Radiolabelling and Biological Apprisal of ^{99M}Technetium Labeled Tamoxifen for Anticancer Activity

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ABSTRACT

The aim of the present study was to develop the procedure for radiolabeling of an anticancer drug tamoxifen with 99mTc for tumor diagnosis and to evaluate the antitumor effect of tamoxifen in EAC bearing mice. The Ehrlich ascites carcinoma (EAC) cells were injected invivo in Balb-C mice by intraperitoneal (i.p.) inoculation of $2x10^6$ cells/mice after every 10 days. The study included the radiolabeling of tamoxifen, in vitro stability of radiolabeled drug, invitro binding of radiolabeled drug with plasma protein, partition coefficient and biodistribution of radiolabeled drug in mice. MTT assay, mean survival time and % increase in life span, body weight, tumor size, volume and hematological parameters (Red blood cells, Haemoglobin, Haematocrit, Mean corpuscular haemglobin (MCH), Mean cellular heamoglobin concentration, Platelets, Lymphocytes, Monocytes, Eosinophils, Granulocytes and Red cell distribution width) were also evaluated. Radiolabeling of tamoxifen with technetium-99m (^{99m}Tc) was 97%. Invitro stability of the labeled complex was increased with time and found maximum at 2 hours (98.1.± 0.17). Partition coefficient of the labeled drug showed that it was highly lipophilic. Biodistribution study in tumor bearing mice exhibited high uptake in tumor cells. Tamoxifen significantly increased the life span and mean survival time of tumor bearing mice and significantly reduces the tumor weight, size and volume, body weight as compared to the control (EAC bearing) group. In conclusion, the present study indicates that ^{99m}Tc labeled tamoxifen is a potentially strong tumor diagnostic agent with low uptake in normal tissues and has anticancer activity in solid tumors.

Key words: *Tamoxifen; Ehrlich ascites carcinoma; Radiolabeling; Technetium-99m; Solid tumor*

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INTRODUCTION

Cancer is a leading cause of death worldwide accounting for 7.6 million deaths i.e., about 13% of all deaths. About 70% of all deaths occurred due to cancer in low- and middle-income countries. About 30% of cancer deaths are due to the five leading behavioural and dietary risks i.e., high body mass index, low fruit and vegetable intake, lack of physical activity as well as tobacco and alcohol use. By 2020, the world population is expected to have increased to 7.5 billion; of this number, approximately 15 million new cancer cases will be diagnosed, and 12 million cancer patients will die. In India, about 2.0-2.5 million people are suffering from cancer in which about 0.7 million new cases coming in each year and nearly half of people die in each year.[1]

Cancers are primarily an environmental disease with 90-95% of cases attributed to environmental factors and 5-10% due to genetics. Common environmental factors that contribute to cancer death include tobacco (25-30%), diet and obesity (30-35%), infections (15-20%), radiation (both ionizing and non-ionizing, up to 10%), stress, lack of physical activity and environmental pollutants.[2]

Ehrlich ascites (ET) tumor cell is a spontaneous murine mammary adenocarcinoma adapted to ascites form and carried in outbred mice by serial intraperitoneal passage. This cell has specific lectin agglutinability patterns. ET cell has ability to produce ascites tumors and solid tumour due to growth on basement membranes and purified extracellular matrix molecules.[3] Ascites liquid is gray-white or sometimes has a light bloody viscose liquid and contains 10 million neoplastic cells in 0.1 cc.[4] EAC is used as ascites or a solid form. When ascites fluid that contains the tumor cell, if injected by i.p., the ascites form is obtained but if injected by S.C., a solid form is obtained.[5]

Tamoxifen (TAM), a triphenylethylene derivative, is a selective estrogen receptor modulator (SERM) that has become the treatment of choice for women diagnosed with all stages of hormone-responsive breast cancer. Since its introduction for the treatment of advanced breast cancer, its indications have increased to include the treatment of early breast cancer, ductal carcinoma *in situ*, and more recently for breast cancer chemoprevention it is also efficacious in both pre- and postmenopausal women.[6]

One of the most important tasks in the fight against cancer is its early detection and localization so that treatment may be starts at an early stage. To facilitate

the detection of tumors which transport drugs, immunomodulators or other agents out of tumor cells, it would be desirable to have radiopharmaceuticals that is not readily pumped out in the tumor cells and could be used to identify the location of the resistant tumors. Therefore, early detection of tumors using a radioactive pharmaceutical is one of the most attractive and efficient non-invasive method.[7]

Although, tamoxifen has been studied in different models of tumor but its ^{99m}Tc radiolabelling study for evaluation of antitumor activity has not been explored yet. Hence, aim of this study is to evaluate the antitumor activity of tamoxifen by radiolabelling with ^{99m}Tc. ^{99m}Tc is a radioisotope used in 80% of all nuclear medicine. It is an isotope of the artificially produced element technetium and it has almost ideal characteristics for a nuclear medicine scan.[8]

MATERIAL AND METHODS

Animals: Female Swiss albino mice weighing 20-25 g (8 weeks old) and rabbit 2.5-3.5 kg (6-8 months old) were procured from the Institute of Nuclear Medicine and Allied Science (INMAS), Defence Research and Development Organization (DRDO), Delhi. The mice were kept in polypropylene cages (38 cm × 23 cm × 10 cm) under standard laboratory conditions (12 h light and 12 h dark cycle) and had a free excess of commercial diet and water add libitum. The animal house was maintained at 25±2°C and 50-60% relative humidity. All the described procedures were reviewed and approved by the Institutional Animal Ethics Committee of DRDO.

Drugs and Chemicals: Tamoxifen, Technetium-99 metastable (^{99m}Tc), dimethyl sulphoxide (DMSO), n-octanol, formaldehyde (37%). All these reagents were obtained from the Institute of Nuclear Medicine and Allied Sciences (INMAS), DRDO, Delhi.

