International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (2): 1260-1265
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved
UGC Approved Journal

In Vivo Anti Cancer Activity of Novel 2-Phenyl-1-Benzofuranone Derivatives on Sarcoma Induced Mice by Histopathological Examination

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Received: 12 Jan 2019 / Accepted: 11 Mar 2018 / Published online: 1 Apr 2019
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Abstract
The newly synthesized compounds are being tested for *in vitro* anti-cancer activity. Method used for In Vitro testing is Sulforhodamine B assay also known SRB assay. The best resulted compounds were tested for In Vivo studies in sarcoma induced mice. Sarcoma induced to mice by using direct induction ascites to the mice. Doxorubicin was the standard drug for the comparison results of the synthesized drugs. The obtained samples were examined hematoxylin with eosin staining and under rotary microscope. Samples PG1A & HLVC exhibited optimized results.

Keywords
Sarcoma, Histopathology, eosin staining.

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INTRODUCTION:
Sarcoma are tumours at connecting the tissues and the body organs.[1] There are about 50 types of soft tissue sarcoma. Pain may occur depending on the location and organs nearby.[2] They can occur at any age, with a median age at presentation of 65 but can affect young people, including children.[3] The histopathology is the study of normal/infected tissue under microscope.[4] The tissue that is studied comes from a biopsy or surgical procedure whereby a sample of the suspect tissue is selected and sent to the laboratory. It is then processed and cut into very thin layers (called sections), stained, and examined under microscopes to characterize the details of the cells in the tissue. Histopathology reports on surgical cancer specimens are getting more and more complex. They may include: The microscopic appearance of the involved tissue, Special stains, Molecular techniques, Other tests.[5-7]

METHODOLOGY:
In vivo screening of the of selected drugs:
Swiss albino mice were collected from Sanzyme laboratories, Hyderabad, Telangana, India. Animals were maintained at room temperature 23-25°C with a maintenance fresh air of 100% quality and humidity of 50-60%. The mice were store in polycarbonate containers with meshed on the top were fed with pelleted feed (M/s Sanzyme industries Hyderabad) and autoclaved water.
Selection of Mice:
Mice of same sex, strain, healthy of 18-23 gm with disease free condition are selected for the experiments. The procedures selected for

experiments are approved by institutional animals ethics committee.

Grouping of Animals:

The tumour infected animals were divided in to different as per treatment procedure. Two other groups included control group with normal saline (0.2ml/animal) and standard group of doxurubicin (20mg/kg).

Group I Disease control group:

The mice in this group are injected with a normal saline (0.2ml/animal) daily of n=3.

Group II Standard group:

The tumour infected animal in this group are injected with a doxurubicin (200mg/kg) daily of n=3.

Group III Sample dosing:

The tumour infected animal in this group are injected with a samples **IA, IB, ID, IE, IF, IIB, IIC, IIIA, IVB, IVF, VA, VC, VD, VE.** (100mg/kg) daily of n=3/each samples.

Group IV Sample dosing:

The tumour infected animal in this group are injected with a samples **IA, IB, ID, IE, IF, IIB, IIC, IIIA, IVB, IVF, VA, VC, VD, VE.** (200mg/kg) daily of n=3/each samples.

Group V Sarcoma 180:

The tumour infected animal in this group are of n=3/each samples.

Day 13 The shortest and longest diameters of tumors were measured with a vernier caliper and the tumor volume was determined according to established procedure: Percent inhibition of tumor:

Body weight and tumour volume analysis:

Body weights of the experimental mice were recorded in all the groups at day 0 and on every day during the protocol. % increase in body weight was calculated daily.[8]

$$\% \text{ Increase in Body weight} = \frac{\text{Body wt. of animal on } 15^{th} \text{ day - Body wt. of animal on 0 day}}{\text{Body wt. of animal on 0 day}} \times 100$$

Tumor volume = (length × width2) /2, where length and width are in centimetres.

Haematology parameters

Blood was collected from the retro-orbital plexus of animals into di-potassium EDTA coated vacutainers and analysed using a veterinary blood cell counter (PCE-210 VET, ERMA Inc., Tokyo, Japan).[9]

Histopathology

Haematoxylin and eosin staining

Mammary tissues were fixed in 10% neutral buffered formalin (NBF), dehydrated with alcohol and cleared thoroughly using xylene. The tissues were impregnated with paraffin wax and 5 µm sections were cut using a rotary microtome (RM2245, Leica Microsystems GmbH, Wetzlar, Germany), mounted on slides, de-waxed with xylene followed by rehydration through graded series of alcohol. The tissues were then stained with haematoxylin and eosin. The slides were observed under a microscope, analysed by a pathologist who was blinded to the samples, and photographs taken. The slides were observed for the change in the tubular–alveolar pattern of the mammary gland for identification of the type of carcinoma, infiltration of immune cells; necrosis and haemorrhage.[10]

