



Original Article

## Preclinical meritorious anticancer effects of Metformin against breast cancer: An *In vivo* trial



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### المخلص

**أهداف البحث:** يهدف هذا البحث إلى تقييم الآثار قبل السريرية المضادة للسرطان للميتفورمين في نموذج طعم أجنبي لسرطان الثدي.

**طرق البحث:** أجريت هذه التجربة التداخلية خلال فترة محددة مدتها خمسة أشهر (أغسطس ٢٠١٦ - يناير ٢٠١٧). حيث استخدمنا نموذج طعم أجنبي من الفئران بلب/ج عارية. وكان حجم العينة ٥٠ فأراً، وزعت لمجموعتين المجموعة أ والمجموعة ب لمجموعتي الميتفورمين والتحكم السلبية، على التوالي. وتم تقييم النشاط السرطاني للميتفورمين من خلال مقارنة حجم الورم، ووزن الورم، ونسبة تراجع الورم، ونسبة التراجع المنوية ومعدل البقاء على قيد الحياة.

**النتائج:** بالمقارنة مع مجموعة التحكم، خفض الميتفورمين بشكل كبير تطور الورم في نموذج الطعم الأجنبي لسرطان الثدي الناتج حسب مؤسسة ميتشغان للسرطان-٧. وقد انعكس ذلك من خلال اختلافات كبيرة في حجم الورم في المتابعة النهائية. وقد تم دعم النتائج التي توصلنا إليها من خلال انخفاض كبير في معدل نمو الورم ووزن الورم في مجموعة الميتفورمين مقارنة بمجموعة التحكم. وبالمثل، كان معدل البقاء الكلي وتراجع الورم مرتبطين بشكل أكبر في مجموعة الميتفورمين.

**الاستنتاجات:** أثبتت هذه الدراسة أن الميتفورمين يمكن أن يقلل بشكل كبير من نمو الورم ويمكن أن يزيد من معدل البقاء على قيد الحياة في نموذج الطعم الأجنبي لسرطان الثدي.

**الكلمات المفتاحية:** ميتفورمين؛ حجم الورم؛ معدل البقاء على قيد الحياة؛ مؤسسة ميتشغان للسرطان-٧؛ سرطان الثدي

### Abstract

**Objective:** This research aims to evaluate the preclinical meritorious and anticancer effects of Metformin in a Xenograft model of breast cancer.

**Methods:** This interventional trial was conducted during a defined period of 5 months (August 2016 January 2017). We used a Xenograft model of nude BALB/c mice. A sample size of 50 mice, allocated into two groups and designated as Group A and Group B for Metformin and negative control groups, respectively. The anticancer activity of Metformin has been evaluated by comparing the tumour volume, tumour weight, tumour regression ratio, percentage regression, and survival rate.

**Results:** Compared with the control group, Metformin can significantly reduce the progression of tumour in the Xenograft model of breast cancer induced by MCF-7. This is reflected by significant differences in tumour volume at the final follow-up ( $p < 0.001$ ). Our findings are further supported by a significant reduction of the tumour growth rate ( $p < 0.001$ ) and tumour weight ( $p < 0.001$ ) in the Metformin group than in the control group. Similarly, the total survival rate and tumour

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regression are more significantly correlated in the Metformin group.

**Conclusion:** This study demonstrates that Metformin can significantly reduce the tumour growth and can increase the survival rate in a Xenograft model of breast cancer.

**Keywords:** Athymic; CDX model; MCF-7; Metformin; Tumour Volume

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## Introduction

Breast cancer is the third most frequently recognized cancer worldwide following stomach and lung cancers, and the second supreme reason for loss of life. Incidence rates worldwide vary significantly from 19.3/100,000 women in East Africa to 89.7/100,000 women in Western Europe.<sup>1</sup>

A higher prevalence of breast cancer has been observed in Pakistan in comparison with other Asian states. Nearly one in every nine female patients are diagnosed with breast cancer and the prevalence rate was close to 24.4%.<sup>2,3</sup>

Although there are numerous chemotherapeutic agents available, the fact is that though they work on the tumours, their exceptional control on most tumours are quiet missing, which is mostly due to the upsurge in the resistance of these chemotherapeutic drugs.<sup>4</sup> Hence, there is a persistent plea for novel, potent, and more secure anticancer drugs that may help in contending with this dilemma.<sup>5</sup>