Transplantation of tumor cells: Ehrlich ascites carcinoma (EAC) cells were obtained from the INMAS. The ascites fluid was drawn out from EAC tumor bearing mice at the log phase (days 7-8 of tumor bearing) of the tumor cells. The EAC cells were maintained *in vivo* in Balb-C mice by i.p., inoculation of $2x10^6$ cells/mice after every 10 days and were used for both *in vivo* and *in vitro* study.[9]

Assay for in vitro cytotoxicity study

MTT [3-(4, 5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assay [10]

Method- EAC cells were cultured in 96 well plates with growth medium and 10% FCS (Fetal calf serum). Increasing concentrations of tamoxifen (1, 5, 7.5, 10, 15, 20 µg/ml) was added to the cells and incubated at 37ºC for 14 h in CO2 incubator with 5% CO2. The media was replaced with a fresh growth medium along with 20 µl of MTT. Again it was incubated for 4 h at 37ºC. The reaction resulted in the reduction of MTT by the mitochondrial dehydrogenase of viable cells to a purple formazan product. The MTT- formazan product was dissolved in DMSO and estimated by measuring the absorbance at 570 nm in an ELISA plate reader. Experiments were repeated at thrice. The mean was calculated, and compared with the control test samples. The percentage growth inhibition was calculated using the following formula:

% Growth inhibition/ % cytotoxicity = Control OD – Treated OD / Control OD X 100

In vivo Antitumor Activity

Treatment Schedule: Female Swiss albino mice (20-25 g) were randomly divided into 4 groups, each group contains 12 mice. Group I (normal control): Animals received dimethyl sulfoxide (4 ml/kg/day in 70:30 of DMSO and normal saline, i.p., for 14 days), Group II (EAC control): Animals received EAC cells (0.1 ml of 2×10^6 cells/mouse, i.m., into the right hind limb, single dose), Group III (EAC + TAM10): Animals received EAC cells (0.1 ml of 2×10^6 cells/mouse) and tamoxifen (10 mg/kg/day, i.p., for 14 days), Group IV (EAC + TAM20): Animals received EAC cells (0.1 ml of 2×10^6 cells/mouse) and tamoxifen (20 mg/kg/day, i.p., for 14 days).

After treatment, animals of each group were kept fasting for 18 h and blood was collected by direct retro-orbital plexus for the estimation of hematological parameters. Animals were then sacrificed for antitumor activity. The remaining six animals of each group were kept alive for 40 day to check the mean survival lime and percent increase in life span of the tumor bearing animals.

Tumor growth response: Antitumor effect of tamoxifen was assessed by observation of change in body weight, ascitic tumor volume, tumor size, tumor weight, mean survival time (MST) and percentage increase life span (% ILS).[11]

Antitumor Parameters

Mean survival time (MST) and percentage increase life span (% ILS)

The effect of tamoxifen on tumor growth was observed by MST and % ILS. MST of each group containing 6 mice were monitored by recording the mortality daily for 6 weeks and % ILS was calculated by using following equation. [11]

MST = (Day of first death + day of last death) / 2

% ILS = [(MST of treated group/ MST of control group) – 1] x 100

Body weight measurement: Body weight of the experimental mice was recorded both in the treated and control groups at the beginning of the experiment (day 0) and sequentially on 3, 5, 7, 9, 11, 13th day during the

treatment period and final body weight calculated on 15th day.[12]

Tumor size: Tumor size of the experimental animals was measured on 3, 5, 8, 11 and 13th day during the treatment using vernier calliper and final tumor size calculated on 15th day by using the following formula: Tumor size = ¾

Tumor volume: Tumor volume of the experimental animals was measured on 3, 5, 8, 11 and 13th day during the treatment using vernier calliper and final tumor volume was calculated on 15th day by using the following formula: V = (3.14/6) × D1 × D2 × D3

Where D1, D2 and D3 represent three orthogonal diameters.[12]

Tumor weight: The mice were dissected on the 24 h later of the last day of treatment and the tumor were removed from the right hind paw of the mice, wash with saline, air dried and weigh accordingly.

Radiolabelling: Tamoxifen was labeled with ^{99m}Tc by the method of direct labeling.

PROCEDURE

To an amount of 3.4 mg of tamoxifen in 0.7 ml of dimethyl sulfoxide (DMSO) and 0.3 ml of normal saline, 50 µl aliquots of stannous chloride solution (2mg/ml) in 10% acetic acid was added and contents were shaken gently. This was followed by adjustment of pH to 7.0 using $NaHCO_3$ (0.5 M) solution. The contents were filtered through 220 nm membrane Millipore filter. Subsequently 0.3ml Tc-99m pertechnetate solution (2mCi) was added to the filtrate and shaken gently incubated at room temperature for 10 minutes.

The pH of reaction mixture was varied from 5.0 to 8.0 before adding requisites volume of pertechnetate. The labeling efficiency and stability of labeled product were measured with time. In of another set of experiments, the pH (7.0) of the reaction mixture was kept constant while $SnCl_2$ concentration was varied from 50µl to 500µl measured at different time intervals.

Evaluation of radiolabeling efficiency: The radiolabeling efficiency was evaluated using the method of instant thin layer chromatography-silica gel. (ITLC-SG) impregnated strip as stationary phase and 100% acetone as the mobile phase. The free technetium (Tc) moves with the solvent and the reduced or hydrolysed Tc, if any, along with the conjugated one, stays at the base thereby effecting partial separation vis-a-vis the purification.[13]

$$\% \text{ Radiolabeling} = \frac{\text{Radioactivity (counts) retained in the lower half of strip}}{\text{Radioactivity associated (total counts) with the strip}} \times 100$$

Evaluation of in vivo/ in vitro stability: In vitro stability of the ^{99m}Tc-tamoxifen complex was estimated in the serum by incubating 50µl of the complex with 450µl of human serum at 37˚C. Aliquots at different time periods were applied on ITLC-SG strip and allowed to run in 100% acetone to check any dissociation and degradation of the labeled complex. The dissociation was estimated as the % radiolabeled complex remaining after the incubation time intervals of 0h, 1h, 2h, 4h and 24h.[14]

$$\% \text{ Radiolabeled complex} = \frac{\text{Radioactive counts at lower half of the strip at specified time}}{\text{Corresponding total counts of strip at that time}} \times 100$$

