME SOFTWARE:

Table 5: Pharmacological properties of constituents

Compound	Mol Wt (g/mol)	No of heavy atom	No of aromatic heavy atom	Fraction Csp3	No of rotatable Bond	No of H bond acceptor	No of H bond donor	Molar refractivity	TPSA	$Logp_{o/w}$	Log s
Indomethacin	357.79	25	15	0.16	5	4	1	96.12	68.53	2.76	-4.86
D-(+)-Ribonic acid. Gamma-lactone	148.11	10	0	0.80	1	5	3	28.81	86.99	-0.21	0.20
5-Hexadecyne	222.41	16	0	0.88	10	0	0	77.19	0.00	4.59	-5.41
Eicasanoic acid	312.53	22	0	0.95	18	2	1	100.03	37.30	4.56	-6.44
1,2- Dioctylcyclopropene	264.49	19	0	0.89	14	0	0	90.86	0.00	4.58	-5.77
Pentadecanoic acid, 2-hydroxy-1-(Hydroxymethyl)ethyl ester	314.48	22	0	0.94	17	4	2	92.25	66.76	4.09	-4.21
9,17-Octadecadienal,(Z)	264.45	19	0	0.72	15	1	0	87.89	17.07	4.38	-4.70
7,11-Hexadecadienal	236.39	17	0	0.69	12	1	0	78.28	17.07	3.93	-3.78
2-Tridecenal,(E)-	196.33	14	0	0.77	10	1	0	64.33	17.07	3.35	-3.74
Stigmasterol	412.69	30	0	0.86	5	1	1	132.75	20.23	5.01	-7.46
Beta-sitosterol	414.71	30	0	0.93	6	1	1	133.23	20.23	4.79	-7.90
9,12-Octadecadienoic acid(z,z)-	280.45	20	0	0.72	14	2	1	89.46	37.30	0	0
4-cyano-2,2,5,5-tetramethyl-3-imidazoline-3-oxide-1-oxyl	183.21	13	0	0.75	0	4	1	56.20	76.01	0.00	-0.61
1,10-Decanediol	174.78	12	0	1	9	2	2	52.51	40.46	2.65	-1.90
Piperazine,1,2,4-Trimethyl	128.22	9	0	1	0	2	0	47.27	6.48	2.21	-0.96
2,2-dimethyl-N-Tert-Butylaziridine-1-Carboxamide	170.25	12	0	0.89	3	1	1	53.54	32.11	2.41	-1.28
Acetamide, N-methyl-N-(4-(1-Pyrrolidinyl)-2-Butynyl)-	193.29	14	0	0.75	3	2	0	62.86	20.31	2.76	-1.79
Campesterol	400.68	29	0	0.93	5	1	1	128.42	20.23	4.92	-7.54
1-methylcyclooctene	124.22	9	0	0.78	0	0	0	42.79	0.00	2.46	-2.79
1,9-nonanediol	160.25	11	0	1	8	2	2	47.70	40.46	2.35	-1.63
4-Tridecene(z)-	182.35	13	0	0.85	9	0	0	64.13	0	3.88	-4.36
1 Pyrrolidineethamine	110.16	8	5	0.33	2	1	1	33.21	30.95	1.49	-0.64

COMPOUND	GI Absorption	BBB Permeate	PGP Substrate	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
D-(+)-Ribonic acid. Gamma-lactone	Low	No	No	No	No	No	No	No
5-Hexadecyne	Low	No	No	Yes	No	No	No	No
Eicasanoic acid	Low	No	No	Yes	No	No	No	No
1,2- Dioctylcyclopropene	Low	No	No	Yes	No	No	No	No
Pentadecanoic acid, 2-hydroxy-1-(Hydroxymethyl)ethyl ester	High	Yes	No	No	No	No	Yes	No
9,17-Octadecadienal,(Z)	High	No	No	Yes	No	Yes	No	No
7,11-Hexadecadienal	High	Yes	No	Yes	No	No	No	No
2-Tridecenal,€-	High	Yes	No	Yes	No	No	No	No
Stigmasterol	Low	No	No	No	No	Yes	No	No
Beta-sitosterol	Low	No	No	No	No	No	No	No
9,12-Octadecadienoic acid(z,z)-	Low	-	-	-	-	-	-	-
4-cyano-2,2,5,5-tetramethyl-3-imidazoline-3-oxide-1-oxyl	High	No	No	No	No	No	No	No
1,10-Decanediol	High	Yes	No	No	No	No	No	No
Piperazine,1,2,4-Trimethyl	Low	No	No	No	No	No	No	No
2,2-dimethyl-N-Tert-Butylaziridine-1-Carboxamide	High	Yes	No	No	No	No	No	No
Acetamide, N-methyl-N-(4-(1-Pyrrolidinyl)-2-Butynyl)-	High	Yes	No	No	No	No	No	No
Campesterol	Low	No	No	No	No	No	No	No
1-methylcyclooctene	Low	Yes	No	No	No	No	No	No
1,9-nonanediol	High	Yes	No	No	No	No	No	No
4-Tridecene(z)-	Low	No	No	Yes	No	No	No	No
1 Pyrrolidineethamine	High	No	No	No	No	No	No	No