RESULTS

Table No 1: *In Vivo* studies results of the selected drugs of 100mg/kg

S.No	Treatments	Dose (mg/Kg)	Body weight (g) Day 1	Body weight (g) Day 5	Body weight (g) Day 12	Weight of tumour (g)	Volume of ascitic fluid (ml)	Number of tumour cells (X107)	Tumour Growth inhibition	Mortality
1	Normal Saline	0.2ml	2.20 ± 0.49	24.00 ± 0.55	29.60 ± 0.93	8.74 ± 0.52	8.92 ± 0.51	686.79 ± 54.40	0	0
2	Doxorubicin	20	21.80 ± 0.58	21.20 ± 0.92	18.00 ± 1.82	0.52 ± 0.24	0.50 ± 0.22	14.00 ± 5.74	97.96	0
3	PG 1A 100mg	100	22.98±0.23	22.18±0.45	18.76±0.24	2.09±0.65	1.76±0.82	77.87±18.76	89.97	13.28
4	PG 1B 100mg	100	22.98±0.03	21.87±0.23	18.28±0.34	0.54±0.56	0.84±0.21	5.09±27.89	56.72	17.87
5	PG 1D 100mg	100	20.98±0.98	22.98±0.42	21.24±0.39	8.08±0.43	8.17±0.13	218.98±13.28	19.82	16.58
6	PG 1E 100mg	100	19.87±0.04	18.29±0.45	22.76±0.98	7.98±0.26	7.88±0.22	187.91±19.87	34.47	18.34
7	PG 1F 100mg	100	18.87±0.12	19.87±0.76	21.34±0.18	3.16±0.43	3.16±0.17	68.98±28.76	87.76	19.54
8	RS 1B 100mg	100	21.27±0.23	21.19±0.87	18.92±0.12	2.13±0.32	1.78±0.19	76.34±19.83	87.65	18.54
9	RS 1C 100mg	100	22.09±0.87	24.98±0.98	22.34±0.23	7.45±0.34	7.32±0.09	189.09±24.39	0	16.87
10	OR 1A 100mg	100	18.79±0.09	21.29±0.67	19.28±0.15	3.14±0.54	3.03±0.76	70.19±22.36	38.76	18.65
11	HQ 1B 100mg	100	22.98±0.23	19.87±0.32	18.19±0.35	2.98±0.38	2.87±0.88	68.79±29.87	58.46	17.52
12	HQ 1F 100mg	100	21.29±0.22	18.76±0.45	21.87±0.47	7.87±0.23	7.5±0.64	176.98±22.10	78.98	18.43
13	HL 1A 100mg	100	22.98±0.32	19.29±0.32	22.98±0.19	5.87±0.95	5.73±0.32	154.93±19.87	65.08	16.54
14	HL 1C 100mg	100	21.27±0.23	21.19±0.87	18.92±0.12	2.13±0.32	1.78±0.19	76.34±19.83	87.65	18.54
15	HL 1D 100mg	100	20.09±0.98	18.11±0.23	20.98±0.34	4.32±0.43	4.03±0.54	86.69±20.98	65.72	18.75
16	HL 1E 100mg	100	18.98±0.03	19.81±0.87	21.87±0.45	3.45±0.54	3.12±0.32	67.98±19.65	85.48	19.87

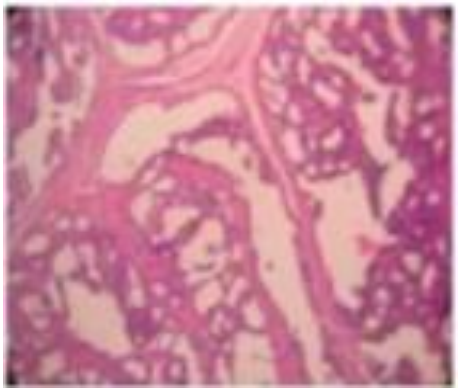
Table No 2: *In Vivo* studies results of the selected drugs of 200mg/kg