Bio-distribution studies: Biodistribution of ^{99m}Tc-tamoxifen was carried out in normal mice as well as in Ehrlich Ascites Tumor (EAT) bearing mice of 2-3 months of age and weighing about 25-30 g. An aliquot of labeled tamoxifen (100 µCi) injected i.p. to the mice and dissected at 1h, 2h, 4h and 24h of post administration. Various organs/ tumour were removed, made free from adhering tissues and weighed. The radioactivity was measured in each organ and expressed as percent injected dose per whole organ.[15]

Scintigraphy in EAT mice: For scintigraphy in mice tumor was implanted in Balb/C mice weighing approximately 25-30g. EAT cell line was maintained in the ascites form by serial weekly passage. Exponentially growing EAT cells were harvested and resuspended in phosphate buffer saline. 1-1.5 million cells were injected intramuscularly in the thigh of the right hind leg of the mice and the mice were used after 10 days when the solid tumour had grown to about 1cm in diameter. The mice were administered 100ml (3.7 MBq) of the labeled complex intravenously through

the tail vein and imaging was performed starting from 15 min to 24 hr using a planar gamma camera equipped with pinhole collimator.[15]

Hematological studies: The effect of tamoxifen on peripheral blood was investigated. RBC, WBC counts, and estimation of Hemoglobin were done with standard procedures.

Statistical Analysis: The experimental results were expressed as the mean ± SEM. Data was analyzed by one way analysis of variance followed by post hoc analysis.

RESULTS

Tamoxifen was labeled with high efficiency by the direct labeling technique using reduced 99mTc. Data on radiochemical purity and stability of the labeled complex were obtained by ascending chromatography using saline or 100% acetone as the solvent. The radiochemical impurities are free pertechnetate and reduced/hydrolysed ^{99m}Tc (radiocolloids) in the 99mTc-labeled complexes.

In vitro stability of ^{99m}Tc-tamoxifen complex: In vitro stability of the labeled complex with time was studied in human serum at 37˚C, is shown in Table 1. The stability of the labeled complex was increased with time and found maximum at 2 hours. Even after 24 hours of incubation, there was no significant reduction in the radiolabeled compound, indicating its high stability and suitability for *in vivo* use.

In vitro cytotoxicity assay: TAM showed direct *in vitro* cytotoxic effect on the EAC cell line in a concentration dependent manner (Fig. 1).

In vivo antitumor activity

Influence of tamoxifen on body weight: There was a significant (P<0.5, P<0.01 and P<0.001) decreased in the body weight of animals in TAM treated groups when compared with EAC control (Table 2).

Influence of tamoxifen on tumor weight, MST and %ILS: There was a significant (P<0.001) decreased in the tumor weight of animals in TAM treated groups when compared with EAC control and there was a significantly (P<0.001) increase in mean survival time (MST) to 38 ± 1.98 (% ILS=51.03) and 41 ± 2.73 (% ILS=62.95) on administration of tamoxifen in a dose dependant manner compared to EAC control (25.16 ± 1.51) (Table 3).

Influence of tamoxifen on tumor size and volume: There was a significant (P<0.01 and P<0.001) decreased in tumor size (Fig. 2) and significant (P<0.05, P<0.01 and P<0.001) decreased in tumor volume (Fig. 3) of animals in TAM treated groups when compared with EAC control.

Influence of tamoxifen on hematological analysis: There was a significant (P<0.001) increase in the total WBC and eosinophils as well as a significant (P<0.001) decrease in the total RBC, lymphocytes and monocytes in EAC control compared to normal control. On treatment with TAM (10 and 20 mg/kg), these levels come near to normal.

We also observed a significant (P<0.001) decrease in the Hb, MCHC and HCT in EAC control compared to normal control. Treatment with TAM (10 and 20 mg/kg) significantly ((P<0.5, P<0.01 and P<0.001) increased these levels compared to EAC control (Table 4).

Biodistribution studies of ^{99m}Tc-tamoxifen: The biodistribution of ^{99m}Tc-tamoxifen in different organs of normal and tumour mice after 1h, 2h, 4h and 24 h is shown in (Figure 4.1-4.10). After administration of ^{99m}Tc-tamoxifen, percentage injected dose/whole organ was found as follows: muscles (0.67%) at 1 hour, heart (0.58%), kidney (15.67%), spleen (2.25%) and stomach (1.89%) at 2 hours and blood (3.24%), lungs (1.0%), liver (15.67%), brain (0.054%) and intestine (2.2%) at 4 hours. The distribution of drug in tumour at 1 hour was 1.35% (Figure 5). The maximum distribution of ^{99m}Tc-tamoxifen was observed in liver and kidney when ^{99m}Tc-tamoxifen was administered in EAC. Scintigraphy study of radiopharmaceutical in Tumour induced mice was observed after 2 hours and 4 hours (Fig. 6).

Table 1: In vitro stability of ^{99m}Tc-tamoxifen complex in human serum at 37˚C

Time (Hours)	% Labeling stability
0 h	94.16 ± 0.60
1 h	96.46 ± 0.37
2 h	98.1 ± 0.17
4 h	97.2 ± 0.72
24 h	91.33 ± 0.63

The samples were subjected to instant thin layer chromatography using silica gel-coated fibre sheets with 100% acetone as the mobile phase. Data are expressed as mean ± standard error of mean, where n = 6.