Table 6: PREDICTION OF TOXICITY BY USING PROTOX SOFTWARE:

SI.NO	COMPOUNDS	PREDICTED LD50 mg/kg	PREDICTED TOXICITY CLASS	HEPATO TOXICITY	CARCINO GENICITY	IMMUNO TOXICITY	MUTA GENICITY	CYTO TOXICITY
Std	Indomethacin	12	2	Active	Inactive	Active	Inactive	Inactive
1	D-(+)-Ribonic acid. Gamma-lactone	10600	6	Inactive	Inactive	Inactive	Inactive	Inactive

2	5-Hexadecyne	300	3	Inactive	Inactive	Inactive	Inactive	Inactive
3	Eicasanoic acid	900	4	Inactive	Inactive	Inactive	Inactive	Inactive
4	1,2- Dioctylcyclopropene	5000	5	Inactive	Inactive	Inactive	Inactive	Inactive
5	Pentadecanoic acid, 2-hydroxy-1-(Hydroxymethyl)ethyl ester	5000	5	Inactive	Inactive	Inactive	Inactive	Inactive
6	9,17-Octadecadienal,(Z)	5000	5	Inactive	Inactive	Inactive	Inactive	Inactive
7	7,11-Hexadecadienal	5000	5	Inactive	Inactive	Inactive	Inactive	Inactive
8	2-Tridecenal,(E)-	5000	5	Inactive	Inactive	Inactive	Inactive	Inactive
9	Stigmasterol	890	4	Inactive	Inactive	Active	Inactive	Inactive
10	Beta-sitosterol	890	4	Inactive	Inactive	Active	Inactive	Inactive
11	9,12-Octadecadienoic acid(z,z)-	10000	6	Inactive	Inactive	Inactive	Inactive	Inactive
12	4-cyano-2,2,5,5-tetramethyl-3-imidazoline-3-oxide-1-oxyl	227	3	Inactive	Active	Inactive	Inactive	Inactive
13	1,10-Decanediol	1000	4	Inactive	Inactive	Inactive	Inactive	Inactive
14	Piperazine,1,2,4-Trimethyl	2030	5	Inactive	Inactive	Inactive	Inactive	Inactive
15	2,2-dimethyl-N-Tert-Butylaziridine-1-Carboxamide	1500	4	Inactive	Inactive	Inactive	Active	Inactive
16	Acetamide, N-methyl-N-(4-(1-Pyrrolidinyl)-2-Butynyl)-	150	3	Inactive	Inactive	Inactive	Inactive	Inactive
17	Campesterol	890	4	Inactive	Inactive	Active	Inactive	Inactive
18	1-methylcyclooctene	2760	5	Inactive	Inactive	Inactive	Inactive	Inactive
19	1,9-nonanediol	1000	4	Inactive	Inactive	Inactive	Inactive	Inactive
20	4-Tridecene(z)-	2760	5	Inactive	Inactive	Inactive	Inactive	Inactive
21	1 Pyrrolidinethamine	423	4	Inactive	Inactive	Inactive	Inactive	Inactive

Toxicity predictions indicated that most compounds fall under Classes 4–6, suggesting low acute toxicity and a favorable safety margin. Sterols such as β-sitosterol, stigmasterol, and campesterol were placed under Class 4 (LD ~890 mg/kg), indicating relative safety with only mild immunotoxic potential. Fatty acids and aldehydes were largely non-toxic with higher LD values, while a few compounds such as 4-cyano derivatives were categorized under Class 3, reflecting comparatively higher toxicity.

