Research Article

In vitro, In vivo and In silico Radioprotective Activity of Ethanol and Aqueous Extracts of Pterocarpus Marsupium

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ABSTRACT

Development of radioprotective agents has been a subject of intense research to alleviate the aftermath of exposure to radiation. The aim of the study was to determine the radioprotective potentials of the ethanol (EtPm) and aqueous (AqPm) extracts of *Pterocarpus marsupium* (Pm). *In vitro* experiments on protection of DNA damage by EtPm and AqPm extracts against electron beam (EB) radiation was carried out on pBR322 plasmid DNA. Comparatively EtPm significantly ($P < 0.05$) showed good protection at low dose rate. Swiss Albino mice treated orally with different concentrations of EtPm and AqPm extracts, exposed to 6 Gy were screened for *in vivo* radio-protective effects using comet assay. Significant reduction of comet parameter in mice lymphocyte cells were observed in both the extract treated mice, but when compared to AqPm, EtPm exerted good protection. *In silico* experiment on high throughput screening of the constituents of Pm was done by 2D quantitative structure activity relationship (QSAR) analysis using 20 compounds of Pm, various geometric, lipophilic, electronic and spatial descriptors were correlated with radioprotective activity using genetic function approximation (GFA). ADMET properties was analysed using Accelrys Discovery Studio and Molecular docking studies by means of FlexX tool. The compounds revealed statistically significant structural activity and most of the compounds were not toxic in nature. The constituents eventually inhibited p53 protein by binding to Arginine 248 and Arginine 273 amino acid. The results demonstrate that Pm can protect DNA from radiation-induced damage *in vitro, in vivo* and *in silico* without recognizable toxic effects.

Keywords: 2D QSAR; ADMET; GFA; FlexX; *Pterocarpus marsupium;* EB radiation; Radioprotective activity

INTRODUCTION

Exposure of cellular component to ionizing radiation inflicts deleterious effect on living system through the generation of superoxide anion, hydroxyl radicals, and hydrogen peroxide, which are known as radical oxygen species (ROS). These ROS are mainly produced in cellular respiration which on accumulation in the body can lead to cumulative damage of bio-molecules such as proteins, lipids and DNA, resulting in oxidative stress[1]. Although almost all organisms possess antioxidant defense mechanism and repair systems to protect them from oxidative damage, in some high extents these systems are insufficient to entirely prevent such damage[2]. Damage to DNA caused by radiation leads to activation of tumor suppressor p53 protein. This protein acts as a DNA sequence-specific transcription factor for regulating and activating the expression of a wide range of target genes in response to genotoxicity stress and then leads to apoptosis[3]. The amino acids like Arg248 and Arg273 are the main residues of p53 protein that interact with DNA[4] and hence suppressions of these two residues are proposed to be responsible for inhibition of the p53-DNA binding integrations upon radiation to avoid apoptosis. Due to the increased use of ionizing radiation in radiotherapy, there is a need to develop an effective and non-toxic radioprotector to minimize its adverse effects.

In radiotherapy of cancer, normal tissues need to be protected while cancers are exposed to high doses of radiation. A large number of compounds, natural and synthetic, have been evaluated for this purpose[5]. However, most of them failed clinically because of toxicity and side effects. Hence search for an ideal radioprotector is a compelling urgency.

Pterocarpus marsupium Roxb (Leguminosae) one of the large deciduous trees commonly found in hilly regions of India, especially in Deccan Peninsula. It is distributed in Karnataka, Bihar, Gujarat, Madhya Pradesh, Uttar Pradesh, Orissa and Tamil Nadu. Various parts of this plant are widely used in traditional ayurvedic medicine for the treatment of diabetes[6] mellitus and other disease. The gum obtained from the stem is used as astringent, in diarrhoea and for toothache; the leaves are useful as external applications for boils, sores and skin diseases[7]. The flavonoid constituents, marsupin, pterosupin and liquiritigenin, isolated from the heart wood of the plant are studied for its anti-cancerous property by Raimando.[8] Traditionally it is difficult to select the best chemical moiety of compound that plays an effective role as radioprotectors, so we used computational strategies that include quantitative structure activity relationship (QSAR) modelling, ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) studies and

Molecular Docking studies to specifically identify potential compounds from Pm that could act as radioprotector. In this study evaluation of radioprotective activity of EtPm and AqPm against EB radiation induced DNA damage *in vitro*, *in vivo* and *in silico* was carried out.

