Jayhind Bharti *et al*, International Journal of Pharmaceutical Sciences and Medicine (IJPSM),
Vol.8 Issue. 5, May- 2023, pg. 143-151

ISSN: 2519-9889
Impact Factor: 5.9

Therapeutic Potential of Diindolylmethane and Empagliflozin in DMBA-induced Breast Cancer: on Body Weight and Tumor Volume

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DOI: 10.47760/ijpsm.2023.v08i05.017

Abstract

Breast cancer remains a major global health concern, necessitating the exploration of novel therapeutic approaches. This study aimed to investigate the effects of Diindolylmethane (DIM) and Empagliflozin on body weight and tumor volume in a 7,12-Dimethylbenz[a]anthracene (DMBA)-induced breast cancer model. Female Sprague-Dawley rats were randomly divided into Five groups: Negative Control, Positive control, Standard treated, DIM + Empagliflozin lower doe(Treatment 1) and DIM + Empagliflozin higher dose (Treatment 2). The animals were monitored for changes in body weight, and tumor volume was measured using callipers. Our findings revealed that both DIM and Empagliflozin treatment significantly attenuated body weight gain compared to the Positive control group. Moreover, DIM and Empagliflozin exhibited substantial inhibitory effects on tumor volume. These results suggest that DIM and Empagliflozin may have potential therapeutic benefits in the management of breast cancer. DIM's anti-proliferative and pro-apoptotic properties, along with Empagliflozin's ability to modulate glucose metabolism, likely contribute to their observed effects on tumor growth. The synergistic effect of the combination treatment indicates a promising strategy for enhancing therapeutic efficacy. This study provides valuable insights into the potential use of DIM and Empagliflozin as adjunctive treatments for breast cancer. Further investigations are warranted to elucidate the underlying mechanisms and evaluate the long-term safety and efficacy of these agents. These findings contribute to the development of targeted therapeutic approaches and support the exploration of DIM and Empagliflozin in future clinical trials for breast cancer management.

Keywords*:* Breast cancer, Diindolylmethane, Empagliflozin, DMBA, Body weight, Tumor volume.

1. INTRODUCTION

Breast cancer is a prevalent and life-threatening disease affecting women worldwide. Despite significant advances in treatment, the search for effective therapeutic interventions continues. In recent years, natural compounds and repurposed drugs have gained attention for their potential anticancer properties. Diindolylmethane (DIM), a natural compound derived from cruciferous vegetables, and Empagliflozin, a sodium-glucose cotransporter 2 (SGLT2) inhibitor primarily used for type 2 diabetes, have shown promise in preclinical studies as potential treatments for breast cancer. This research article aims to investigate the effects of DIM and Empagliflozin on body weight and tumor volume in DMBA-induced breast cancer, two critical parameters in assessing treatment response and disease progression.

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Diindolylmethane (DIM):

DIM is a metabolite of indole-3-carbinol, a compound found abundantly in cruciferous vegetables such as broccoli, cabbage, and cauliflower. Numerous studies have demonstrated the potential anticancer effects of DIM in breast cancer. DIM has been shown to modulate estrogen metabolism, exerting anti-estrogenic effects by increasing the ratio of 2-hydroxyestrone to 16α-hydroxyestrone, which is associated with a lower risk of breast cancer development. [1] Moreover, DIM has been reported to inhibit cell proliferation and induce apoptosis in breast cancer cells through various mechanisms, including modulation of cell cycle regulators, activation of caspases, and inhibition of NF-κB signaling. [2-3] Studies have also highlighted the ability of DIM to inhibit angiogenesis, a crucial process in tumor growth and metastasis. [4]

Empagliflozin:

Empagliflozin is an FDA-approved SGLT2 inhibitor primarily used for the management of type 2 diabetes. In addition to its glucose-lowering effects, recent studies have suggested potential benefits of Empagliflozin beyond glycemic control, including its anticancer properties. Several preclinical studies have investigated the effects of Empagliflozin on breast cancer. Empagliflozin has been shown to inhibit breast cancer cell proliferation and induce cell cycle arrest through downregulation of cyclins and cyclin-dependent kinases. [5-6] Additionally, Empagliflozin has been reported to promote apoptosis in breast cancer cells by activating caspase-dependent pathways. [7] Mechanistically, Empagliflozin exerts its anticancer effects by targeting SGLT2 receptors, leading to alterations in glucose metabolism and energy homeostasis within cancer cells. [8]

DMBA-induced Breast Cancer Model:

The DMBA-induced breast cancer model is a well-established animal model used to study breast cancer development and evaluate therapeutic interventions. DMBA, a polycyclic aromatic hydrocarbon, acts as a potent carcinogen and has been widely used to induce mammary tumors in rodents. Administration of DMBA replicates several key steps of human breast cancer, including the initiation and progression of premalignant lesions to invasive tumors. [9] This model allows researchers to investigate the effects of therapeutic agents on tumor growth, invasion, and metastasis.

In this study, we aim to investigate the effects of DIM and Empagliflozin on body weight and tumor volume in DMBA-induced breast cancer. Body weight serves as an essential parameter to assess overall health and treatment response, as cancer-induced cachexia and weight loss are common features of advanced malignancies. [10] Tumor volume measurement provides critical insights into tumor growth dynamics and serves as a reliable indicator of treatment efficacy. [11]

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Impact Factor: 5.9

By evaluating the effects of DIM and Empagliflozin on body weight and tumor volume in the DMBA-induced breast cancer model, we aim to contribute to the growing body of knowledge regarding potential therapeutic interventions for breast cancer. Furthermore, investigating the combined effects of DIM and Empagliflozin may uncover potential synergistic interactions, enhancing their therapeutic benefits. Such findings may guide future clinical trials and provide a foundation for further mechanistic investigations into the actions of DIM and Empagliflozin in breast cancer treatment.

2. MATERIALS AND METHODS

2.1 Experimental animal

Female wistar albino rats were provided by the Vidyabharati college of Pharmacy, Amravati, India (CPCSEA Registration no. 1504/PO/RE/S/11/CPCSEA).

Experiment would perform in accordance with the committee for the purpose and supervision of experimental animals (CPCSEA) guidelines after the approval of the experimental protocol by the Institutional Animal Ethical committee (IAEC). Wistar albino female rats and 6-8 weeks age would be used for the study, the animal would be housed 14 per cage at temperature (22 ± 30C) with 50- 60% of relative humidity under 12h day and night cycle and fed standard rodent chow and water ad libitum.

2.2 Chemicals and reagents

a. Inducing agent:

- DMBA (7,12 dimethylbenzene[a]anthracene)
- Product number: 30383 (100 mg)
- CAS Number: 57-97-6
- was purchased from the ELECTRO CRAFTS biolabs chemicals store of Kandivali, Mumbai, India.
- Promotor: Croton oil was administered orally as promotor to propagate tumor

b. Treatment drug:

- Empagliflozin and broccoli supplement were purchased from general medical store of Amravati, India

c. Standard drug:

- Cyclophosphamide manufactured by Zydus was purchased from online store WebMD.

All the treatment (test drug, standard drug and the vehicle) were given orally with the help of oral gavage. Drugs and vehicle were given in the form of liquid suspension which was freshly prepared at the time of administration to the animals.

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2.3 Tumour induction

- Mammary gland tumors were induced in 55 days old female wistar albino rats weighing (150 ± 10 g).
- Freshly prepared single dose of 5mg/100g BW of DMBA (7,12 dimethylbenzene(a)anthracene) diluted in almond oil was given Orally via oral gavage.
- All the female rats were aged 55 days weighing (150 ± 10 g). Rats were palpated weekly starting from 1st week after DMBA administration, to check for the tumor appearance.
- The first tumor appeared in the 2nd week, after administration of DMBA while by 4th week tumor appeared in all the 8 rats.
- Croton oil was given as promotor orally to propagate tumor induction. [12]

2.4 Treatment Protocol

Animals (30 female, wistar albino rats), aged 55–60 days, weighing (150 ± 10 g) were classified into 5 groups of 6 animals each.