Considering all the findings, β-sitosterol emerged as the best compound, followed closely by campesterol and stigmasterol. These sterols showed the strongest docking scores, favorable ADME properties, and acceptable safety profiles, making them the principal contributors to the anti-inflammatory activity of Erythrina variegata seeds. Although their limited oral absorption presents a challenge, this can be overcome through modern formulation strategies such as nanoemulsions or lipid-based carriers.

ANTI-INFLAMMATORY ACTIVITY-CARRAGEENAN INDUCED INFLAMMATION

Acute Toxicity Testing:

Method:

OECD Guideline 203 - Fish, Acute Toxicity Test (Conducted Study)

Result Interpretation:

- The LC50 (lethal concentration to kill 50% of the test population) was determined by plotting the concentration of the test substance against the percentage of fish that died at each concentration.
- Since no mortality or significant effects were observed at the highest tested dose, the LC50 was determined to be >40 mg/kg, indicating that the test substances (Ethanolic Extract & Chloroform Extract of EV) exhibited no acute toxicity within the tested concentration range.

Result

CL50 (mg/L): Since no mortality was observed at the highest tested concentration (100 mg/L), the LC50 is >100 mg/L

IV: Confidence interval; not applicable in this case as no mortality occurred.

Anti Inflammatory Screening

Based on the acute toxicity testing, the low, medium and high doses were fixed to be 25 mg/L, 50 mg/L, 100 mg/L respectively for Ethanolic Extract of *Erythrina variegata* & Chloroform Extract of *Erythrina variegata*.

Table 7: Assessment of Body weight

S.No	Groups	Body weight (g)
1	Normal Control	0.99 ± 0.1
2	Indomethacin (10 mg/kg)	0.65 ± 0.2
3	Ethanolic Extract of EV (25mg/L)	0.66 ± 0.1
4	Ethanolic Extract of EV (50mg/L)	0.54 ± 0.1
5	Ethanolic Extract of EV (100mg/L)	0.50 ± 0.2
6	Chloroform Extract of EV (25mg/L)	0.60 ± 0.2
7	Chloroform Extract of EV (50mg/L)	0.51 ± 0.1
8	Chloroform Extract of EV (100mg/L)	0.47 ± 0.2

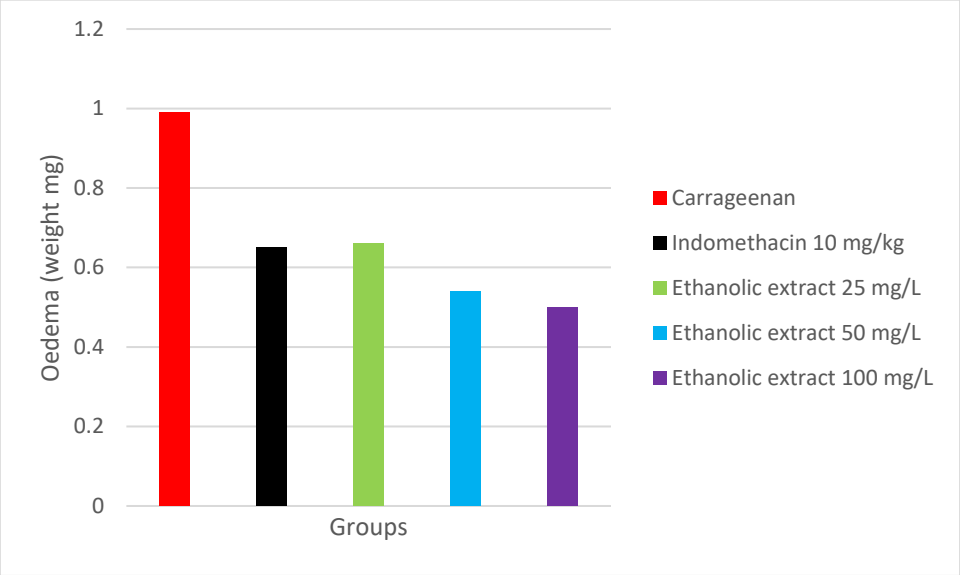


Figure 1: Graphical representation of inhibition of carrageenan induced abdominal edema of Ethanolic extract