MATERIALS AND METHODS

Chemicals

pBR322 plasmid DNA was purchased from Bangalore Genei. Low melting agarose, high melting agarose, TRIS base, disodium EDTA, TritonX-100, sodium sarcosinate, DMSO and propidium iodide for performing comet assay were obtained from Sigma Aldrich (St. Louis, Missouri).

Plant Material Collection and Extraction

Stem bark of Pm was collected from Western Ghats region, Karnataka. The plant was identified and authenticated by the expert Taxonomist, Department of Botany, Mangalore University, India. The material was shade dried for 6-8 weeks and powdered. The powdered material was defatted with petroleum ether and extracted by refluxing with ethanol in soxhlet apparatus for 48 hrs. Simultaneously aqueous extract was prepared by boiling stem bark powder in distilled water for 30 min, kept for 3 days with intermittent shaking and filtered to remove insolubles. Both the filtered extracts were concentrated in vacuum using rotary evaporator at 35°C. Solvents leftover were completely removed on water bath, dried and used for further analysis.

Estimation of DNA damage *in vitro*

pBR322 plasmid DNA was used to study the protective effect of extract against EB radiation induced DNA damage, at Microtron centre, Mangalore University, Mangalore, Karnataka, India, at a dose rate of 72 Gy/min. The vials containing pBR322 plasmid DNA (250ng in 0.01M sodium phosphate buffer) was divided into 10 different tests. Vial I served as normal control (i.e. only DNA), vial II exposed to EB radiation at 4Gy served as irradiation control, wherein intensity of EB radiation was standardized by exposing DNA to different dose of EB radiation (i.e. 2Gy, 4Gy, 6Gy and 8Gy) which showed damage from 4Gy onwards, vial III, IV, V and VI were treated with (50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml) of EtPm and vial VII, VIII, IX and X were treated with same concentrations of AqPm. All the vials were exposed to EB radiation, and then the irradiated samples were loaded on 1% agarose gel and electrophoresed with TAE buffer (pH 8) at 100 V for 50min. The gel was stained with ethidium bromide and documented using UV transilluminator.

Estimation of DNA damage studies *in vivo*

Animals

The animal study was conducted on Swiss albino mice aged 10-12 weeks old (30±5g) chosen from animal house of Justice K.S. Hegde Medical College, Deralakatte, Mangalore. Animal care and handling was carried out according to the guidelines set by World Health Organization; Geneva, Switzerland. The studies were performed in accordance with the institutional animal ethical committee. Animals were randomly divided into 8 groups of 6 animals each, group I: Served as a control, fed with normal diet and double distilled water, group II: Fed with normal diet and exposed to EB radiation, group III, IV, V: Administered with EtPm at 100, 200 and 400mg/kg body weight respectively. Group IV, VII and VIII: Administered with AqPm at 100, 200, 400 mg/kg body weight, respectively, once daily for 15 consecutive days. On the 16th day, all the group of mice except control were exposed to 6Gy EB radiation[9], at Microtron centre, Mangalore University, Mangalore, Karnataka, India. After radiation all the groups of animals were observed for 15 days and they were sacrificed by cervical dislocation on the 16th day. The blood was drawn and the lymphocytes were separated using histopaque.

Estimation of *in vivo* DNA damage by Comet Assay

The comet assay was conducted under alkali conditions according to the procedure followed by Madhu[10]. The quantification of the DNA strand breaks of the stored images was performed using CASP software[11] by which the percentage of DNA in the tail (% T), tail length (TL) and olive tail moment (OTM) could be obtained directly.

In silico Studies

QSAR Modeling

QSAR studies were performed using Discovery Studio 3.5, a commercial tool performed on windows 7-64 bits system. The data set of 20 compounds from Pm was retrieved from literatures[12-14]. From the original data set of 20 compounds Table 1, first 14 compounds were selected as members of the training set for QSAR model development, and the remaining 6 compounds were considered as members of the test set for external validation.

