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Research Article
Impact of Metformin on Hypothyroidism Induced by Cyclophosphamide in Rats

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Abstract

Background and Objective: Cyclophosphamide (CYP), a commonly used chemotherapeutic agent, is administered to patients to treat various cancer types. The toxic effects of this substance are widely known and can cause damage to the liver, kidneys, nervous system and bone marrow. Metformin (MET) is frequently prescribed medication for diabetes mellitus type-2, known for its effectiveness in managing blood sugar levels. Present research aims to study the effect of co-administering MET on CYP-induced hypothyroidism by determining the level of thyroid hormones. **Materials and Methods:** Forty rats, with a body weight ranging from 220 to 250 g, were divided into four groups of ten animals, each for control and treatment purposes. Saline was given to animals in control group. The CYP-treated group was administered four doses of CYP (100 mg/kg each) by intraperitoneal route. The MET administered animals received 3 mg/mL dose mixed in their drinking water, starting one day preceding to the CYP jab and continued until the last dose. Further, the combination group of animals was administered four doses of a CYP and a daily MET through their drinking water. Daily observations were made on the animals for any signs of mortality, while blood samples were collected to measure TSH as well as thyroid hormones (free and total T3 and T4 concentrations). **Results:** The study's data suggested that the administration of CYP resulted in a higher mortality rate than the control animals. Additionally, when combined with MET, CYP further decreased the survival rate. Furthermore, a significant reduction in the concentrations of free and total T3 and T4 were observed in animals treated with CYP and CYP+MET upon comparison with control group. On the other hand, no significant changes in TSH level were recorded across all groups. **Conclusion:** The findings of the study revealed that the induction of hypothyroidism by CYP was not reduced by co-treatment with MET. This suggests that while MET does have anti-proliferative properties but the toxic effects of CYP was augmented, when used together.

Key words: Cyclophosphamide, hypothyroidism, metformin, thyroid, rats

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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

Thyroid hormones such as T3 (Triiodothyronine) and T4 (Thyroxine) is known to control various physiological functions[1]. Their levels are crucial for the development and functioning of the body[1]. The thyroid gland produces T3 and T4, which are then released into the bloodstream[2]. These hormones can travel to all organs, including the brain and binds to thyroid hormone receptors (THRs)[3]. The THRs are classified as nuclear receptors and exists in two isoforms (THRα and THRβ) with varying distributions in different tissues[4]. According to previous studies, THRα1 is mostly located in heart and skeletal muscle, while liver, kidneys and brain predominantly contains THRβ1[5]. Imbalances in the thyroid hormones and THRs expressions can disrupt normal bodily functions[5]. It is clear that mice with THRα-knockout in the hippocampus show a cognitive deficiency, providing further evidence of the importance of THRα in brain and cognitive function[6]. Thus, hypothyroidism is marked by irregular concentrations of thyroid hormones, which in turn affect various organs, including the receptors for THRs, leading to changes in their functioning[7]. Neuroimaging studies have further identified variations in brain structure and function in hypothyroidism diagnosed patients[8].

Cyclophosphamide commonly abbreviated as CYP is an anticancer medication obtained by synthetic reaction. This drug is an alkylating agent belonging to the nitrogen mustard class[9]. It is the liver that is responsible for the activation of CYP, which ultimately leads to the transformation of mustard into alkylating phosphoramide[10]. The drug accomplishes its action by alkyl group affixation to the DNA guanine base, which ultimately leads to the formation of cross-links that are irreversible in the DNA strands[11]. In the end, this results in the death of cells that is mostly observed at S and G2 cell cycle phases[12]. Because of its cytotoxic effects, it is widely utilized in the treatment of various types of carcinomas, either on its own or in conjunction with other medications[12]. At lower doses, CYP is also prescribed for certain autoimmunity conditions (multiple sclerosis, rheumatoid arthritis, glomerulonephritis, systemic lupus erythematosus) and as a preventive measure for organ rejection after transplantation[13,14]. The treatment with CYP can lead to various negative effects, including hair loss, low platelet count, ulcers in the mucous membranes, changes in skin color, scarring of the lungs, facial injuries, low white blood cell count, blood in the urine, bleeding in the lungs and small intestine, as well as problems with thinking and an underactive thyroid[15-17]. These complications have been found to significantly impact the treatment's prognosis, often resulting in unfortunate outcomes.

