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Original Article

RADIOPROTECTIVE ACTIVITY OF *FICUS RACEMOSA* ETHANOL EXTRACT AGAINST ELECTRON BEAM INDUCED DNA DAMAGE *IN VITRO, IN VIVO AND IN SILICO*

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ABSTRACT

Objective: To investigate the radioprotective effect of *Ficus racemosa* (Fr) ethanol stem bark extract against electron beam radiation (EBR) induced DNA damage using *in vitro, in vivo* and *in silico* models.

Methods: The extract of Fr was tested against radiation induced DNA damage by exposing pBR322 plasmid to different EBR dose rates. Comet assay was conducted using mice which were exposed at 6Gy EBR. *In silico* study was performed by inhibiting p53 protein C-chain (1TUP C) using phyto chemicals of Fr.

Results: The *in vitro* results revealed that, Fr at lower concentration (50µg) showed inhibitory effect on radiation induced DNA damage compared with control. Exposure of mice to 6Gy EBR increased comet parameters like TL (Tail length), OTM (Olive tail moment) and %T (percentage of DNA in the tail) of blood lymphocytes. Fr ethanol extract given orally prior to irradiation at a dose of 400 mg/kg body weight protected the DNA from the radiation damage. The phytochemicals of Fr showed clear interaction with p53 protein chain C, specifically binding to Arginine 248 (ARG248) and Arginine 273 (ARG273) amino acid residues thereby inhibiting the p53 protein-DNA interaction upon radiation.

Conclusion: The present study indicates that Fr ethanol extract significantly reduced radiation induced DNA damage *in vivo* and *in vitro.* It also showed that the biologically active compounds of Fr have ability to inhibit wild p53 protein which is responsible for apoptosis; these compounds can be used as radioprotectors during chemotherapy to protect normal tissues surrounding cancerous tissue.

Keywords: *Ficus racemosa*, Radioprotective activity, Molecular docking, p53 protein.

INTRODUCTION

Radiation therapy is usually applied to the different tumors because it uses high energy radiation to shrink tumors and kill cancer cells. Ionizing radiation can induce oxidative stress at the cellular level which results in the damage of biologically important macromolecules such as DNA, proteins, lipids and carbohydrates in various organs [1]. DNA lesions can be induced either by direct ionization of DNA or indirectly through the reaction of aqueous free radicals [2]. Highly reactive oxygen radicals produced by ionizing radiation mainly damage DNA which leads to cell killing and mutations. Damage to DNA caused by radiation in turn leads to activation of p53 protein. This protein acts as a DNA sequence-specific transcription factor regulating and activating the expression of a range of target genes in response to genotoxic stress [3]. This in turn initiates a cascade of signal transduction pathways leading to altered cellular responses including cell-cycle arrest and apoptosis that are well known to prevent cancer development. Amino acids i.e. Arg248 and Arg273 are the core residues of p53 protein that interact with DNA and suppressions of these two residues are proposed to be responsible for inhibition of the p53 protein binding to the DNA upon radiation to avoid apoptosis [4].

Irradiation of normal tissues (wild p53) which are surrounding cancerous tissue (mutated p53) during the course of therapeutic radiation can result in side effects including mild chronic symptoms, acute toxicities, or severe organ dysfunction [5]. Therefore it is important to protect DNA from radiation induced damage. There have been lots of attempts to find an agent for use in radiation therapy that can preferentially protect the normal tissues from radiation damage [6]. However, the fact remains that there is not a single radio protective drug available which meets all the prerequisites of an ideal radioprotector, which has no toxicity, remains stable for a number of years without losing shelf life and can be easily administered [7]. In view of this, the search for less toxic and more potent radio protective drugs continues.

Ficus racemosa Linn. (Family: Moraceae) an indigenous medicinal plant is used in traditional system of medicine for the treatment of several disorders and it is one of the herbs mentioned in all ancient scriptures of ayurveda, siddha, unani and homeopathy. Diverse plant parts such as bark, root, leaf, fruits and latex are used as astringent, carminative, vermifuge and anti-dysentery. The phytochemicals like sterols, triterpenoids, alkaloids, tannins, flavonoid and phenols isolated from different source of plant was studied [8, 9]. In continuation of this line of investigation, the *in vivo* and *in vitro* protective activity was studied by using EBR as an oxidative DNA damaging agent. The *in silico* radioprotective activity of the constituents of Fr was investigated by high throughput screening and molecular docking approach.

MATERIALS AND METHODS

Chemicals

TRIS base, high melting agarose, low melting agarose, disodium EDTA, tritonX-100, sodium sarcosinate, DMSO and propidium iodide for performing comet assay were obtained from Sigma Aldrich (St. Louis, Missouri).

All biological assay reagents, SGPT, SGOT, total bilirubin, ALP, albumin, total protein kits and pBR322 plasmid DNA were purchased from Bangalore Genei, India.