S.No	Treatments	Dose (mg/Kg)	Body weight (g) Day 1	Body weight (g) Day 5	Body weight (g) Day 12	Weight of tumour (g)	Volume of ascitic fluid (ml)	Number of tumour cells (X107)	Tumour Growth inhibition	Mortality
1	Normal Saline	**0.2ml**	2.20 ± 0.49	24.00 ± 0.55	29.60 ± 0.93	8.74 ± 0.52	8.92 ± 0.51	686.79 ± 54.40	0	0
2	Doxorubicin	20	21.80 ± 0.58	21.20 ± 0.92	18.00 ± 1.82	0.52 ± 0.24	0.50 ± 0.22	14.00 ± 5.74	97.96	0
3	PG 1A 200mg	200	22.43±0.45	20.76±0.74	21.65±0.34	2.87±0.53	6.54±0.32	165.56±16.45	76.58	16.72
4	PG 1B 200mg	200	21.45±0.54	20.87±0.34	21.98±0.34	0.65±0.45	0.87±0.65	5.87±20.87	43.28	16.57
5	PG 1D 200mg	200	21.98±0.48	21.54±0.54	20.23±0.43	7.66±0.54	5.76±0.43	154.98±21.25	65.37	18.35
6	PG 1E 200mg	200	22.26±0.65	21.98±0.76	22.09±0.54	5.76±0.96	7.87±0.54	176.09±21.25	0	17.86
7	PG 1F 200mg	200	19.43±0.87	18.76±0.67	18.87±0.74	5.64±0.45	4.23±0.76	86.75±18.65	68.56	17.46
8	RS 1B 200mg	200	21.98±0.93	19.54±0.52	19.45±0.21	3.45±0.65	5.32±0.23	149.76±17.65	57.48	18.45
9	RS 1C 200mg	200	23±0.65	21.14±0.48	22.87±0.26	2.76±0.34	4.65±0.37	88.97±19.54	63.29	18.09
10	OR 1A 200mg	200	21.54±0.91	21.76±0.79	22.98±0.36	4.37±0.55	5.81±0.41	134.65±19.05	52.39	19.54
11	HQ 1B 200mg	200	22.45±0.55	20.54±0.28	21.65±0.65	2.76±0.32	4.87±0.35	76.58±22.28	63.48	16.47
12	HQ 1F 200mg	200	21.87±0.63	21.65±0.94	20.98±0.32	5.48±0.23	5.32±0.62	143.76±27.45	57.87	19.46
13	HL 1A 200mg	200	20.81±0.32	20.65±0.37	22.81±0.98	3.76±0.44	5.98±0.98	152.39±21.51	65.39	18.32
14	HL 1C 200mg	200	18.46±0.35	20.98±0.45	23±0.56	8.56±0.65	3.71±0.56	65.49±19.27	75.49	19.45
15	HL 1D 200mg	200	21.98±0.43	23±0.35	22.18±0.54	3.37±0.65	7.54±0.45	176.58±19.65	54.87	18.43
16	HL 1E 200mg	200	20.09±0.32	21.65±0.65	20.98±0.67	5.42±0.65	4.32±0.34	84.38±14.37	62.98	16.43

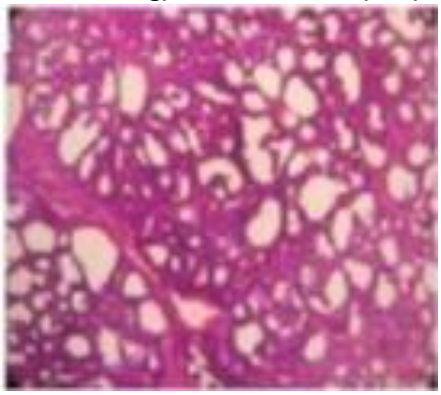
Histopathology of tumor samples

Disease Control sections showed mammary tissue with tumor composed of malignant cells arranged in tubular and small glandular pattern seen infiltrating the stroma. At places, ducts showed varying degree of intra-ductal proliferation and features of ductal carcinoma insitu features. Mild peritumoral lymphocytic infiltration was seen. Findings are of tubular carcinoma of breast. Mammary histology sections of PG1A and HL1C treated animals at both doses showed breast tissue with large area of fibrosis, hyalinization, patchy necrosis and atrophic lobules. No evidence of malignancy was seen. Doxorubicin treated animals had breast tissue with large area of fibrosis, hyalinization, patchy necrosis and atrophic lobules. No evidence of malignancy was observed.