Anticancer therapy has commonly been directed via parenteral administration, which is the common reason for the poor compliance of anticancer therapy (apart from economic burden). Thus, there has been a constant increment in the demand for access to ideal oral anticancer drugs, providing clear benefits as a means of consolation and ease of use, in addition to the sufferers' proclivity for oral treatment.<sup>5</sup>

An additional decisive element is to boost the eminence of life and to enable the affected person to be unconstrained from the monetary burdens of current cancer therapies. In a recent observation, it has been determined that a couple of anticancer drugs, which are still evolving as anticancer agents, can be easily administered (mostly via the oral route).<sup>6</sup>

Metformin is a frequently recommended hypoglycaemic agent for treating diabetes mellitus type 2 and has gained appreciation due to its diversity of effects, which includes assistance in augmenting weight reduction, maintaining regular plasma levels of lipids in conjunction with the safety it offers in opposition to cardiovascular complications.<sup>7</sup>

Furthermore, its confirmed and useful healing consequences in polycystic ovarian diseases (PCO), currently

Metformin gains greater attention due to its potential to inhibit the growth of tumour cells.<sup>8</sup>

In recent times, it has been determined that Metformin can preclude tumour progression via the inhibition of the mTOR pathway.<sup>9</sup> mTOR plays a vital role in the development of various cancer cells, most commonly by either satisfying the nutritional necessities of tumour cells either by dint of endorsing the nutritional uptake by the extended amino acids and glucose transporter expressions.<sup>10</sup>

mTOR may also induce angiogenesis through increased hypoxia-inducible factor 1 and 2 expressions, thus replenishing the dietary needs of irregularly multiplied tumour cells.<sup>11</sup> This pathway can also persuade tumour advancement with the aid of overwhelming apoptosis via its impact at the cancer suppressor's genes (p53 and p27).<sup>12</sup> Additionally, mTOR has supervisory actions on the synthesis of cyclin D1 that is a crucial aspect of checkpoint in cell growth cycle.<sup>13</sup>

## Materials and Methods

This interventional study has been conducted in the Pharmacology Department of BMSI, in alliance with PCMD, Karachi, and the institutional ethical committee of JPMC, Karachi, approved this protocol (No. F 5–89/2015/GENL/599/JPMC).