Plant material and extraction

Stem bark of Fr was collected from ethno medical practioner near Mala region of Udupi district and was identified and authenticated by the expert taxonomist from the Department of Botany, Mangalore University, India. The material was shade dried and powdered. The powdered sample was extracted by refluxing with the ethanol in soxhlet apparatus for 48 hrs. The final extract was filtered, concentrated, dried and used for further analysis.

Irradiation

The irradiation work was conducted at Microtron centre, Mangalore University, Mangalore, Karnataka, India. The pBR322 plasmid DNA containing vials for *in vitro* study and the animals restrained in well-ventilated perspex boxes were exposed at a distance of 30 cm from the beam exit point of the Microtron accelerator at a dose rate of 72 Gy/min.

Estimation of DNA damage *in vitro*

To study the protective effect of Fr on DNA strand breaks, the pBR322 plasmid DNA sample (250ng in 0.01M sodium phosphate buffer) was exposed to EBR at a dose rate of 2Gy/min, 4Gy/min, 6Gy/min, and 8Gy/min for dose fixation in the absence of drug. After irradiation, the DNA samples were electrophoresed on 1% agarose gel stained with Gold View containing TAE buffer at 100V for 50 min and photographed using UV transilluminator. After dose fixation (i.e. 4Gy/min) another sets of vials containing pBR322 DNA samples was randomly divided into 6 vials. Vial I: normal control i.e. untreated DNA, vial II: radiated control exposed to electron beam, vial III, IV, V and VI: treated with 50, 100, 150 and 200µg of Fr extract respectively and all the vials were exposed to EBR at 4Gy except control. After exposure the DNA samples were electrophoresed on 1% agarose gel stained with Gold View containing TAE buffer and photographed using UV transilluminator.

***In vivo* study**

Animals

Animal care and handling were carried out according to the guidelines set by the World Health Organization; Geneva, Switzerland. The institutional animal ethical committee has approved this study. Swiss albino mice aged 6-8 weeks (30±5g) taken from an inbred colony, were used for this study. The animals were maintained under controlled conditions of 12h light 12 h dark. Animals were housed in a polypropylene cage containing sterile paddy husk as bedding throughout the experiment. They were provided a standard mouse feed and water *ad lithium*.

Subchronic toxicity studies

Subchronic toxicity study of Fr was conducted as described by Miller and Tainter [10]. Swiss Albino mice were divided into two groups of six animals each and were starved for 18 hr prior to the experiment. Group I: control received only distilled water; group II: were fed orally with Fr extracts suspended in distilled water at the concentration of 2000 mg/kg body weight [11]. Food and water intake was monitored daily. After 30 days of treatment animals were sacrificed; blood was collected by cardiac puncture. Whole blood was centrifuged at 3,500 rpm for 5 min using a table centrifuge (Remi. India). The serum was collected and used for the enzyme estimation. SGOT and SGPT were estimated according to the methods of Reitman and Frankel [12]. ALP was analyzed by the method of King and Armstrong [13] while the total bilirubin level was estimated as described by Malloy and Evelyn [14].

Estimation of DNA damage by comet assay

To carry out *in vivo* DNA damage studies, animals were randomly divided into 5 groups of 6 animals each. Group I served as control fed with normal diet and double distilled water, group II animals were exposed to EBR, group III animals were administered orally with Fr stem bark extract at a dose of 100 mg/kg body weight, group IV with 200 mg/kg body weight and group V with 400 mg/kg body weight respectively once daily for 15 consecutive days. On the 16th day, mice were exposed to 6Gy EBR [15]. After irradiation they were observed for the next 15 days. Finally on the 16th day the animals were sacrificed by cervical dislocation, blood was collected and lymphocytes were separated using histopaque and comet assay was conducted under alkali conditions according to Singh *et al.* [16].

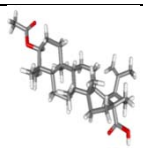
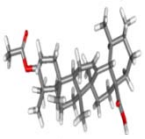
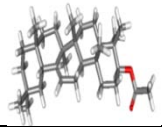
In order to estimate DNA damage in blood lymphocytes, 10µl sample was mixed with 200µl of low melting agarose at 37 °C and layered on frosted slides pre-coated with 200µl high melting agarose. After solidification, the cover slips were removed and the slides were kept in pre-chilled lysing buffer for 1 hr. The slides were removed from the lysis solution and placed on a horizontal electrophoresis tank filled with the alkaline buffer and then equilibrated with the same buffer for 20 min. Electrophoresis was carried out for 20 min at 25 V and 300 mA and neutralized with 0.4 M Tris, pH 7.4, stained with 50µl of ethidium bromide and observed using fluorescence microscope (Olympus. 40x objective). The level of DNA damage was assessed from the DNA migration distance by subtracting the diameter of the nucleus from the total length of the comet. 50 randomly selected cells were examined for each replicate and for each sample. The quantification of the DNA strand breaks of the stored images was performed using CASP software (www. casplab. com) by which the percentage of DNA in the tail, tail length and OTM could be obtained directly.

