International Journal of Pharmacology

ISSN 1811-7775

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OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2020.276.281

Research Article
Effect of Acute Chemotherapy on Glucose Levels in Rats

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Abstract

Background and Objective: Diabetes Mellitus (DM) occurs when body cannot produce enough insulin or develops resistance to insulin at the level of insulin-sensitive tissues. This leads to high blood glucose (hyperglycemia) and the complications of DM. Anticancer chemotherapeutic agents producing cytotoxicity can cause DM by damaging pancreatic β-cells, reducing the production of insulin or its sensitivity in the body. The study was designed to evaluate the effect of acute treatment of common chemotherapeutic agents on the blood glucose levels in rats. **Materials and Methods:** The effects of acute chemotherapy with doxorubicin (DOX), cyclophosphamide (CYP) or fluorouracil (5-FU) on blood glucose levels and induction of DM were tested in female rats. Experimental animals were divided onto four groups such as; control (normal saline), DOX (2 mg kg^{-1} b.wt.), CYP (50 mg kg^{-1} b.wt.) and 5-FU (40 mg kg^{-1} b.wt.). A single dose of anticancer drugs was administered by intra-peritoneal route for five days. The parameters such as; survival rate, blood glucose level, insulin tolerance and normalized body weight were determined. The data obtained was statistically compared by one-way ANOVA followed by Tukey's test to find the significance. **Results:** The data from the present study indicated that acute treatment of anticancer chemotherapeutic agents did not produce mortality nor significantly altered blood glucose level and insulin tolerance. However, a significant ($p<0.001$) decline in normalized body weight was observed at last day of the experiment. **Conclusion:** The result of the study suggests that single dose treatment of DOX, CYP and 5-FU for five days did not alter the blood glucose in rats.

Key words: Chemotherapy, diabetes, blood glucose levels, metabolic disorders, hyperglycemia, cytotoxicity

Citation: Ahmad H. Alhowail, Gena S. Alfawzan, Maha A. Aldubayan and Lolwah S. Alsalamah, 2020. Effect of acute chemotherapy on glucose levels in rats. Int. J. Pharmacol., 16: 276-281.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder resulting from the failure of tissues to respond to insulin and use glucose, resulting in chronic hyperglycemia. Diabetes interrupts carbohydrate, fat and protein metabolism with severe consequences, resulting in long-term complications [1]. In 2014, there were up to 387 million diabetic patients worldwide and it is estimated that this number will increase by more than 200 million by 2035[2]. In T2DM, the main problem is not the disease itself but the comorbidities that plague patients as the disease progresses. These complications affect other physiological systems and organs, resulting in cerebrovascular dysfunction[3], liver failure[4], renal failure[5,6] and neuropathy[7].

Previous studies have revealed that insulin signaling plays a vital role in cell survival[8]. Insulin signaling is implicated in the regulation of many cellular signaling pathways, such as; the phosphoinositide 3-kinase (PI3K) and Mitogen Activated Protein Kinase (MAPK) pathways[9,10]. Binding of insulin to the insulin receptor causes phosphorylation of insulin receptor substrates that activate the PI3K/AKT pathway, which is involved in glucose transporter trafficking to the cell surface, thus causing glucose influx from the bloodstream and reducing hyperglycemia[11]. Alterations in insulin signaling can also modify other functions[12]. For example, insulin signaling is critical for brain function, changes in insulin signaling can alter glucose uptake and modulate neurotransmitter release in the brain, producing cognitive dysfunction or heart arrhythmia[13,14].

Hyperglycemia caused by T2DM is also associated with the development of cancer[15]. Cancer and T2DM share common risk factors: older age, male sex, obesity, lack of physical activity, a high-calorie diet and tobacco smoking[16,17]. Acute stress caused by cancer itself or by chemotherapeutic agents used to treat cancer exacerbate insulin resistance, causing hyperglycemia[18]. Chemotherapy induces cytotoxic effects that can cause hyperglycemia via two mechanisms: first, by damaging pancreatic b-cells and thus reducing the production of insulin[19]; second, by affecting other cells, triggering insulin resistance[20]. However, previous studies investigating chemotherapy-induced hyperglycemia are controversial[21].

The aim of this study was to assess chemotherapy-induced hyperglycemia by testing the effects of multiple chemotherapeutic agents on glucose levels and insulin tolerance in experimental rats. Animals were injected with doxorubicin (DOX), cyclophosphamide (CYP) or fluorouracil (5-FU) daily and glucose levels were assessed one day after the last dose.