Chemical structures were drawn using ChemSketch tool (http://www.acdlabs.com) and molecular properties of compounds were retrieved from the Pubchem database (http://pubchem.ncbi.nlm.nih.gov/). The half maximal inhibitory concentration values (IC_{50}) of the compounds tabulated, converted to the corresponding pIC_{50} ($-logIC_{50}$) and used as dependent variables in the QSAR investigations as represented in Tables 1. It is necessary to select numerical descriptors for a set of molecule in order to build QSAR models.
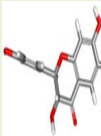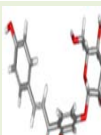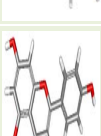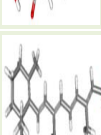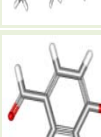
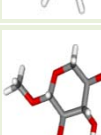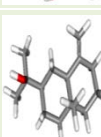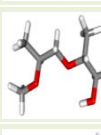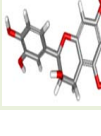A descriptor can be a quantitative property that depends on the structure of the molecule. In this study totally 5 descriptors such as LogP, Molecular Properties, Molecular Property Counts, and Surface Area Volume and Topological descriptors were used as input molecular properties that could describe the molecules[15,16].
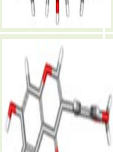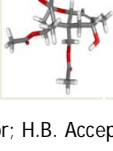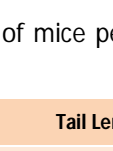
These descriptors were used for generating the set of molecular properties with the help of calculate molecular properties protocol under QSAR protocol cluster. Genetic

Function Approximation Algorithm was used for generating QSAR models, in GFA[17], pIC50 was considered as dependent variable and descriptors were considered as independent variables of molecule.

Table 1: Structure, molecular properties and IC50 values of compounds of Pm
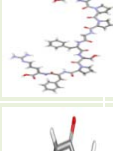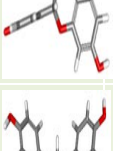
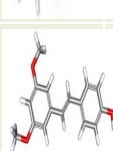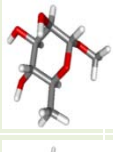| S. No	Name	Accession No	Structure	IC50	pIC50	Molecular formula	Molecular weight g/mol	H.B. donor	H.B. Acceptor	log p	TPSA	N atoms
1	Carpusin	CID 134369		100	4.00	$C_{16}H_{14}O_6$	302.27	2	3	2	96.2	22
2	Garbanzol	CID 442410		18.2	4.73	$C_{15}H_{12}O_5$	272.25	3	4	1.6	87	20
3	Isoliquiritigenin	CID 25245575		0.43	6.36	$C_{21}H_{22}O_9$	418.39	6	9	1.7	77.8	30
4	Pterosupin	CID 133775		0.012	6.90	$C_{21}H_{24}O_{10}$	436.40	8	10	-0.6	188	31
5	4',5,7-Trihydroxyflavone	CID 5280443		0.7	6.15	$C_{15}H_{10}O_5$	270.23	3	5	1.7	87	20
6	Tretinoin	CID 444795		70	4.15	$C_{20}H_{28}O_2$	300.43	1	2	6.3	37.3	22
7	4-Hydroxybenzaldehyde	CID 126		16.5	4.78	$C_7H_6O_2$	122.12	1	2	1.4	37.299	9.0
8	Arbinopyranoside	CID 99057		0.39	6.40	$C_6H_{12}O_5$	164.15	3	5	-2	69.2	11
9	Beta-eudesmol	CID 91457		1.49	5.82	$C_{15}H_{26}O$	222.36	1	1	3.7	20.228	16.0
10	Dipropyleneglycolmonomethyl ether	CID 25485		100	4.00	$C_7H_{16}O_3$	148.20	1	3	0.1	38.696	10.0
11	Epicatechin	CID 72276		1.59	5.79	$C_{15}H_{14}O_6$	290.26	5	6	0.4	110.374	21.0

12	Erythrodiol	CID 101761		14	4.85	$C_{30}H_{50}O_2$	442.71	2	3	7.6	40.5	32
13	Ethanedione	CID 123072		0.23	6.63	$C_{14}H_8F_2O_2$	246.20	0	4	3.6	34.1	18
14	Kinoin	CID 123643		50	5.16	$C_{59}H_{89}N_{17}O_{14}$	1260.44	17	34	-5	497	90
15	Liquirtigenin	CID 114829		0.32	6.49	$C_{15}H_{12}O_4$	256.25	4	1	2.2	66.8	19
16	Propterol	CID 185124		12.5	4.90	$C_{15}H_{16}O_3$	244.28	3	3	2.6	60.7	18
17	Pseudobaptigenin	CID 5281805		0.34	6.46	$C_{16}H_{10}O_5$	282.24	1	5	2.6	65	21
18	Pterostilbene	CID 5281727		200	4.69	$C_{16}H_{16}O_3$	256.29	1	3	3.8	38.7	19
19	Rhamnopyranoside	CID 84695		6.5	5.18	$C_7H_{14}O_5$	178.18	3	5	-1.6	79.2	12
20	Sesquiterpene	CID 72650		150	4.82	$C_{19}H_{26}O_8$	382.40	2	8	-0.9	115	27