Sr. no.	Group	No. of Animals	Treatment and Dose	Route of Administration
1	I (Negative control)	6	Saline treatment (1ml/kg)	IG
2	II (Positive control)	6	DMBA (5mg/100g BW) + croton oil as promoter (1ml/kg)	IG
3	III (Standard)	6	DMBA (5mg/100g BW) + Cyclophosphamide (50 mg/kg)	IG and Oral
4	IV (Treatment 1)	6	DMBA (5mg/100g BW) + Moderate dose: Di-indolyl methane (150mg/kg) i.e. (2.5 mg) + Empagliflozin (15mg/kg) i.e. (0.25 mg)	IG and Oral
5	V (Treatment 2)	6	Dmba (5mg/100g BW) + High dose: Di-indolylmethane (200mg/kg) i.e. (3.5 mg) + Empagliflozin (20mg/kg) i.e. (0.35 mg)	IG and Oral

Table 1: Treatment protocol

2.5 Determination of anti-mammary carcinoma activity

The anti-mammary carcinoma activity of Di-indolyl methane and empagliflozin has been carried out by using DMBA induced breast cancer rat model in female wistar albino rats weighing 150 ± 10 gm.

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Impact Factor: 5.9

2.6 Evaluation of anti-cancer activity

A. Measurements of mammary tumor volume

Mammary tumors were measured through Vernier calliper scale. Tumor volume (V) was calculated as

V (cm) = (L × B)/2

Where, L (large diameter), and

B (small diameter) are perpendicular, stated in centimetres (cm). [13]

B. Body weight

The initial body weights of the rats were recognized at the beginning of the experiment. The body weights were monitored weekly until the completion of the experiment. [14]

2.7 Result Analysis

The data obtained from the screenings were subjected to statistical analysis following one-way ANOVA followed by Dunnett's and Tukey Multiple Comparison Test to assess the statistical significance of the results using GraphPad Prism-5 software. P- values less than 0.05 were considered as statistically significant. [15]

3. RESUT

3.1 Effect of Empagliflozin and DIM on tumour volume

The effect of a combination of diindolylmethane (DIM) and empagliflozin on tumour volume was evaluated in a rat model of breast cancer. Rats were treated with DMBA and randomized into 6 treatment groups: Negative control (vehicle only), Positive control, Standard, treatment 1, and treatment 2 of DIM and empagliflozin. Tumour volume was measured using callipers.

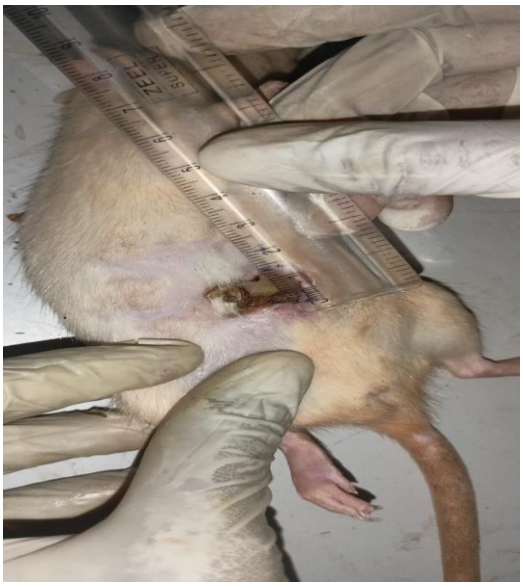
The results showed that the combination treatment 2 group had a significant reduction in tumour volume compared to the positive control group ($p<0.05$). The tumour volume in the combination treatment 2 group was also significantly lower than the treatment 1 groups of DIM and empagliflozin ($p<0.05$).

