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IMPACT OF DAPAGLIFLOZIN ON APOPTOSIS AND EFFECTOR CYTOTOXIC CELLS USING BREAST CANCER CELLS IN MICE

Shimaa M. Elkhyat[1*], Nageh A. El-Mahdy[2], Mayada E. Elhusseiny[3], Abdel-Aziz A. Zidan[4]

[1]*Department of Pharmacy, Tanta UniversityEducational Hospital, Tanta, Egypt*
[2]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tanta University, Tanta, Egypt*
[3]*Department of Histology and Cell Biology, Faculty of Medicine, Tanta University, Tanta, Egypt*
[4]*Department of Zoology, Faculty of Science, Damanhour University, Damanhour, Egypt*

Background:*Dapagliflozin is a sodium-glucose cotransporter-2 (SGLT2) inhibitor with anti-inflammatory, antioxidant, and apoptosis modulator activity. Our purpose of this study was to assess the immediate impact of dapagliflozin on cytotoxicity and apoptosis of Ehrlich Ascites Carcinoma (EAC) cell lines in vitro and investigate its effect with a standard or high insulin diet in solid EAC-bearing mice compared to cyclophosphamide (CTX) chemotherapy.* ***Methods:*** *EAC cell lines were seeded for in vitro cytotoxicity by MTT assay and apoptosis after treatment with serial concentrations of dapagliflozin (1, 4, 10, 25, 50, and 100 M) for 24 hours. After that, solid EAC-bearing mice were inoculated subcutaneously at tumor growth, Mice were divided and treated with dapagliflozin in the presence of a low or high insulin diet or with a combination of dapagliflozin and CTX.* ***Results:****In vitro results showed that dapagliflozin at 25 M had a cytotoxic effect on EAC cell viability at 13.8% compared to untreated cells at 100%. Dapagliflozin at (25, 50, and 100) M gradually increased late apoptotic cell percentage (21,33, and 46% respectively).In vivo results showed that the administration of EAC-bearing mice with an average glucose diet in the presence of dapagliflozin increased the percentage of effector CD8 T cells population ($CD8^+$ $KLRG1^+$) to 13.6% compared to CTX, and control group 2.8%, 7.7% respectively.* ***Conclusions:*** *Dapagliflozin and cyclophosphamide can be used together to increase antitumor activity and enhance immunologic and pathological outcomes.*

Keywords*:Antitumor,Cytotoxicity,Dapagliflozin, andEffector cells*

INTRODUCTION

Traditional cancer cures are being selected according to each subject's medical condition, type, stage of cancer, general health, and lifestyle. Chemotherapy, radiation, and sometimes their combining are among the several expensive therapies methods. Alopecia, reduced appetite, vomiting, nausea, diarrhea, sores in the mouth, pancytopenia, and occasionally suppression of bone marrow are only a few of the most common adverse reactions of these medications that may cause morbidity[1].

Cancer still threatens the public's health despite considerable advancements in its diagnosis and therapy[2]. Immunotherapy is an appealing anti-cancer strategy, which may selectively target and produce antitumor impacts while having fewer side effects on normal cells. However, results from clinical trials testing different vaccine approaches have been rather disappointing. Therefore, current cancer immunotherapeutic paradigms must be significantly improved by developing alternative approaches[3].

Many animal models have been established to investigate the precise molecular processes that underlie the etiology of cancer.

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*Corresponding author: Shimaa M. Elkhyat, E-mail: shimaamahmoudelkhyat@gmail.com

Because of its high transplantable capability, inexpensive price, rapid proliferation, ease of convenience, and high rate of similarity to human malignancies[4], Solid Ehrlich carcinoma (SEC) formed as one of the most frequently utilized transplantable tumors models in studies on cancer[5].

An effective treatment for a non-insulin-dependent form of diabetes is the sodium-glucose cotransporter-2 (SGLT2) inhibitor dapagliflozin[6]. Several animal studies have shown that dapagliflozin has effective anti-inflammatory and antioxidant characteristics[7,8]. According to research, dapagliflozin may also alter the signaling pathways that control apoptosis and autophagy[9]. These results could point to a possible use of dapagliflozin in cancer treatment.

The ability of glucose to enter cancer cells is crucial for their survival. As a result, cancer cells express more glucose transporter-1 (GLUT1)[10]. Numerous cancer cell types, including those of cancer of the colon, have recently been shown to possess SGLT2 along with GLUT1.1 Although the inhibition of GLUT1 is a promising possibility for cancer treatment, this strategy has had difficulties since normal cells additionally express GLUT1, and inhibition would probably have several adverse impacts on healthy tissues[11]. However, given that SGLT2 expression is more limited than the widely expressed GLUT1, it may be feasible for SGLT2 inhibitors to have a selective decrease in tumor development[12]. We aim to address the associated effects of using dapagliflozin as an anti-cancer drug on tumor progression, effector T cells, and histological changes.

METHODS

Dapagliflozin Forxiga (5 mg) was purchased from Astra Zeneca, Cairo, Egypt, andcyclophosphamide (CTX) (Catalog # C0768-10G0) was obtained from Sigma-Aldrich Company, CA, USA. PBS, and diluted to 4 mg/150µl with PBS for IP administration at a dose of 200 mg/kg. The following supplies were bought from Sigma (Cairo, Egypt): buffered phosphate saline (PBS), comprehensive RPMI-1640 medium, which is enriched with amino acid-containing, dimethyl sulfoxide (DMSO), antibiotics, and MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide). The apoptosis analysis chemicals, propidium iodide (PI), and annexin V were bought from BD Biosciences in Ca, USA.

Ehrlich Ascites Carcinoma (EAC), a breast cancer cell line, was bought from Cairo University's National Cancer Institute in Egypt. Holding Company for Biological Products and Vaccines (VACSERA), Cairo, Egypt, sold 90 female BALB/c mice 6 to 8 weeks old. At the start of the trial, the rats weighed between 22 and 25 gm. At the Tanta University Faculty of Pharmacy, mice were treated and kept in an environment free of pathogens upon the approval of the institutional ethical committee. The mice had been adjusted for two weeks before the commencement of the experiment.