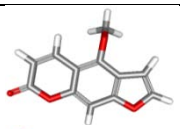
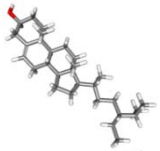
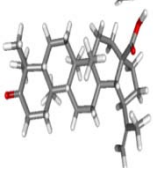
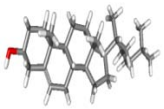
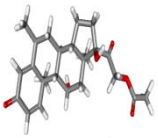
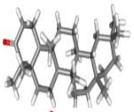
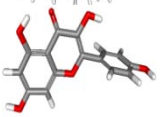
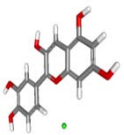
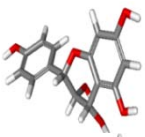
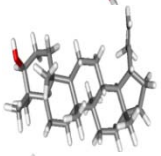
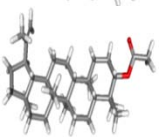
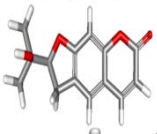
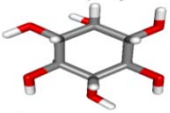
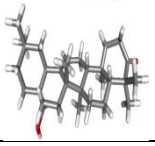
***In silico* studies**

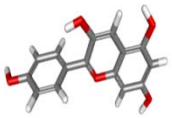
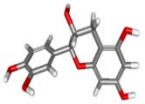
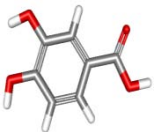
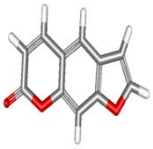
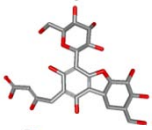
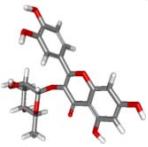
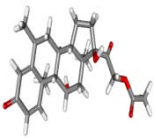
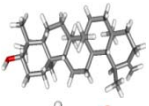
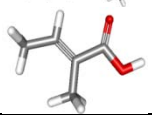
QSAR Analysis

Data sets of 25 compounds of Fr for QSAR analysis were selected from the literature (Pubchem database). Chemical structures and biological activity for the complete set of compounds were divided into training and test sets. The half maximal (50%) inhibitory concentration of compounds (IC_{50}) employed in this study (varying from 0.021 to 100 µM), were converted to the corresponding pIC_{50} (-$logIC_{50}$) and used as dependent variables in the QSAR investigations. All the details of compounds were represented in (tables 1). From the original data set of 25 compounds, 19 compounds were selected as members of the training set for QSAR model development, and the remaining 7 compounds were considered as members of the test set for external validation. Discovery Studio2.5 software (DS 2.5) was used to carry out QSAR studies [17].

Table 1: Structures, molecular properties and IC50 values of Fr constituents

S. No.	Name	Accession ID	IC50 (µM)	pIC50	Structures	Mol WT (g/mol)	H. B. donor	H. B. Acceptor	LogP	TPSA
1	Acetylbetulinic acid	CID 289984	4.6	1.7		498.7	1	4	7.7	63.6
2	Acetlyursolic acid	CID 25032406	14.2	1.8		498.7	1	4	7.4	63.6
3	α-Amyrin acetate	CID 92156	50.0	2.0		468.7	0	2	9.6	26.3

4	Bergaptan	CID 2355	1.7	2.1		216.1	0	4	2.3	48.7
5	Betasitosterol	CID 222284	1.9	2.1		414.7	1	1	8.6	20.2
6	Betulonic acid	CID 9933683	3.3	2.2		454.6	1	3	7.9	54.3
7	Campesterol	CID 173183	2.2	1.92		400.6	1	1	2.2	43.7
8	Depomedrol	CID 5877	26.6	2.07		416.5	2	6	2.7	100.9
9	Friedeline	CID 91472	50.0	1.9		426.7	0	1	7.85	17.0
10	Kampferol	CID 5280863	0.02	1.8		286.2	4	6	2.1	111.1
11	Leucoantho cyanidin	CID 68247	12.0	2.0		322.6	5	6	4.3	102
12	Leucopelargonin	CID 3286789	1.2	1.7		290.2	5	6	0.7	110
13	Lupeol	CID 259846	10.4	2.0		426.7	1	1	9.9	20.2
14	Lupeal acetate	CID 92157	22.7	2.1		468.7	0	2	10.4	26.3
15	Marmesin	CID 334704	67	1.8		246.2	1	4	1.9	55.8
16	Mesoinositol	CID 892	0.4	2.0		180.1	6	6	-3.7	121
17	Oleanonic acid	CID 12313704	2.03	2.0		454.6	1	3	7.2	54.4