Table 2: Influence of tamoxifen changes body weight in Ehrlich ascites carcinoma mice

Days	EAC control (2×10^6 cells/mice)	EAC + TAM (10 mg/kg)	EAC + TAM (20 mg/kg
0	26.5 ± 0.76	23.83 ± 0.58*	23.66 ± 0.73*
3	26.83 ± 0.64	23.91 ± 0.65*	23.5 ± 0.91*
5	27.08 ± 0.66	24.75 ± 0.38*	23.41 ± 0.78**
7	26.75 ± 0.38	23.5 ± 0.68**	22.16 ± 0.57***
9	27.41 ± 0.30	22.83 ± 0.64***	21.41 ± 0.47***
11	27.91 ± 0.35	22.08 ± 0.74***	20.58 ± 0.56***
13	28.58 ± 0.39	21.25 ± 0.80***	20 ± 0.53***
15	29.16 ± 0.33	20.66 ± 0.77***	19.66 ± 0.44***

EAC, ehrlich ascites carcinoma; TAM, tamoxifen, Data are expressed as mean ± standard error of mean, where n = 6, *P < 0.05, **P < 0.01 and ***P < 0.001 versus EAC control

Table 3: Influence of tamoxifen on tumor weight, median survival time and percentage increase in life span

Parameters	EAC control (2×10^6 cells/mice)	EAC + TAM (10 mg/kg)	EAC + TAM (20 mg/kg)
Tumor weight (g)	4.02 ± 0.13	2.58 ± 0.2***	1.72 ± 0.24***
MST (days)	25.16 ± 1.51	38 ± 1.98***	41 ± 2.73***
% ILS	-	51.03	62.95

EAC, Ehrlich ascites carcinoma; TAM, tamoxifen; MST, median survival time; % ILS, percentage increase in life span. Data are expressed as mean ± standard error of mean, where n = 6, ***P < 0.001 versus EAC control.

Table 4: Influence of Tamoxifen on Hematological analysis in ehrlich ascites carcinoma mice

Parameters	Normal saline (0.5 ml/kg)	EAC control (2×10^6 cells/mice)	EAC + TAM (10 mg/kg)	EAC + TAM (20 mg/kg)
Hemoglobin (g)	12.86 ± 0.27	8.16 ± 0.42***	9.88 ± 0.29**	11.26 ± 0.29***
Total RBC (10^6cells/mm^3)	6.94 ± 0.25	2.4 ± 0.46***	3.05 ± 0.16*	3.73 ± 0.16***
Total WBC (10^3cells/mm^3)	7.2 ± 0.35	20.3 ± 0.54***	11.04 ± 0.34***	8.35 ± 0.21***
Lymphocyte (%)	70.75 ± 1.44	43.3 ± 1.42***	49.58 ± 0.88*	57.56 ± 1.59***
Monocyte (%)	1.9 ± 0.09	0.6 ± 0.11***	1.09 ± 0.08*	1.58 ± 0.51***
Eosinophills (%)	3.98 ± 0.20	9.57 ± 0.17***	6.65 ± 0.25***	4.86 ± 0.32***
Granulocytes (%)	21.41±38	54.31 ± 1.65	47.05 ± 0.91**	34.33 ± 1.73***
HCT (%)	41.41 ± 1.12	33.08 ± 1.01***	36.66 ± 1.01*	38.08 ± 0.87**
MCHC (g/dl)	29.81 ± 0.92	16.91 ± 0.49***	19.75 ± 0.62*	20.91 ± 0.72**

EAC, ehrlich ascites carcinoma; TAM, tamoxifen. Data are expressed as mean ± standard error of mean, where n = 6, *P < 0.05, **P < 0.01 and ***P < 0.001 versus EAC control.

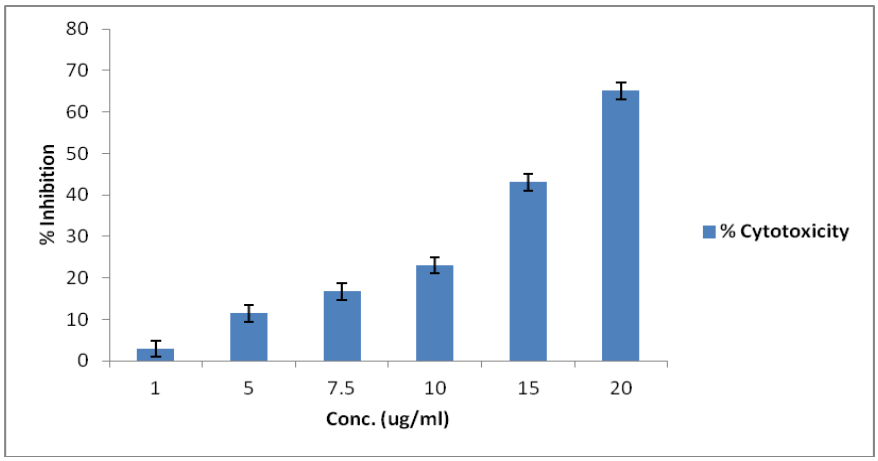

Fig. 1: Effect of tamoxifen on percentage cell viability

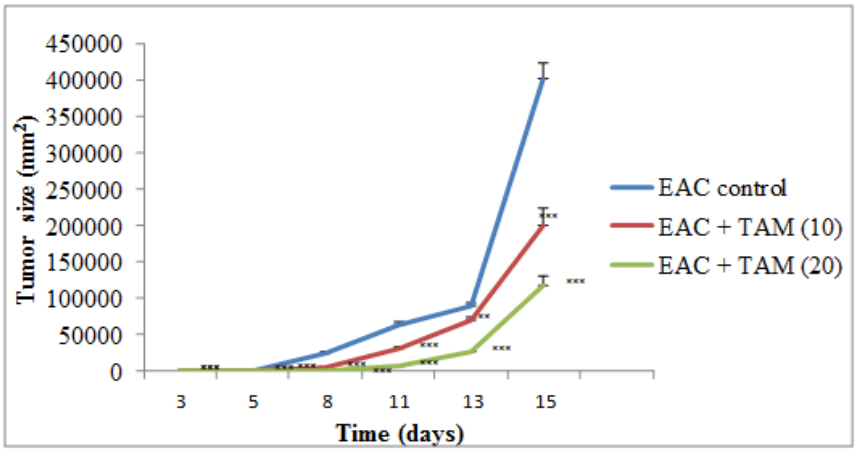

Fig. 2: Influence of tamoxifen on tumor size

EAC, ehrlich ascites carcinoma; EAC + TAM (10), ehrlich ascites carcinoma + tamoxifen 10 mg/kg; EAC + TAM (20), ehrlich ascites carcinoma + tamoxifen 20 mg/kg. Data are expressed as mean ± standard error of mean, where n = 6, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus EAC.