Cell culture preparation

The Ehrlich ascites fluid was collected from Ehrlich female with ascites BALB/c mice and then suspended in PBS before being centrifuged at 1200 rpm for 10 minutes at 4° C. The supernatant was abandoned, PBS was added, and cells were recently refuge for further washing. The trypan-blue-exclusion-assay assessed cell viability, then at a seeding density of 2×10^6 cells per flask, EAC cells were grown in T-25 tissue culture flasks with 7 mL of complete culture media. Cells were left in a CO_2 incubator at 37° C for 24 hours. After that, cells were examined under an inverted microscope to check for confluence. Once the cells were 80% confluent, drug treatment was started.

The clarification of ascetic fluid induction and collection was added in cell culture preparation methodology section as the following:

The parent cell line cells were obtained from the national cancer institute at Cairo University in Egypt. EAC cells were injected intraperitonealy (I.P) 2.5×105 cells .within 16-18 days following the tumor injection, the ascetic fluid was developed after that mice were scarified by cervical dislocation, and EAC cells were extracted from the peritoneal cavity and centrifuged for 10 minute at 1200 rpm at 37° C. At least two rounds of washing were performed with 30 ml phosphate buffer saline (PBs).

Then the trypan blue viability test was used to assess the total number of tumor cells

MTT cytotoxicity assay on EAC cells

Various concentrations of dapagliflozin (1, 4, 10, 25, 50, and 100 M) were prepared for cytotoxicity evaluation by MTT assay compared to untreated EAC as a negative control as EAC cells were seeded into 96-well plates in 200 µl at 1 x 10^3 cells per well. Cells receiving therapy were kept at 37°C in 5% CO_2 in an incubator for a single day. Following removing the media, the cells were rinsed with 100 µl of PBS, 20 ml of MTT (5 mg/ml) was applied to each thoroughly, and the plate was subsequently allowed to sit for 4 hours. After that, 150 µl of DMSO was transferred to each well after the cells were cleaned with PBS. A plate reader was used to determine the absorbance at 570 nm. Utilizing GraphPad Prism 6.0, the examination of cell viability was carried out.

EAC cell apoptosis by flowcytometry measurement

From cultivated cells that were earlier exposed to dapagliflozin doses of 1, 4, 10, 25, 50, and 100 M, EAC cells were gathered. The cells had been resuspended in 1X annexin-binding buffer to a final density of 1 × 106 cells/ml after being cleansed twice with ice-cold PBS, their density being measured. In 1.5 ml Eppendorf tubes, the cell suspensions were placed with 5 µL of annexin V-fluorescein isothiocyanate (FITC) and 1 µL of PI (100 µg/ml) working solution. After 15 minutes of room-temperature incubation with the stained EAC cells, 400 µL of 1X annexin-binding buffer was added, gently mixed, and the samples were then preserved on ice. Flow cytometry was then used to evaluate the cells.

Induction of solid Ehrlich carcinoma in vivo

Ehrlich cancer cells were put in saline suspension and measured via the trypan blue exclusion method using a hemocytometer under an inverted microscope. 250×10^3 cells were administered subcutaneously (s.c.) into the mammary gland in each mouse for all 25 mice. After the tumors had reached a palpable size, mice in each group were then further split into five groups (n =10 /group) to be treated at D9 with 200 mg/kg of CTX, 0.1 mg/kg Dapagliflozin in the presence of low or enriched diet-based glucose, and combination of CTX and Dapagliflozin, contrasted to un-treated EAC-bearing mice.

Blood samples collection

Blood specimens were taken on day 28 from a mouse's tail vein and placed in tubes containing EDTA for flow cytometric evaluation and glucose measurement. All mice were anesthetized by ether and sacrificed by cervical dislocation.

Phenotypic analysis of effector CD8 T cells in blood samples isolated from EAC-bearing mice

Anti-mouse antibodies CD8 (PE-Cy7) and KLRG (APC) were used to stain the blood sample (100 µl), which was provided by BioLegend, San Diego, CA, US. After the stained samples had been left to sit in the dark for 20 to 30 minutes, the erythrocytes were lysed for 15 minutes using BD FACS lysis buffer (1X). The specimens were subsequently centrifuged for 5 minutes at 1250 rpm. PBS was used to wash and resuspend the cells twice. At Tanta University's Center of Excellence in Cancer Research (CECR), cells were obtained using a FACSCanto II (BD Biosciences), and BD FACS Diva software (BD Biosciences) was utilized for data processing.

Glucose Measurement

Plasma was separated from withdrawn blood samples from treated mice. Using direct and automation-ready procedures following glucose kits data sheet.For measuring glucose level after incubation for 8 minutes with glucose working reagent, then the absorbance was directly observed at 630nm using an ELISA reader at the research unit, faculty of pharmacy, Tanta University, Egypt.

Measuring The tumor volume and calculating the tumor growth rate

Tumor sizes were routinely measured by a Vernier digital caliper from day seven up to day 28. The formula determined the tumor's volume: volume (mm3) = (length × width2)/2.

Tissue sampling for histological examination

The malignant masses were removed, weighted, and dissected. Some of the dissected tumors were promptly preserved in 10% buffered formalin solutions for histological analysis. The formalin-fixed tumors have been managed using progressively stronger alcohols, followed by xylene. Following serial sectioning of the tissues into 3-5 m thick blocks of paraffin, they were subsequently stained with H&E. Using a digital camera enabling photography and a light microscope, all of the stained slices of tissues were inspected.