Metformin (MET) is classified as an insulin sensitizer and is commonly prescribed to manage non-insulin dependent diabetes mellitus[18]. The medication effectively lowers blood sugar levels by targeting various mechanisms, including reducing glucose production in hepatic and enhancing glucose uptake in organs such as liver, intestine as well as muscles[19]. Reported pharmacological properties of MET include reducing fat deposits and anti-tumor cell proliferation, even in non-diabetic individuals[18]. Previous research has shown that combining MET with a well-established anticancer drug can have positive effects[20]. Nevertheless, the studies have not provided any information regarding the impact of using CYP and MET in combination on thyroid function. Hence, present study aims to assess the role of MET on thyroid function in rats undergoing chronic treatment with CYP. The concentrations of T3 and T4 (free and total) as well as TSH were measured using Electrochemiluminescence (ECL) method.

MATERIALS AND METHODS

Study area: The study was carried out at the Pharmacology Laboratory in the College of Pharmacy, Qassim University, Saudi Arabia, from September 2019 to October, 2019.

Chemicals and drugs: Drugs required for the research were purchased from market such that Endoxan® (Baxter Ltd., Germany) and Metfor® (Tabuk Pharmaceutical Company of Saudi Arabia) were selected for cyclophosphamide and metformin hydrochloride, respectively.

Animals: A group of forty rats, all 10-12 weeks old, obtained from animal house in the College of Pharmacy, Qassim University, were kept in separate housing units within an environment that followed a light/dark cycle of 12 hours, with the illumination switching on at 6:00 in the morning. The animals were provided with unrestricted admittance to pelleted food and water in the 24 hrs. The experimental rats were observed each day for the survival rate. The studies were conducted following the necessary ethical procedures for animal experimentation, as required by the institution.

Experimental design and treatment protocol: For the purpose of this investigation, the animals were separated into four groups, with ten animals belonging to each group. Intraperitoneal administration of normal saline serves as the control. Treatment 1: CYP is to be administered

intraperitoneally at a concentration of 100 mg/kg, 4 times/day, on alternate days. The second treatment consisted of MET (3 mg/mL) dissolved in drinking water and induced for a period of 10 days. The fourth was a combination treatment, wherein MET was given for a period of 10 days and on the starting day of MET induction, CYP was administered in four doses on alternate days. Following the conclusion of the treatments, the animals were put to sleep and blood samples were taken. The experimental rats were monitored every day to ensure that their survival/mortality rates were accurately recorded.

Electrochemiluminescence (ECL): During the tenth day, after the rats had been put under hypnosis with Carbon Dioxide (CO_2), they were put to euthanized in a humane manner by having their heads severed from their necks. This occurred following the last administration of the drug. Shortly after, blood samples were gathered from the control/untreated group and the treatment groups such as those received MET, CYP and CYP+MET. Samples collected were subsequently positioned into tubes having Ethylenediaminetetraacetic Acid (EDTA). After the mentioned procedure, the vials with the blood samples underwent centrifugation (12,000× g) for ten mins. This process led to the isolation of the plasma component. The levels of biomarkers such as TSH, T4, FT3 and T3 were measured by means of an automatic analyzer that relies on electrochemiluminescence (ECL) technique based on immunoassay concept. The task was carried out in compliance with the procedures described by the maker, Roche Diagnostics, headquartered in Germany[21].

Statistical analysis: The values collected from the *in vivo* study underwent ANOVA (One-way Analysis of Variance) test. The outcomes of the research were reported as the Mean±SEM (Standard Error of the Mean). Subsequently, a Tukey analysis was employed to compare the data individually, with statistically significance values being determined by a p-value below 0.05.