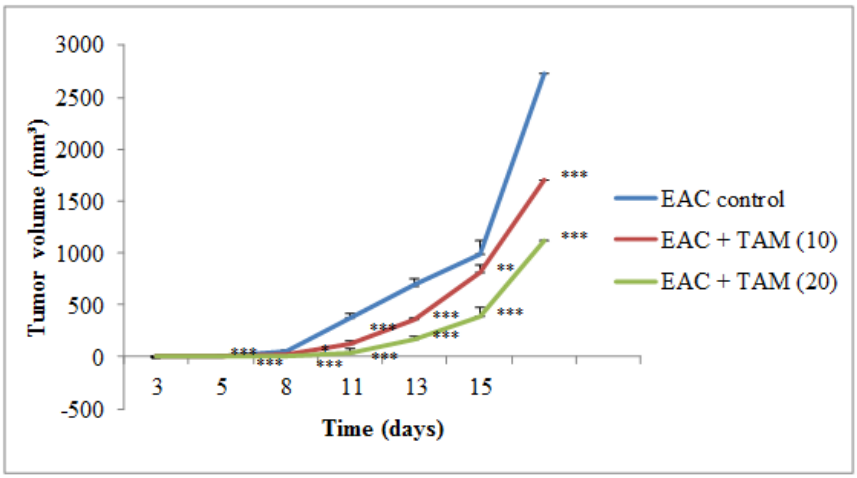

Fig. 3: Influence of tamoxifen on tumor volume

EAC, ehrlich ascites carcinoma; EAC + TAM (10), ehrlich ascites carcinoma + tamoxifen 10 mg/kg; EAC + TAM (20), ehrlich ascites carcinoma + tamoxifen 20 mg/kg. Data are expressed as mean ± standard error of mean, where n = 6, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus EAC.

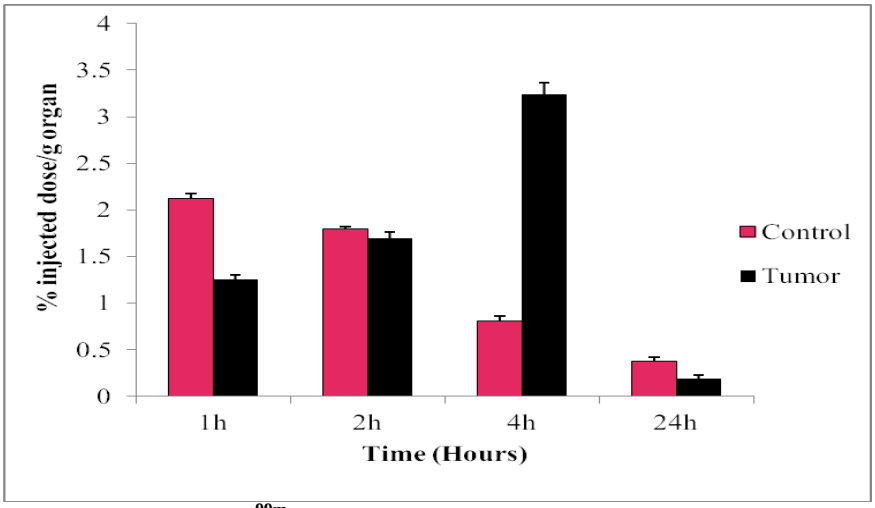

Fig. 4.1: Distribution of ^{99m}Tc bound tamoxifen in blood tumor and normal mice

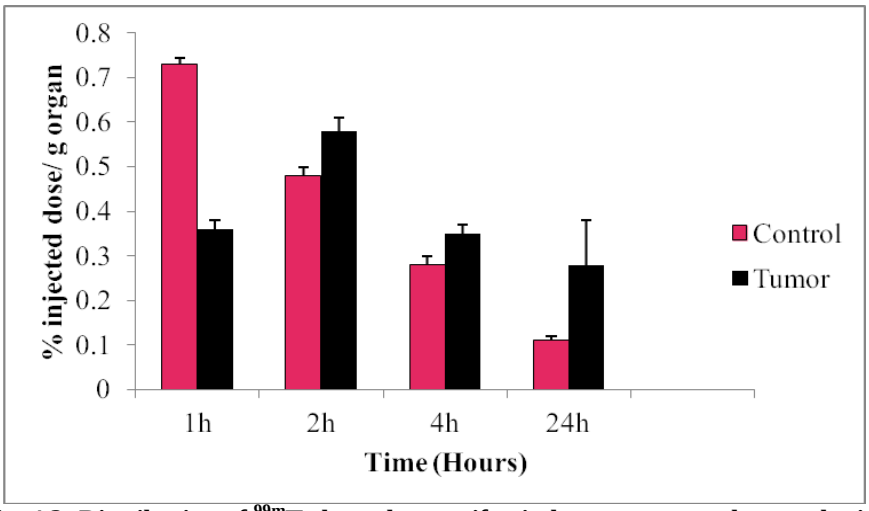

Fig. 4.2: Distribution of ^{99m}Tc bound tamoxifen in heart tumor and normal mice

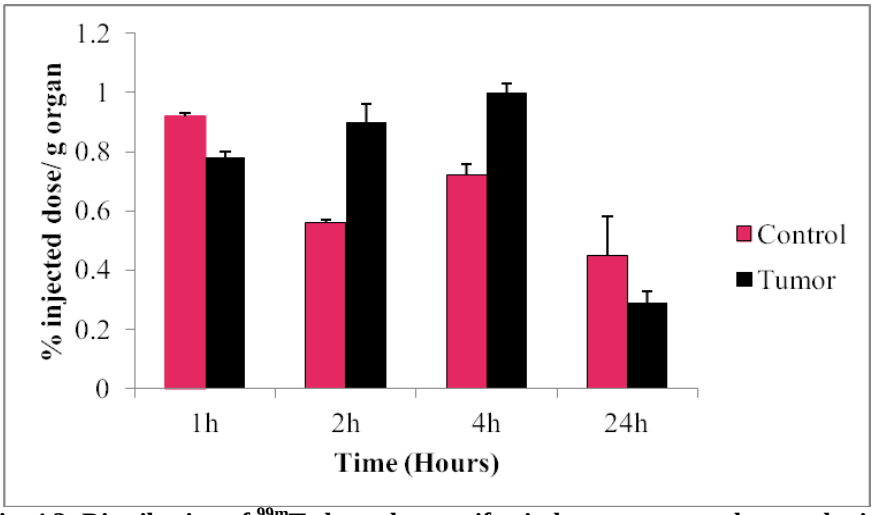

Fig. 4.3: Distribution of ^{99m}Tc bound tamoxifen in lungs tumor and normal mice

