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Quercetin inhibits steroid-induced hypergluconeogenesis in *Saccharomyces cerevisiae*

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Abstract

Background Steroid-induced hypergluconeogenesis is a significant contributor to hyperglycemia, often complicating the therapeutic use of steroids. This study investigates the potential of quercetin, a naturally occurring flavonoid, to mitigate steroid-induced hypergluconeogenesis in *Saccharomyces cerevisiae*. The levels of glucose, total proteins, free amino acids, pyruvate, lactate and antioxidants were assessed in the quercetin-treated yeast cells induced with betamethasone at different time intervals. The glucose uptake potential of yeast cells treated with quercetin was also studied and also the effect of steroids and quercetin on cell viability was analyzed.

Results Our results show that quercetin effectively reduces gluconeogenesis by normalizing the levels of metabolites involved in the process and alleviates the hyperglycemic effects associated with steroid exposure. Quercetin-treated yeast cells also demonstrated a better uptake of glucose. Additionally, quercetin was found to improve the overall cell viability highlighting its role in modulating glucose metabolism.

Conclusion These outcomes suggest that quercetin can serve as a promising adjunct therapy for managing steroid-induced metabolic disturbances, providing a natural and effective approach to counteracting steroid-induced hyperglycemia.

Keywords Steroids, Betamethasone, Gluconeogenesis, *Saccharomyces cerevisiae*, Glucose

Background

In mammals, glucose remains a key supply of energy for active tissues like the brain and blood cells, and its levels are kept constant within a narrow range. This is achieved by the liver since it plays a vital role in glucose homeostasis, functioning as the primary organ for storing glucose as glycogen and producing glucose endogenously through glycogenolysis and gluconeogenesis [1]. Short-term fasting conditions lead to the production and release of glucose through glycogenolysis, the process of breaking down glycogen into glucose. During prolonged fasting or starvation, glycogen stores are exhausted, and gluconeogenesis, the synthesis of glucose from noncarbohydrate precursors, becomes the primary mechanism for sustaining blood glucose levels [2]. However, an abnormally elevated rate of hepatic gluconeogenesis is a significant factor contributing to the hyperglycemia seen in both type I and type II diabetes [3]. The most frequently used substrates for gluconeogenesis in the liver include lactate, pyruvate and glycerol where they are transformed into glucose [4].

Glucocorticoids are steroid hormones crucial for survival under stress. Glucocorticoids also called corticosteroids are extensively used as anti-inflammatory and immunosuppressive medications to treat a broad spectrum of diseases. While these medications are effective

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in managing various conditions, including autoimmune disorders, asthma and certain cancers, their use has been associated with numerous side effects including steroid-induced hyperglycemia or diabetes [5]. Glucocorticoids stimulate gluconeogenesis in the liver by upregulating key enzymes participating in gluconeogenesis, such as glucose-6-phosphatase and phosphoenolpyruvate carboxykinase, ultimately leading to insulin resistance and increased gluconeogenesis [6]. Though gluconeogenesis is a normal physiological process, the excessive production of glucose-induced by steroids can lead to hyperglycemia [7]. This is particularly concerning in patients with existing insulin resistance or those who are predisposed to diabetes, as the increased hepatic glucose output can exceed the body's capacity to regulate normal blood glucose levels [8]. The incidence of hyperglycemia and diabetes in hospitalized patients treated with glucocorticoids (GCs) without a prior history of diabetes exceeds 50%. GCs increase the risk of hyperglycemia and diabetes in nondiabetic individuals by two to four times. Moreover, the administration of exogenous GCs significantly disrupts glycemic control in patients with preexisting diabetes [9].

Betamethasone is a potent glucocorticoid steroid used to treat allergic reactions, skin disorders, autoimmune diseases and respiratory issues like asthma. It mimics cortisol, reducing inflammation and suppressing the immune system [10]. Betamethasone works by binding to glucocorticoid receptors, altering gene expression to inhibit pro-inflammatory cytokines and increase anti-inflammatory proteins. This leads to a decrease in inflammation, immune cell activation and tissue damage [11]. However, betamethasone is associated with a range of side effects, especially when used at high doses or long-term including stimulation of appetite, weight gain, elevated glucose levels and blood pressure. Among these, one of the notable metabolic effects of betamethasone is the stimulation of gluconeogenesis by increasing the expression of key enzymes like phosphoenolpyruvate carboxykinase and glucose-6-phosphatase [12]. This effect can lead to steroid-induced hyperglycemia and even the development of diabetes in some patients [13].

Corticosteroids including betamethasone were extensively used during the COVID-19 pandemic, to manage severe inflammation and prevent immune overreactions, particularly in patients experiencing cytokine storms, an excessive immune response that can cause severe damage to the lungs and other organs [14]. However, the use of steroids, while lifesaving, also led to significant adverse effects, particularly in diabetic patients or those with undiagnosed diabetes. One of the primary side effects was extreme hyperglycemia, with blood glucose levels spiking dangerously high, leading to a higher risk of complications and mortality [15]. Moreover, steroids weaken the immune system, making patients more vulnerable to infections like mucormycosis, a rare but aggressive fungal infection caused by a group of fungi called mucormycetes. This opportunistic infection, often referred to as black fungus, severely affected the patient's eyesight and in many cases, was life-threatening, particularly when it invaded the sinuses or brain [16]. Despite these risks, steroids remained crucial in combating severe COVID-19-related immune disorders, including acute respiratory distress syndrome and cytokine release syndrome, both of which could have proven fatal if left unchecked [17]. In such a complex scenario, natural compounds could be employed as a potential adjunct therapy. Phytochemicals, with anti-inflammatory and antioxidant properties, may offer protective benefits, reducing inflammation and oxidative stress [18]. Thus, phytochemicals could play a pivotal role in mitigating both hyperglycemia and mucormycosis risks while still supporting the immune system during COVID-19 management.

Phytochemicals, naturally occurring compounds found in plants, have garnered significant attention for their potential role in reducing gluconeogenesis and managing diabetes [19]. Phytochemicals can be valuable in neutralizing the metabolic effects of betamethasone and may help regulate blood glucose levels and prevent steroid-induced hyperglycemia. These bioactive substances, including flavonoids, polyphenols, alkaloids and terpenoids, possess various mechanisms that help regulate glucose metabolism and insulin sensitivity [20]. Phytochemicals can inhibit key enzymes involved in gluconeogenesis, thereby reducing the excessive production of glucose in the liver. Additionally, they may enhance insulin signaling pathways, promote glucose uptake in peripheral tissues and lower oxidative stress and inflammation; the factors that contribute to insulin resistance and hyperglycemia [21]. Numerous researches have established the antidiabetic potential of specific phytochemicals like flavonoids found in common herbs, fruits, vegetables and spices as they have shown promise in lowering blood glucose levels and improving overall metabolic health [22].

