Attenuation of Oxidative Stress-Associated Hematological Alterations and Hepatic Injury Induced by D-Dalactose or γ -Irradiation Using β -Hydroxybutyrate in Male Rats

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Research Article

Keywords: D-galactose, gamma irradiation, oxidative stress, β -hydroxybutyrate, trace elements

Posted Date: February 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-166169/v1>

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Abstract

This work aims to investigate the possible inhibitory action of β -hydroxybutyrate (β OHB) against hematological alterations and hepatic injury associated with oxidative stress caused by D-galactose or γ -irradiation in rats. Six groups of male rats were used as the control, irradiated group (5 Gy), D-galactose (150 mg/kg b.wt), β -hydroxybutyrate (72.8 mg/kg b.wt), γ -irradiation plus β OHB, and D-galactose plus β OHB. Complete blood count and glucose-6-phosphate-dehydrogenase (G6PD) activity were determined in whole-blood samples. In addition, the hepatic malondialdehyde (MDA), nitric oxide (NO), and glutathione (GSH) levels and the superoxide dismutase (SOD) activity were evaluated. Moreover, certain elements were measured in liver tissue (iron (Fe), copper (Cu), and zinc (Zn)). The G6PD activity significantly diminished post exposure to D-galactose or γ -irradiation. In the β OHB, D-galactose, or γ -irradiation groups, liver MDA levels and SOD activity were significantly increased. Meanwhile, NO and GSH levels were significantly increased relative to normal control levels in the γ -irradiation group. The findings showed that β OHB alleviated hematological alterations, enhanced the altered biochemical indices, and modulated the change in Cu, Fe, and Zn elements in D-galactose or γ -irradiation group. These results highlight the role of β OHB as a powerful protective agent against hematological alterations and liver impairment by reducing G6PD-mediated oxidative stress and controlling the measured elements.

Introduction

The accumulation of reactive oxygen species (ROS) with final oxidative stress will result in D-galactose or γ -irradiation (Chen et al. 2007; Kim and Lee 2014). Moreover, oxidative stress is considered to be important in the pathophysiology of numerous diseases including cancer, Parkinson's and cardiovascular diseases, and the aging process. Cellular lipids, proteins, and DNA have been damaged by ROS, affecting their normal functions. Thus, cell aging and mortality can result in an excess of ROS death (Butterfield and Boyd-Kimball 2018).

There was a naturally reducing sugar in the body which is completely metabolized at normal levels, was called D-galactose. Conversely, excess D-galactose is converted to galactitol by galactose reductase, which can relate to osmotic stress. Additionally, galactose oxidase induces the metabolism of high D-galactose levels to produce hydrogen peroxide. Consequently, high levels of hydrogen peroxide increase the oxidative stress and finally disturb the function of macromolecules and cells (Kumar et al. 2010). Therefore, the treatment of rats with D-galactose gives similar signs with those aged naturally. D-galactose injection was used widely to form a model for anti-aging research (Wei et al. 2005).

Ionizing radiation (IR) is an oxidative stress and DNA damage inducer, which encourages the aging process. In addition, IR forms premature senescence through apoptosis and growth arrest both *in vitro* and *in vivo* as a result of DNA damage response (Mavragani et al. 2017; Nguyen et al. 2018). Interestingly, *in vivo* IR response is tissue-specific and dose-dependent, and the proliferative capacity and structural function of the affected tissues seem to be determining factors regulating IR responses (Schofield and Kondratowicz 2018).