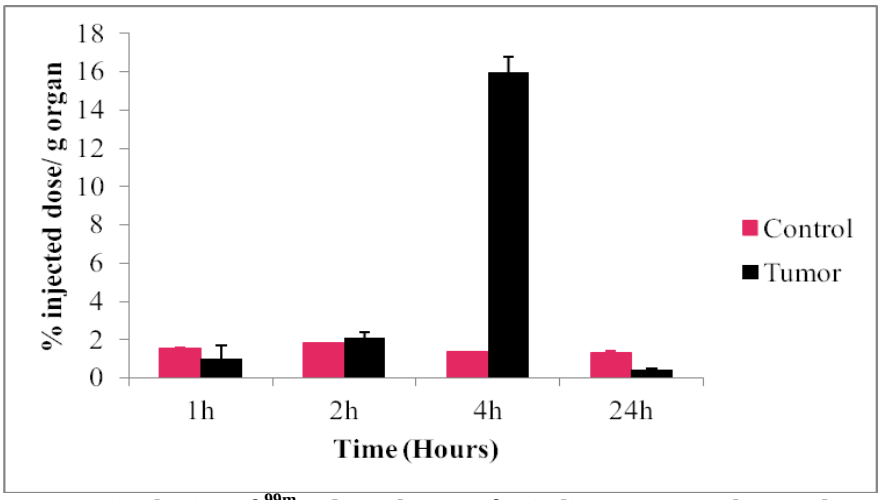

Fig. 4.4: Distribution of ^{99m}Tc bound tamoxifen in liver tumor and normal mice

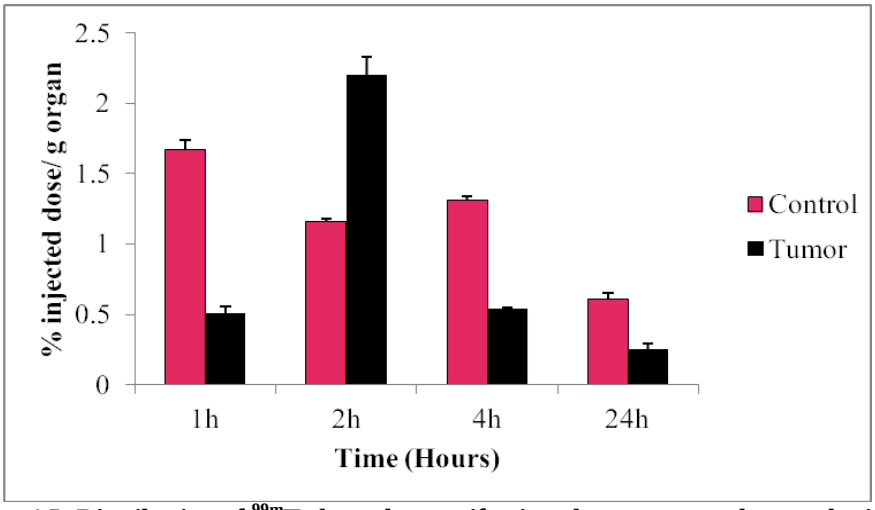

Fig. 4.5: Distribution of ^{99m}Tc bound tamoxifen in spleen tumor and normal mice

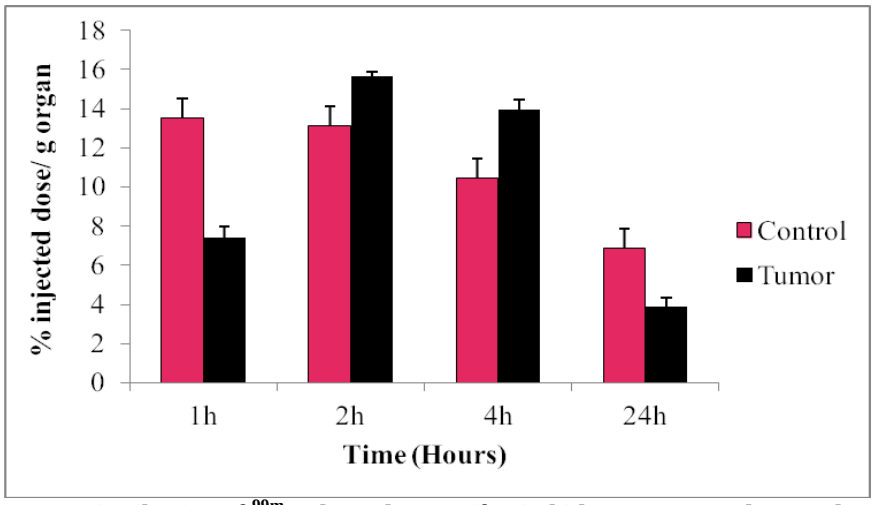

Fig. 4.6: Distribution of ^{99m}Tc bound tamoxifen in kidney tumor and normal mice

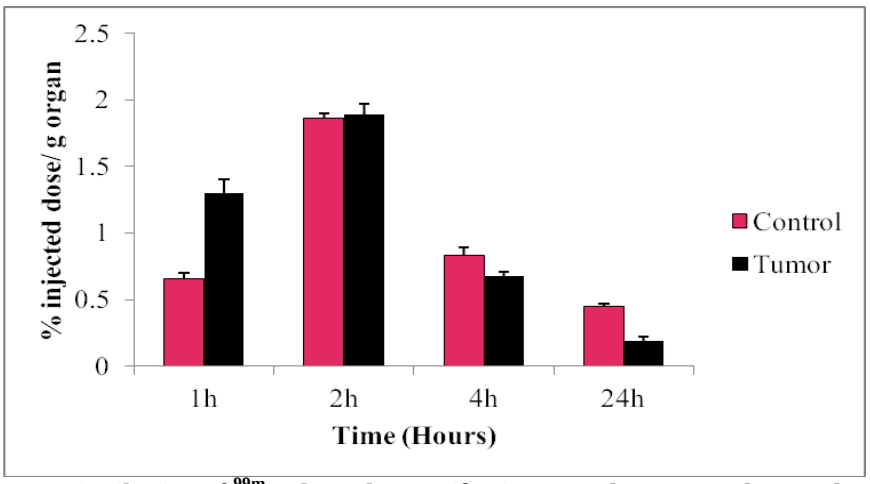

Fig. 4.7: Distribution of ^{99m}Tc bound tamoxifen in stomach tumor and normal mice.