Foot Note: H.B. Donor: Hydrogen Bond donor; H.B. Acceptor: Hydrogen Bond Acceptor; Log P: Partition coefficient; TPSA: Topological Polar Surface Area; N atoms: Heavy atoms.

Table 2: Protection of cellular DNA of mice peripheral blood lymphocytes exposed to EB radiation in the presence of EtPm and AqPm extracts.

Groups	Tail Length	%T	OTM
Control	7.3 ± 1.61	0.60 ± 0.066	0.52 ± 0.181
Irradiated control	234.20 ± 1.4	39.62 ± 1.64	16.623 ± 1.639
EtPm 100 mg/kg	140.18 ± 1.326	20.61 ± 1.992	10.98 ± 0.75
EtPm 200 mg/kg	134.17 ± 1.004**	16.13 ± 1.167*	7.14 ± 0.095**
EtPm 400 mg/kg	97.51 ± 1.118***	12.29 ± 1.396***	7.13 ± 0.527***
AqPm 100mg/kg	165.80 ± 1.624	32.31 ± 1.233	13. 07 ± 0.821
AqPm 200 mg/kg	143.21 ± 1.125**	25.48 ± 1.217*	8.73 ± 0.190**
AqPm 400 mg/kg	136.76 ± 1.041*	17.34 ± 1.613**	7.51 ± 0.935***

Foot note: Values were reported as mean ± S.E.M. for group of six animals. The data was analyzed by Prism 3 software. Asterisks indicated statistically significant values when compared to radiation, *p<0.05, **p<0.01, ***p<0.001.
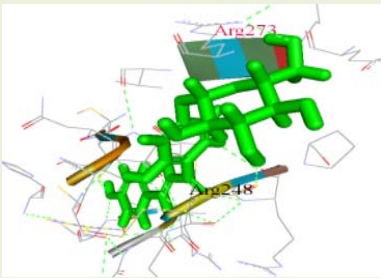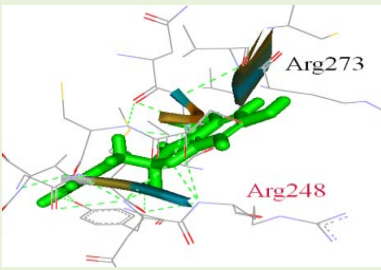
Table 3: Statistical analysis of training set compounds of Pm.

	Friedman LOF	R–square	Adjusted R–square	Cross validated R–squared	Significant Regression	Significance of regression F–value	Critical SOR F–value (95%)	Lack of fit points	Min. expt. error for non significant LOF (95%)
Equation 1	0.051	0.994	0.987	0.900	Yes	92.68	2.56	22	0.091

Table 4: Predicted ADME and TOPKAT profiles of Pm compounds.

ADME Profile								
S. No	Name	BBB Level	Absorption Level	Solubility Level	Hepatotoxicity Probability	CYP2D6 Probability	PPB Level	AlogP98	ADMET PSA_2D
1	Carpusin	4	1	3	0.898	0.205	2	3.21	141.63
2	Garbanzol	4	0	2	0.961	0.237	2	3.53	134.60
3	Isoliquiritigenin	4	0	3	0.941	0.148	2	4.92	114.58
4	Pterosupin	4	0	3	0.973	0.133	2	4.96	171.67
5	4',5,7-Trihydroxyflavone	4	0		0.921	0.223	1	4.07	120.18
6	Tretinoin	4	1	3	0.841	0.237	0	3.46	160.05
7	4-Hydroxybenzaldehyde	4	0	3	0.947	0.192	0	4.41	188.67
8	Arbinopyranoside	2	0	3	0.980	0.203	0	4.39	164.90
9	Beta-eudesmol	4	0	3	0.894	0.134	0	4.02	143.00
10	Dipropyleneglycol monomethyl ether	4	1		0.973	0.128	1	4.45	156.07
11	Epicatechin	3	0	3	0.961	0.105	0	4.23	197.60
12	Erythrodiol	3	0	3	0.986	0.257	2	4.61	167.86
13	Ethanedione	3	0	3	0.921	0.143	0	4.99	162.44
14	Kinoin	4	0	2	0.962	0.168	0	3.91	120.81
15	Liquirtigenin	4	0	2	0.960	0.128	0	4.53	148.31
16	Propterol	4	0	4	0.985	0.179	2	4.34	138.11
17	Pseudobaptigenin	4	1	5	0.966	0.129	0	4.59	180.30
18	Pterostilbene	4	0	2	0.902	0.168	0	4.71	120.81
19	Rhamnopyranoside	3	0	2	0.960	0.237	0	4.09	119.45
20	Sesquiterpene	3	0	5	0.852	0.159	0	4.13	138.67