The body responds to the prolonged reduction of glucose availability, insulin suppression, and liver glycogen depletion by increasing the ketone bodies to act as alternate metabolic substrates for extrahepatic tissues, in many cases such as hunger, fasting, intense exercise, calorie restriction, or the ketogenic diet (KD). Although KD has a strong therapeutic potential, its efficacy and usefulness as a metabolic therapy for widespread clinical use is restricted by several factors. Due to severe dietary restriction, patient compliance with KD can be limited, and the diet is widely shown as unpalatable and intolerant to high-fat ingestion. As a result, ketosis may be difficult to manage as even a small amount of carbohydrates or excess protein may readily inhibit ketogenesis through intake. (Amari et al. 1995; Halevy et al. 2012). Moreover, it may take several weeks for the ketone body to enhance tissue regeneration and tissue usage (keto-adaptation), and patients may develop moderate hypoglycemic symptoms during this transitional phase (Zhang et al. 2013).

Given both the great therapeutic potential and limitations of KD, an oral exogenous ketone supplement capable of inducing sustained therapeutic ketosis without the need for dietary restriction would serve as a practical alternative. Furthermore, several supplements of natural and synthetic ketones capable of inducing nutritional ketosis have been identified. Desrochers et al. (Desrochers et al. 1995) demonstrated that exogenous ketone supplements: (R,S)-1,3-butanediol and (R,S)-1,3-monoesters and diester butanediol-acetoacetate have been proposed to be used in elevated pig blood ketone bodies (> 0.5 mM). Therefore, the safety and efficacy of chronic oral administration of β -hydroxybutyrate (β OHB) ketone monoester in rats and humans has been shown by Clarke et al. (Clarke et al. 2012). Subjects maintained high blood ketones without dietary restriction and recorded little to no adverse side effects, demonstrating the potential to eliminate the restrictive diet typically needed to achieve therapeutic ketosis.

Numerous studies suggest that certain KD benefits are responsible for the effects of ketone body metabolism. Interestingly, in research on type 2 diabetes patients, improved glycemic regulation, enhanced lipid markers, and retraction of insulin and other medicines occurred before weight loss became important. In parallel, the growth of mitochondrial ROS species is reduced by both β OHB and acetoacetate (Kim et al. 2007; Maalouf et al. 2007). In addition, it has been shown that ketone bodies improve the heart's hydraulic performance by 28% while decreasing oxygen consumption and elevating ATP production (Kashiwaya et al. 1994). High ketone bodies have thus increased metabolic efficiency and, as a consequence, reduced superoxide production and glutathione production (Veech et al. 2001).

Sullivan et al. (Sullivan et al. 2004) found that rats fed KD for 10–12 days showed increased hippocampal uncoupling proteins, suggesting a decreased ROS produced by mitochondria. In addition, mitochondrial biogenesis increased in rats maintained on KD for 4–6 weeks (Bough and Rho 2007; Bough et al. 2006). Consequently, Shimazu et al. (Shimazu et al. 2013) stated that β OHB is a class-I histone deacetylases exogenous and unique inhibitor, which provides oxidative stress safety. By reducing inflammatory biomarkers (e.g., tumor necrosis factor- α (TNF- α), interleukin (IL)-6, IL-8, E-selectin, I-CAM, and PAI-11), ketone bodies have also been shown to suppress inflammation (Paoli et al. 2015; Ruskin et al. 2009). It is also assumed that exogenous ketone bodies themselves impart many of the advantages associated with KD. Furthermore, the effect of β OHB on hematological alteration and hepatic

injury caused by chronic administration of D-galactose or by an acute dose of γ -irradiation in male rats has been investigated in the present research.

Material And Methods

Experimental animals

Throughout the experiment in this study, 48 male rats aged 3 months were used and collected from the animal house of the Egyptian Atomic Energy Authority. In addition, the animals were fed commercial pellets and given tap water ad libitum throughout the experimental stages. Rats from the Animal Ethics Committee (IAEC) of the Faculty of Science, Tanta University, Tanta, Egypt, were treated in compliance with the proposed national ethical guidelines instructions.

Irradiation Process

Acute 5 Gy (0.708 rad/s) whole-body γ -irradiation dose was performed using the facilities provided by the National Center for Radiation Research and Technology. The 137 irradiation unit (gamma cell, 40) provided by Candy Minimal Atomic Energy was used. It is defined by a uniform distribution of rays to small biological materials that do not pose any external hazards to operators.