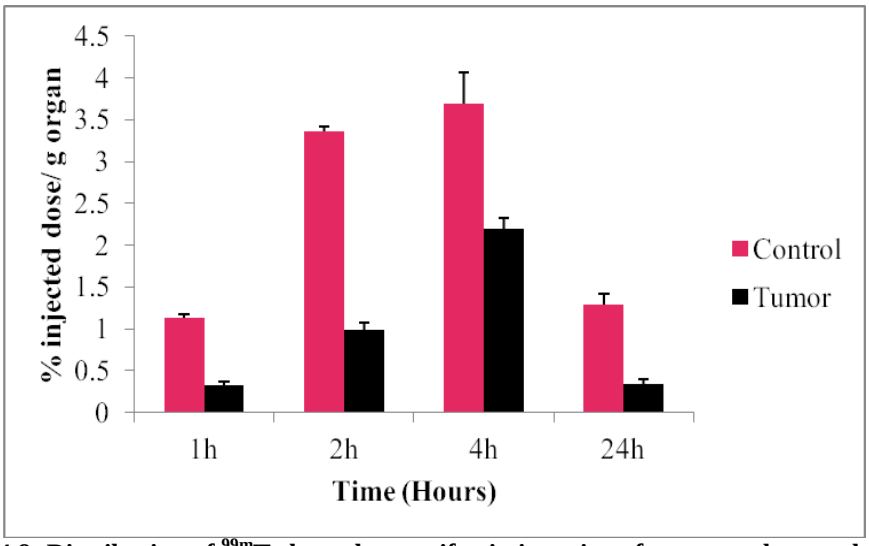

Fig. 4.8: Distribution of ^{99m}Tc bound tamoxifen in intestine of tumor and normal mice

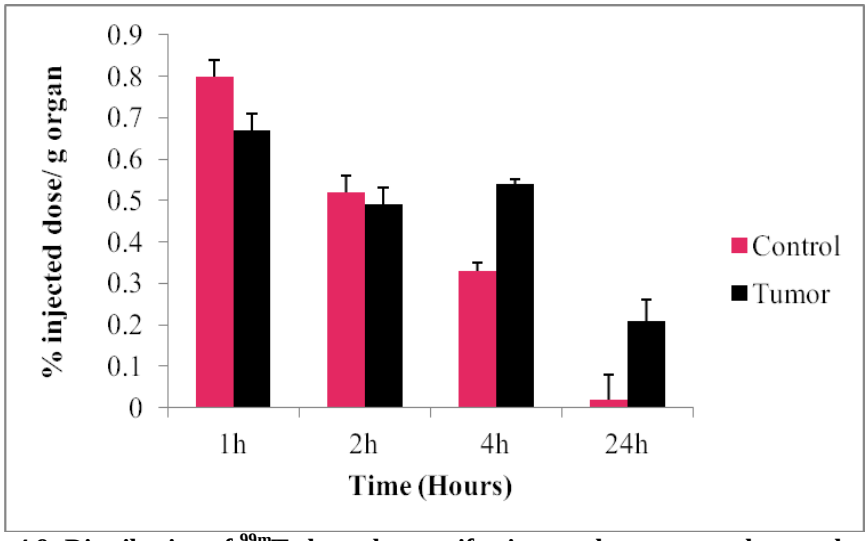

Fig. 4.9: Distribution of ^{99m}Tc bound tamoxifen in muscles tumor and normal mice

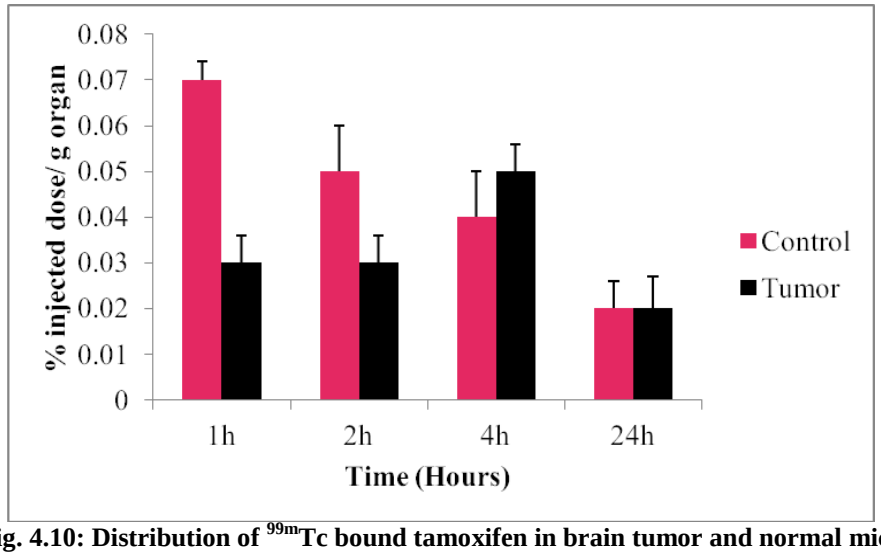

Fig. 4.10: Distribution of ^{99m}Tc bound tamoxifen in brain tumor and normal mice