Statistical analysis

The Statistical Package for Social Science (SPSS) version 18 was used for the statistical analysis. Following a one-way analysis of variance (ANOVA), Tukey's post hoc test was conducted. The tumor volume, growth rate, and other data involving two independent parameters were analyzed using a two-way ANOVA. Fisher's least-significant differences (LSD) were then used as a post hoc analysis. The difference between mean values is considered statistically significant at $p < 0.05$. The findings were displayed as mean ± SD.

RESULTS AND DISCUSSION

Results

Effect of different concentrations of Dapagliflozin on Ehrlich ascites carcinoma (EAC) cellsMTT cytotoxicity assay.

EAC cell viability was examined after treatment with (1, 4, 10, 25, 50, and 100 M) dapagliflozin compared to untreated EAC for 24 hours using an MTT assay. In-vitro, findings revealed that the different concentrations of dapagliflozin reduced cell viability by various percentages. In contrast, the 25M concentration showed the lowest viability, which decreased from 100% to 13.8% compared to untreated EAC cell viability (100%), as shown in **Fig. 1**. Therefore, the calculated concentration of dapagliflozin to be used at IC50 was 7.4 M.

Evaluation of apoptosis of EAC tumor cells after treatment

EAC cells were cultivated and exposed to different doses of dapagliflozin (1, 4, 10, 25, 50, and 100 M) to evaluate the apoptosis of EAC tumor cells obtained from culture. 24-hour contrast with untreated EAC cells, Cells were harvested for the assessment using annexin/PI staining by flow cytometry. The findings revealed that dapagliflozin at (25, 50, and 100 M) produces a substantial gradual rise in late apoptotic cell percentage (21, 33, and 46%), respectively, compared with untreated EAC cells (0.1%). At the same time, dapagliflozin at (1 and 25 M) enhanced a remarkable increase in necrotic cell percentage (14 and 15.9%) compared with untreated EAC cells (7.8%). As well, other treated cells with dapagliflozin at 4, 10, 50, and 100) M showed non-significant differences in necrotic cell percentage (9.5, 9.8, 6.6, and 7.3%), respectively, as shown in **Fig. 2**.

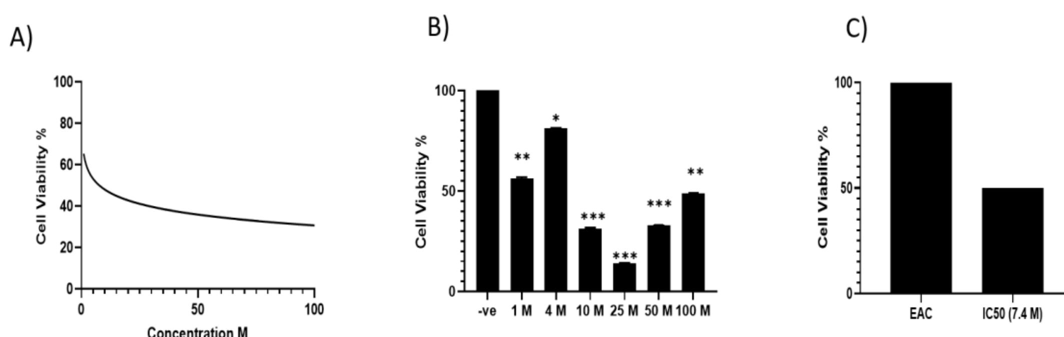

Fig. 1: The effect of dapagliflozin on the EAC viability. (A and B) showing the percentage of EAC cell viability after treatment with various concentrations (1, 4, 10, 25, 50, and 100 M) of dapagliflozin. C) showing the calculated percentage of EAC cell viability of IC50 concentration of dapagliflozin. EAC was seeded in complete RPMI-1640 medium at 37° C, 5%CO_2, then treated with different concentrations of dapagliflozin. EAC cell viability was measured using an MTT assay. Data were represented as mean ± SE (n = 3). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $P < 0.0001$ statistically significant comparison of control group.