RESULTS

CYP and MET impact on the survival rate in rats: A equivalent rat method was established in order to gain a improved understanding of whether or not MET induced an effect on reducing the survival/endurance rate of rats that were administered with CYP. This was accomplished through the utilization of a Kaplan-Meier test. According to the findings of the survival rate analysis that was carried out between rats that were induced with CYP as well as rats that were treated with CYP and MET. The results suggested that the treatment with CYP and MET did, in fact, considerably reduce the survival rate when related to the treatment with CYP alone. Therefore, the findings of the study demonstrated that the inclusion of MET to CYP has the possibility to bring about the lethal effect of CYP. Each of the saline as well as MET groups maintained a survival rate of one hundred percent (Fig. 1).

Impact of CYP and MET on TSH concentrations: The findings of the ECL test revealed no discernible variation in the concentrations of TSH amid the groups that were given either saline or those that were given treatments. As a result, both MET as well as CYP treatments appear to had no observable consequence on the concentrations of TSH (Fig. 2).

Impact of CYP and MET on free and total T3 and T4 concentrations: Based on the results of the ECL study, the

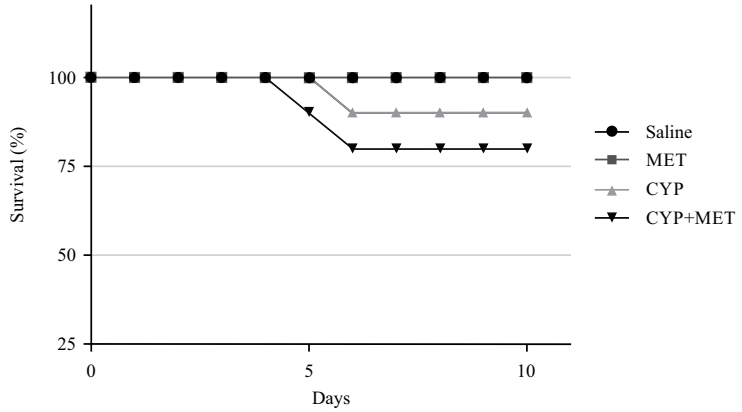

Fig. 1: CYP and MET impact on the survival/endurance rate

Survival rate of the rats treated with CYP is decreased when combined with MET. The impact of MET on survival was assessed in rat models. The treatment regimen consisted of four dosages of CYP (400 mg/kg), while MET was given by oral route after it was dissolved into the drinking water on a continuous basis

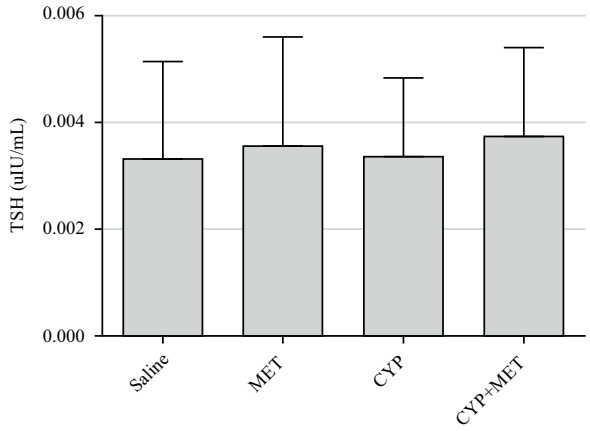

Fig. 2: Exploring the effects of CYP, MET and their combined usage on TSH levels

Graph indicates that no notable variations were recorded among the four rats' groups in the study. The value is presented as the standard error with mean for six rats in each group and it was analyzed using a statistical analysis method