18	Perlargonidin	CID 440832	4.4	1.9		271.2	4	4	3.2	81.9
19	Transcatechin	CID 73160	5.3	1.9		490.4	5	6	0.4	110
20	Protocatechuin	CID 72	0.479	2.1		154.1	3	4	1.1	77.7
21	Psoralen	CID 6199	3	1.9		186.1	0	3	2.3	39.4
22	Racemosic acid	-	19	1.7		504.3	5	1	-2.68	67.2
23	Rutin	CID 5280805	1.7	1.8		414.7	1	1	9.3	20.2
24	Stigmasterin	CID 5280794	43.4	2.0		412.6	1	1	8.6	20.2
25	Taraxasterol	CID 115250	0.19	2.1		426.7	1	1	9.1	20.2
26	Tiglic acid	CID 125468	1.3	1.9		100.1	1	2	1	37.3

Footnote: Mol WT: Molecular Weight; H. B. donor: Hydrogen Bond donor; H. B. Acceptor: Hydrogen Bond Acceptor; Log P: Partition coefficient; TPSA: Topological Polar Surface Area

ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity)

The ADME properties of Fr were calculated using Accelrys Discovery Studio software tool in which various pharmacokinetics parameters like blood brain barrier penetration (BBB), absorption level solubility level, hepatotoxicity, CYP2D6, plasma protein binding (PPB) levels were estimated for the compounds listed in (table 1). Obtained results were cross checked with standard values [18]. The toxicity profile of compounds was predicted using TOPKAT which uses a range of quantitative structure toxicity relationship (QSTR) models for assessing special toxicological endpoints such as aerobic biodegradability, mutagenicity, developmental toxicity prediction and skin irritation test [19].

Molecular docking

Molecular docking studies were performed using the phyto constituents present in Fr which have a promising role as radioprotectors [20]. As stated in the literature, 26 pharmacologically relevant compounds of Fr were retrieved from NCBI-Pubchem database and were chosen for docking studies. The structures of the 26 ligands were sketched using Chem. sketch tool (http://www. acdlabs. com). The X-ray crystallographic structure of p53protein was retrieved from protein data bank (http://www. rcsb. org/pdb/files/1TUP. pdb) with PDB ID: 1TUP C-chain. All the phytoconstituents were converted into appropriate formats, suitable for docking exercises and molecular docking was performed using FlexX tool [21].

Statistical analysis

All results were expressed as mean±Standard Deviation (S. D). Statistical significance was determined using one-way analysis of variance (ANOVA). P values<0.05 were considered as significant. All statistical analysis was carried out using Dunnett's test using instant statistical package (Graph Pad Prism version 3.0 software).

RESULTS

Estimation of DNA damage *in vitro*

EBR induced DNA damage occurred 4Gy onwards and at 6 Gy it was found irreversible damage and hence 6Gy was considered as toxic dose. During exposure to ionizing radiation, the plasmid DNA suffered strand breaks, which converted the super coiled (sc) form of plasmid DNA to open circular form (oc) (fig. 1).

The presence of Fr extract during irradiation protected pBR322 DNA from radiation damage. 50µg concentrations of the Fr extracts showed good protective effect (fig. 2. Lane 3) when compared to

irradiate control, indicating that prior treatment of the extracts may able to protect the DNA damage during irradiation.

Fig. 1: Damage of pBR322 DNA by EBR; C: Control, R: Radiated, OS: Open Circular, SC: Super Coiled

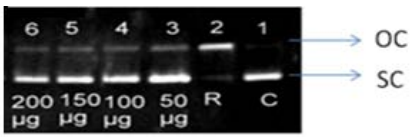


Fig. 2: *In vitro* radioprotective activity of Fr at different concentrations; C: Control, R: Radiated, OS: Open Circular, SC: Super Coiled

Subchronic toxicity studies

The effect of Fr extract on serum biochemical parameters are presented in (table 2). The levels of serum enzymes such as SGOT, SGPT, ALP; albumin, total protein and bilirubin content were not significantly different compared to control groups.

Estimation of DNA damage by comet assay

Exposure of control mice to EBR increased comet parameters like TL, OTM and %T of blood lymphocytes, suggesting radiation-induced damage to DNA.

Table 3 showed that when the Fr treated mice were exposed to EBR, the comet parameters in lymphocytes cells were increased compared to control groups i.e. from 7.3±1.61, 0.6037±0.066 and 0.5126±0.181 to 234.2±1.4, 39.62±1.64 and 16.623±1.639 respectively.

An administration of Fr extracts orally prior to irradiation at a dose of 400 mg/kg body weight protected the DNA from damage when compared to other lower concentrations i.e. 100 and 200 mg/kg body weight respectively which indicates the concentration dependent DNA protection.