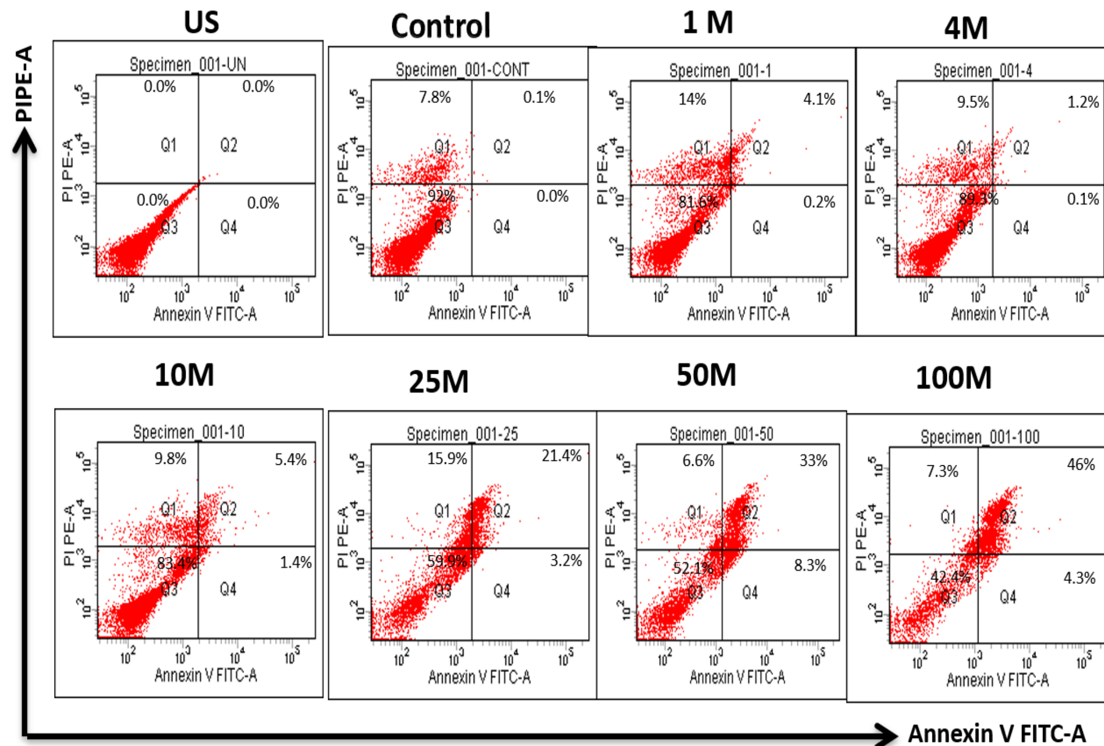

Fig. 2: The effect of dapagliflozin on EAC apoptosis. EAC was seeded in complete RPMI-1640 medium at 37° C, 5% CO_2, then treated with different concentrations of dapagliflozin. EAC cell apoptosis was measured using flow cytometry. Data were represented as mean ± SE (n = 3). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $P < 0.0001$ statistically significant comparison of control group.

Effects of Dapagliflozin on phenotypic analysis of effector CD8 T cells CD8+ KLRG1+ in blood samples

Phenotypic analysis of effector CD8 T cells displaying a CD8+KLRG1+ phenotype in EAC-bearing mice treated with a standard or high glucose and dapagliflozin or with a combination of dapagliflozin and CTX. The findings displayed that administration of EAC-bearing mice with an average glucose diet with dapagliflozin significantly increased the percentage of effector CD8 T cells population(CD8+ KLRG1+) to 13.6% compared to CTX, and control group 2.8%, 7.7%, respectively; however, the administration of EAC-bearing mice with high glucose diet and dapagliflozin, and a combination of CTX and dapagliflozin showed approximately the same percentage of effector CD8 T cells population compared to CTX 2.7%, 2.7%, and 2.8% respectively as shown in **Fig. 3**.

Effect of Dapagliflozin on tumor progression in tumor-bearing mice

CTX and dapagliflozin together are more efficient in the Ehrlich solid tumor model. Following mice treatment with high glucose diet and dapagliflozin or normal glucose diet and dapagliflozin, the findings revealed that combining CTX and dapagliflozin showed a significant effect in limiting the propagation of tumors based on the tumor size in comparison to CTX and EAC; however, a reduction in tumor progression with high glucose diet and dapagliflozin has existed, normal glucose diet and dapagliflozin groups compared to CTX and EAC. In addition, the combination group was more efficient at reducing tumor progression, as shown in **Fig. 4**.

- At day 28 after treatment there was significant decrease in tumor size by 95.2%. Between control group (EAC) and glucose enriched diet +dapagliflozin.
- At day 28 after treatment there was significant decrease in tumor size by 94.2%. Between cyclophosphamide (CTX) and glucose enriched diet +dapagliflozin.
- At day 28 after treatment there was significant decrease in tumor size by

97.4%. Between control group (EAC) and normal diet +dapagliflozin.

- At day 28 after treatment there was significant decrease in tumor size by 96.9%. Between cyclophosphamide (CTX) and normal diet + dapagliflozin.
- At day 28 after treatment there was significant decrease in tumor size by 99.8%. Between control group (EAC) and combination CTX+ dapagliflozin.
- At day 28 after treatment there was significant decrease in tumor size by 99.8%.Between cyclophosphamide (CTX) and combination CTX+ dapagliflozin.
- At day 28 after treatment there was significant decrease in tumor size by 96.6%.Between glucose enriched diet+dapagliflozin and combination CTX+ dapagliflozin.
- At day 28 after treatment there was significant decrease in tumor size by 93.5% between normal diet+ dapagliflozin and combination CTX+ dapagliflozin.