Treatments

At a dosage of 150 mg/kg per bodyweight (bw) diluted in distilled water, D-galactose (D-Gal) was supplied to the animals (Liu et al. 2009). DL- β hydroxybutyrate from the ketone body was administered to certain classes of rats at a dosage of 75.5 mg/kg bw dissolved in saline (Choragiewicz et al. 2008). Both D-galactose and β -hydroxybutyric acid sodium salt were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Experimental design

The rats were randomly divided into six groups each consisting of eight rats after 2 weeks of acclimatization to the home cage (group I), normal control group; group II; rats subjected to 5 Gy as a single dose of whole-body γ -irradiation; group III, rats received 150 mg D-galactose/kg bw intraperitoneally for 42 consecutive days; group IV, rats received 72.8 mg DL- β hydroxybutyrate/kg bw intraperitoneally for 42 consecutive days; and group V, rats exposed to β OHB 5 Gy (whole-body irradiation) as in group IV and treated with an intraperitoneal injection for 14 consecutive days with β OHB 72.8 mg/kg bw; group VI, rats received 150 mg D-galactose/kg bw for 42 consecutive days and 72.8 mg/kg bw intraperitoneal injection with β OHB for the last 14 consecutive days with D-galactose. Rats were anesthetized with an intraperitoneal injection of 1.2 mg/kg bw of urethane on the day after final treatment (Flecknell 1993). Consequently, the rats were sacrificed after the whole-blood and liver tissues were collected for biochemical analyses.

Samples collection

Blood sampling

Whole blood was collected via cardiac puncture after anesthesia. In an ethylene diamine tetraacetic acid (EDTA) tube, blood was collected from each rat. In addition, EDTA blood samples were used for the determination of activity and a complete blood count of glucose-6-phosphate dehydrogenase (G6PD), where hematological parameters including red blood cells (RBCs), white blood cells (WBCs), mean corpuscular volume (MCV), platelet counts, and hemoglobin (Hb) concentration were evaluated using blood counters (Techo, Miami, FL, USA).

Liver tissue sampling

Liver tissue was prepared following the method of Li et al. (Li et al. 2014). Excised liver tissue was washed with ice-cold isotonic 0.9% NaCl after dissection, blotted between two filter papers, and weighed. Liver tissue was divided into two portions (part 1, 10% (W/V) homogenate in ice-cold 0.9% NaCl was prepared for biochemical analysis using a Tri-R STIR-R model K41 homogenizer; part 2, was washed for determination of certain elements with deionized water).

Biochemical analysis

Lipid peroxidation was quantified in liver homogenate according to Yoshioka et al. (Yoshioka et al. 1979). The estimation of nitric oxide was according to Montgomery and Dymock (Montgomery and Dymock 1961). Liver glutathione (GSH) was calculated according to the updated procedure of Ellman (Ellman 1959). Moreover, superoxide dismutase (SOD) activity in tissue homogenates was assessed according to the method of Masayasu and Hiroshi (Masayasu and Hiroshi 1979). Consequently, the biochemical analysis was done using a Helios γ UV-VIS spectrophotometer.

Preparation of tissue samples for atomic absorption

Trace elements have been determined in the liver tissue of rats. Following digestion at 5:1 ratio (v/v) in pure concentrated nitric acid and hydrogen peroxide (Nada et al. 2012), a Landmark MLS-1200 Super High-Performance Microwave Digestor Unit was used for sample digestion. Moreover, the selected elements were estimated using Thermo Science ICE 3000 Series Atomic Absorption Spectrometry, fitted with deuterium background correction. All the solutions were prepared with ultrapure water obtained from ELGA with a specific resistance of 18 U/cm. Consequently, the biochemical analysis was done using a Helios γ UV-VIS spectrophotometer.