MATERIALS AND METHODS

The study was conducted at the Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Kingdom of Saudi Arabia, during the month of February, 2020.

Chemicals: The CYP (Endoxan®) was obtained from Baxter (Germany), DOX (ADRIM®) was obtained from Fresenius Kabi Oncology Ltd. (India); 5-FU (Utoral®) was from Korea United Pharm Inc., South Korea.

Animals: Albino female rats (12 weeks old) with body weight (between 170-220 g) bred in the animal facility at Qassim University were used in this study. Rats (n = 6 per group) were divided into four groups and received a single dose of DOX (2 mg kg^{-1}), CYP (50 mg kg^{-1}) or 5-FU (40 mg kg^{-1}) by intraperitoneal injection every day for 5 days. Rats were housed in Qassim University's animal house in a controlled and pathogen-free environment (25°C) with free access to water and standard chow diet. The experiment was conducted as per the standard guidelines for animal studies and after obtaining the approval from Institutional Animal Ethics Committee (Approval ID 2020-CP-3).

Insulin tolerance test: The insulin tolerance test was performed after 3 h fasting state as described previously[22]. Blood samples were collected from both chemotherapy-treated rats and control rats through the tail vein every 10 min for 1 h after intraperitoneal injection of insulin (2 U kg^{-1}). Plasma glucose was determined by dropping blood into blood glucose meters obtained from ACCU-CHEK company.

Other parameters recorded in the study were survival rate, blood glucose level and normalized body weights at the start and end of the experiment.

Data analysis: Data were analyzed by using Graphpad Prism 5 software by one-way analysis of variance, followed by Tukey's test to compare all variables. The $p \leq 0.05$ was considered to indicate the significance of the results.

RESULTS

Effect of chemotherapy on survival rate: Data to determine the influence of acute treatment of chemotherapeutic agents is represented in Fig. 1. All four experimental groups including DOX, CYP and 5-FU at the tested dose and duration of exposure did not produce mortality. A 100% survival rate was

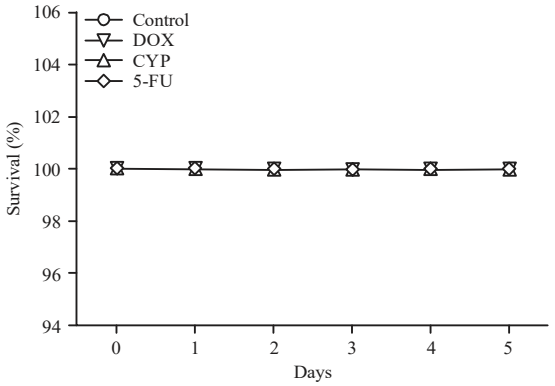

Fig. 1: Survival rate of rats following treatment with doxorubicin (DOX), cyclophosphamide (CYP) or fluorouracil (5-FU)

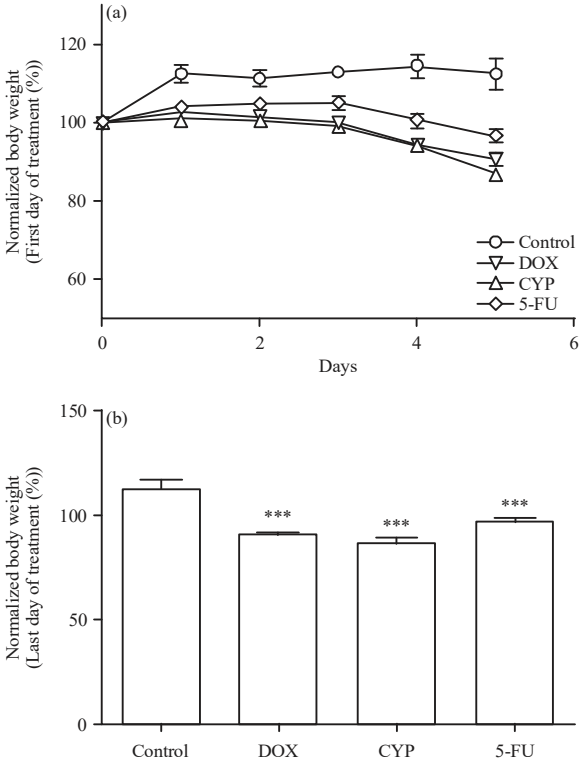

Fig. 2(a-b): Effects of chemotherapy on normalized body weight at (a) First day of treatment and (b) Last day of treatment
DOX: Doxorubicin, CYP: Cyclophosphamide, 5-FU: Fluorouracil

observed during the duration of experiment. Therefore, acute DOX, CYP or 5-FU treatment did not affect the survival rate of rats.