Table 5: Docking score of the p53 protein with respective constituents of Pm along with interacting residues (Arg248 and Arg273)

SI No	Compound	Docking Score	Residues Interacting	Distance	Interactions
1	Isoliquiritigenin	-12.27	R248 R273 R249 N247	2.39 A° 2.89 A° 3.42 A° 3.04 Ao	
2	Carpusin	-11.62	R248 R273 V274 N247 R249	2.62 A° 4.48 A° 2.43 A° 3.21 A° 4.07 A°	

Pharmacokinetics Parameters

ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of the molecules were predicted using Discovery Studio 3.5 (Accelrys). In this module, six mathematical models such as aqueous solubility, blood–brain barrier penetration (BBB), cytochromeP450 2D6 (CYP2D6) inhibition, hepatotoxicity, human intestinal absorption, and plasma protein binding were used to quantitatively predict ADMET characteristics of the chemical structure of the molecules[18].

The toxicity profile of the compounds were 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

Preparation of Protein and Ligand Structure

The 3D crystal structure of p53 protein (chain C) (PDB ID: 1TUP-C) for conducting Molecular docking studies was downloaded from the Protein Data Bank (http://www.rcsb.org/pdb/files/1TUP.pdb). Before docking, all the water molecules were removed and H-atom were added to protein file for correct ionization and tautomeric states of amino acid residues such as aspartic acid, serine, glutamine, arginine and histidine[20]. A dataset of first 14 compounds represented in Table 1 were used as lead compounds for docking studies.

Determination of Active Site and Molecular Docking Studies

Q site finder server was used for the identification of the most potential active site on p53 protein where the ligand can bind and interact[21]. The docking of the prepared ligands with p53 protein receptor was determined using Biosolve-IT FlexX a commercial tool, to identify active potential drug[22].

Statistical Analysis

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

RESULTS AND DISCUSSION

Effect of Pm extracts on EB radiation induced DNA Damage

Protecting cellular DNA during radiotherapy might result in prevention of normal cell damage surrounding cancerous cell[23]. When pBR322 DNA was exposed to 4 Gy dose of EBR, the covalently super coiled (sc) form DNA disappeared and the intensity of open circular (oc) form of the plasmid DNA increased Figure 1 lane 2 because of the induction of strand breaks in the DNA as worked and

observed by Wei and Hinterman[24,25]. Pre-treatment of plasmid pBR322 DNA for 1hr with different dose of EtPm (Lane 3-6) and AqPm (Lane 7-10) before 1hr of radiation exposure gives a dose dependent protection by decreasing the intensity of oc form of DNA and increase in intensity of sc form band. Both EtPm and AqPm treated group rendered good protection to DNA when exposed to EB radiation.

The EtPm extract showed excellent protection even at 50µg concentration Figure 1 lane 3 compared to all other concentrations of AqPm Figure 1 lane 8. From these results it can be deduced that EtPm was able to inhibit DNA damage more effectively at lower concentration when compared to AqPm. This may be due to possible involvement of some endogenous cellular components present in ethanol extract[26].
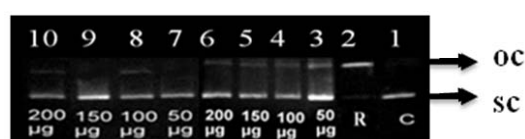
C: Normal Control, **R**: Radiated control, **OS**: Open circular form, **SC**: Super coiled form