Table 2: Effect of Fr extract on the function of liver enzymes

Tests	SGOT (U/l)	SGPT (U/l)	ALP (U/l)	Total protein (g/dl)	Albumin (g/dl)	Total bilirubin (mg/dl)
Control	19.525±0.4	27.55±0.4	77.69±0.09	7.26±0.46	2.153±0.37	2.1815±0.27
Fr	19.54±0.36	26.92±0.45	77.525±0.95	7.32±0.31	2.320±0.34	2.57±0.41

Footnote: Values are expressed as mean±SD

Table 3: *In vivo* radioprotective activity of Fr extract using comet assay

Groups	Tail Length	%T	OTM
Control	7.3±1.61	0.6037±0.066	0.5126±0.181
Irradiated control	234.2±1.4	39.62±1.64	16.623±1.639
Fr 100 mg/kg	137±0.9	19.17±1.137	9.545±1.983
Fr 200 mg/kg	103.4±1.685	6.641±0.7315**	4.1928±0.494**
Fr 400 mg/kg	81.5±0.921***	6.516±0.7227***	3.015±0.770***

Footnote: Values are reported as mean±S. E. M. for a group of six animals. Asterisks indicated statistically significant values when compared to radiation. *p<0.05, **p<0.01, ***p<0.001

Table 4: Predicted and actually values of 19 training set and 7 test set compounds

S. No.	Fr constituents	pIC50 (Actual)	GFATemp Mdel_1(Predicted)
Training set			
1	Acetylbetulinic acid	1.73	1.72
2	Acetylursolic acid	1.81	1.81
3	α-Amyrin acetate	2.01	2.48
4	Bergaptan	2.13	2.17
5	Betasitosterol	2.15	2.17
6	Campesterol	1.92	1.94
7	Leucantcyanidin	2.04	2.10
8	Leucopelargonidn	1.78	1.80
9	Lupeol	2.09	2.10
10	Lupeol acetate	2.10	2.13
11	Mesoinositol	2.00	2.03
12	Perlargonidin	1.92	1.95
13	Protocatechin	2.11	2.13
14	Psoralen	1.91	1.93
15	Racemosic acid	1.73	1.73
16	Rutin	1.92	1.95
17	Stigmasterin	2	1.95
18	Taraxasterol	2.11	2.15
19	Tiglic acid	1.91	1.92
Test set			
1	Kampferol	1.87	1.86
2	Oleanonic acid	2.32	2.31
3	Marmesin	1.98	1.97
4	Transcatechin	1.95	1.95
5	Friedeline	2.40	2.38
6	Betulonic acid	2.00	2.01
7	Depomedrol	2.86	2. 84

***In silico* studies**

QSAR analysis

The results of external validation for Fr were listed in (table 4) and the graphical representation for the experimental activity versus GFA Temp Mode_1 (predicted activity) of both training set and test set were displayed in (fig. 3, fig. 4). All the descriptors were taken as independent variables on X-axis and biological active values of compounds were taken as dependent variable on Y-axis, and GFA model was performed for all the phytoconstituents and statistical analysis of the training set compounds were represented in (table 5).

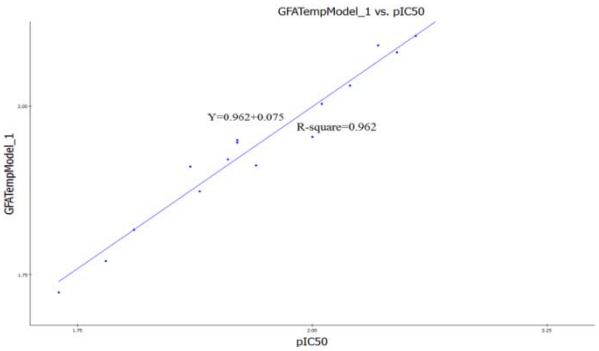


Fig. 3: Activity values predicted by GFA model of training set compounds of Fr

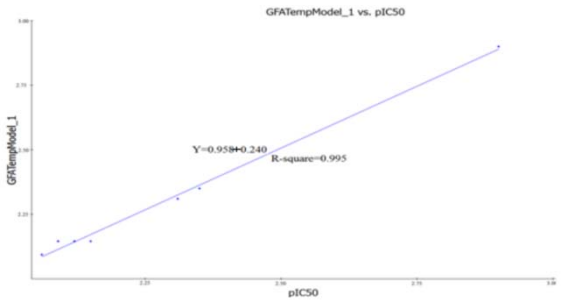


Fig. 4: Activity values predicted by GFA model of test set compounds of Fr

Table 5: Statistical analysis of training set compounds of Fr

	r^2	(adj)r^2	(pred)r^2	RMS residual error	Fried man LOF	S. O. R. p-Value
Equation 1	0.995	0.998	0.992	0.018	0.095	1.33E-05

Footnote: r^2 is coefficient of determination; r^2 (adj) is r^2 adjusted for the number of terms in the model; r^2 (predicted) is the prediction (PRESS) r^2, equivalent to q^2 from a leave-1-out cross-validation; Friedman L. O. F. is the Friedman lack-of-fit score; S. O. R. p-value is the p-value for significance of regression.

The first best equation bearing the relevant descriptors are given as GFA Template model1 below

GFATempMode1= 2.0233+0.54829; ALogP–0.93868; Molecular fractional polar surface area–0.80254; Molecular weight–0.54158; Number of hydrogen donor-1.5164; Number of hydrogen acceptor+2.8333; number of rotatable bonds–1.1912; number of rings–0.98855; Number of aromatic rings+0.00257542

From the GFATempMode1, we can deduce that the 2D descriptors showing good correlation (as+sign indicate positive correlation) of the molecules structure properties with its biological activity. Although the classical 2D QSAR model provided some useful information and showed a good predictive ability, on which moieties are particularly important to p53 protein inhibition.