Flavonoids, an important group of polyphenols produced by plants through secondary metabolism, are known for their aromatic and therapeutic properties, as well as their role in plant adaptation [23]. Quercetin is a naturally occurring flavonoid present in various plants and fruits. Quercetin has multiple health benefits, including antidiabetic, anti-inflammatory, antioxidant, anti-arthritic, anti-Alzheimer's and wound-healing effects. Flavonoids, including quercetin, have been linked to the prevention of diseases such as type 2 diabetes and cardiovascular disorders [24]. Polyphenols, like quercetin,

interact with molecular pathways crucial for glucose regulation, particularly insulin signaling [25]. Quercetin has been shown to reduce hepatic glucose production by lowering the production of essential enzymes involved in gluconeogenesis. This suggests that bioflavonoids can effectively decrease gluconeogenesis in the liver [26].

Saccharomyces cerevisiae is a versatile eukaryotic model organism that has undergone significant evolutionary development [27]. Yeast, a type of fungus, shares a similar life cycle and cellular architecture with multicellular eukaryotes like plants and animals. Despite the phylogenetic distance between yeast and human or animal cells, many regulatory mechanisms are remarkably conserved across these species. This makes the yeast model invaluable for studying disease development processes [28]. Since the yeast genome was fully sequenced in 1996, it has continued to be a cornerstone in research, contributing extensively to the understanding of cell biology, systems biology and metabolic studies [29].

The present study focuses on the effect of steroid-induced gluconeogenesis in yeast culture and the inhibitory effect of quercetin on steroid-induced elevated gluconeogenesis. Elevation of gluconeogenesis was evaluated by assessing various biochemical components. Oxidative stress induced during steroid induction was assayed by assessing antioxidants in the yeast cell. Glucose uptake assay and cell viability assays were also performed.

Materials and methods

Chemicals

All chemicals used in the present study were purchased from HiMedia and Sigma-Aldrich and were of analytical grade.

Yeast growth media

Saccharomyces cerevisiae, i.e., baker's yeast is generally used to grow under aerobic and substrate-limiting conditions for efficient growth. 100 mg of Baker's yeast was suspended in 10 ml of sterile water and kept at room temperature overnight. Yeast was then cultured in 100 ml of yeast peptone dextrose (YPD) broth medium.

Experimental design

Yeast was grown on yeast peptone dextrose broth at 37 °C for 48 h and then separated into 4 groups. Betamethasone (10 mg/ml) was added to the culture medium of all groups except the normal control group. Insulin (1000 µU/ml) and quercetin (100 µmol/L) were added to the respective groups 20 min after induction with betamethasone.

Induction & treatment

GROUP I—Normal control: normal yeast culture.
GROUP II—BT—Negative control: yeast cells were treated with betamethasone 10 mg/ml to induce gluconeogenesis [30]
GROUP III—BT+Insulin—Positive control: yeast cells induced with betamethasone were treated with Insulin (1000 µU/ml).
GROUP IV—BT+Quercetin: yeast cells induced with betamethasone were treated with quercetin (100 µmol/L).

Collection of cell-free extract

The yeast culture belonging to different groups was centrifuged at 4000 rpm for 10 min, and the pellet with cells was washed with Tris buffer 5 mmol/L, pH 7.5. The cells were then lysed by the osmotic shock cell disintegration method. The cells were suspended in 20 ml of hypotonic solution containing 50 mmol/L Tris HCl buffer pH 7.5 with 10 mmol/L EDTA and 30% sucrose and shaken gently for 20 min. The cell suspension was then centrifuged, and the pellet was disintegrated by adding 20 ml of 10 mmol/L $MgCl_2$ for 20 min. Ultimately, the cells were removed by centrifugation, and the supernatant was stored for further analysis. This procedure was performed at different time gaps of 0, 20, 40, 60 and 80 min during the treatment period. The cell-free extract was stored at 4 °C for further analysis of metabolites.

Estimation of glucose

Glucose in the cell-free extract was estimated using the glucose oxidase assay kit method [31].

Estimation of proteins

The total protein content in the cell-free extract was estimated by Lowry's method [32]. To 0.2 ml of cell-free extract, 0.8 ml of distilled water and 5 ml of Lowry's reagent were added and mixed. The solution was incubated at room temperature for 10 min after which 0.5 ml of Folin's phenol reagent was added and further incubated for 20 min. The blue color developed was read at 640 nm.

Estimation of free amino acids

0.5 ml of cell-free extract was made up to the final volume of 1 ml with distilled water. 1.5 ml of ninhydrin reagent was added and after mixing kept in a boiling water bath for 20 min. After cooling down, 8 ml of 50% propanol diluent solvent was added, and the absorbance was measured at 570 nm against a blank [33].

Estimation of pyruvate

0.1 ml of the cell-free extract was taken, and added 0.5 ml of DNPH reagent was incubated for 20 min at room temperature. Then, 5 ml of 0.4N NaOH was added and mixed well, and the absorbance was recorded at 540 nm [34].

Estimation of lactate

50 µl of cell-free extract and 2 ml of 0.2% $FeCl_3$ are mixed well. After incubating for 15 min at room temperature, the absorbance was recorded at 390 nm [35].

Estimation of antioxidants

Estimation of catalase

The yeast cell-free extract was added to 1 ml of 0.01 M phosphate buffer (pH 7.4), 0.5 ml of 0.2 M H_2O_2 and 0.4 ml of H_2O and incubated for 10 min. The reaction was terminated by adding 2 ml of dichromate acetic acid reagent. To the control, the enzyme was added after the acid reagent. All the tubes were heated for 10 min, and absorbance was read at 610 nm against blank [36].

Estimation of superoxide dismutase

1.2 ml of sodium pyrophosphate buffer, 0.1 ml of PMS, 0.3 ml of NBT, 0.2 ml of the yeast cell-free extract and water in a total volume of 2.8 ml were taken and added 0.2 ml of NADH. The solution was incubated at 30 °C for 90 s, and the reaction was arrested by the addition of 1 ml of glacial acetic acid. It was then mixed with 4 ml of n-butanol, allowed to stand for 10 min and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560 nm in a spectrophotometer [37].

Estimation of vitamin C

0.5 ml of cell-free extract was taken and made up to 2 ml with 4% TCA. 0.5 ml of DNPH reagent was added to all the test tubes, followed by 2 drops of 10% thiourea solution and incubated at 37 °C for 3 h. The formed osazones were dispersed in 2.5 ml of 85% cold sulfuric acid. The tubes were incubated for 30 min at room temperature, and the absorbance was read spectrophotometrically at 540 nm [38].