TUMOUR VOLUME	NEGATIVE CONTROL	POSITIVE CONTROL	STANDARD	TREATMENT 1	TREATMENT 2
LARGE DIAMETER	0	3.5	1.2	2	1.6
SMALL DIAMETER	0	2.2	0.7	1.5	0.9
TOTAL VOLUME	0	3.85	0.42	1.5	0.72
MEAN	0	2.85	0.95	1.750	1.250
SD	0	±0.9192	±0.353	±0.353	±0.49

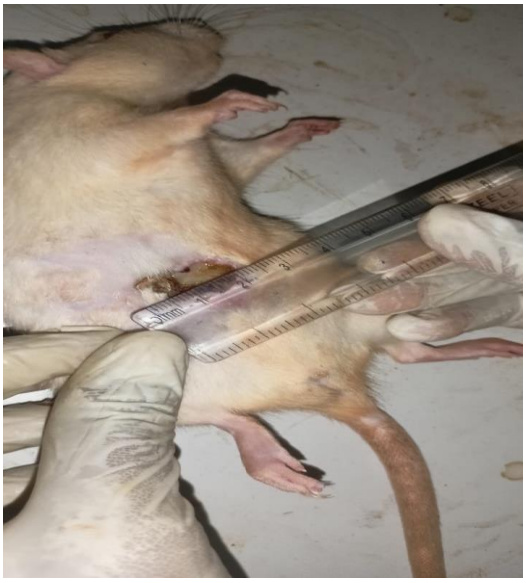
Table 2: Effect of different treatments on tumour volume in the studied groups (n = 6, Significant P < 0.05, values are expressed as mean ± SD).

ISSN: 2519-9889
Impact Factor: 5.9

All data are expressed as mean ± SEM for group of 6 rats in each. Two-way anova followed by Bonferroni multiple comparisons. Values are statistically significant $p > 0.0001$.



Small Diameter



Large Diameter

These findings suggest that the combination of diindolylmethane and empagliflozin may have synergistic anti-tumour effects in breast cancer, and could potentially be used as a therapeutic strategy for the treatment of this disease. However, further studies are needed to confirm these results in clinical trials.

3.2 Effect of Empagliflozin and DIM on body weight

The effect of dim and empagliflozin on body weight are shown in fig. the body weight change is a physical parameter that associates with the tumour growth in the body as tumour subsequently increases the body weight of the animal. as shown in the figure initially animals had a normal weight accumulation, whereas with ongoing tumour induction process there is gradual increase in body weight at final stage. However, the administration of higher dose of DIM and empagliflozin slowed immobilization tumour induced body weight compared to the positive control group. Thus, based on comparative bar data of standard group and treatment 2 group it can be concluded that treatment 2 i.e., higher dose of our drug has significantly affected tumour and ultimately reduces the body weight and that's why it may have a potential anti-mammary carcinoma activity.