Figure 1: Protection of plasmid pBR322 against EBR induced strand breaks by crude extract of Pm at 50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml

Effect of Pm extracts on EB radiation induced DNA Damage by Comet Assay

The exposure of control mice compared to treated EB radiation increased comet parameters like TL, OTM and %T of blood lymphocytes as damaged cell takes the appearance of a comet, with head and tail regions, because DNA in cells undergoing damage is cleaved in to fragments. By CASP software, a variety of geometric and densitometry parameters are provided, which estimates the amount of DNA in the head and tail regions and even the extent of migration into the tail region. As the TL and density reflects the number of single-strand breaks in the DNA, the percentage of DNA in the tail provides a quantitative measure of the damaged DNA[27,28]. It is considered that a bimodal distribution with very low and very high comet parameters within a population of cells indicates the onset of radiation effect on DNA damage[29].

No significant changes were observed in lymphocytes cells of unirradiated animals (control) as determined by scoring comet parameters like %T, OTM and TL. In mice exposed to 6 Gy (irradiated), the comet parameters significantly increased, when compared to control group i.e. the TL increased from 7.3±1.61 to 234.20±1.4, %T extended from 0.60±0.066 to 39.62±1.64 and OTM from 0.52±0.181 to 16.623±1.639 respectively. Significant decrease in comet parameter was observed in EtPm treated groups at different concentrations as presented in Table 2. Especially at 400mg/kg body weight EtPm showed good protection when compare to other concentrations.

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Similarly there was also significant reduction (P< 0.05) of DNA damage observed in mice administered with AqPm at 400 mg/kg body weight. Comparatively EtPm treated mice have effective protection when compared to AqPm. It may be attributed to phytoconstituents in the ethanol extract that combats against free radical mediated degradation to the deoxyribose sugar moiety of DNA[30].

QSAR Studies

The structure activity relationship denoted by the QSAR model yielded a high activity descriptors relationship accuracy of 99.4% referred by regression coefficient (r^2 =0.994) and a high activity prediction accuracy of 98%. Several QSAR models were generated for the compounds using GFA, among those the best QSAR model was selected on the basis of statistical parameters like r^2 (square of correlation coefficient for training set of compounds), q^2 (cross-validated r^2), and pred-r^2 (predictive r^2 for the test set of compounds). All QSAR models were validated and tested for its predictability using external test set compounds. The analysis showed statistically best model for the compounds were represented in Table 3. The model when validated using LOF method showed a cross-validated correlation coefficient (q^2) value of 0.994, and a good predictive value (adj r^2, external validation) of 0.987. In this QSAR model, 99.4% of the variance in biological activity was predicted, as indicated by r^2 value multiplied by 100. The graphical representation of the results was represented in Figure 2.

From the equation below we can deduce that among the 2D descriptors, Molecular weight, angle energy and aromatic rings group showed good correlation (as + sign indicate positive correlation) of the molecule structure with its biological activity. Although the classical 2D QSAR model provided some useful information and showed a good predictive ability, the topological descriptors convey little information, on which moieties are particularly important to inhibit p53 protein.
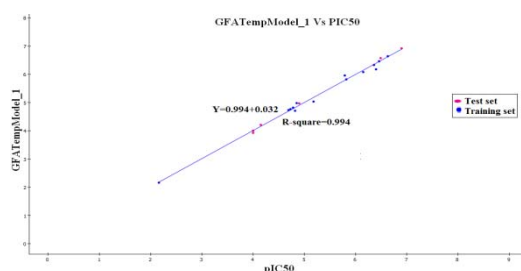
Equation: GFATempModel_1 =

2.1796 + 0.2131 * ALogP – 0.1924 * Molecular Fractional Polar Surface Area – 0.093034 * Molecular Weight – 0.1447 * Num6_H_Donors + 0.00257546 * Molecular_Weight * HBA_Count – 0.33038 * Num_RotatableBonds + 121.165 * AngleEnergy * Num_Rings 6 + 0.76181 * Num_Aromatic Rings.

Figure 2: Predicted versus observed values of training and teat set compounds.