Cell viability

The viability of yeast cells was determined as follows (Table 1). Experimental yeast cells in media were divided into four groups:

Yeast cells were centrifuged at 2500 rpm for 5 min, and the pellet was resuspended in phosphate buffer saline. The cells were washed thrice with PBS. An equal amount of cell suspension and trypan blue dye solution was combined simultaneously. The live cells will be unstained and dead cells appear blue because intracellular proteins bind to trypan blue and render blue color to it. The cells were then counted using a hemocytometer under a light microscope.

Table 1 Experimental design for cell viability analysis

Group	Yeast suspension (µl)	Betamethasone (µl)	Quercetin	Insulin
Group I	100	10	–	–
Group II	100	10	–	–
Group III	100	10	–	5 µl
Group IV	100	10	10 µl	–

Glucose uptake assay

Quercetin in five different concentrations was prepared (100–500 µg/ml) and to each tube, 1 ml of glucose solution was added and incubated for 10 min at 37 °C, after which 100 µl of yeast cell suspension was added and mixed thoroughly and incubated for 1 h at room temperature. After incubation mixtures were centrifuged at 2500 *g* for 5 min. The separated supernatant was used to estimate glucose uptake by estimating the amount of glucose in the supernatant using the glucose oxidase method. Metronidazole was taken as a standard drug, and the same procedure was followed for the standard [39].

Statistical analysis

The experiment was performed in triplicates, and the results are represented as mean ± SD. The values were statistically analyzed by one-way ANOVA followed by the Bonferroni test. '*P*' value of less than 0.05 was considered significant.

Results

Biochemical parameters

After administration of betamethasone, the levels of glucose in the BT-treated groups were significantly higher ($p < 0.05$) at the 20th minute compared to the normal control. In the negative control, the increase in glucose levels continued to increase till the end of the experimental period indicating elevated gluconeogenesis (Fig. 1). The quercetin-treated group displayed higher glucose levels at the 40th minute and it was lowered at the 80th minute.

The protein levels of the groups treated with betamethasone elevated at the 20th minute, and in the negative control group, it remained significantly ($p < 0.05$) elevated till the 80th minute. At the end of 80 min, the

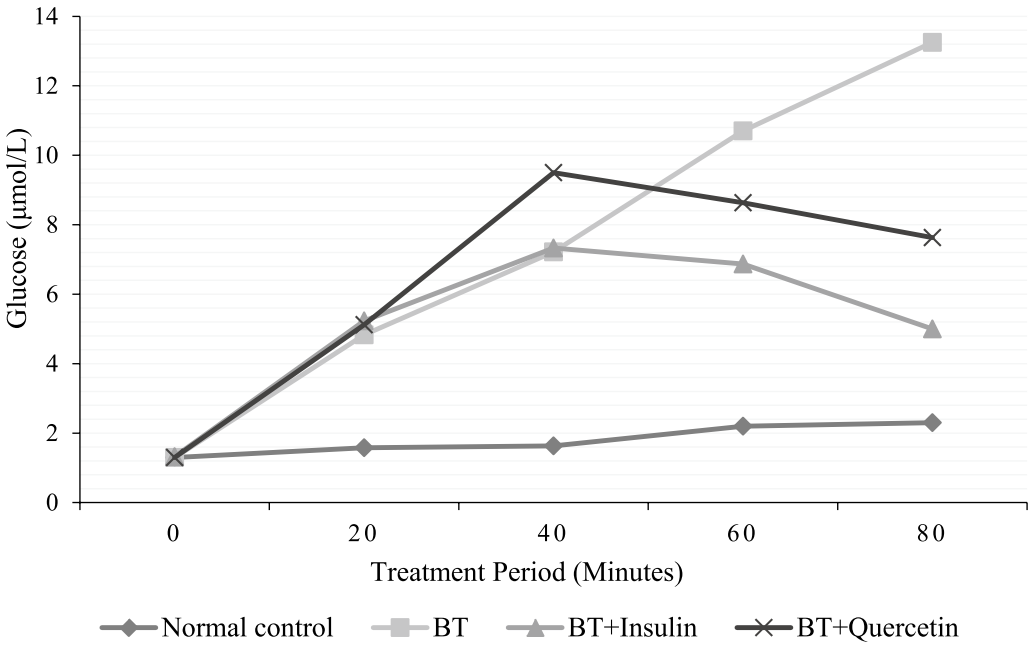

Fig. 1 Effect of quercetin on glucose levels in steroid-induced yeast cells. (Values are expressed in µmol/L as mean ± S.D ($n = 3$))

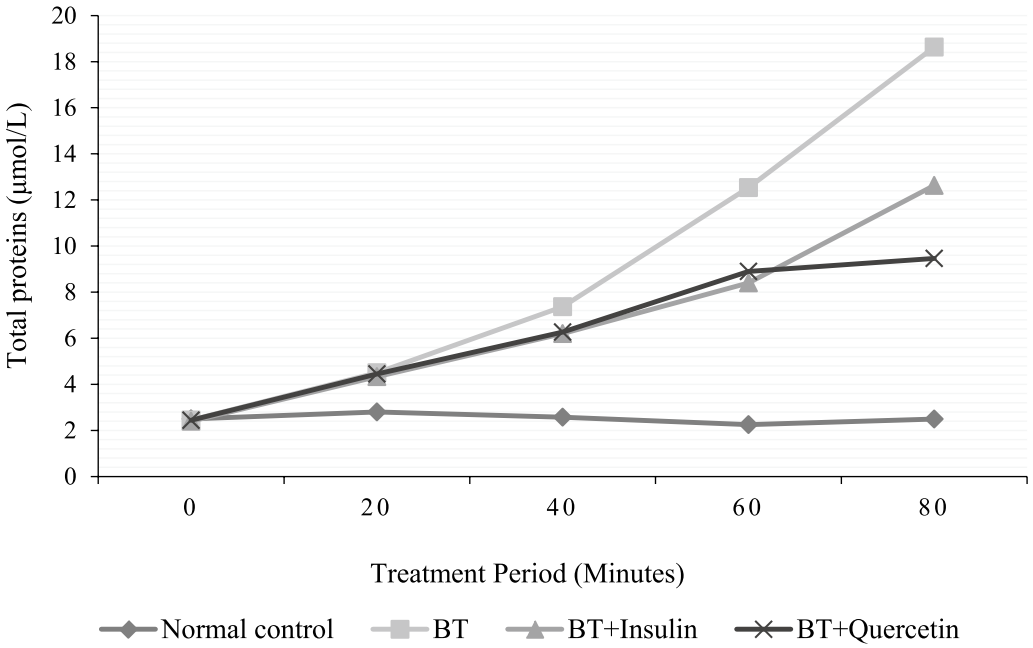

Fig. 2 Effect of quercetin on total protein levels in steroid-induced yeast cells. (Values are expressed in µmol/L as mean ± S.D ($n = 3$))

quercetin-treated group had a significantly ($p < 0.05$) lower protein level than the negative control and insulin-treated groups (Fig. 2).