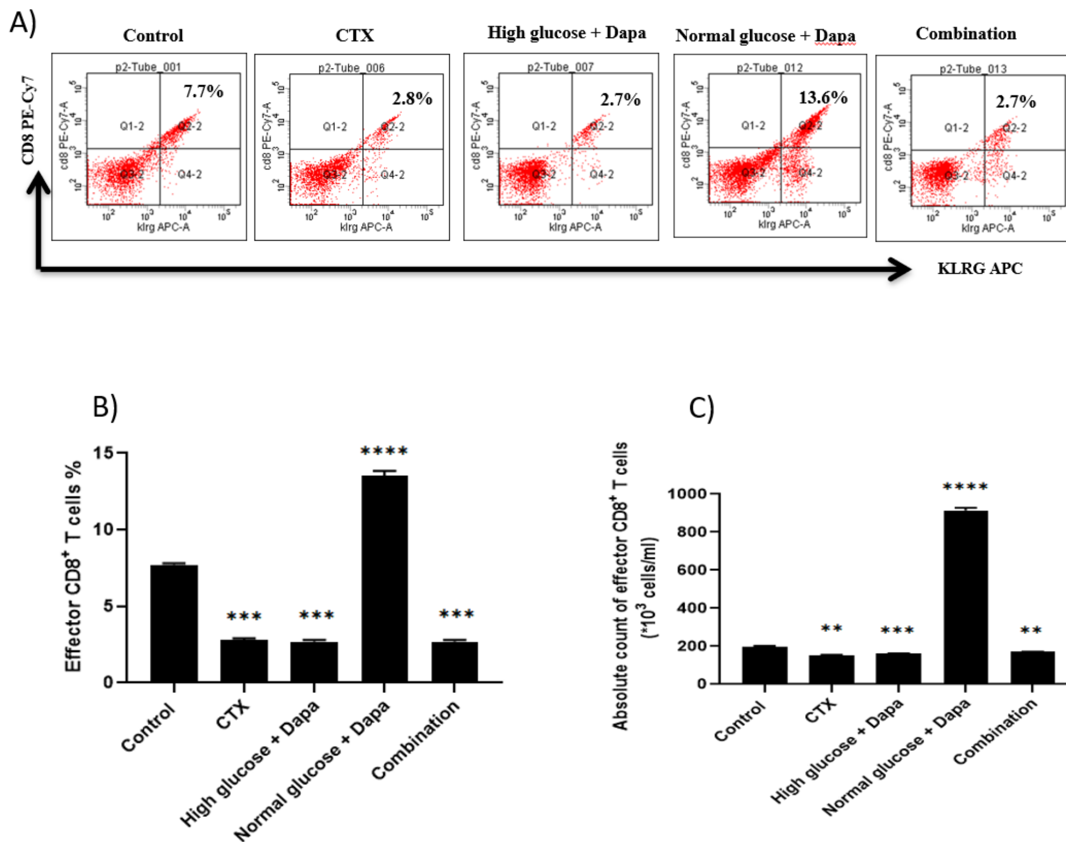

Fig. 3: The effect of dapagliflozin on phenotypic analysis of effector CD8 T cells CD8+ KLRG1+ in blood. Mice were challenged with EAC cells on day 0, treated with dapagliflozin with normal and high glucose dietary and a combination of Dapagliflozin with CTX on day one and sacrificed at day then phenotypic analysis was assessed using flow cytometry. Data were represented as mean ± SE (n = 3)., ** $p \leq 0.01$, *** $p \leq 0.001$, **** $P < 0.0001$ statistically significant comparisons of the control group.

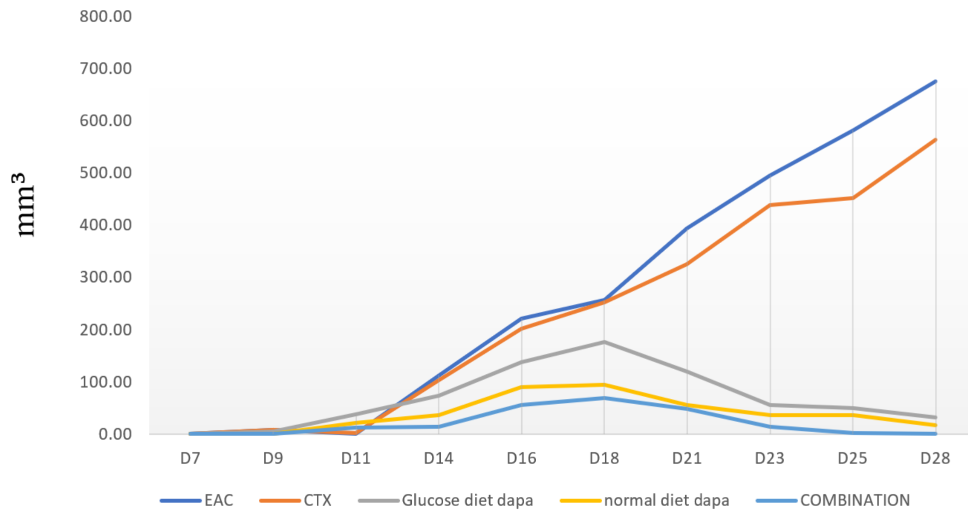

Fig. 4: The effect of dapagliflozin on tumor progression. Mice-bearing tumors were observed frequently for measuring the tumor size three times a week. The data shown in this figure represents a minimum of seven individual observations with ten animals per group.

Effect of dapagliflozin on Glucose level in tumor-bearing mice

Glucose level analysis showed that no significant variation existed in the untreated EAC group or treated with the high diet and a combination of dapagliflozin and CTX. Of note, a significant reduction in glucose levels in CTX and normal diet and dapagliflozin group existed as compared to untreated and other treated groups, as shown in **Fig. 5**.

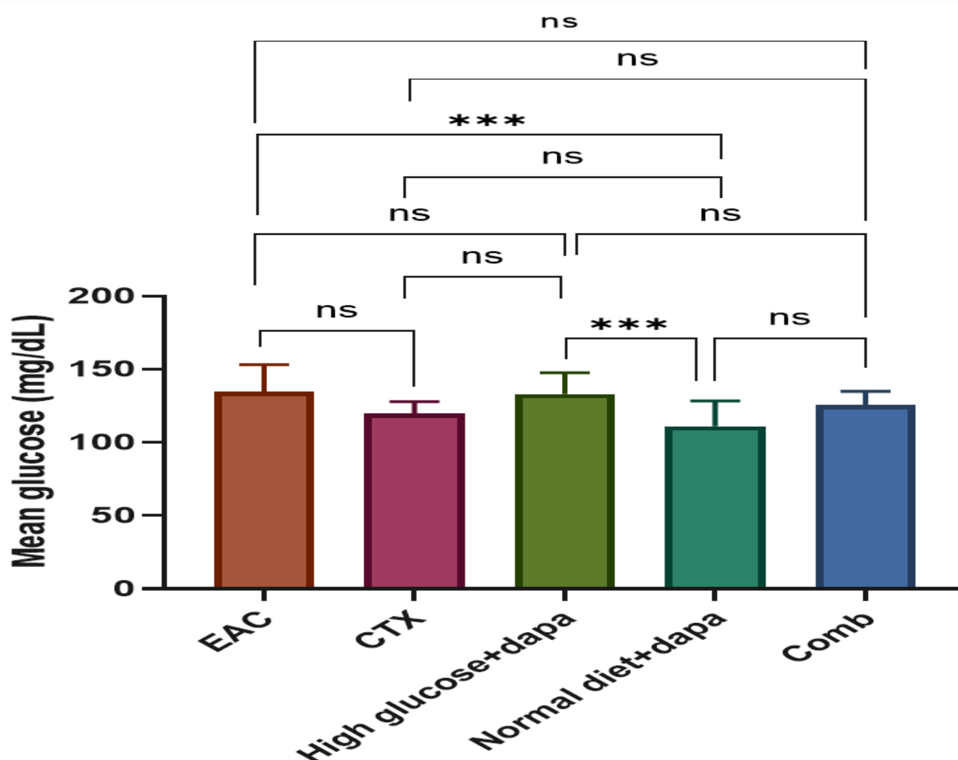

Fig. 5: The effect of dapagliflozin on glucose level in blood. Mice were challenged with EAC cells on day 0, treated with dapagliflozin with normal and high glucose dietary and a combination of Dapagliflozin with CTX on day one and sacrificed at day then glucose level was assessed using ELISA reader. Data were represented as mean ± SE (n = 3)., ** $p \leq 0.01$, *** $p \leq 0.001$, **** $P < 0.0001$ statistically significant comparisons of the control group.