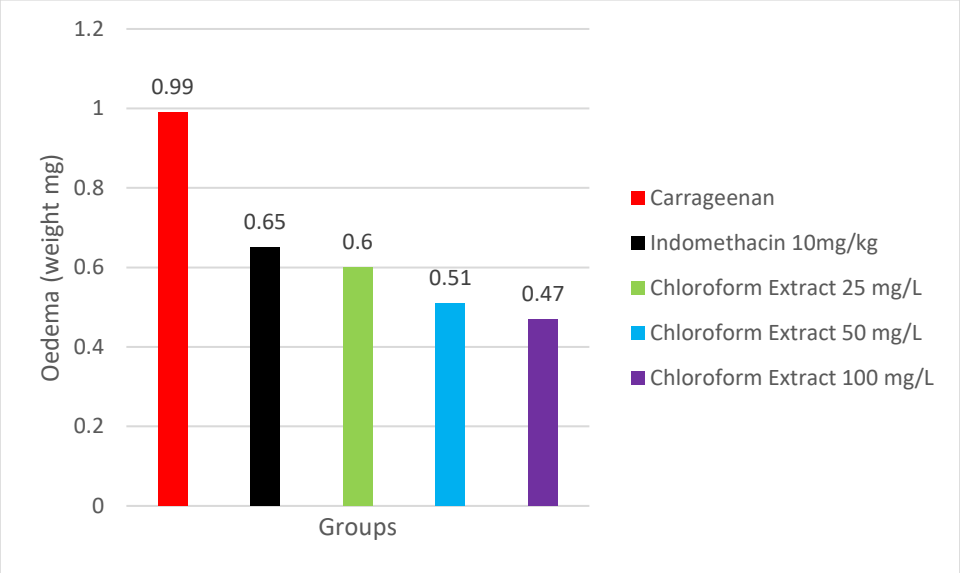


Figure 2: Graphical representation of inhibition of carrageenan induced abdominal edema of Chloroform extract

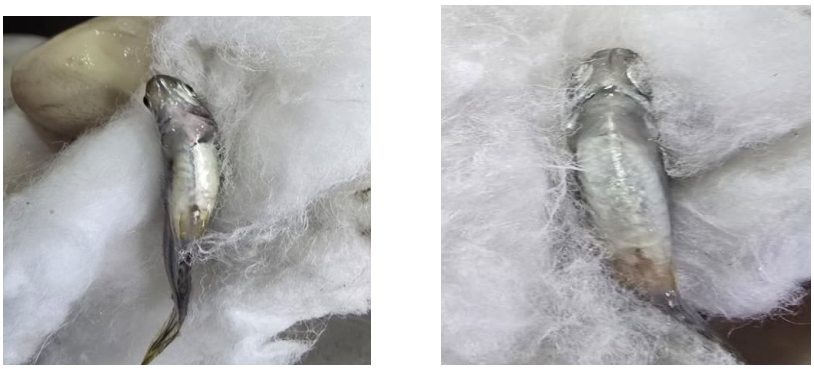


Figure 3: Macroscopic view of Adult Zebrafish (*Danio rerio)*

Table 8: Behavioural Analysis

S.No	Groups	Stage I	Stage II	Stage III	Total
1	Normal Control	1/2	0/2	0/2	1/6
2	Indomethacin (10 mg/kg)	1/2	0/2	1/2	2/6
3	Ethanolic Extract of EV (25mg/L)	1/2	0/2	1/2	2/6
4	Ethanolic Extract of EV (50mg/L)	1/2	1/2	0/2	2/6
5	Ethanolic Extract of EV (100mg/L)	2/2	1/2	1/2	4/6
6	Chloroform Extract of EV (25mg/L)	1/2	0/2	1/2	2/6
7	Chloroform Extract of EV (50mg/L)	2/2	0/2	1/2	3/6
8	Chloroform Extract of EV (100mg/L)	1/2	1/2	0/2	2/6

Histopathological Changes:

The formula for calculating the IHC value is:

$$I = \frac{\sum_{i-1}^{na} ai + 10 \sum_{i-1}^{nb} bi + 102 \sum_{i-1}^{nc} ci}{N}$$

Where:

- a_i represents observations of level I damage (score 0-10)
- b_i represents observations of level II damage (score 11-20)
- c_i represents observations of level III damage (score 21-50)
- N is the total number of observations.