Steroid induction also significantly decreased ($p < 0.05$) the free amino acid levels in yeast cells (Fig. 3). The group treated with quercetin showed improvement in the free amino acid levels starting at the 40th minute but decreased thereafter.

The betamethasone-induced group showed a marked reduction in the pyruvate levels beginning from the

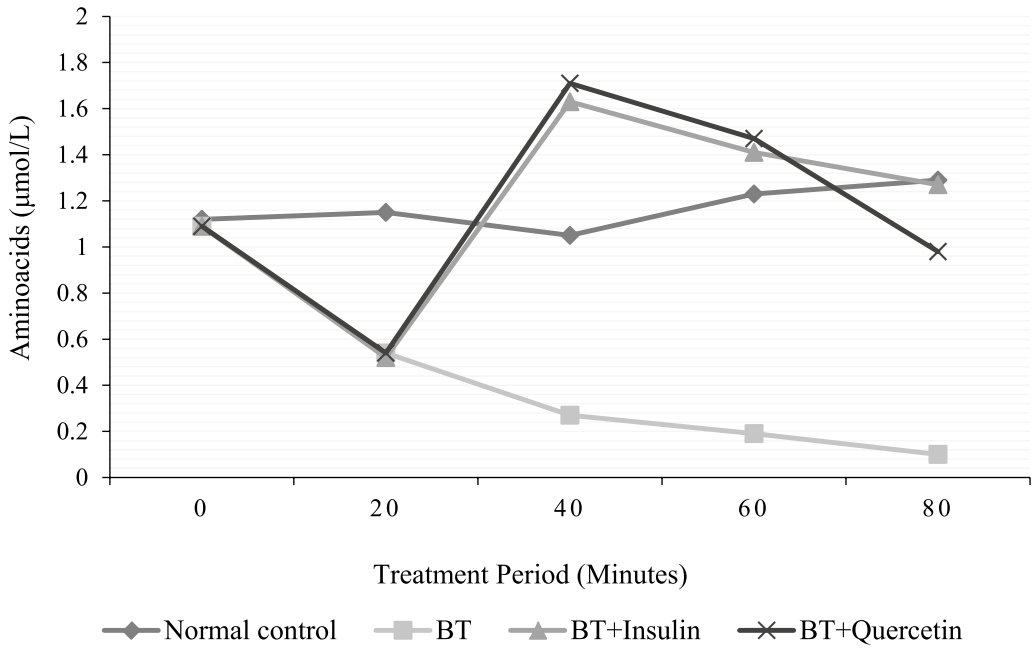

Fig. 3 Effect of quercetin on free amino acid levels in steroid-induced yeast cells. (Values are expressed in µmol/L as mean ± S.D ($n = 3$))

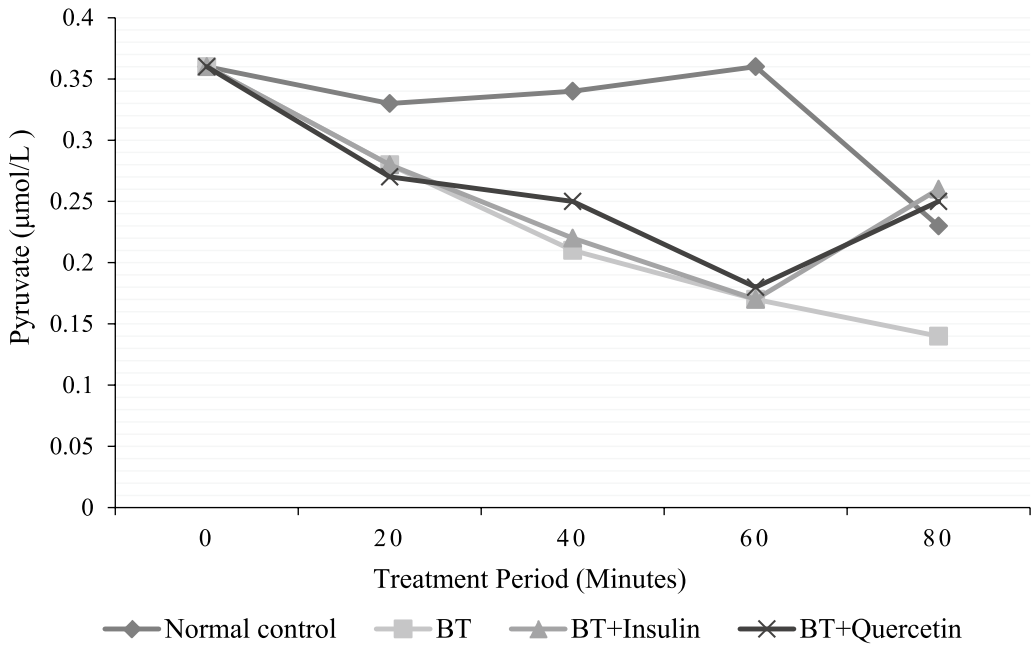

Fig. 4 Effect of quercetin on pyruvate levels in steroid-induced yeast cells. (Values are expressed in µmol/L as mean ± S.D (n = 3))

20th minute that went on till the 80th minute (Fig. 4), whereas the BT-induced yeast cells treated with quercetin initially had significantly decreased ($p < 0.05$) pyruvate levels from the 20th minute to the 60th minute, but the levels improved by the 80th minute nearer to that of the normal control. Yeast cells induced with betamethasone showed significantly increased ($p < 0.05$) lactate levels with an increase in time, and in the negative control, the lactate levels remained elevated when compared to normal control. Quercetin treatment considerably decreased the levels of lactate by the 80th minute (Fig. 5).