ADMET

Various pharmacokinetics and pharmacodynamics properties like aqueous solubility, human intestinal absorption, PPB, BBB penetration, cytochromep450 inhibition and hepatotoxicity levels were predicted for Fr compounds and represented in (table 6).

The interpretation of the values was done using standards provided by Accelrys Inc. The graphical representation of ADMET properties are shown in (fig. 5). TOPKAT studies predicted aerobic biodegradability, mutagenicity, developmental toxicity potential (DTP), skin irritant and carcinogenicity of the compounds of Fr were represent in (table 7).

Molecular docking studies

The docking of phytoconstituents from plant Fr to 1TUP C protein reveal that the stretch of amino acid residues from Lys 132 to Glu 285 in the p53 protein broadly interact with the ligands and interestingly coincide with the p53-DNA binding interacting residues i.e. Arg248 and Arg273. The docking scores were obtained from the compounds with 1TUP C as the receptor. The output of all the ligands is indicated by energy values in kcal/mol. Least the energy values strongest is the interaction. Among 26 ligands, 7 ligands inhibited p53 protein like Kampferol, Oleanonic acid, Marmesin, Transcatechin, Friedeline, Betulonic acid and Depomedrol were represented in the (table 8).

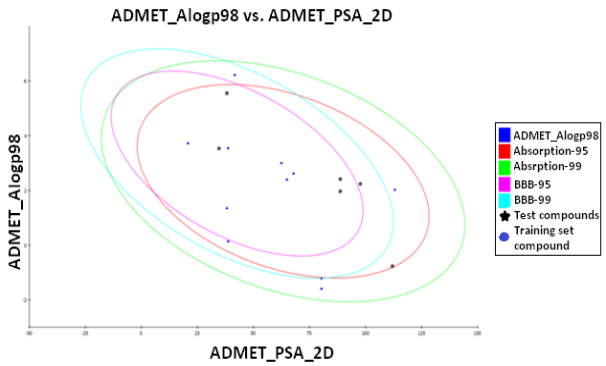


Fig. 5: ADMET properties of Fr ethanol extract

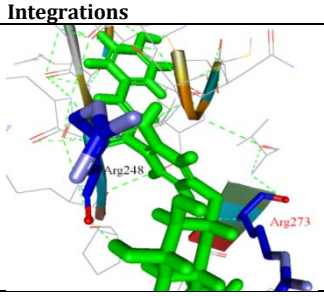
Table 6: Predicted ADME profiles of Fr compounds

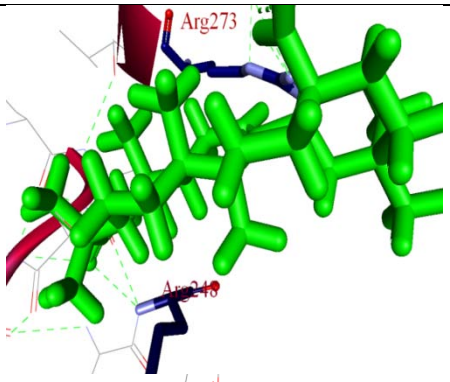
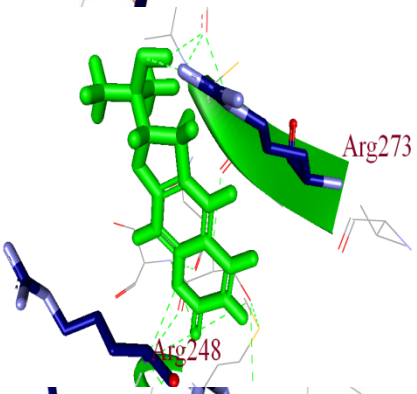
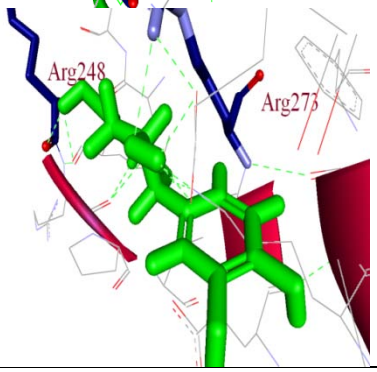
S. No.	Name	BBB level	Absorption level	Solubility level	Hepatotoxicity	CYP2D6	PPB level
1	Acetylbetulinic acid	4	1	0	0	0	2
2	Acetlyursolic acid	4	1	0	1	1	2
3	α-Amyrin acetate	4	1	0	0	0	2
4	Bergapten	2	0	3	0	0	1
5	Betasitosterol	4	1	0	0	0	2
6	Betulonic acid	4	0	1	0	0	2
7	Campesterol	4	0	3	1	1	1
8	Depomedrol	4	0	3	0	0	2
9	Friedeline	4	0	3	0	0	2
10	Kampferol	4	0	3	0	0	2
11	Leucoantho cyanidin	4	1	5	1	0	0
12	Leucopelargonin	3	0	3	1	1	2
13	Lupeol	3	0	4	0	0	1
14	Lupeal acetate	2	0	3	0	0	2
15	Marmesin	4	0	3	0	0	0
16	Mesoinositol	4	3	3	1	0	2
17	Oleanonic acid	3	0	3	0	0	0
18	Perlargonidin	4	3	1	1	0	2
19	Transcatechin	2	0	3	0	0	1
20	Protocatechuin	4	3	0	0	0	2
21	Psoralen	2	0	4	0	0	0
22	Racemosic acid	4	2	0	0	0	2
23	Rutin	4	2	0	0	0	2
24	Stigmasterin	4	3	0	0	0	2
25	Taraxasterol	3	0	3	0	0	2
26	Tiglic acid	4	0	3	0	0	0