Table 9: IHC values of Liver, Intestine, Kidney:

Group	IHC Value of liver	IHC Value of Intestine	IHC Value of Kidney
Group 1: Carrageenan	510	30	510
Group 2: Indomethacin + Carrageenan	5	5	5
Group 3: Ethanolic Extract (25mg/L) + Carrageenan	116	15	116
Group 4: Ethanolic Extract (50mg/L) + Carrageenan	4	15	4
Group 5: Ethanolic Extract (100mg/L) + Carrageenan	4	15	4
Group 6: Chloroform Extract (25mg/L) + Carrageenan	510	30	510
Group 7: Chloroform Extract (50mg/L) + Carrageenan	5	15	5
Group 8: Chloroform Extract (100mg/L) + Carrageenan	5	15	5

LIVER

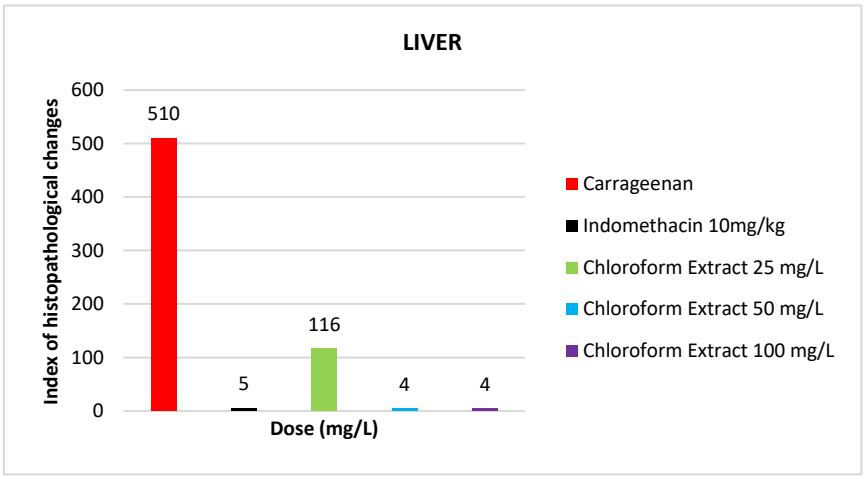


Figure 4: Graphical representation of liver in Ethanolic extract

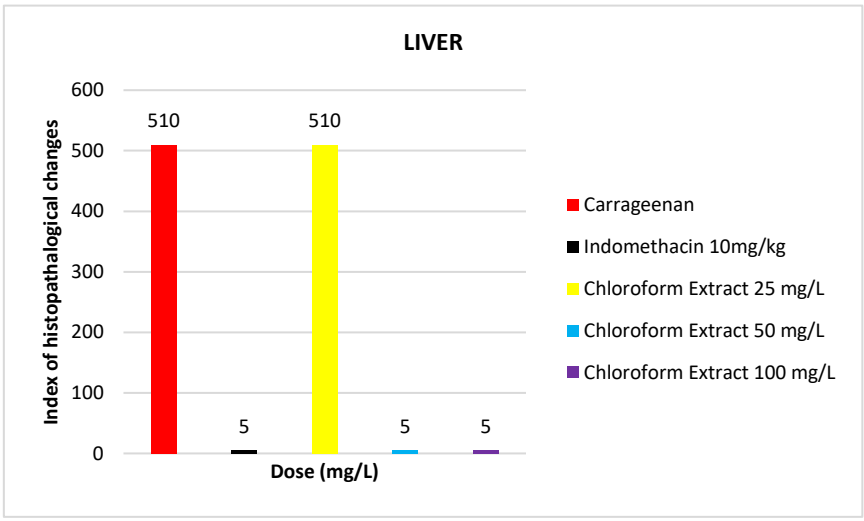


Figure 5: Graphical representation of liver in Chloroform extract

INTESTINE

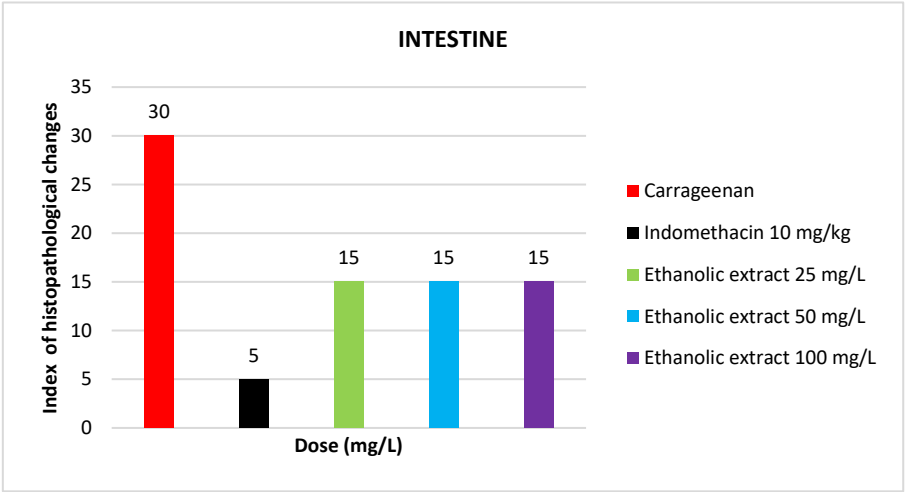


Figure 6: Graphical representation of intestine in Ethanolic extract

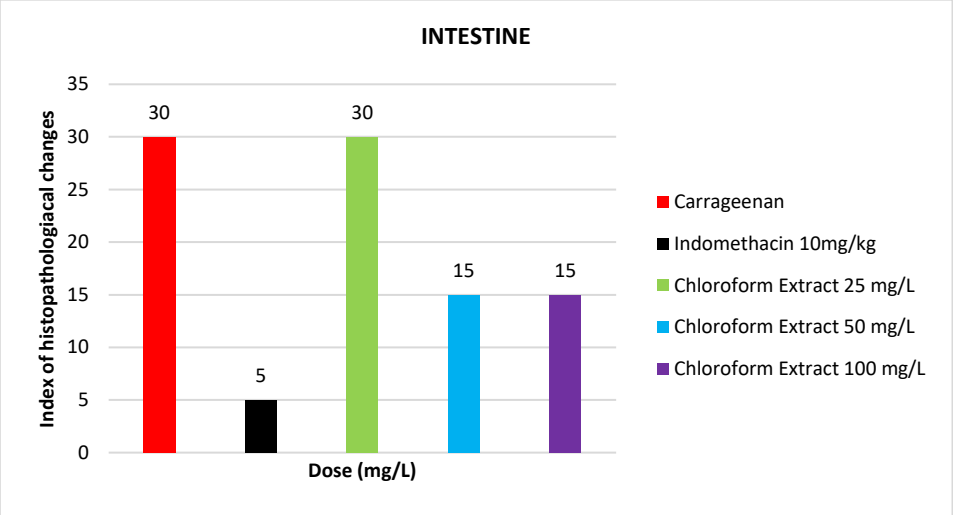


Figure 7: Graphical representation of intestine in Chloroform extract