Effect of dapagliflozin on Histopathological changes in tumor-bearing mice

In order to assess the effect of dapagliflozin on the histopathological changes in tumor-bearing tumor mice, different sections were taken from the mammary gland in all the experimental groups, as shown in **Fig. 6**.

A photomicrograph of a section of EAC showed the following: A) Positive control group I showed disturbed histological architecture of mammary gland, diffuse infiltration with tumor cells (thin arrow), pleomorphism, hyper-chromatism, and elevated N/C ratio, admixed with few sites of necrosis (thick arrow). B) Group II (EAC tumor treated with dapagliflozin on high glucose diet) showing disturbed architecture, tumor cells (thin arrow), few sites of necrosis (thick arrow), and apoptotic bodies (curved arrow). C) Group III (EAC tumor treated with cyclophosphamide) showing little effect. Some cells were normal with pale stained nuclei (arrowhead), while still; there were focal areas of tumor cells (thin arrow) and wide areas of necrosis (thick arrow). D) Group IV (EAC tumor treated with dapagliflozin on a normal diet) showed mild improvement with an increasing number of normal cells with pale stained nuclei (arrowhead) while still there were a focal area of tumor cells (thin arrow) and necrosis (thick arrow). E) Group V (EAC tumor treated with combined cyclophosphamide and dapagliflozin) showing marked improvement of the histological architecture of the mammary gland, arrangement of lobules (L) of normal cells with pale stained nuclei except for areas of necrosis (thick arrow) and focal area of ghost cells (G).

NB: tumor cells (thin arrow), area of necrosis (thick arrow), apoptotic bodies (curved arrow). (H&E Mic. Mag. x 200)

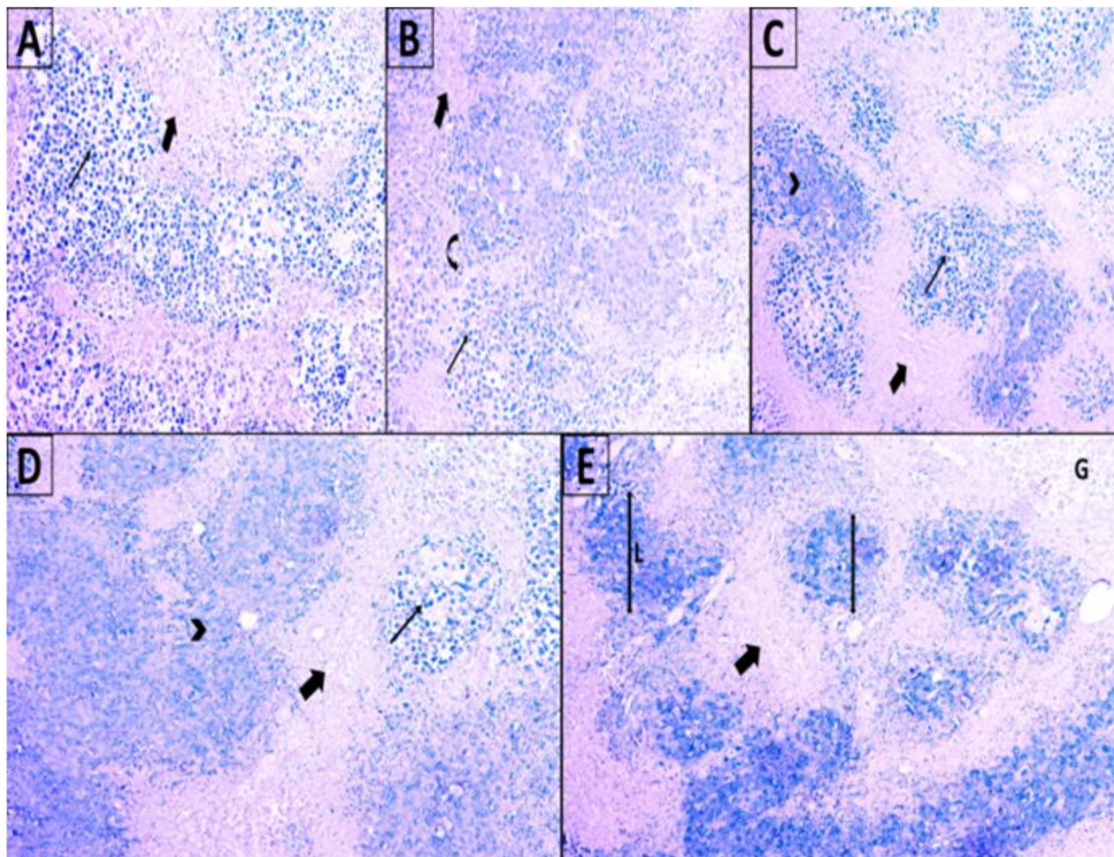

Fig. 6: A photomicrograph of a section of Ehrlich Ascites carcinoma (EAC) showed A) Positive control group. B) Group II (EAC tumor treated with Forxiga on high glucose diet). C) Group III (EAC tumor treated with cyclophosphamide). D) Group IV (EAC tumor treated with Forxiga on a normal diet). E) Group V (EAC tumor treated with combined cyclophosphamide and Forxiga). (H&E Mic. Mag. x 200).