Effect of chemotherapy on normalized body weight: The effect of various treatments on the normalized body weight

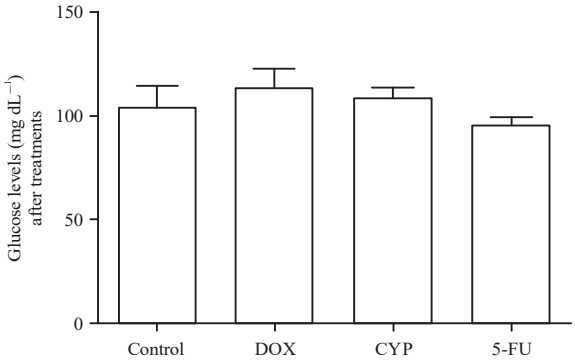

Fig. 3: Effect of DOX, CYP and 5-FU treatments on the blood glucose levels on female rat's models
DOX: Doxorubicin, CYP: Cyclophosphamide, 5-FU: Fluorouracil

determined at first and last day of the study is represented in Fig. 2a-b, respectively. The observation of normalized body weight at the start of the study revealed that there is a non-significant change in the percentage body weight calculated in reference to the average body weight of the group of animals. However, the normalized body weight at the end of the study indicated a significant ($p<0.001$) decline in comparison with the control animals, DOX and CYP treated animals exhibiting more reduction in body weight than 5-FU treated group.

Blood glucose test: The estimation of blood glucose level after acute treatment of DOX produced non-significant variation in the blood glucose level compared to the control group. The treatment of other two chemotherapeutic agents such as; CYP and 5-FU also did not showed significant alteration in the blood glucose level in comparison to control animals (Fig. 3). Thus, the result of blood glucose testing revealed the relationship between acute DOX, CYP or 5-FU treatments and glucose levels showing that glucose levels were not altered following chemotherapy treatments. Therefore, no significant alterations in glucose levels were observed between chemotherapy-treated and non-treated rats.

Insulin tolerance test: The observation obtained for the insulin tolerance test is recorded as blood glucose level and normalized blood glucose level with reference to the first day value. The values of blood glucose after the administration of insulin (2 U kg^{-1}) indicated a progressive decline, however, the comparison indicated non-significant variation between treatment and the control group. The normalized blood glucose level also revealed progressive fall for all the groups and non-significant alteration between control and treatment group (Fig. 4a-b).

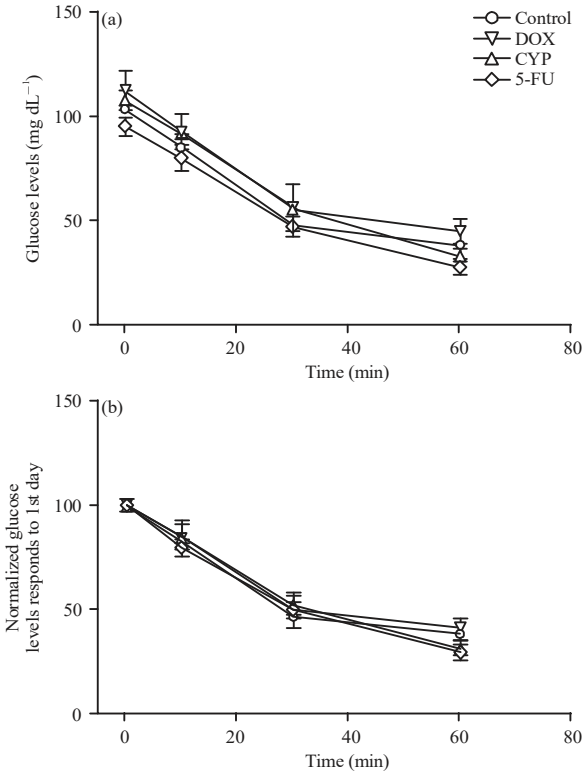

Fig. 4(a-b): Effect of chemotherapy on (a) Blood glucose levels in rats and (b) Normalized blood glucose level respond to 1st day
DOX: Doxorubicin, CYP: Cyclophosphamide, 5-FU: Fluorouracil