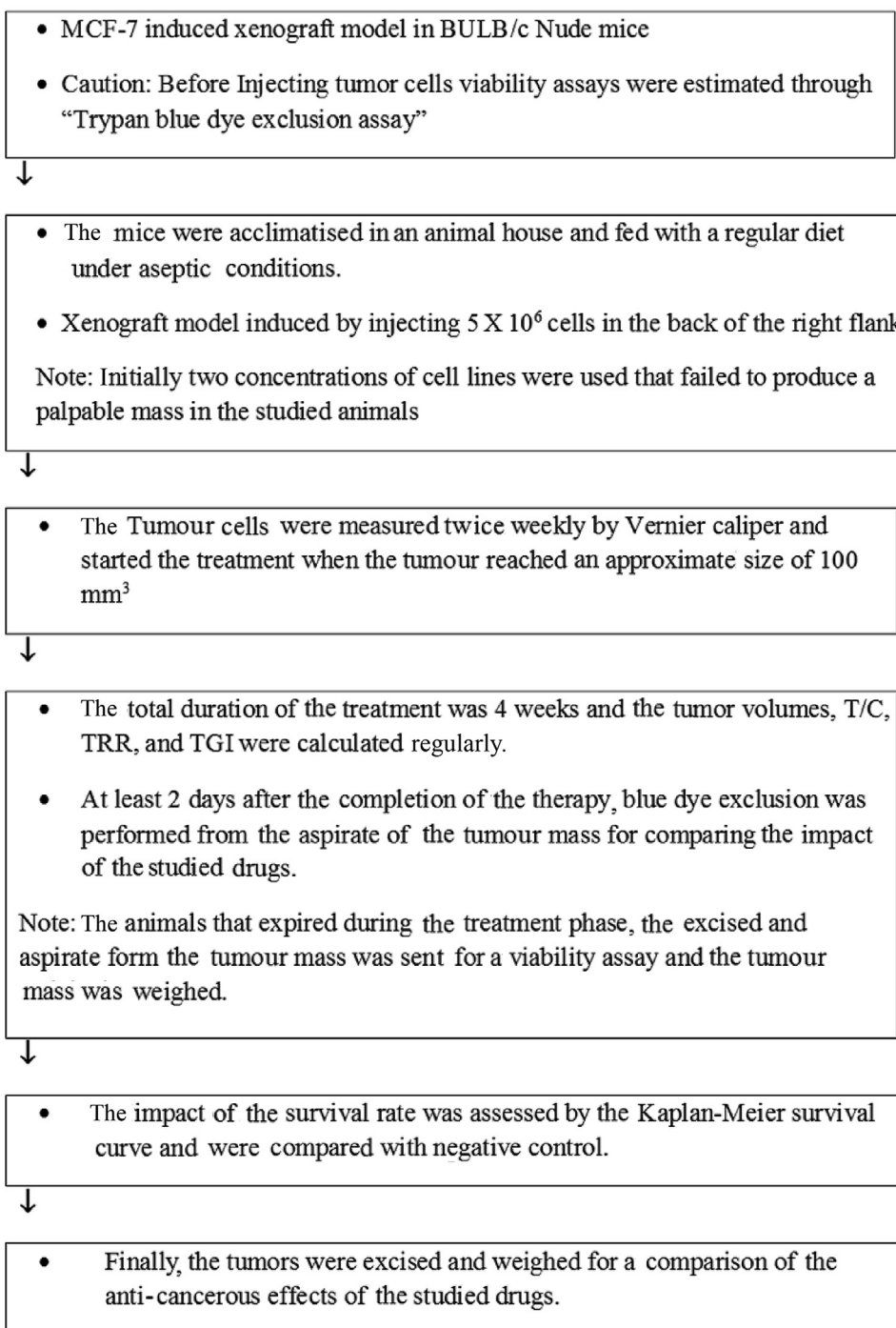
For the evaluation of the anticancer activity of the study drug, we have used the cell line derived Xenograft Model (CDX model), and the MCF-7 cell line (representative of luminal A breast cancer). MCF-7 cells have been refined in DMEM supplemented by antimicrobial and antifungal (Streptomycin and Penicillin and Amphotericin B), Sodium Pyruvate and L-Glutamine (both 1%) and 10% Foetal Bovine Serum (FBS) at 37 °C in a 5% CO<sub>2</sub> atmosphere. Before the inoculation of MCF-7 cells into the studied mice, the cells have been washed with PBS, trypsinized, resuspended in BMEM, and a trypan blue dye exclusion assay has been used for analysing the viability of MCF-7. About 98% viability is required for the inoculation of tumour cell cultures.<sup>14</sup>

A total sample size of 50 athymic BALB/c mice (25 in each treatment group) with ages ranging between 6 and 8 weeks has been utilized for this trial. The sample size is calculated by the 'resource equation' technique as defined by Charan and Kantharia (2013).<sup>15</sup> Fifty mice, divided equally into two groups; group A assigned to receive Metformin 150 mg/kg/day<sup>16,17</sup> PO for 30 days and group B assigned only to receive normal saline 30 mL/kg/day PO for 30 days. The mice have been cared for in a specific pathogen-free environment at room temperature with free admittance to food and water and a 12/12 h light/dark cycle.

A cell volume of approximately  $5 \times 10^6$  cells in PBS and Matrigel (0.5 mL) is infused into the back of the right flank of the mice. Tumour development is observed over the long run utilizing a Vernier calliper and the treatment is begun once the tumour reaches an approximate size of 100 mm.<sup>3,18–20</sup>

The study period has been for 3 months (12 weeks), and in the beginning, the animals have been screened on a daily groundwork and examined until the tumour volume surpassed about 100 mm<sup>3</sup> compared with when the therapy was started.

### Schematic flow chart for *in vivo* trial



Once the tumour reaches 100 mm<sup>3</sup>, the therapies are started, after which the tumour volumes are analysed every third day to evaluate tumour progress. As much as the discernible size is achieved in this experiment on day 7 in the tumour volumes of all species. Hence, management starts on day 7 and later a minimum of 10 animal follow-ups (after every third day) are conducted.

The total treatment period is 30 days; however, the animals were not sacrificed for survival rate assessment on day 36. Earlier at the baseline tumour inoculation, cell viability was assessed through a trypan blue dye exclusion assay. This has then been repeated at the end of the study.

The anticancer activity of the study drugs is observed through tumour volume, tumour regression ratio (TRR),

tumour control ratio (T/C ratio), tumour growth ratio (V/V<sub>0</sub>), tumour growth rate, Log cell kill (LCK), tumour weight, percent regression, and survival assessment with the help of the survival curve of the Kaplan–Meier method.