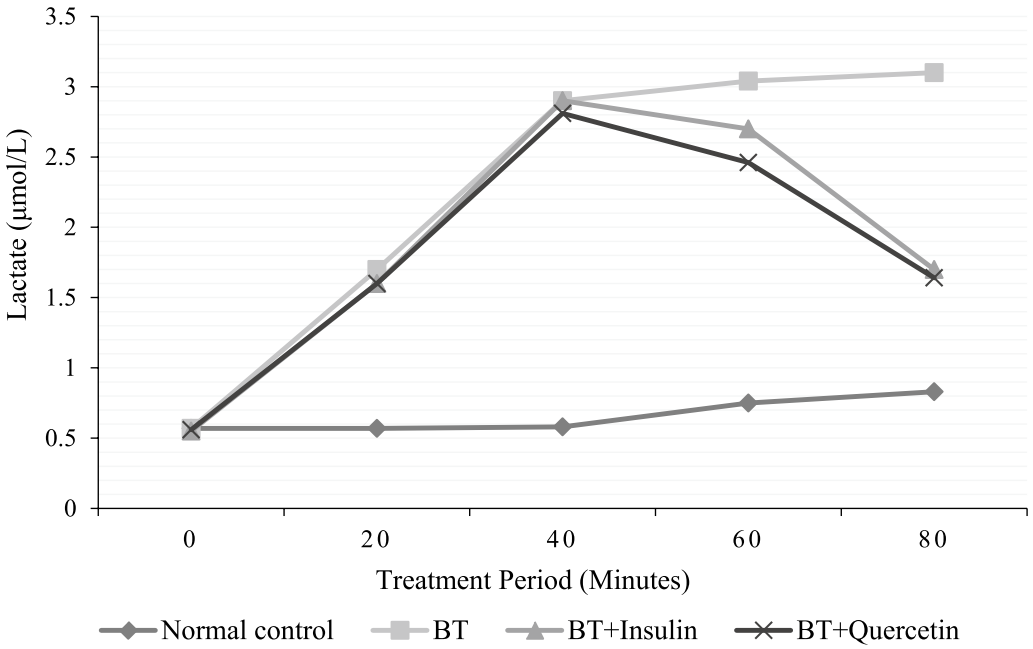

Fig. 5 Effect of quercetin on lactate levels in steroid-induced yeast cells. (Values are expressed in µmol/L as mean ± S.D ($n = 3$))

Estimation of antioxidants

The induction of yeast cells with betamethasone led to a significant decrease ($p < 0.05$) in the catalase levels starting from the 20th minute (Fig. 6). Treatment of BT-induced yeast cells with quercetin instigated a significant improvement ($p < 0.05$) in the catalase levels by restoring the catalase activity. Betamethasone induction also produced a reduction in the superoxide dismutase activity as seen in the negative control group. Quercetin treatment to BT-induced yeast cells produced a significant

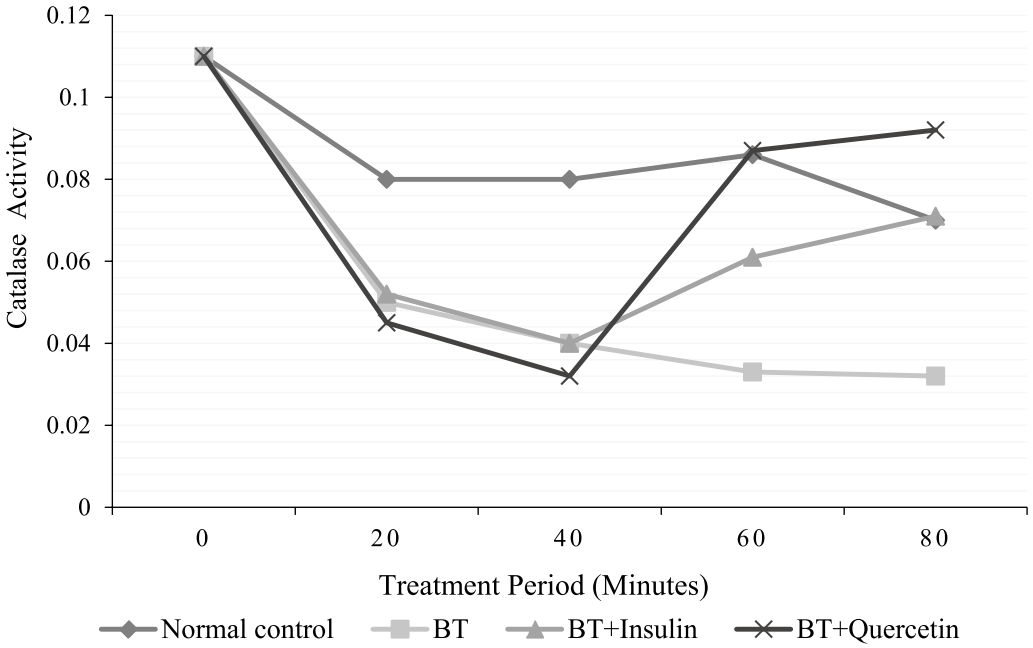

Fig. 6 Effect of quercetin on catalase levels in steroid-induced yeast cells. (Values are expressed as µmol of H_2O_2 consumed/min/mg protein and given as mean ± S.D ($n = 3$))

improvement ($p < 0.05$) in the enzyme levels starting from the 60th minute. Quercetin treatment significantly restored the reduced levels of SOD, suggesting that quercetin may modulate antioxidant activity in betamethasone-treated yeast cells (Fig. 7).

Steroid induction drastically reduced the vitamin C levels in the yeast cells compared to the normal control group. In the current study, treatment of BT-induced yeast cells with quercetin and insulin led to a significant rise ($p < 0.05$) in vitamin C levels starting from the 40th

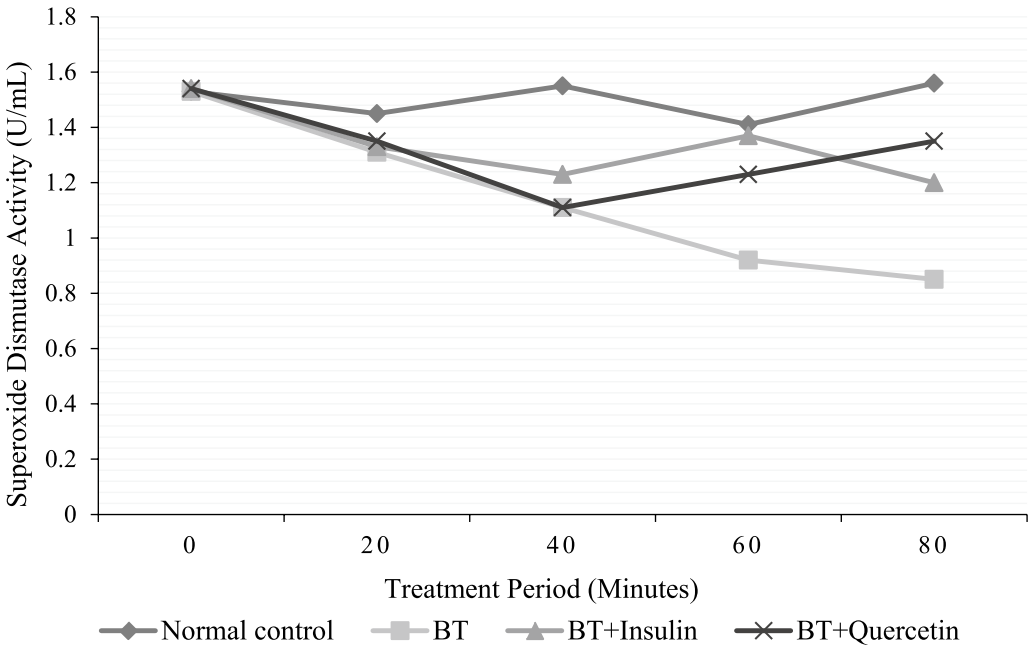

Fig. 7 Effect of quercetin on superoxide dismutase levels in steroid-induced yeast cells. (1Unit: amount of enzyme that causes a 50% reduction in NBT oxidation; values are expressed as mean ± S.D ($n = 3$))