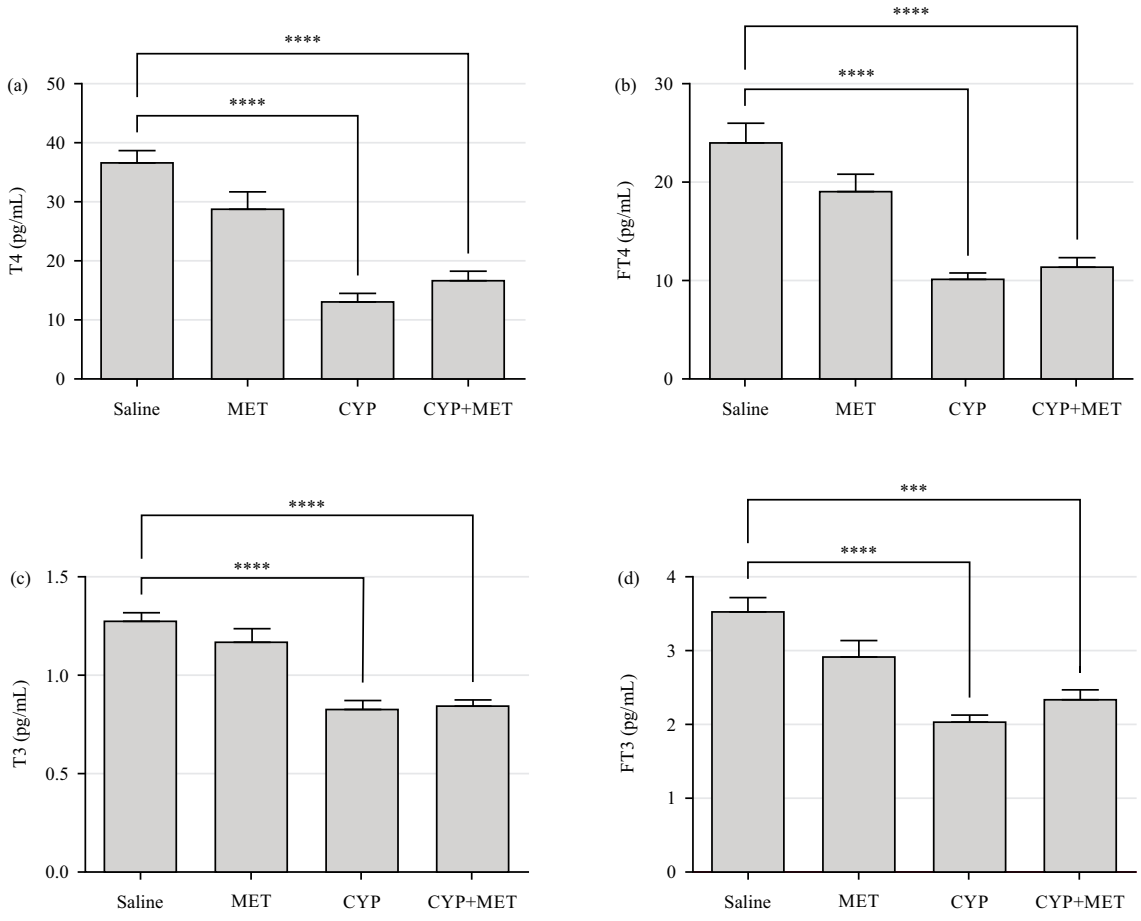

Fig. 3(a-d): Effects of CYP, MET and their combination on total (a) T4, (b) Free T4, (c) Total T3 and (d) Free T3 levels

A significant reduction in the values of free and total thyroid hormones T4 and T3 was brought about by the administration of CYP (400 mg/kg, intraperitoneal injection). However, the administration of MET did not have any effect on the levels of both free as well as total T4 or T3. In contrast to the saline group, the CYP+MET group did not demonstrate any indication of improvement in either the T4 or T3 levels. The SEM is used to express the data. ***p<0.001 and ****p<0.0001 when it compared with animals that were given saline

concentrations of T4 and T3 in both their free and total forms were significantly lower in the animal groupings that was treated with CYP in comparison to those given saline. The T4 and T3 concentrations were not improved by the co-treatment with MET; rather, they were decreased by the treatment with CYP (Fig. 3a-d).