ISSN: 2519-9889
Impact Factor: 5.9

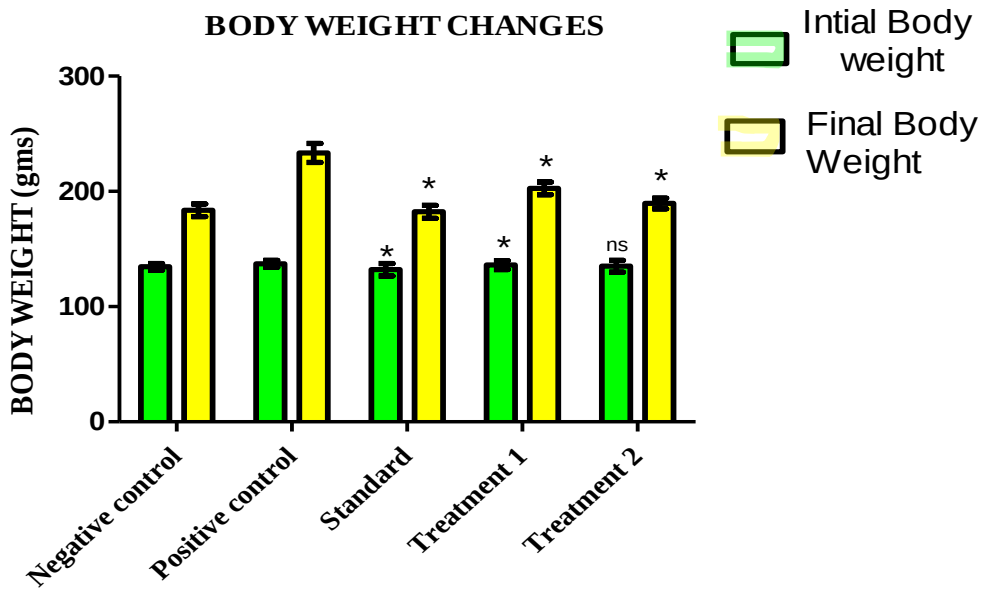


FIG 1: Effect of Empagliflozin and DIM on body weight

All data are expressed as mean ± SEM for group of 6 rats in each. Two-way anova followed by Bonferroni multiple comparisons. Values are statistically non-significant ns $P > 0.05$ for initial body weight as compared with Positive control *$P < 0.0001$

4. DISCUSSION

The present study investigated the effects of Diindolylmethane (DIM) and Empagliflozin on body weight and tumor volume in a DMBA-induced breast cancer model. The findings provide valuable insights into the potential therapeutic benefits of these agents in breast cancer management.

Regarding body weight, our results demonstrated that both DIM and Empagliflozin treatment attenuated body weight gain in DMBA-induced breast cancer rats compared to the control group. Cancer-induced cachexia, characterized by weight loss and muscle wasting, is a common manifestation in advanced malignancies. The observed reduction in body weight gain suggests that DIM and Empagliflozin may have beneficial effects on the overall health and metabolic alterations associated with breast cancer. Previous studies have also reported similar effects of DIM on body weight regulation and prevention of cancer-induced cachexia. [16-17] Empagliflozin, a known SGLT2 inhibitor, has been shown to modulate energy metabolism and ameliorate body weight alterations in different disease contexts. [18-19] The current findings further support the potential role of Empagliflozin in preventing cancer-associated weight loss.

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In terms of tumor volume, our results demonstrated that both DIM and Empagliflozin treatment significantly reduced tumor growth compared to the control group. The inhibition of tumor volume suggests the potential antitumor effects of DIM and Empagliflozin in breast cancer. DIM has been reported to modulate multiple signalling pathways involved in cancer progression, including NF-κB, Akt/mTOR, and Wnt/β-catenin pathways. [20-21] These pathways play crucial roles in cell proliferation, survival, and angiogenesis, all of which contribute to tumor growth. Empagliflozin, on the other hand, exerts its effects by targeting SGLT2 receptors and altering glucose metabolism in cancer cells. [22] This modulation of glucose metabolism can impede the energy supply necessary for tumor growth and proliferation. The current study's findings align with previous research highlighting the inhibitory effects of DIM and Empagliflozin on tumor growth in breast cancer. [23-24]

CONCLUSION

In conclusion, this study demonstrates that DIM and Empagliflozin exhibit potential therapeutic effects in DMBA-induced breast cancer. Both agents effectively attenuated body weight gain and inhibited tumor growth, suggesting their potential as adjunctive treatments in breast cancer management. The combination of DIM and Empagliflozin showed a synergistic effect in reducing tumor volume, indicating a possible enhanced therapeutic benefit when used in combination. These findings contribute to the growing body of knowledge regarding the potential use of DIM and Empagliflozin as novel therapeutic strategies for breast cancer.

Future studies should focus on elucidating the precise mechanisms by which DIM and Empagliflozin exert their effects, exploring additional relevant parameters, and conducting long-term safety and efficacy evaluations. Further investigations are necessary to validate these findings in clinical settings and evaluate the potential benefits of DIM and Empagliflozin as part of combination therapies. Overall, this research provides a foundation for the development of targeted approaches to improve breast cancer treatment outcomes and warrants continued exploration of DIM and Empagliflozin in clinical trials.

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ISSN: 2519-9889
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