Pharmacokinetic Properties

ADMET properties of all designed Pm compounds were predicted and compared with predicted ADMET properties of standard compounds using DS, Accelrys software. ADMET prediction was used to screen for sorting out those compounds represented in Table 1 that already followed Lipinski's rule of 5. In the present work, all the compounds were fallen outside the 99% ellipse as the values of most of the compounds was 4 except arbinopyranoside (2). Hence the compounds may not be able to penetrate the BBB, so the chances of CNS side effects are low or absent[31]. Intestinal absorption levels of most of the compounds were 3 and 4 which infers that the constituents are expected to posse's good human intestinal absorption[32]. ADME aqueous solubility logarithmic level of most of the compounds was found to be 2, 1 or 0 which indicates very little aqueous solubility[33]. The CYP2D6 score predicts the inhibitory and non-inhibitory character of the given query chemical structure on CytochromeP450 2D6 enzyme. The closer the CYP2D6 scores to either 0 or 1, the more closely the predictions agree with each other. All compounds were predicted as non-inhibitors of CYP2D6. The ADME CYP2D6 probability values of all the compounds lies in the range of 0.1-0.23 so the results are reliable. Hence the side effects (i.e., liver dysfunction) are not expected upon administration of these compounds as represented in Table 4. The hepatotoxicity score predicts the hepatotoxic nature of the chemical compounds. The score of hepatotoxicity probability of all the compounds infer non-toxic properties. The pin plot analysis was represented in Figure 3. TOPKAT studies predicted the aerobic biodegradability, mutagenicity, developmental toxicity potential (DTP), skin irritant and carcinogenicity of the compounds of Pm. From toxicity studies it was found that most of the ligands were non-mutagenic except arbinopyranoside and ethanedione; rhamnopyranoside and dipropylene glycol monomethyl ether showed carcinogenicity. Hence the finding reveals that most of the compounds were non-mutagenic, non-carcinogenic, non-toxic and non-irritant. The similar TOPKAT parameters were observed by Venkataramana.
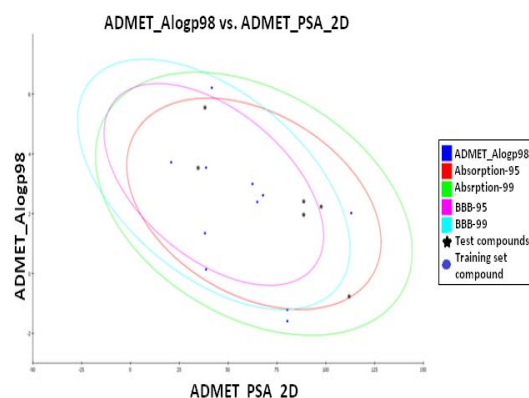

Figure 3: Graphical representation of ADMET properties of Pm.

Molecular Docking Studies

Binding Site Prediction of p53 Protein

The active pocket residues for p53-DNA binding domain are Lys132, Asn239, Ser240, Ser241, Cys242, Asn247, Arg248, Arg249, Pro250, Glu271, Val272, Arg273, Val274, Cys275, Ala276, Asp281, and Glu285 identified using the interaction energy between the protein and simple Vander Waals probe to locate energetically favorable binding sites. Especially Arg248 and Arg278 are the residues mainly responsible for binding of p53 to DNA, so blocking the residues to interact with DNA give radioprotection[34].

Protein-ligand Docking

The docking of phytoconstituents of Pm to p53 protein reveal that the stretch of amino acid residues from Lys132 to Glu285 broadly interact with all 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 using 1TUP C as the receptor. The output of all the ligands were given by energy values in kcal/mol. Least the energy values strongest is the interaction[35], among 20 ligands, isoliquiterigenin inhibited p53 protein strongly with binding score -12.27 followed by carpusin with score of -11.62. The compounds interaction to p53 protein along with docking score was represented in the Table 5.

CONCLUSION

From the obtained results it can be concluded that the presence of EtPm and AqPm extract during EB radiation protected DNA damage *in vivo* and *in vitro*. In this study we have designed a set of 20 novel molecules of Pm and performed docking simulations in order to identify best ligand to inhibit p53 protein and tested for QSAR, ADME and toxicity profiles using *in silico* tools.

Among 20 compounds isoliquiritigenin has shown best dock score of -12.27 followed by carpusin -11.62 with better ADMET profiles. Binding energies in the protein ligand interactions explains how ligand fits with target protein. Examination of the binding interactions of the ligands helps in elucidating the reasonable and appropriate structural features of ligand which increase the binding affinity and therapeutic efficacy. Hence through *in silico* studies it may be concluded that isoliquiritigenin can be used as a novel drug as radioprotector for protecting DNA from radiation.

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