**Tumour volume:** The solid tumour volume is measured on day 5 after the tumour inoculation using Vernier callipers and are repeated until the end of the study every 3 days. The following formula is used for the calculation of the tumour volume<sup>21,22</sup>:

$$V = 0.5236 \times W \times L \times T$$

where L represents the length and W represents the Width.

**Tumour growth ratio (V/V<sub>0</sub>):** V/V<sub>0</sub> can be calculated by the treatment group tumour volume divided at a particular time using preliminary tumour boom at baseline (earlier than starting remedy).<sup>23,24</sup>

**Tumour control ratio:** The formula used to calculate the tumour control ratio is as follows<sup>25</sup>:

$$T/C \text{ Ratio} = \frac{\text{Cancer Growth volume of the experiment group}}{\text{Cancer Growth volume of the control group}}$$

Agents generating a T/C at around 15% are considered highly active against the tumour, agents with a mean tumour volume T/C of about 45% but >15% are considered to have intermediate activity, and those with mean T/C values > 45% are considered to have low activity levels.<sup>26</sup>

**Log cell kill:** This can be calculated by multiplying the tumour growth delay with tumour Growth rate. According to LCK reference values, the drug can be classified as highly active to inactive drugs<sup>27,28</sup>:

**Tumour regression ratio (TRR):** By dividing the growth rates of investigational group with the control group, we can calculate the tumour regression ratio.<sup>29</sup>

**Percentage Regression:** The following formula is used for calculating the percentage regression<sup>29</sup>:

$$\text{Percentage regression} = (T_0 - T_t) \times 100 / T_0$$

where T<sub>0</sub> is tumour volume on day 0; and T<sub>t</sub> is tumour volume of the same group at a particular time.

**Tumour Growth rate:** The tumour growth rate can be calculated by dividing the tumour weight (after excision) and total survival days of the mice.<sup>30</sup>

**Tumour delay (T – C):** This is calculated by the differences in time (in days) to reach 30% of its initial tumour volume in both groups (treated and control groups).<sup>31</sup>

**Tumour growth index (TGI):** The following formula is used to calculate the TGI (A TGI value > 50% is considered significant).<sup>32</sup>

$$\%TGI = (1 - \{T_t / T_0 / C_t / C_0\}) / 1 - \{C_0 / C_t\} \times 100$$

where,

T<sub>t</sub> = median tumour volume of the treated group at time t

T<sub>0</sub> = median tumour volume of the treated group at time 0 (at baseline)

C<sub>t</sub> = median tumour volume of the control group at time t

C<sub>0</sub> = median tumour volume of the control group at time 0 (at baseline).

**Kaplan–Meier survival curve:** This curve is an upfront, handy, and useful analytical device for measuring the treatment group's survival overall performance and the use of control as a reference to measure the studied group's survival rate, and may additionally play a remarkable role in producing fact-based survival time figures.<sup>33</sup>

**Trypan blue dye exclusion assay:** Before inducing the CDX xenograft model for the evaluation of the anticancer activity of the studied drugs, the viability of cancer cell culture should be determined by a trypan blue dye exclusion assay because before the inoculation of the tumour cell culture, its viability should be >95%.<sup>34</sup> Hence, in this study we perform a viability assay twice during the entire study period (before injecting the tumour cells and finally 2 days after the completion of the therapy by aspirating from the tumour mass).

The assay is based on the principle that live cells have intact cell membranes that exclude certain dyes, such as trypan blue, eosin, or propidium, while dead cells do not. A cell suspension is combined with a dye in this test and then visually examined to determine whether the dye is taken up or excluded by the cells. A viable cell would have a clear cytoplasm, while a blue cytoplasm will have a nonviable cell.

Blend 1 part of 0.4% trypan blue and 1 part of cancer cell suspension (cultured without FBS as this may mislead the results). This permits the combination to incubate for about 3 min at room temperature.

Note: Cells ought to be counted within 3 to 5 min of blending in with trypan blue, as longer brooding periods will prompt cell demise and decreased feasibility checks.