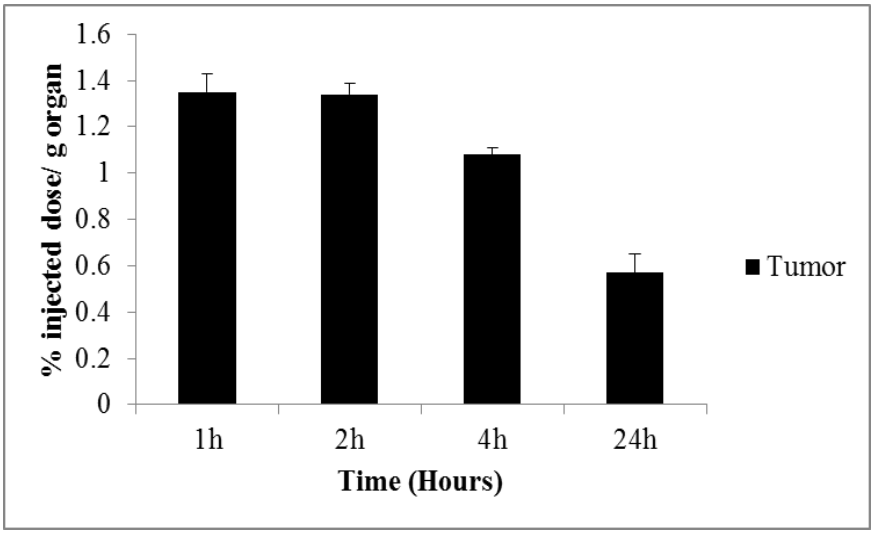

Fig. 5: Distribution of 99mTc bound tamoxifen in tumor of EAC

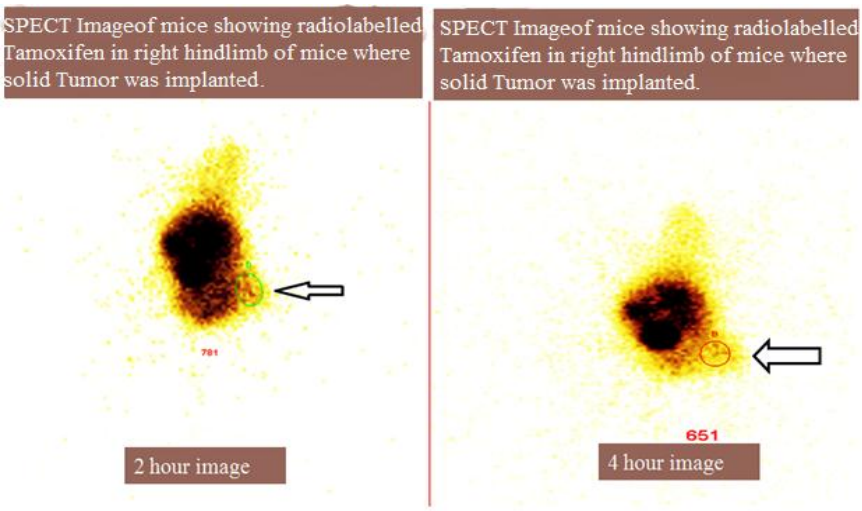

Fig. 6: Scintigraphy study of radiopharmaceutical in Tumor induced mice

DISCUSSION

Cancer is a disease of misguided cells which have high potential of excess proliferation without apparent relation to the physiological demand of the process. It is world's second killer after cardiovascular disease and it ever was killed 7.6 million people in 2005, out of them three quarters were from in low and middle income countries. That number is expected to increase to 9.0 million up to 2015 and rise further to 11.5 million in 2030. New cancer patients in India are estimated between 7 to 9 lakhs.[16] Chemotherapy, radiotherapy and surgery are only three major existing modes of treatment in modern medicine. Chemotherapy is still a major challenge to the cancer patients because such highly potent drug can be toxic and less than 1% of injected drug molecules can reach their target cells, whereas the rest may damage healthy cells and tissue.[17]

Murine type tumors are common malignant tumors. The primary therapy for these tumors includes surgery, radiation therapy and chemotherapy. These therapies have been extremely successful in the treatment of early carcinoma, the prognosis for advanced and recurrent diseases.

The present study was carried out to evaluate the antitumor effect of TAM in Ehrlich ascites carcinoma (EAC) bearing mice. The EAC cells were initially described as a spontaneous murine mammary rapidly growing adenocarcinoma with a very aggressive behavior and can proliferate in almost all strains of mice.[18] The Ehrlich ascites tumor implantation *per se* a local inflammatory reaction with increasing vascular permeability which results in an intense edema formation, cellular migration, progressive ascitic fluid formation which is essential for tumor growth since the ascites fluid is the direct nutritional source for tumor cells, and the faster increase in ascites fluid with tumor growth could possibly be a means to meet the nutritional requirements of tumor cells.[19]

Ehrlich Ascites Carcinoma is widely used for the detection of anticancer effect of various compound like acetone semicarbazone, doxorubicin. From the present experiment, it is limpid that treatment with TAM at the doses of 10 and 20 mg/kg significantly reduces the tumor volume, body weight, tumor weight and tumor size when compared to the tumor control group.

The reliable criteria for judging the value of any anticancer drug are the prolongation of life span inhibition of gain in average body weight and the decrease in WBC.[20] The increase of life span of tumor bearing mice by reduction of tumor volume and size of the tumor growth is a positive result and further corroborates the antitumor effect of tamoxifen in solid tumor.

The results of the present study showed an antitumor effect of Tamoxifen against EAC in Swiss albino mice. A significant ($p < 0.001$) enhancement of MST and decrement of gain in average body weight was observed.

The major problems encountered in cancer chemotherapy are myelosuppression and anemia.[21] Anemia occurred in tumor bearing mice is mainly due to the reduction of RBC or Hemoglobin production and this may occur either due to the iron deficiency or hemolytic or myelopathic conditions.[22] The analysis of the hematological parameters showed minimum toxic effect in the mice treated with TAM. After 14 days of transplantation, TAM was able to reverse the changes in the hematological parameters consequent to tumor inoculation. This indicates that TAM has a protective effect on the hemopoietic system.

CONCLUSION

In the present study cancer was successfully induced by EAC cell in Balb-C mice and it is concluded that ^{99m}TC labeled tamoxifen has significant anticancer activity in solid tumor bearing mice with low uptake in normal tissues. Tamoxifen can also be used as a potential anticancer agent for the treatment of solid tumors.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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