KIDNEY

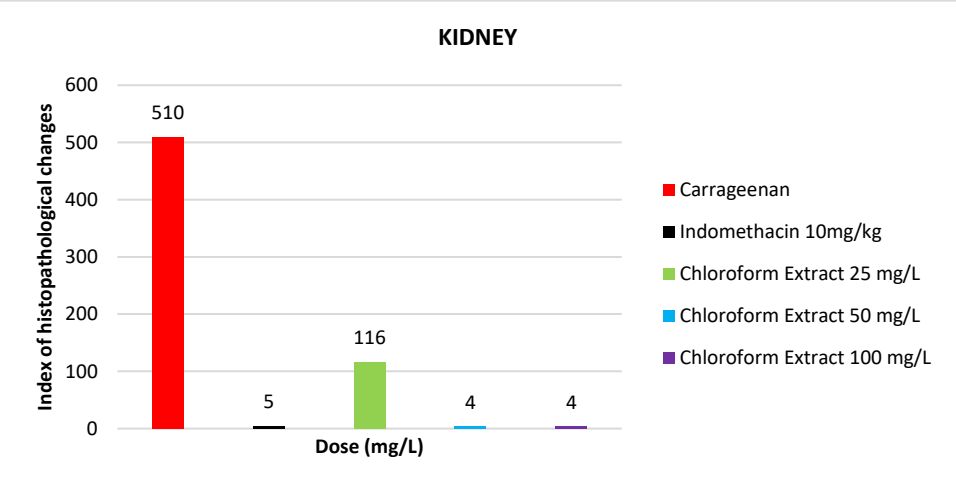


Figure 8: Graphical representation of kidney in Ethanolic extract

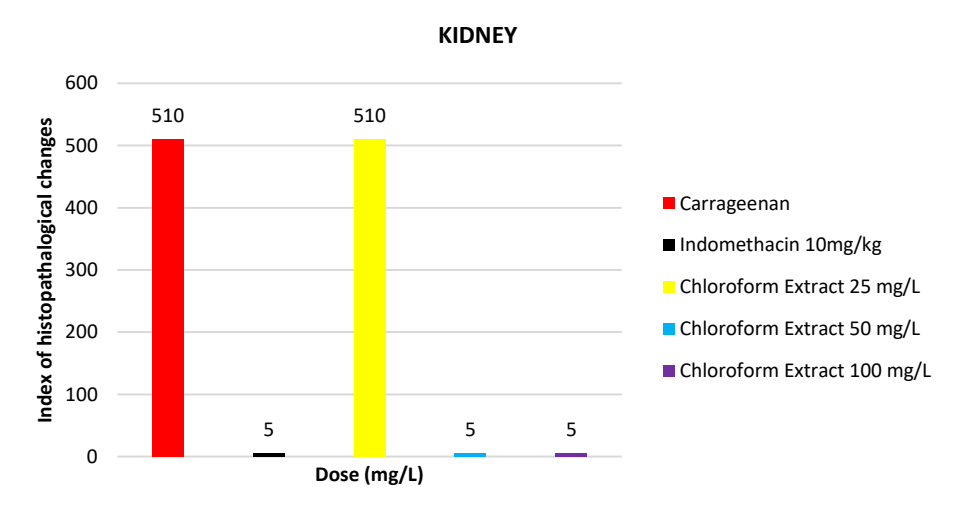


Figure 9: Graphical representation of kidney in Chloroform extract

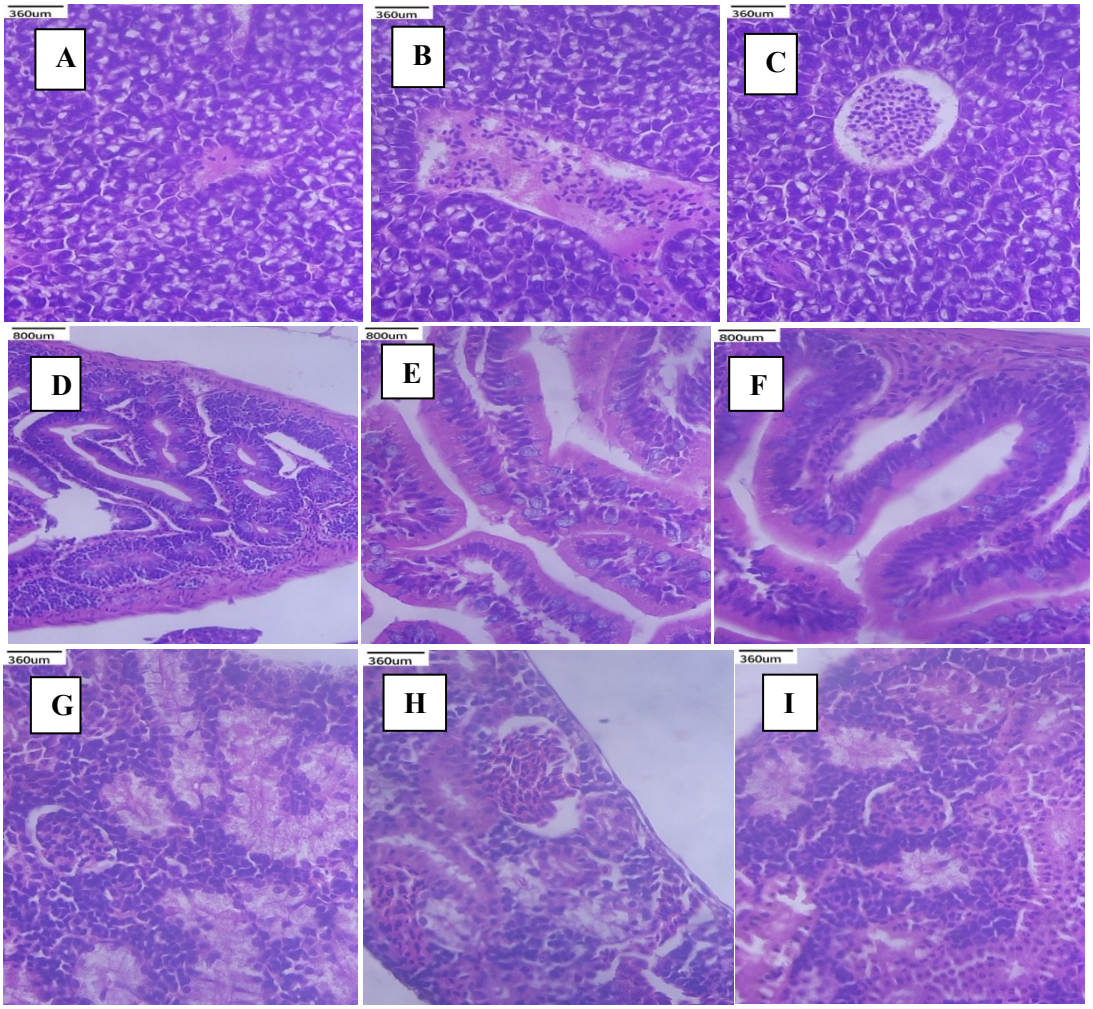


Figure 10: Histopathological changes of Liver, Intestine, Kidney of Adult Zebrafish. In (A-C), histopathological changes of liver cause Mild degenerative changes, reduced neutrophilic infiltration Normal mucosal linin and normal muscular layers. In (D –F), histopathological changes of intestine cause Mild degenerative changes in epithelium, reduced neutrophilic infiltration, normal muscular layers. In (G-I), histopathological changes of kidney cause Mild degenerative changes in epithelium, reduced neutrophilic infiltration, normal muscular layers, complete resolution of degenerative changes.

Table 10: Observatoins level of liver, intestine and kidney

GROUPS	LIVER	INTESTINE	KIDNEY