Pour a drop of the trypan dye and cancer cell blend to a haemocytometer. Spot the haemocytometer on the phase of a binocular magnifying instrument and spotlight on the cells (to check the viable and non-viable cells). Viable cells appear clear because the intact membrane dye was unable to penetrate inside the cell. While dead cells appear as stained as blue because of the lack of membrane dye that penetrated inside the cells.<sup>34</sup>

As per protocol for the calculation of viable cells, the dilution factor or element of trypan can be reduced by multiplying the total viable cells to 2. Similarly, for the calculation of total cell count, add the stained and unstained cells and multiplying it with 2. Cell viability percentage can be calculated with the following formula<sup>34</sup>:

$$\text{Cellular Viability (\%)} = \left\{ \frac{(\text{total cells} - \text{dead cells})}{\text{total cells}} \right\} \times 100$$

### Statistical analysis

Version 24.0 of IBM SPSS is used to analyse the data. Session variables, including tumour volume, treatment-control ratio (T/C), tumour regression ratio (TRR) and tumour growth index, are compared using the Repeated Measure ANOVA among various follow-ups. All variables among both groups are compared by using the Student's t-test.

Trypan blue dye exclusion assay variables including total cell count, viable cell count, death cell count, and percentage viability are compared between the baseline and the end of study using a paired t-test.

## Results

Comparison of tumour volume between both groups reveal that compared with the control group Metformin meaningfully reduces the progression of tumour in the MCF-7 transplant model of mice. As presented in Table 1, on day 7, no noteworthy variances are noted for tumour volume between both groups ( $p = 0.222$ ) and the mean tumour volume for Metformin and the control groups are  $109.34 \pm 4.073$  and  $110.90 \pm 4.802$  respectively. While at final follow up (at day 36), major differences ( $p = <0.001$ ) have been found among both groups and mean tumour volume for metformin and control groups have been  $172.316 \pm 6.497$  and  $715.989 \pm 31.59$ .

The anticancer effects of Metformin against MCF-7 induced breast cancer are further supported by the tumour control ratio (TCR) and TGI. Metformin significantly reduced the TCR at final follow-up from  $0.794 \pm 0.031$  to  $0.240 \pm 0.014$ . As per recommended TCR values, Metformin acts as an intermediate acting agent against MCF-7 induced breast cancer, because at final follow-up the TCR value was about 24%, which falls in the range between  $>15\%$  and  $<45\%$ .

**Table 1: Evaluation of Tumour dimensions between treated Groups.**

Follow ups	Group A Mean $\pm$ SD N = 25	Group B Mean $\pm$ SD N = 25	p-value
7th day	$109.34 \pm 4.07$ (107.57–111.02)	$110.90 \pm 4.80$ (145.5–150.5)	0.222
9th day	$119.38 \pm 5.93$ (116.93–121.83)	$150.31 \pm 5.76$ (141.16–165.92)	$<0.001^{**}$
12th day	$129.38 \pm 5.34$ (127.18–131.59)	$193.27 \pm 7.67$ (177.82–212.74)	$<0.001^{**}$
15th day	$137.46 \pm 6.58$ (134.75–140.18)	$231.35 \pm 6.89$ (217.00–244.72)	$<0.001^{**}$
18th day	$148.46 \pm 4.35$ (146.66–150.26)	$319.07 \pm 15.71$ (276.82–345.22)	$<0.001^{**}$
21st day	$155.78 \pm 4.81$ (153.80–157.77)	$388.95 \pm 26.39$ (339.17–428.72)	$<0.001^{**}$
24th day	$167.14 \pm 5.75$ (164.77–169.52)	$444.59 \pm 22.30$ (487.0–564.73)	$<0.001^{**}$
27th day	$174.78 \pm 4.92$ (172.75–176.81)	$514.18 \pm 21.29$ (487.00–564.73)	$<0.001^{**}$
30th day	$183.16 \pm 5.95$ (180.69–185.61)	$580.67 \pm 27.96$ (528.30–627.00)	$<0.001^{**}$
33rd day	$180.68 \pm 6.87$ (177.84–183.51)	$642.66 \pm 30.39$ (607.81–697.0)	$<0.001^{**}$
36th day	$172.32 \pm 6.50$ (169.63–174.99)	$715.99 \pm 31.59$ (656.81–787.0)	$<0.001^{**}$
p-value	$<0.001^{**}$	$<0.001^{**}$	

Mean  $\pm$  SD.

(Min–Max).

\*\* Significant at 1%.