Table 7: Predicted TOPKAT profile of Fr compounds

S. No.	Name	Aerobic biodegradability	Mutagenicity	DTP	Skin irritant	Carcinogenicity		
						Rodent	Female mouse	Male mouse
1	Acetylbetulinic acid	No	Non-Mutagen	No	No	No	No	No
2	Acetlyursolic acid	No	Non-Mutagen	No	No	No	No	No
3	α-Amyrin acetate	No	Non-Mutagen	No	No	No	No	No
4	Bergaptan	No	Non-Mutagen	No	No	No	No	No
5	Betasitosterol	No	Non-Mutagen	No	No	No	No	No
6	Betulonic acid	No	Non-Mutagen	No	No	No	No	No
7	Campesterol	No	Non-Mutagen	No	No	No	No	No
8	Depomedrol	No	Non-Mutagen	No	No	No	No	No
9	Friedeline	No	Non-Mutagen	No	No	No	No	No
10	Kampferol	No	Non-Mutagen	No	No	No	No	No
11	Leucoantho cyanidin	Yes	Non-Mutagen	No	No	No	No	No
12	Leucopelargonin	No	Non-Mutagen	No	No	No	No	No
13	Lupeol	Yes	Non-Mutagen	No	No	No	Yes	Yes
14	Lupeal acetate	Yes	Non-Mutagen	No	No	No	No	No
15	Marmesin	No	Non-Mutagen	No	No	No	No	No
16	Mesoinositol	No	Non-Mutagen	No	No	No	No	No
17	Oleanonic acid	No	Non-Mutagen	No	No	No	No	No
18	Perlargonidin	No	Non-Mutagen	No	No	No	No	No
19	Transcatechin	Yes	Non-Mutagen	No	No	No	No	No
20	Protocatechuin	Yes	Non-Mutagen	No	No	No	No	No
21	Psoralen	Yes	Mutagen	No	No	No	No	No
22	Racemosic acid	Yes	Mutagen	No	No	No	No	No
23	Rutin	Yes	Non-Mutagen	No	No	No	Yes	Yes
24	Stigmasterin	No	Non-Mutagen	No	No	No	No	No
25	Taraxasterol	No	Non-Mutagen	No	No	No	No	No
26	Tiglic acid	No	Non-Mutagen	No	No	No	No	No

Table 8: Docking of constituents of Fr to 1TUP C protein

Compound	Docking score (Kcal/mol)	Residues interacting	Distance	Integrations
Kampferol	**-14.87**	R248	2.99 A°	
		R273	2.69 A°	
		D281	4.24A°	
		V274	3.83A°	

Oleanonic acid	-9.67	R248 R273 C242	3.76 Aº 2.73 Aº 3.69Aº	
Marmesin	-8.95	R248 R273 N247 D281	3.03 Aº 3.07 Aº 3.30Aº 3.22Aº	
Transcatechin	-7.72	R248 R273 E285 R249 E271	2.07 Aº 2.89 Aº 2.93Aº 3.13Aº 3.58Aº	

DISCUSSION

Radiotherapy one of the treatments for cancer, faces a major drawback as it inevitably involves exposure of normal tissues to the deleterious effects of ionizing radiation. Damage to DNA and membrane lipids is the critical factors in radiation-induced cellular damage and reproductive cell death [22]. Herbal medicines have been gaining importance in radioprotective drug discovery owing to lesser side effects as reviewed extensively by many authors [23]. Protective effect of Fr extract and its constituents on radiation induced DNA damage was studied using *in vitro, in vivo* and *in silico* models. When pBR322 plasmid DNA exposed to 4 Gy EBR, super coiled form of DNA converted in to open circular form because of the induction of strand breaks in the DNA. Treatment with Fr extract with different concentrations before 1 hr of radiation exposure gives a dose dependent protection which can be explained by checking the depletion of oc form of DNA to sc form (fig 2). This result is similar to work reported by Nitin *et al.* [24], who showed that diethyldithicarbamate along with γ irradiation resulted in a significant protection of pBR322 DNA damage in a dose-dependent manner compared with control mice.