Statistical analysis

Statistical analysis was conducted using one-way variance analysis (ANOVA) followed by the honestly relevant difference test of post hoc Tukey using the GraphPad Prism 5 software package. Data were provided with an appropriate significance level of $p \leq 0.05$ as mean $\pm$ SEM ($n = 8$ rats/group). The approach used for analyzing the findings was given by Milton et al. (Milton et al. 1986).

Results

The β OHB administration per se resulted in a substantial increase ($p \leq 0.05$) in the PLT count by 54.39% relative to the standard control values (Table 1). The administration of D-galactose resulted in a significant 45.28% rise ($p \leq 0.05$) in WBCs relative to the usual control value. β OHB administration improved all hematological parameters to normal control levels along with D-galactose.

Neither β OHB nor D-galactose administration had a significant effect relative to the normal control value on Hb, MCV, and RBCs. Moreover, β OHB administration along with irradiation significantly increased ($p \leq 0.05$) WBCs compared with the irradiated group, achieving a percentage increase of 124.35%.

Table (1): Effect of DL- β -hydroxybutyrate (72.8 mg/kg) on complete blood count (CBC) in rats intoxicated D-galactose (150 mg/kg bw) or exposed to γ -irradiation (5 Gy)

Groups	Hb (g/dl)	MCV (fl)	PLT (*10 ⁹ /l)	RBCs (*10 ¹² /l)	WBCs (*10 ⁹ /l)
Control	17.04 $\pm$ 0.199	50.78 $\pm$ 0.852	555.6 $\pm$ 38.38	8.916 $\pm$ 0.07	4.56 $\pm$ 0.318
β OHB	16.44 $\pm$ 0.625 – 3.52 %	53.08 $\pm$ 0.62 – 4.52 %	857.8 $\pm$ 45.56 ^a 54.39 %	8.566 $\pm$ 0.22 – 3.9 %	3.67 $\pm$ 0.27 – 19.51 %
D- Gal	16.94 $\pm$ 0.297 – 0.58 %	50.92 $\pm$ 0.755 – 0.27 %	532 $\pm$ 23.01 – 4.24 %	8.806 $\pm$ 0.193 – 1.23 %	6.625 $\pm$ 0.396 ^a 48.54 %
D-Gal + β OHB	15.56 $\pm$ 0.367 – 8.68 % – 8.14 %	52.32 $\pm$ 0.575 – 3.03 % 2.74 %	535.8 $\pm$ 32.15 – 3.56 % 0.71 %	8.75 $\pm$ 0.16 – 1.86 % – 0.63 %	5.82 $\pm$ 0.503 27.63 % – 12.15 %
IR	11.95 $\pm$.5 ^a – 29.87 %	70.87 $\pm$ 1.1 ^a 39.56 %	133.3 $\pm$ 4.6 ^a – 76.00 %	5.78 $\pm$ 0.229 ^a – 35.17 %	1.56 $\pm$ 0.067 ^a – 65.78 %
IR + β OHB	14.1 $\pm$ 0.28 ^{ac} – 17.25 % (17.99 %)	77.86 $\pm$ 2.89 ^{ac} 53.32 % (9.86 %)	347 $\pm$ 16.62 ^{ac} – 37.54 % (134.77 %)	6.8 $\pm$ 0.2 ^{ac} – 23.73 % (17.64 %)	3.5 $\pm$ 0.122 ^c – 23.24% (124.35 %)

Data are presented as mean $\pm$ SE (n = 8). **a**, **b**, and **c** indicate significant changes from control, D-Gal, and IR, respectively, at $p \leq 0.05$ using ANOVA followed by Tukey–Kramer as a post-ANOVA test. Normal percentage indicates percentage change from the control group. Underlined percentage indicates percentage change from the D-galactose group. Percentage in round brackets indicates percentage change from the IR group. β OHB β -hydroxybutyrate, D-Gal D-galactose, IR γ -irradiation.