**Table 2: Effects of Metformin on Tumour Control Ratio (TCR) and TGI.**

Follow ups	TCR Mean $\pm$ SD N = 25	TGI Mean $\pm$ SD N = 25
9th day	$0.79 \pm 0.03$ (0.78–0.81)	$20.55 \pm 3.126$ (19.26–21.85)
12th day	$0.67 \pm 0.04$ (0.65–0.69)	$32.95 \pm 3.88$ (31.35–34.55)
15th day	$0.59 \pm 0.02$ (0.59–0.60)	$40.59 \pm 2.06$ (39.74–41.44)
18th day	$0.47 \pm 0.03$ (0.46–0.48)	$53.37 \pm 2.49$ (52.34–54.40)
21st day	$0.40 \pm 0.03$ (0.39–0.41)	$60.35 \pm 4.38$ (58.55–62.16)
24th day	$0.38 \pm 0.02$ (0.37–0.39)	$62.85 \pm 3.57$ (61.38–64.32)
27th day	$0.34 \pm 0.02$ (0.33–0.35)	$66.43 \pm 2.63$ (65.35–67.52)
30th day	$0.31 \pm 0.02$ (0.31–0.32)	$68.99 \pm 2.40$ (68.01–69.99)
33rd day	$0.311 \pm 0.02$ (0.30–0.32)	$72.19 \pm 1.91$ (71.40–72.98)
36th day	$0.24 \pm 0.014$ (0.23–0.25)	$75.98 \pm 1.45$ (75.38–76.58)
p-value	$<0.001^{**}$	$<0.001^{**}$

Mean  $\pm$  SD.

(Min–Max).

\*\* Significant at 1%.

**Table 3: Comparison of Tumour Growth Ratio (V/V0) among treated Groups.**

Follow ups	Group A Mean $\pm$ SD N = 25	Group B Mean $\pm$ SD N = 25	p-value
9th day	$1.09 \pm 0.04$ (1.08–1.11)	$1.36 \pm 0.072$ (1.23–1.49)	0.003
12th day	$1.18 \pm 0.04$ (1.17–1.20)	$1.75 \pm 0.12$ (1.53–1.95)	$<0.001^{**}$
15th day	$1.26 \pm 0.051$ (1.24–1.28)	$2.09 \pm 0.11$ (1.88–2.29)	$<0.001^{**}$
18th day	$1.36 \pm 0.06$ (1.34–1.38)	$2.88 \pm 0.21$ (2.38–3.24)	$<0.001^{**}$
21st day	$1.43 \pm 0.06$ (1.40–1.45)	$3.51 \pm 0.29$ (2.92–4.02)	$<0.001^{**}$
24th day	$1.53 \pm 0.08$ (1.499–1.56)	$4.02 \pm 0.25$ (3.503–4.47)	$<0.001^{**}$
27th day	$1.60 \pm 0.08$ (1.57–1.63)	$4.62 \pm 0.29$ (4.19–5.23)	$<0.001^{**}$
30th day	$1.68 \pm 0.09$ (1.64–1.72)	$5.24 \pm 0.34$ (4.69–5.81)	$<0.001^{**}$
33rd day	$1.66 \pm 0.09$ (1.62–1.69)	$5.79 \pm 0.39$ (5.22–6.55)	$<0.001^{**}$
36th day	$1.58 \pm 0.09$ (1.54–1.62)	$6.45 \pm 0.42$ (5.64–7.23)	$<0.001^{**}$
p-value	$<0.001^{**}$	$<0.001^{**}$	

Mean  $\pm$  SD.

(Min–Max).

\*\* Significant at 1%.

**Table 4: Comparison of Tumour weight, Growth rate, Percentage regression among the treated groups.**

Variables	Treated Groups		p-value
	Group A Mean $\pm$ SD N = 25	Group B Mean $\pm$ SD N = 25	
Tumour weight (gm)	0.39 $\pm$ 0.03 (0.38–0.40)	0.55 $\pm$ 0.02 (0.52–0.58)	<0.001**
Growth rate	0.01 $\pm$ 0.001 (0.01–0.01)	0.02 $\pm$ 0.002 (0.01–0.02)	0.001
Percentage Regression	–57.80 $\pm$ 9.30 (–77.60 to –42.80)	–549.42 $\pm$ 41.55 (–623.44 to –464.19)	<0.001**
Growth Delay (Days)	12.40 $\pm$ 1.580 (9–15)	4.08 $\pm$ 1.91 (0.00–9.00)	<0.001**
LCK	0.11 $\pm$ 0.02 (0.07–0.15)	0.07 $\pm$ 0.03 (0.00–0.17)	<0.001**
Tumour Regression Ratio	0.57 $\pm$ 0.12 (0.41–1.00)	– –	

Mean  $\pm$  SD.

(Min–Max).

\*\* Significant at 1%.