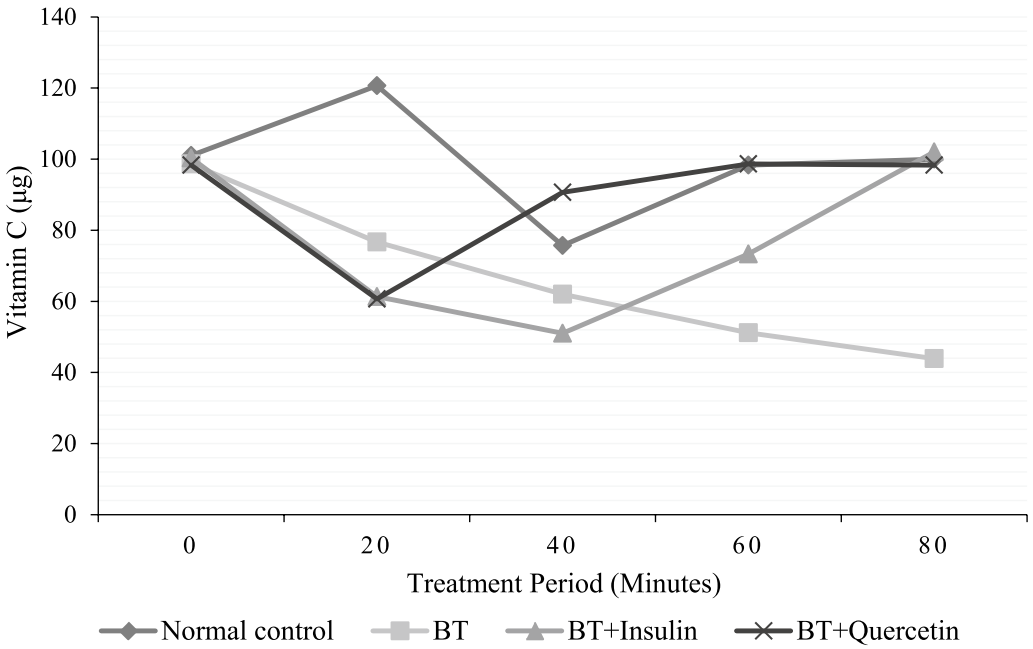

Fig. 8 Effect of quercetin on vitamin C levels in steroid-induced yeast cells. (Values are expressed as mean ± S.D ($n = 3$))

minute and continued till the end of the experimental period (Fig. 8).

Cell viability

Cell viability analysis indicated that betamethasone induction decreased the viability of cells (Group II). There was also a significant increase ($p < 0.05$) in the number of dead cells in the negative control group. Quercetin improved the viability of yeast cells (Fig. 9) as indicated by the presence of more live cells in the quercetin-treated group comparable to the insulin-treated group.

Glucose uptake assay

The rate of glucose uptake by yeast cells treated with quercetin was studied, and the results indicate that both quercetin and the positive control metronidazole showed a dose-dependent increase in the uptake glucose, where the rate of uptake increased as the concentration increased (Fig. 10). In the present study, quercetin improved the uptake of glucose indicating its effect on key signaling pathways in glucose metabolism.

Discussion

Corticosteroids play a critical role in the treatment of conditions like asthma, autoimmune diseases including rheumatoid arthritis, lupus and severe allergic reactions, as well as critical illnesses like sepsis or complications of COVID-19, including cytokine storms due to their significant anti-inflammatory and immunosuppressive effects [40]. However, prolonged or high-dose steroid usage can cause hyperglycemia, raising the risk of diabetes by upregulating gluconeogenesis in the liver. This increases the likelihood of developing steroid-induced diabetes, particularly in individuals with prediabetes [15]. Throughout history, humans have relied on plants and their metabolites to treat various health issues. Phytochemicals, a broad range of bioactive compounds derived from plants, have gained significant attraction for their health-promoting effects [41]. Among these, flavonoids are one of the most ubiquitous and widely distributed categories renowned for their health benefits. Quercetin is one such flavonoid compound known for its health-promoting benefits ranging from antidiabetic, antioxidant and anti-inflammatory to immune-modulating properties [42]. Employing phytochemicals to alleviate the side effects of steroids is a promising strategy due to their natural beneficial properties. This method may also enhance the effectiveness of steroids while reducing adverse effects, causing improved patient outcomes. In this study, the effect of quercetin on mitigating steroid-induced gluconeogenesis was studied using yeast cells as a model organism.

In the present study, betamethasone treatment increased the glucose levels in the yeast cells. Previous studies have also indicated that hyperglycemia induced by steroids is dose-dependent and establishes rapidly, often within minutes to hours of administration similar to our results [43]. This increase in glucose levels is due

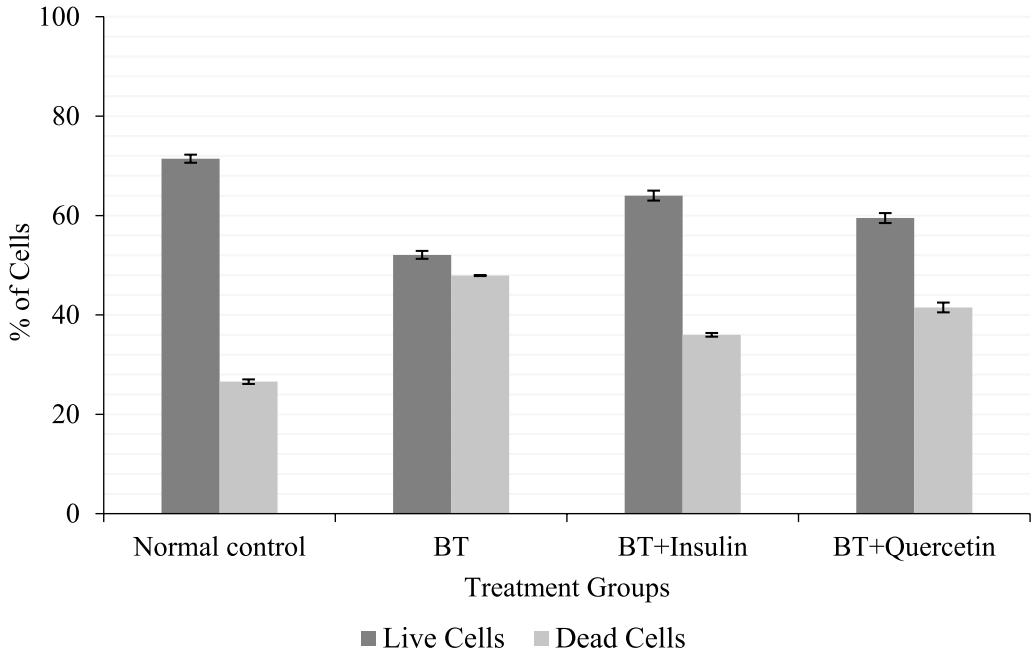

Fig. 9 Effect of quercetin on cell viability. (Values are expressed as mean ± S.D ($n = 3$))