Figure 1 shows that G6PD activity was significantly reduced by 22.14% in rats treated with D-galactose ($p \leq 0.05$) compared with the control group. In addition, β OHB administration with D-galactose resulted in a substantial 21.29% increase in G6PD activity relative to the D-galactose group.

In contrast with the control group, γ -irradiation caused a significant decrease ($p \leq 0.05$) in blood G6PD activity among γ -irradiated animals by 34.98%. In contrast with the γ -irradiated group, β OHB administration with γ -irradiation significantly increased ($p \leq 0.05$) G6PD activity by 46.4%. Furthermore, G6PD activity was restored to normal control levels by β OHB administration along with D-galactose or γ -irradiation.

Data are presented as mean $\pm$ SE ($n = 8$). **a**, **b**, and **c** indicate significant changes from control, D-Gal, and IR, respectively, at $p \leq 0.05$ using ANOVA followed by Tukey–Kramer as a post-ANOVA test. Normal percentage indicates percentage change from the control group. Underlined percentage indicates percentage change from the D-galactose group. Percentage in round brackets indicates percentage change from the IR group. β OHB β -hydroxybutyrate, D-Gal D-galactose, IR γ -irradiation.

As highlighted in Fig. 2, β OHB administration per se did not significantly alter liver GSH and nitric oxide (NO) contents. Meanwhile, it significantly ($P \leq 0.05$) increased malondialdehyde (MDA) levels and SOD activity by 318.31% and 26% relative to the normal control value, respectively. Administration of D-galactose significantly ($P \leq 0.05$) increased hepatic MDA content and SOD activity by 302.19% and 28.2%, respectively, in comparison with the normal control levels. However, D-galactose-treated groups with β OHB induced a significant ($P \leq 0.05$) decrease in hepatic MDA and SOD activity by 45.76% and 66.45%, respectively, compared with the D-galactose groups.

Exposure to γ -irradiation significantly ($P \leq 0.05$) increased the MDA, NO, and GSH levels and SOD activity by 558.24%, 101.73%, 210.06%, and 102.9%, respectively, compared with the normal control value. However, the rat model that received β OHB with γ -irradiation recorded remarkable percentage decrease in MDA, NO, GSH levels and SOD activity by 58.04%, 25.85%, 48.92%, and 33.35%, respectively, compared with γ -irradiation group.

Data are presented as mean $\pm$ SE ($n = 8$). **a**, **b**, and **c** indicate significant changes from control, D-Gal, and IR, respectively, at $p \leq 0.05$ using ANOVA followed by Tukey–Kramer as a post-ANOVA test. Normal percentage indicates percentage change from the control group. Underlined percentage indicates percentage change from the D-galactose group. Percentage in round brackets indicates percentage change from the IR group. β OHB β -hydroxybutyrate, D-Gal D-galactose, IR γ -irradiation.

Figure 3 pinpoints that β OHB administration significantly increased ($p \leq 0.05$) liver copper content by 28.21% relative to the corresponding control group. In addition, the D-galactose group significantly exhibited a 22.29% liver iron increase and 19.61% zinc decrease compared with the control group. Meanwhile, treatment with β OHB for D-galactose-exposed rat significantly increased copper content by 24.7% and showed nonsignificant change ($p \leq 0.05$) in liver iron and zinc concentration compared with the D-galactose group.

Rats submitted to γ -irradiation (5 Gy) exhibited a significant decrease in copper (Cu) and zinc (Zn) by 34.15% and 20.55%, respectively, and a nonsignificant decrease in iron content compared with the normal control value. Meanwhile, a significant recovery exists in copper concentration in γ -irradiation co-treated with the β OHB group compared with the γ -irradiation group. However, liver iron and zinc significantly decreased ($p \leq 0.05$) by 20.26% and 18.84%, respectively, compared with the normal control value.