**Table 5: Evaluation of the Survival analysis with Kaplan–Meier survival curve of the treated groups.**

Variables	Group A	Group B	P value
	Mean $\pm$ SD N = 25	Mean $\pm$ SD N = 25	
Total survival Days	44.28 $\pm$ 2.48 (39–49)	34.56 $\pm$ 4.17 (24–39)	<0.001**

Mean  $\pm$  SD.

(Min–Max).

\*\* Significant at 1%.

This is further supported by the fact that Metformin significantly reduced the tumour progression compared with negative control as Metformin significantly increased the TGI from  $20.555 \pm 3.126$  to  $75.979 \pm 1.446$  at final follow-up, which is presented in Table 2.

The anticancer effects of Metformin are further supported by its effect on the tumour growth ratio. Compared with the control group, Metformin can significantly reduce the tumour growth as there were statistically noteworthy differences at final follow-up ( $p = <0.001$ ) and mean  $V/V_0$  were  $1.578 \pm 0.093$  for Metformin and  $6.449 \pm 0.421$  for the control group, which is depicted in Table 3.

Table 4 indicates that Metformin therapy significantly reduced tumour progression and its effects on tumour weight, growth rate, percentage regression, and growth delay.

Similarly, compared with the control group, Metformin has a significant effect on the survival of MCF-7 induced breast cancer model of mice as assessed by the Kaplan–Meier survival analysis. A comparison of tumor viability as measured by the trypan blue dye exclusion assay revealed that the percent viability of the tumor was significantly lower in Metformin-treated groups (see Tables 5–7)

**Table 6: Evaluation of the effects on Trypan blue dye exclusion assay among the treated groups at Baseline.**

Variables	Treated Groups		P value
	Group A Mean $\pm$ SD N = 25	Group B Mean $\pm$ SD N = 25	
Viable cells count	266.10 $\pm$ 5.47 (263.20–269.00)	266.63 $\pm$ 5.89 (255.24–277.10)	0.056
Total cells count	271.00 $\pm$ 5.67 (268.20–273.90)	271.69 $\pm$ 5.88 (260.10–283.03)	0.053
Death cells Count	4.94 $\pm$ 1.73 (0.84–8.06)	5.47 $\pm$ 1.16 (3.73–7.74)	0.330
Percentage Viability	98.18 $\pm$ 0.64 (97.03–99.69)	98.04 $\pm$ 0.42 (97.23–98.70)	0.50

Mean  $\pm$  SD.

(Min–Max).

**Table 7: Evaluation of the effects on Trypan blue dye exclusion assay among the treated groups at final follow up.**

Variables	Treated Groups		P value
	Group A Mean $\pm$ SD N = 25	Group B Mean $\pm$ SD N = 25	
Viable cells count	123.20 $\pm$ 4.76 (121.30–125.00)	248.65 $\pm$ 7.07 (231.45–261.20)	<0.001**
Total cells count	266.20 $\pm$ 6.41 (263.40–269.00)	272.20 $\pm$ 7.53 (251.90–289.12)	0.001**
Death cells Count	143.09 $\pm$ 7.51 (129.50–162.20)	24.56 $\pm$ 3.11 (17.80–30.31)	<0.001**
Percentage Viability	46.29 $\pm$ 1.98 (41.67–49.65)	91.02 $\pm$ 1.08 (89.06–93.55)	<0.001**

Mean  $\pm$  SD.  
(Min - Max).

\*\* Significant at 1%.

## Discussion

Breast cancer is one of the paramount perplexing issues universally, principally in terms of diagnosis, hindrance, and in particular its treatment.<sup>35</sup> Presently, the mortality related complications of breast carcinoma continues to increase, which is predominantly attributable to late management of the disease and poor compliance of the patients. The poor compliance connected with anticancer medication was the result of monetary difficulties, solemn side effects related to conventional chemotherapeutic agents, and apprehension towards injectable therapy.<sup>36</sup> Hence, today there is an additional specialisation in search of economical medical care that effectively reduces the growth of cancer cells.<sup>37</sup>

Tumour cell lines are typically utilized for analysis functions and are concluded as being a helpful convenience within the genomic approach, and its portrayal exhibits that they are really a renowned expression for the analysis of the common segments, which are prerequisites in the disease.<sup>38</sup>

Metformin is one of the foremost routinely prescribed economical medicament.<sup>39</sup> Recently, it had been found that by its direct or indirect (insulin mediated) actions, this drug is useful in treating many cancers. Metformin employs its anticancer effects by using a galvanizing AMPK pathway that plays as the protagonist in cellular physiological circumstances.<sup>40</sup>