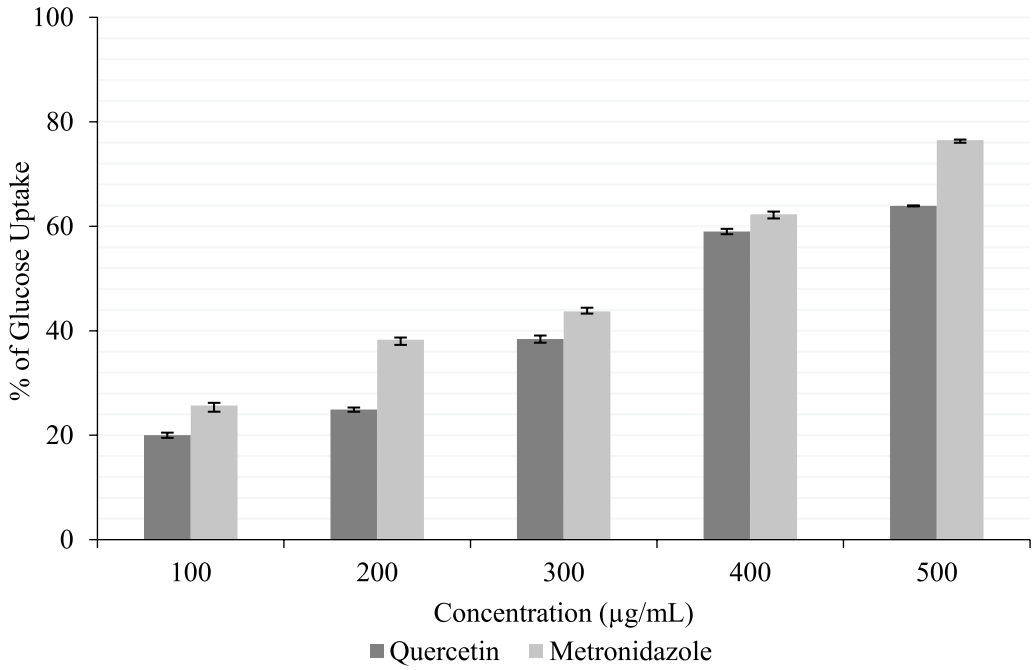

Fig. 10 Effect of quercetin on glucose uptake in yeast cells. (Values are expressed as mean ± S.D (*n* = 3))

to the neutralization of insulin's action by glucocorticoids in the liver and the stimulation of gluconeogenesis, leading to an overall increase in hepatic glucose production [44]. Another mechanism through which glucocorticoids influence liver glucose regulation is by antagonizing the effects of insulin through an increase in the expression of the pseudo-kinase Trb3, which inhibits AKT phosphorylation, leading to hyperglycemia and insulin resistance [45]. This can exacerbate preexisting conditions such as diabetes or lead to new-onset hyperglycemia, increasing the risk of cardiovascular complications, infections and delayed wound healing. Hence, reducing glucose levels after steroid treatment is clinically important to prevent complications associated with steroid-induced hyperglycemia [7]. Treatment of steroid-induced yeast cells with quercetin led to a significant decrease in glucose levels. This shows that quercetin was able to bring down the glucose levels. Suppression of gluconeogenesis is particularly important in type 2 diabetes mellitus where it contributes significantly to elevated endogenous glucose production. Therefore, regulating gluconeogenesis is essential for effective blood glucose management [3]. Previous studies in animal models have established that flavonoids reduce blood glucose levels by controlling liver gluconeogenesis [46]. The results indicate that quercetin is effective in mitigating steroid-induced hypergluconeogenesis. Steroid induction also increased the levels of total proteins. Similar to our results, Moore et al. [47] have reported that mean serum total protein was significantly increased in glucocorticoid-treated dogs at the fourth week of treatment. Glucocorticoids also both increase skeletal muscle catabolism and decrease muscle synthesis which increases free protein levels [48]. Additionally, glucocorticoids are also shown to cause muscle atrophy by inhibiting mTOR, a kinase that phosphorylates S6K1 and 4E-BP1, two proteins essential for the initiation of mRNA translation [49]. The increase in protein levels in BT-induced groups could also be due to the upregulation in the expression of gluconeogenic enzymes like phosphoenolpyruvate carboxykinase and glucose-6-phosphatase [50]. In this study, quercetin treatment effectively reduced the total protein levels in steroid-induced yeast cells.

Steroid induction led to decreased free amino acid levels in yeast cells indicating that free amino acids are diverted to the synthesis of glucose through gluconeogenesis. Marconi et al. [51] demonstrated that betamethasone significantly increases the concentration of amino acids probably by stimulating protein catabolism in skeletal muscles. During elevated gluconeogenesis, amino acids are broken down, and their carbon skeletons are used to form glucose. This causes a decrease in the levels of these amino acids in the body, as they are being consumed for glucose production [52]. Quercetin treatment has improved the free amino acid levels in the steroid-induced yeast cells. As previously mentioned, quercetin has been shown to inhibit key gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase and