Data are presented as mean $\pm$ SE ($n = 8$). **a**, **b**, and **c** indicate significant changes from control, D-Gal, and IR, respectively, at $p \leq 0.05$ using ANOVA followed by Tukey–Kramer as a post-ANOVA test. Normal percentage indicates percentage change from the control group. Underlined percentage indicates percentage change from the D-galactose group. Percentage in round brackets indicates percentage change from the IR group. β OHB β -hydroxybutyrate, D-Gal D-galactose, IR γ -irradiation.

Discussion

The present work focuses on testing another mechanism for the induction of hepatic aging by D-galactose or γ -irradiation following the study by Habieb et al. (Habieb et al. 2021). Biomarkers of oxidative stress were used with those essential elements in the biological system

The results of this study clearly showed that a rise in the WBC counts exists, followed by a substantial decrease in the G6PD activity. Glycation end-products may result in the elevation of white blood cell counts by D-galactose (Hofmann et al. 1999; Yang et al. 2007). Moreover, D-galactose interacts with the amino protein group creating derivatives called advanced glycation end-products. These glycation end derivatives have been shown to improve some angiogenic and inflammatory cytokine expression, including vascular endothelial growth factor, TNF- α , IL-6. Moreover, this method could be the reason for leukocyte count activation and elevation (Costa et al. 2007).

Current research has shown an increase in platelets based on β OHB administration, which reflects an improvement in the process of differentiation by β OHB per se. Platelet count elevation could be associated with increased liver production of thrombopoietin, which in turn may be responsible for the proliferation of megakaryocytes and their conversion to platelets (Lee and Bergmeier 2017; Subenthiran et al. 2013).

Exposure to γ -irradiation significantly increased MCV. This result is consistent with Abdelhalim et al. (Abdelhalim et al. 2015) who indicated that a change in morphology and deformation of RBCs can be attributed to an increase in the size of RBCs by γ -irradiation, which has been confirmed by a slight increase in red distribution width.

A significant decline in RBCs, WBCs, platelets, and hemoglobin content in irradiated rats could be due to erythrocyte precursor destruction in bone marrow (Ashry and Hussein 2007). In addition, decreased production of kidney erythropoietin hormone, which is essential for the production of RBCs or erythropoiesis, can be attributed to increased destruction of RBCs by γ -irradiation (Hassan et al. 2016). Free radicals are known to be responsible for RBCs because they contain extraordinary membrane

content of polyunsaturated fatty acids (PUFAs) and oxygen transport linked to redox-active hemoglobin molecules, which are efficient promoters of activated oxygen species (Ebrahimzadeh et al. 2009). Exposure to γ -irradiation significantly increased MCV. This result coincides with Abdelhalim et al. (Abdelhalim et al. 2015) who suggested that a shift in morphology and deformation of RBCs could be due to an increase in RBC size by γ -irradiation.

The cellular components of the blood are largely susceptible to oxidative stress because the phospholipid bilayer membranes have a high concentration of PUFAs. The decrease in the number of WBCs reported in irradiated rats may therefore be the product of lipid peroxidation caused by radiation and its detrimental impact on the phospholipids of their cell membranes. The drop in hemoglobin content may be related to a decrease in red blood cell counts (Chew and Park 2004; Malhotra and Srivastava 1978).

Glucose-6-phosphate dehydrogenase (EC 1.1.1.4), which catalysis glucose-6-phosphate oxidation to 6-phosphogluconolactone and finally to ribose-5-phosphate, is a major rate-limiting enzyme of the pentose phosphate pathway (PPP). This results in NADPH-equivalent reduction biosynthesis to satisfy cellular needs for biosynthesis of fatty acid reduction and maintenance of cellular redox balance (Maurya et al. 2016).