Discussion

Dapagliflozin, a sodium-glucose cotransporter2 inhibitor, has potential therapeutic advantages in cancer by restricting glucose uptake and altering the tumor microenvironment. In vitro, studies showed that it reduced cell viability and proliferation in Ehrlich cells[13]. A study examined dapagliflozin's impact on breast cancer immune response, finding it reduced immune-suppressive T cells and Myeloid-derived suppressor cells (MDSCs). Further research is needed to understand its effects on Ehrlich cell cytotoxicity and immunity[14].

The present work was aimed to assess the potential antitumor activity of dapagliflozin on cancer cell lines of Ehrlich ascites carcinoma in-vitro and determine the in-vivo antitumor activities of dapagliflozin on tumor-bearing mice.

Our findings showed that the different concentrations of dapagliflozin decreased cell viability by various percentages, while 25 M showed the lowest viability of 13.8% compared to untreated EAC cell viability (100%). Growing evidence shows that dapagliflozin may have anti-cancer effects when apoptosis is analyzed. Dapagliflozin can cause apoptosis in cancer cells, including tumor Ehrlich cells, by inhibiting SGLT2, which regulates glucose absorption. This leads to lower blood glucose levels and increased urine glucose excretion, potentially starving cancer cells and causing apoptosis[15].

Dapagliflozin activates adenosine monophosphate activated protein kinase (AMPK), controlling metabolism and cell development, while inhibiting mTORC1, regulating protein synthesis and growth. This leads to autophagy, which can cause apoptosis if prolonged or severe [16]. Dapagliflozin inhibits non-homologous end joining (NHEJ) which responsible of DNA repair mechanism, increasing cancer cell vulnerability to DNA damage and potentially leading to apoptosis[17].

The findings revealed that all conditions showed a non-remarkable difference in early apoptotic cell percentage. Interestingly, dapagliflozin at 25, 50, and 100 M gradually increased late apoptotic cell percentage (21, 33, and 46%), respectively, compared with untreated EAC cells (0.1%). At the same time, dapagliflozin at 1 and 25 M enhanced a remarkable increase in necrotic cell percentage (14 and 15.9%) compared with untreated EAC cells (7.8%). Also, other treated cells with dapagliflozin (4, 10, 50, and 100 M) showed a non-significant difference in necrotic cell percentage (9.5, 9.8, 6.6, and 7.3%, respectively). These results are in line with Kaung et al.[18] Who found that dapagliflozin's anti-cancer activity as an SGLT2 inhibitor, exhibited cytotoxic effects and regulated apoptosis in human renal cell carcinoma. It also showed hypotonicity in normal cells. It could be explained that dapagliflozin could significantly regulate the phosphorylation of the MAPK pathway[19].

The MAPK pathway is crucial for activating T lymphocytes, which play a central role in cancer immunity. CD8+ T lymphocytes kill malignant cells upon recognition by the T cell receptor (TCR). Recent research suggests CD8+ T cells may express the KLRG1 marker[20]. As a result, we examined the phenotypic traits of effector CD8 T cells with a CD8+ KLRG1+ phenotype in the blood of mice that had Ehrlich Ascites Carcinoma and were given various treatments. Regarding the phenotypic characteristics of these cells, we specifically looked at the impacts of a high glucose diet in the presence of dapagliflozin, a normal glucose diet in the presence of dapagliflozin, the combination of CTX and dapagliflozin, and free CTX as a control. It has been shown that treatment with the combination of CTX and Dapagliflozin led to a significant increase in the frequency of CD8+ T cells expressing the KLRG1 marker compared to the other treatment groups, which is consistent with previous studies[21]. This suggests that this combination therapy may enhance the antitumor immune response by promoting the expansion of effector CD8 T cells. These findings are consistent with Singh et al.[22], who discovered that immuno-stimulatory chemotherapies and targeted immuno-suppressive macrophages in mouse models of Triple negative breast cancer (TNBC) resulted in long-term primary tumor regression in several murine mice models. Combination therapy treated tumors significantly enriching CD8+ T cells, while a high glucose diet with dapagliflozin treatment decreased CD8+ T cell frequency[23]. This finding may suggest that a high glucose diet

may impair the antitumor immune response by altering the phenotype of effector CD8 T cells.

Tumor growth in established EAC tumors was significantly delayed with both normal and enriched diets in the presence of dapagliflozin and combination groups compared to tumor-bearing mice or single CTX doses. This finding is comparable to a work by Nasiri et al.[24] examining the potential of dapagliflozin as an antitumor agent in vivo Poptani et al.[25] found a significant growth delay after CTX treatment in replication timing regulatory factor 1(RIF-1) tumors, possibly due to the high dose and different inoculated tumor cells. The combination group showed the most reduction in tumor growth, suggesting that inhibiting SGLT2 by dapagliflozin may improve CTX therapy efficacy. Furthermore, this study exposed a substantial decrease in the glucose level in the normal diet-dapagliflozin group compared to the EAC group and high glucose-dapagliflozin group. These findings concur with those published by Nasiri et al.[24], who found that the glucose level was substantially decreased in the treated group by dapagliflozin compared to the tumor-bearing mice.

A recent study has demonstrated that dapagliflozin induces apoptosis in human renal and breast tumors cells. Ji hoon jang et al., 2022 demonstrated that dapagliflozin treatment dose-dependently increased cell death in Caki-1 cells (renal cancer cell line). Dapagliflozin treatment also induced apoptosis as confirmed by FITC-conjugated Annexin V/PI staining. Additionally, treatment with dapagliflozin reduced the expression levels of anti-apoptotic proteins, cellular Fas-associated death domain-like interleukin-1-converting enzyme-inhibitory protein (cFLIP)L and cFLIPS in Caki-1 cells. Benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl-ketone inhibited dapagliflozin-induced apoptosis, implying that dapagliflozin–induced apoptosis is regulated by a caspase-dependent pathway. Furthermore, overexpression of cFLIPL, partially inhibited apoptosis induced by dapagliflozin. cFLIPL and cFLIPS mRNA levels remained constant in Caki-1 cells after treatment with 0, 20, 40, 60, 80 and 100 µM dapagliflozin. Notably, it was confirmed that cFLIPS protein levels were reduced due to the increased cFLIPS instability in dapagliflozin-treated Caki-1 cells. dapagliflozin had no effect on HK-2 normal human kidney cells[26] .