Group 1: Carrageenan	Level III	Level III	Level III
Group 2: Indomethacin (10 mg/kg) +Carrageenan	Level I	Level I	Level I
Group 3: Ethanolic extract (25 mg/L) + Carrageenan	Level II	Level II	Level II
Group 4: Ethanolic extract (50 mg/L) + Carrageenan	Level I	Level II	Level II
Group 5: Ethanolic extract (100 mg/L) + Carrageenan	Level I	Level II	Level II
Group 6: Chloroform extract (25 mg/L) + Carrageenan	Level III	Level III	Level III
Group 7: Chloroform extract (50 mg/L) + Carrageenan	Level I	Level II	Level II

Group 8: Chloroform extract (100 mg/L) + Carrageenan	Level I	Level I	Level I

CONCLUSION

The combined phytochemical, GC–MS, docking, pharmacokinetic, and toxicity results demonstrate that *Erythrina variegata* seeds are a rich source of bioactive compounds with significant anti-inflammatory potential. Sterols such as β-sitosterol, stigmasterol, and campesterol play a central role, supported by fatty acid and aldehyde derivatives that contribute to synergistic activity. The favorable docking scores, acceptable pharmacokinetic parameters, and low toxicity predictions justify the traditional use of this plant in inflammatory disorders. Overall, the study highlights *Erythrina variegata* seeds as a promising natural source of anti-inflammatory agents and provides a scientific basis for future clinical investigations aimed at developing novel phytopharmaceuticals. The findings carrageenan induced inflammation and histopathological analysis suggest a dose-dependent effect of both extracts, where higher doses lead to better therapeutic outcomes. The observed improvements in tissue histopathology at higher doses are promising, indicating the potential of EV extracts as a natural anti-inflammatory treatment. In conclusion, both Ethanolic and Chloroform extracts of EV exhibit promising anti-inflammatory effects, with medium and high doses showing significant therapeutic potential.

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