DISCUSSION

Chemotherapeutic agents can induce cytotoxic effects and may damage pancreatic b-cells[19]. This study focused on investigating the effects of multiple chemotherapeutic agents DOX, CYP and 5-FU on blood glucose levels in rats to determine whether these chemotherapeutic agents can induce hyperglycemia. None of the chemotherapy-treated groups differed from the control group in terms of survival or blood glucose level, although a slight decrease in body weight was noted in all of the treatment groups compared to the controls. Rats that received DOX or CYP had a greater reduction in body weight than rats receiving 5-FU treatment (Fig. 2-4). The mortality rate of the experimental groups including the control, DOX, CYP and 5-FU groups were assessed throughout the study period starting from day-0 to the end of the experiment (day 6). The control group received normal saline treatment while the treatment groups received single dose of respective drugs (DOX/CYP/5-FU) by intraperitoneal route for five days. To make the study more clinically relevant, chemotherapy treatment doses used in this study were in the same range typically used in human cancer treatment. A 100% survival rate was observed in all the tested groups of experimental rats (Fig. 1). Similar observations were found in the studies conducted in the past[23,24]. The acute treatment of chemotherapeutic agents did not affect the mortality in the tested animals[23]. The reason suggested is that acute administration of chemotherapeutic agents has insignificant influence on the functioning of the vital organs to cause the mortality[24].

The influence of the treatment on the body weight was recorded as normalized body weight at first day and last day of treatment (Fig. 2a-b). The data from the study indicated that administration of different agents did not produced significant change at the first day of normalized body weight calculated by comparing the individual body weight of animals to the average body weight of the group. However, the normalized body weight at the last day suggested a significant ($p<0.001$) decline. The variation in the body weight was found to be prominent for DOX and CYP treated animals, but less in case of 5-FU administered group. The findings are in accordance with the earlier findings where DOX and CYP were found to be more potent than 5-FU[25]. In general, there are reports which suggested that anticancer chemotherapeutic drugs produces negative influence on the body mass and body index[26].

The observation to find the acute effect of anticancer agents on the blood glucose in rats indicated that none of the drugs in the tested dose and duration altered significantly in comparison to control group (Fig. 3). The insulin tolerance test was performed by injecting insulin (2 U kg^{-1}) to the experimental groups[22]. The blood glucose estimations were done at 0, 10, 30 and 60 min and the data was analyzed as blood glucose level and normalized blood glucose level respond to first day of treatment. The results from the present study suggested that 5 days single dose administration of DOX, CYP and 5-FU as well the control animals showed a progressive decrease in the blood glucose level at all the tested time intervals. However, the comparison of the data between control and treatment groups revealed non-significant variation in blood glucose level at different time intervals. These observations were found to be consistent for both blood glucose level (Fig. 4a) and normalized blood glucose level respond to first day (Fig. 4b). This outcome demonstrated that acute treatment with DOX, CYP or 5-FU did not induce T2DM in a rat model. However, further study will be necessary to evaluate whether chronic chemotherapy treatment negatively affects insulin levels. Some studies conducted earlier reported that anticancer chemotherapeutic agents have the tendency to alter the blood glucose level[15] and insulin tolerance[27] when administered chronically. Several

limitations need to be considered when interpreting the present study findings. First, the animals used in this study were healthy, disease-free female animals and did not have any diseased states such as; cancer. Second, in cancer treatment typically a combination of chemotherapeutic agents is administered, while in this study single agents were administered.

CONCLUSION

Findings of this study indicate that acute treatment of chemotherapeutic agents (doxorubicin, cyclophosphamide or fluorouracil) did not cause any changes in blood glucose levels in rats at the tested dose and duration. Additionally, there was no indication of insulin resistance and the agents did not produce mortality. Further investigations to evaluate the chronic effect is required to confirm that the effect of chronic treatments of chemotherapy on insulin levels.

SIGNIFICANCE STATEMENT

Chemotherapy can produce cytotoxic effects that could cause diabetes by damaging pancreatic β-cells, reducing the production of insulin or by affecting other cells, causing insulin resistance. This study aimed to evaluate the effect of acute treatment of anticancer drugs doxorubicin, cyclophosphamide and fluorouracil on development of diabetes in rats. Present study suggests that the tested anticancer chemotherapeutic agents did not affect the blood glucose level and insulin tolerance. The findings illustrate that there is no clear associations between acute chemotherapy with these agents and induction of hyperglycemic condition.

ACKNOWLEDGMENT

The researchers thank Dr. Syed Rabbani for his help in revising this manuscript.

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