Moreover, our histological results explained that disturbed histological architecture was observed in the mammary gland with diffuse infiltration of the tumor cells,hyperchromatism, pleomorphism, and an elevated N/C ratio(ratio of (N) nucleus to the cytoplasm (C)which elevated in malignancy) admixed with a few necrosis sites and apoptotic bodies. These characteristics were highly observed in the EAC group and EAC tumors treated with high glucose-dapagliflozin compared to the normal control tissues.

Our findings are consistent with Barakat et al.[4], who contrasted the anti-cancer activity of spirulina (200 and 800 mg/kg) on a murine model of EAC to that of a conventional 5-fluorouracil (FU). The results indicated that the untreated EAC-bearing mice produced a palpable tumor that was solid following 13 days, and representative slices taken from these animals revealed leukocyte infiltration, newly developed blood capillaries, and tumor cells infiltrating subcutaneous tissue. Additionally, mice with EAC tumors showed very little necrosis (8–12%).

In the present study, the EAC tumor treated with a normal diet and dapagliflozin showed mild improvement with an increasing number of normal cells but still had focal areas of tumor cells and necrosis. Also, EAC tumors treated with combined CTX and dapagliflozin showed marked improvement in the histological architecture of the mammary gland, the arrangement of normal cell lobules with reduced necrosis areas, and the focal area of ghost cells. Moreover, Sethi, Sen et al.[27] reported similar results in their study. The results showed shrinkage of tumor cells with retrogressive changes. Necrosis was the most common event observed.

Our study demonstrated that, combination use of dapagliflozin simultaneously with cyclophosphamide could enhance the antitumor activity and improve the Immunological and pathological outcomes.

Conclusion

Dapagliflozin and cyclophosphamide can be used as a combination to increase antitumor activity and enhance immunologic and pathological outcomes.

Author contributions

SME, NAE, MEE, AAZ designed, performed experiments, and wrote the paper. SME, NAE performed the experiments. NAE, MEE, AAZ contributed to the preparation of essential materials and commented on the experiments and paper.

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نشرة العلوم الصيدليـــة
جامعة أسيوط

تأثير داباجليفلوزين على موت الخلايا المبرمج والخلايا السامة المناعيه باستخدام خلايا سرطان الثدي فى فئران التجارب

شيماء محمود الخياط[١]* – ناجح المهدي[٢] – ميادة الحسيني[٣] – عبد العزيز عوض زيدان[٤]

[١] قسم الصيدلة، مستشفى جامعة طنطا التعليمي، طنطا، مصر

[٢] قسم علم الأدوية والسموم، كلية الصيدلة، جامعة طنطا، طنطا، مصر

[٣] قسم الأنسجة وبيولوجيا الخلية، كلية الطب، جامعة طنطا، طنطا، مصر

[٤] قسم علم الحيوان، كلية العلوم، جامعة دمنهور، دمنهور، مصر

المقدمه: داباغليفلوزين هو مثبط لناقل الصوديوم والجلوكوزبالكلى وله نشاط مضاد للالتهابات ومضادات الأكسدة ويذيد معدل موت الخلايا المبرمج. كان هدفنا من هذه الدراسة هو تقييم التأثير المباشر للداباجليفلوزين على الخلايا السامه المناعيه وموت الخلايا المبرمج لخلايا سرطان استسقاء إيرليش في المختبر والتحقيق في تأثيره مع اتباع نظام غذائي قياسي أو عالي الجلوكوز في الفئران الحاملة لــ خلايا السرطان مقارنة بالسيكلوفوسفاميد الذى يعتبرالعلاج الكيميائي القياسيى .

الطرق: تم زرع الخلايا السرطانيه فى وحدات زرع لنمو الخلايا من أجل اختبار السمية الخلوية في المختبرلمادة الداباجليفلوزين وموت الخلايا المبرمج بعد العلاج بتركيزات متسلسلة من الداباجليفلوزين (١، ٤، ١٠، ٢٥، ٥٠، و ١٠٠ مول) لمدة ٢٤ ساعة. بعد ذلك، تم تلقيح الفئران الحاملة لــ سرطان الثدى تحت الجلد عند نمو الورم، وتم تقسيم الفئران وعلاجها بالدباغليفلوزين في وجود نظام غذائي منخفض أو مرتفع بالجلوكوز أو بمزيج من داباجليفلوزين والسيكلوفوسفاميد.

النتائج: أظهرت النتائج المختبرية أن الداباجليفلوزين عند ٢٥ مول له تأثير سام للخلايا على حيوية خلايا سرطان الثدى بنسبة ١٣,٨% مقارنة بالخلايا غير المعالجة بنسبة ١٠٠%. الداباجليفلوزين عند (٢٥، ٥٠، و١٠٠) مول زاد تدريجيا نسبة موت الخلايا المبرمج المتأخرة (٢١،٣٣، و ٤٦٪ على التوالي). أظهرت النتائج في الجسم الحي أن إعطاء الفئران الحاملة لــ خلايا سرطان الثدى مع نظام غذائي متوسط الجلوكوز في وجود داباجليفلوزين زادت النسبة المئوية لعدد خلايا CD8 T المستجيبة المناعيه السامه والقاتله للخلايا السرطانيه (CD8+ KLRG1+) إلى ١٣,٦% مقارنة بــ بالسيكلوفوسفاميد ، ومجموعة التحكم ٢,٨%، ٧,٧% على التوالي.

الاستنتاجات: يمكن استخدام داباغليفلوزين وسيكلوفوسفاميد معا لزيادة النشاط المضاد للأورام وتعزيز النتائج المناعية والمرضية.