glucose-6-phosphatase [53]. By inhibiting these enzymes, quercetin reduces the conversion of noncarbohydrate substrates, like amino acids, into glucose. This inhibition decreases the overall gluconeogenic activity, conserving amino acids that would otherwise be used in glucose production. As a result, amino acid catabolism is decreased, leading to higher amino acid levels. This preservation of amino acids may also contribute to increased protein synthesis and muscle mass [54]. The betamethasone-induced group showed reduced pyruvate levels indicating pyruvate utilization for elevated gluconeogenesis. Pyruvate is a crucial precursor for gluconeogenesis. During gluconeogenesis, the enzyme pyruvate carboxylase catalyzes the conversion of pyruvate into oxaloacetate. Oxaloacetate then further transforms eventually to produce glucose [55]. As steroid induction causes elevated gluconeogenesis, pyruvate is continuously used as a substrate for glucose production. Additionally, when the rate of gluconeogenesis is high, there is a reciprocal decrease in glycolysis further reducing the production of pyruvate [56]. Studies reported that glucocorticoid therapy had caused increased blood pyruvate levels in women though the specific effects depend on dosage and duration of treatment. Clinically, glucocorticoids like betamethasone may cause an increase in blood glucose, potentially impacting pyruvate levels through enhanced gluconeogenic activity [57]. The BT-induced yeast cells treated with quercetin had improved pyruvate levels by the end of the treatment period. This shows that by inhibiting gluconeogenesis, quercetin reduces the conversion of pyruvate into glucose, thereby increasing pyruvate levels. Lactate levels were increased in yeast cells induced with betamethasone when compared to normal control. An increase in lactate levels following glucocorticoid administration is attributed to a significant rise in gluconeogenesis. Lactate, produced during anaerobic glycolysis, is sent to the liver, where it is converted back into glucose via gluconeogenesis [58]. Previous studies also indicated that dogs treated with prednisone experienced an increase in blood lactate concentrations similar to our results [59]. Quercetin treatment has caused a reduction in the levels of lactate by the end of the treatment period. It has been reported that dietary polyphenolic intervention can improve insulin signaling. Hence, quercetin could have restored the impaired insulin signaling caused by steroid induction leading to reduced gluconeogenesis and subsequently, lowering lactate levels [60]. Glucocorticoids, including betamethasone, are widely used as adjuvants in cancer therapy to alleviate symptoms like chemotherapy-induced toxicity and intracranial pressure in brain tumors. However, their use is associated with negative outcomes such as suppression of antitumor immunity, therapy resistance and increased metastasis that could be due to elevated lactate levels following steroid use [61]. Lactate production can also occur due to the glycolytic nature of hypoxic tumor cells. Both conditions lead to acidosis that promotes metastasis, angiogenesis and immunosuppression thereby exacerbating the tumor microenvironment [62]. Since quercetin was able to mitigate the increase in lactate levels following steroid administration, it might also help alleviate acidosis in tumors improving clinical prognosis.

Numerous studies have shown that glucocorticoids can negatively impact the antioxidant systems by reducing the activity of key antioxidant enzymes, depleting glutathione levels, increasing oxidative stress and altering the expression of genes concerned with the antioxidant response [63]. In this study, BT-induced yeast cells treated with quercetin showed an improvement in the catalase, superoxide dismutase and vitamin C levels compared to the negative control group II. Studies have also reported that in rats, steroid treatment caused a significant reduction in the catalase and superoxide dismutase activity in the brain [64]. Growing evidence indicates that the superoxide dismutase activity may be significantly reduced in scavenging free radicals during glucocorticoid exposure, without a corresponding increase in the production of this enzyme [65]. Steroid treatment, particularly with glucocorticoids, is generally also associated with decreased vitamin C activity, leading to impaired antioxidant defense and increased oxidative stress [66]. Under conditions of oxidative stress, vitamin C can be rapidly depleted as it neutralizes free radicals. Quercetin, a powerful antioxidant by itself, can also improve the levels of antioxidants and thus protect the cells against steroid-induced oxidative damage [67].

Furthermore, betamethasone induction decreased the viability of cells as there were more dead cells in the negative control group. Studies have indicated that steroids exert cytotoxic effects in cells by downregulating the genes involved in maintaining the structural integrity of cells [68]. In the present study, quercetin improved the viability of yeast cells which could be due to its antioxidant potential that can improve cell viability through modulation of apoptotic pathways, enhancement of mitochondrial function, stimulation of autophagy and protection of DNA [69]. These combined effects could help cells resist damage, maintain their functions and survive under various stress conditions. Studies also suggest that quercetin can extend lifespan in yeast by reducing oxidative stress and influencing carbon metabolism [70]. The analysis of glucose uptake in yeast cells is an important area of research for understanding the effects of phytochemicals found in plants on cellular metabolism. Yeast cells serve as a valuable model organism for investigating how phytochemicals influence glucose metabolism [71].

In this study, glucose uptake assay indicated that quercetin can stimulate glucose uptake in cells, improve insulin resistance and decrease hyperglycemia [72]. These findings suggest that incorporation of quercetin can mitigate the side effects associated with betamethasone treatment, particularly hyperglycemia mediated by hypergluconeogenesis. The limitations of the study include the absence of an in vivo animal model for studying the effect of steroid administration on glycemic control. Nevertheless, this study paves a promising path for future research on the potential role of phytochemicals in mitigating steroid-induced side effects.

Conclusion

The study demonstrates that quercetin effectively inhibits steroid-induced hypergluconeogenesis in *Saccharomyces cerevisiae*. Our findings suggest that quercetin modulates key metabolites involved in gluconeogenesis, thereby mitigating the hyperglycemic effects typically associated with steroid treatment. Briefly, quercetin administration reduces intracellular glucose accumulation driven by steroid-induced gluconeogenesis while also increasing pyruvate levels and lowering lactate concentrations in yeast cells. These effects may help prevent the onset of hyperglycemia. The levels of protein and free amino acids are decreased in steroid-induced cells on quercetin treatment, whereas the levels of antioxidants increased. This modulation may also contribute to improving metabolic imbalances and oxidative stress, particularly in conditions like diabetes, where enhanced gluconeogenesis and oxidative damage exacerbate hyperglycemia and complications. By restoring the balance of gluconeogenesis and glucose uptake in yeast cells, quercetin not only reduces excessive glucose production, but also helps in the maintenance of cellular homeostasis and cell viability. Our findings underscore quercetin's potential as a therapeutic agent for managing steroid-induced metabolic disturbances, providing a promising avenue for further research into its application in metabolic disorders and hyperglycemia.

Abbreviations

GCs	Glucocorticoids
COVID-19	Coronavirus disease 2019
YPD	Yeast peptone dextrose
mg/ml	Microgram/milliliter
µU/ml	Microunits/milliliter
µmol/L	Micromoles/liter
$MgCl_2$	Magnesium chloride
DNPH	2,4-Dinitrophenylhydrazine
NaOH	Sodium hydroxide
$FeCl_3$	Ferric chloride
H_2O_2	Hydrogen peroxide
H_2O	Water
PMS	Phenazine methosulfate
NBT	Nitro blue tetrazolium chloride
NADH	Reduced nicotinamide-adenine dinucleotide
PBS	Phosphate-buffered saline
SD	Standard deviation
BT	Betamethasone
SOD	Superoxide dismutase
DNA	Deoxyribonucleic acid

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Author contributions

VAD contributed to the design and conception of the study. GS performed in vitro experimental work and collected data. The final draft of the manuscript was written by KM. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and will be made available on reasonable request to the corresponding author.

Declarations

Ethics approval and consent to participate

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Consent for publication

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Competing interests

The authors declare that there is no competing interest.

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