DISCUSSION

The principal purpose of current research was to investigate the influence of MET on animals that had been given treatment for CYP-induced hypothyroidism. The TSH, Free Thyroxine (FT4), total Triiodothyronine (T3), Free Triiodothyronine (FT3) and total Thyroxine (T4) were the thyroid hormones that were the focus of this study. The rationale of this research was to estimate the influence that CYP has on these hormones. Additionally, the objective also included to determine whether or not MET could have a beneficial effect on thyroid function by mitigating the negative effects of CYP-induced toxicity.

The Kaplan-Meier method is utilized quite frequently in the process of analyzing data pertaining to the time between events[22]. This method proves to be valuable in survival/endurance rate analysis, as it enables investigators to assess and examine the attrition of test subjects in a particular study[23]. It is commonly employed to evaluate and contrast two sets of participants, such as a placebo grouping and a control grouping or another treatment/induced grouping[23]. Thus, the effects of CYP and MET, as well as their combination, were evaluated in rat models in the present study. The findings indicated a decline in the survival rate observed in the group that received CYP treatment. Furthermore, a greater number of rats exhibited a decrease in survival when MET was administered in conjunction with CYP.

There is a correlation between the existence of hypothyroidism and alterations in a number of physiological activities[24]. These alterations can be linked to the distribution of thyroid receptor (THR) function[25]. Several varieties of THRs, known as THRα and TRβ are identified, which can be found in various organs such as the brain and the heart. The THRs are crucial in the regulation of gene transcription, operating within the nucleus[5]. Typically, the concentrations of TSH and thyroid hormones (T4 and T3) in the bloodstream can provide insight into changes in thyroid hormone concentrations and function, like in hypothyroidism and hyperthyroidism[26]. For instance, the decrease in T4 and T3 concentrations, along with an increase in TSH, indicates the presence of primary hypothyroidism[26,27]. The decline in the concentrations of thyroid hormonal levels in the bloodstream can have an impact on the activity of THR, potentially leading to changes in the functioning of the brain and heart[27,28].

The concentrations of T3, T4, TSH, FT4 and FT3 were examined in the study following treatment with CYP, MET, as well as mixture of CYP with MET. The outcomes of the study showed non-significant changes in TSH concentrations across all treated groups. Significant reductions in T4, FT4, T3 and FT3 concentrations were observed in rats treated with CYP and CYP+MET, as linked to the saline/untreated group. Nevertheless, the levels of T4 and FT4 remained unchanged in rats treated with MET when compared to those treated with saline. According to these findings, it has been discovered that CYP has the ability to impact thyroid function by influencing the concentrations of thyroid hormones. This, in turn, can have an effect on the activity of TR and the proper functioning of organs that rely on thyroid hormones.

CONCLUSION

The treatment with CYP can lead to the development of hypothyroidism, resulting in alterations to various regulatory physiological functions. The study proposed that CYP may cause hypothyroidism by affecting the concentrations of circulating TSH, total and free T4 and T3. Therefore, co-treatment with MET could potentially alleviate the impact of CYP on hypothyroidism. The findings revealed that in rats with CYP-induced hypothyroidism, MET did not alleviate the toxic impact of CYP and did not enhance the significant reduction in the levels of total and free T4 as well as T3.

SIGNIFICANCE STATEMENT

This research aims to estimate the potential amelioratory effects of metformin against hypothyroidism induced by cyclophosphamide. In a previous study, it was discovered that cyclophosphamide can cause a notable reduction in the concentrations of total and free Triiodothyronine (T3) and Thyroxine (T4), leading to hypothyroidism. According to the research, rats that received metformin did not show any enhancements in their T3 and T4 concentrations. The results suggest that metformin does not improve hypothyroidism caused by cyclophosphamide.

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