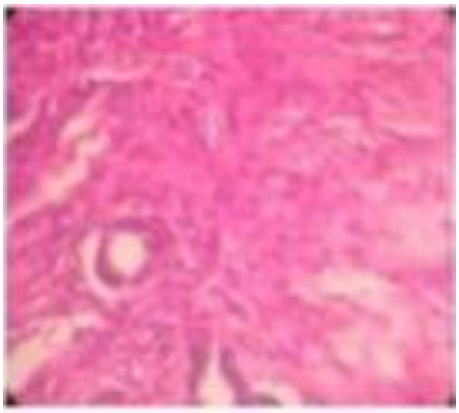
Figure 1 Histopathology (haematoxylin and eosin staining) of breast tumor (40X)



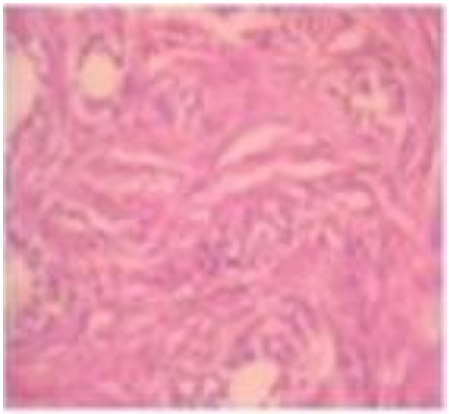
A Disease Control



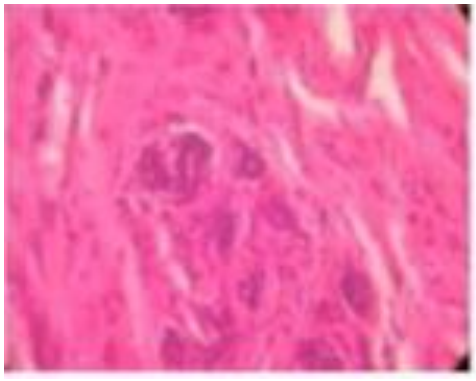
B Disease Control



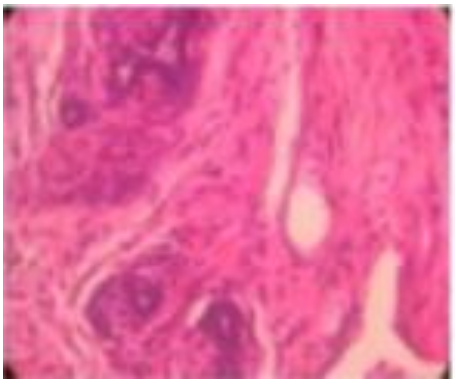
C PG1A 100mg dose



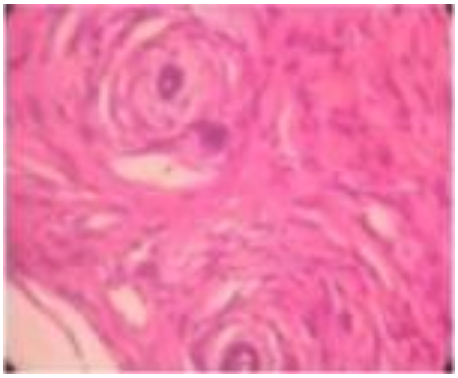
D PG1A 200mg dose



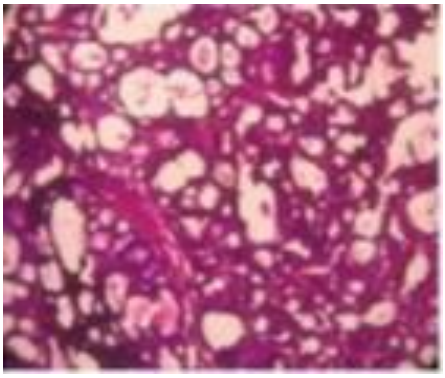
E Doxorubicin 200mg dose



F HLVC 100mg dose



G HLVC 200mg dose



H Doxorubicin 200mg dose

A, B: Disease Control C: PG1A 100 D: PG1A 200 E: Doxorubicin
F: HLVC 100 G: HLVC 200 H: DOXURUBICIN 200

CONCLUSION:

The newly synthesized compounds were screened for their anticancer activity against Human Skin Cancer Cell Line G361 by Sulforhodamine B assay. Doxorubicin was used as a standard reference drug and the results obtained were shown in (Table 1). All compounds (**1-30)** showed low antiproliferative activity. The % Growth inhibition of the compounds **(IA, IB, ID, IE, IF, IIB, IIC, IIIA, IVB, IVF, VA, VC, VD, VE.)** was found to be considerable at a concentration of 10^{-4} M. TGI_{50} (Growth inhibition of 50 % cells, calculated from drug concentration resulting in a 50 % reduction in the net protein increase). The histopathology studies of the selected PG1A and HLVC had shown prominent results in groth inhibition of breast cancer. As benzofuranone derivatives are the most active compounds, it serves as a lead to further optimization in drug discovery process.

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