The alkaline comet assay is an elegant and effective technique to monitor the extent of DNA damage and its protection. When mice were exposed to EBR, the cellular DNA undergoes damage, as reflected by increase of the comet parameters like TL as it shows breaks in DNA and its density, similarly OTM as it indicates fragmentation of DNA and % T because it provides a quantitative measure of the damaged DNA when compared to control [25]. The presence of Fr extracts during EBR exposure showed reduction in comet parameters when compared to irradiated (table 3), indicating its significant role in radioprotection.

QSAR a broadly used tool for developing relationships between the activities and properties of a series of molecules with their structural properties. The QSAR of constituents of Fr was assayed using Discovery Studio 2.5. The analyses showed statistical values for the best models as represented in (table 5). The model when validated using LOF method showed a cross-validated correlation coefficient (q^2) value of 0.992, and a good predictive value (adj r^2, external validation) of 0.998. In this QSAR model, 99.5% of the variance in biological activity was predicted, as indicated by r^2 values of 0.995 multiplied by 100. The QSAR model equation revealed the relationship between experimental activity (i.e. the inhibitory concentration to 50% of the population [pIC_{50}]) as the dependent variable and chemical descriptors as independent variables. Thus, in the present study the QSAR experiment successfully developed a GFA mathematical model for drug activity prediction. This finding was supported by Sarfaraz *et al.* [26] where similar work was carried out on xanthone derivatives for anticancer activity.

Prediction of ADMET properties plays an important role in the drug design process. ADMET is applied at an early phase of drug development process in order to remove the molecules with poor ADMET properties [27]. According to Venkataramana *et al.* [28], aqueous solubility of the compounds helps to predict the percentage

of solubility of drugs in water. Based on the results obtained, the compounds may have good solubility so that they can have complete oral absorption for effective dosage. Reddy *et al.* deduced that blood brain penetration level shows the penetrating efficacy of the compound towards the brain and from the results it is observed that all compounds were fallen outside the 99% ellipse. Hence the compounds may not be able to penetrate the blood brain barrier. So, the chances of central nervous system side effects are low or absent. BBB scores of compounds have shown variable penetrating efficacy. Kampferol, Marmesin, Oleanonic acid, Friedeline scored 0 which implies that these compounds do not inhibit CYP2D6 enzymes when they undergo metabolism via cytochromep450. According to Durga *et al.* [29], PPB level shows whether the compound binds to carrier proteins in the blood [30]. The compounds of Fr showed to have good binding capacity to cross the membrane and bind to plasma protein. Hepatotoxicity level predicts organ toxicity of the molecule and it falls in two levels; zero for non-toxic and one for toxic. Based on these tested levels, the results suggest that the compounds were non-toxic and can be used for further studies.

During radiation induced DNA damage or oxidative stress caused by radiation, transcription factor p53 is activated and stabilized. Consequently, p53 up-regulates the gene expression that facilitates apoptosis or leads to genomic instability [31]. Mutations induced by radiation in p53 protein leads to conformational changes of the protein and also cause loss of its function. Further loss of its apoptotic function can lead to development of radio and drug resistant cancer cells and in turn to severe side effect to normal tissue surrounding cancerous tissue [32].

Hence the protection of normal tissues from radiation damage is of primary importance. Small molecules such as PRIMA-1, RITA, and pifitrin-α, changes the sterical conformation of mutated p53 back to the conformation of wild-type p53 [33]. A novel concept is targeting tumor suppressor p53 as a key player of apoptosis to reach radiosensitization of tumors and in turn radioprotection of normal tissues. Hence inhibition of the function of wild-type p53 using Fr constituents may suppress induction of apoptosis in normal tissues by targeting ARG 248 and ARG 273 and hence, may prevent radiotherapy associated side effects in cancer patients and protect the normal cells damage.

CONCLUSION

The results indicate that the presence of Fr ethanol extract during EBR protected pBR322 DNA damage and even damage of lymphocyte cells of mice. In depth the compounds of Fr mainly Kampferol, Marmesin, Oleanonic acid, Friedeline, Depomedrol and Betulonic acid obtained from literature significantly interacted and inhibited p53 protein thereby inhibiting the process of apoptosis The present studies may be helpful in offering promising leads towards development of radioprotectors in turn protecting the normal tissue from radiation damage.

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ABBREVIATION

Fr: *Ficus racemosa*, EBR: Electron beam radiation, %T: Percent of DNA in tail, TL: Tail length, OTM: Olive tail moment, ADMET: Absorption, Distribution, Metabolism, Excretion and Toxicity, QSAR: Quantitative Structure Activity Relationship, QSTR: Quantitative Structure Toxicity Relationship, GFA: Genetic function approximation, PPB: Plasma protein binding, BBB: Blood Brain-Barrier, DTP: Developmental Toxicity Potential, OC: Open Circular, SC: Super Coiled

CONFLICT OF INTERESTS

Declared None

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