Through actuating AMPK, Metformin will impede the mTOR pathway, which plays a vital role in tumorigenesis.<sup>41</sup> Apart from that, Metformin can have anticancer activity by reversing insulin resistance, because insulin resistance is mostly associated with various diseases including cancers.<sup>42</sup>

In our study, we assessed Metformin's anticancer activity in the MCF-7 model of nude mice induced by a Xenograft model and compared it with a negative control group. Antitumor and cytotoxic activities have been measured by the influence of all therapies on the tumour size, T/C ratio, V/V0, TRR, TGI, tumour weight, tumour growth rate, and

tumour mass cell viability as assessed by trypan blue dye exclusion.

Metformin effectively reduced tumour progression as opposed to negative management as the mean tumour volumes were 172.316  $\pm$  6.497 and 715.989  $\pm$  31.59 for Metformin and normal saline groups at final follow-up (day 36), respectively, and very significant differences were observed among each group. This was further supported by the mean T/C value, which was around 24%, and hence was consistent with the reference ranges of T/C magnitude in relation to Metformin referred as intermediate-acting. In addition, metformin delays the tumour growth compared to the negative management signposted via the tumour growth delay (T-C). These results have been followed by Wang and Wu's (2015) analysis.<sup>43</sup>

Wang and Wu (2015)<sup>43</sup> revealed Metformin's preclinical anti-tumour activity alone and in bladder cancer with Cisplatin. They used BIU-87 and T24 cell lines for the *in vitro* analysis of anti-tumour activity and BIU-87 cells used for *in vivo* analysis of mice transplant model. They established that each medication, used independently or in combination, will effectively decrease cellular viability of *in vitro* cell lines T24 and BIU-87 as evaluated via the MTT assay, whereas Metformin and Cisplatin together ominously diminish the advancement of the tumour as judged by the volume and weight of the tumour.

Correspondingly Orecchioni et al. (2014)<sup>44</sup> evaluates the anti-tumour activity of antidiabetic drugs (Phenformin and Metformin) on immune deficit model of mice. As they appraise the primary tumour progress and metastasis ability, they concluded that Phenformin and Metformin significantly reduced the tumour growth in the mice transplant model induced by MDA-MB-436 compared to the control group. To boot, these drugs effectively reduce the carcinoma transplant model's metastatic capacity as evaluated via a microscopic analysis of various slices of axillary nodes and respiratory organ tissue.

Survival examination by dint of the Kaplan–Meier curve of Metformin indicates that Metformin imperatively increases the survival rate as related to the control group, which was in step with Yin et al. (2013).<sup>45</sup>

In a meta-analysis, Yin et al. (2013)<sup>39</sup> evaluate the favourable effects of antidiabetic drugs on cancer patients' survival by investigating the effects of the combination of antidiabetic drug treatment. They established an inclusive benefit in survival among cancer patients treated with Metformin compared with other antidiabetic drugs.

We conjointly examined the cytotoxic activity of Metformin through trypan blue dye exclusion assays, each at baseline and at the completion of the study. This conjointly demonstrates that Metformin meritoriously declines the viability of breast cancer cells, which was in agreement with the trial shepherded by Griss et al. (2015).<sup>46</sup>

This trial highpoints the useful anticancer effects of economic antidiabetic drugs on breast cancer and offers a route through which researchers will further examine these useful anticancer effects through both preclinical and clinical studies.

## Conclusion

This study revealed that Metformin effectively delays tumour growth in search of an effective economic oral anticancer therapy. Perhaps Metformin, in the treatment of breast cancer cell lines, would be a healthier aspirant to complement the conventional chemotherapeutic agents.

## Recommendations

This study accentuates the anticancer effects of Metformin in the xenograft model of breast cancer. As this trial opens the path for further research, in future there should be more intensive studies in terms of appraising their targeted molecules in larger sample trials.

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## Conflict of interest

The authors have no conflict of interest to declare.

## Ethical approval

Ethical Approval was done by the ethical committee of Jinnah Post Graduate Medical Centre (No. F 5–89/2015/GENL/599/JPMC dated 1 February 2015).

## Authors contributions

RF conceived and design the study configuration, additionally led research, coordinated the gathered data.SL

analysed the collected data and furthermore completed the interpretations of information. AA composed the draft of the manuscript and also proofread the contents. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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