γ -irradiation has a more pronounced effect on G6PD decline activity compared with the decrease induced by D-galactose. The PPP inhibition can then render the exposed RBC more vulnerable to oxidative damage (Demirdag et al. 2015). These changes can result from the vulnerability to oxidation induced by D-galactose or γ -irradiation of free sulfhydryl groups (-SH) necessary for its activity (Giblin et al. 1979). Moreover, D-galactose or γ -irradiation alternation of the redox state of the RBCs subsequently decreases NADPH formation, which in turn inactivates the G6PD activity (Pari and Venkateswaran 2003). Increased RBC exposure to ionizing radiation resulted in a substantial decrease in G6PD activity by γ -irradiation, thereby decreasing survival time (Agarwal et al. 2007). These findings were consistent with those of Van Heyningen et al. (Van Heyningen et al. 1954) who revealed that ionizing radiation such as X-irradiation affected -SH-based enzymes than those enzymes that do not have free -SH groups for operation. Thus, these findings suggest a general improvement in hematological parameters by β OHB by modulation of G6PD activity and decreasing oxidative stress.

Oxygen-derived free radicals enhance membrane lipid peroxidation, enzyme inactivation, DNA fragmentation, and apoptosis activation resulting in disturbance in the living cells (Speakman and Selman 2011). MDA is the end product of lipid peroxidation, which is considered a significant biomarker. Additionally, antioxidant supplementation has an important role to reduce the aging process (Koyama et al. 2013). There are many enzymes in the body such as SOD and glutathione peroxidase which are responsible for scavenging superoxide anion and hydrogen peroxide to prevent ROS-induced damage. Thus, it has been important to determine the oxidative stress status including MDA, NO, GSH levels and SOD activity. Our data recorded that β OHB and D-galactose or γ -irradiation significantly reduced the oxidative stress in the aged rat by restoring GSH levels and SOD activity in the liver and decreasing MDA

level and modulating the trace element alterations, supporting the β OHB mechanism of action and the aging theory of oxidative stress.

The supplementation with DL- β -hydroxybutyrate dramatically increased MDA levels in the liver. However, this finding was not predicted. Thus, this is likely to be because the daily ingestion of ketone salt could cause recurrent bouts of oxidative stress to the liver and this finding requires further mechanistic analysis (Lu et al. 2018). The unusually high level of MDA caused by β OHB alone correlates with Milder et al. (Milder et al. 2010), which could be attributable to the acute increase of hydrogen peroxide (H_2O_2) and 4-hydroxynonanal, an electrophilic lipid peroxidation end-product known to activate nuclear factor erythroid 2-related factor 2 (Nrf-2) detoxification pathway. Moreover, Nrf-2 can upregulate GSH biosynthesis and is a primary response to cellular stress. In addition, another possible explanation for this increase in MDA by β OHB per se may be due to lipid peroxidation mediated by macrophages of liver hepatocytes as an NADPH oxidase (NOX) enzyme upregulated by β OHB that acts as a mediator of the development of reactive oxygen species (Kanikarla-Marie and Jain 2015). The results coincide with Anilkumar et al. (Anilkumar et al. 2008) who reported that high ketone levels were reported to be associated with increased oxidative stress in cell models. Moreover, Baffy (Baffy 2009) correlated an increase in the burden of oxidative stress on macrophage recruitment along with liver resident Kupffer cells, which subsequently raises the levels of hepatic MDA. Furthermore, the findings of this study are in line with the results reported by De Almeida et al. (De Almeida et al. 2010), who showed an increase in protein oxidation and lipid peroxidation in animal model rat livers subjected to acetone-induced ketosis via a free radical dependent mechanism.

In the hippocampus of rats fed a ketogenic diet, Milder et al. (Milder et al. 2010) reported an improvement in H_2O_2 development and lipid peroxidation. In addition, chronic β OHB exposure has also been shown to increase the production of ROS in cardiomyocytes (Pelletier and Coderre 2007).

Liver SOD activity was retained in the β OHB group. These data indicate that β OHB initially generates mild oxidative stress, which through redox signaling can systematically activate the Nrf-2 pathway, leading to an adaptive redox response to oxidative stress (Lu et al. 2018). A significant decrease was not found in SOD activity along with D-galactose administration or γ -irradiation exposure, unlike other research conducted in this field. However, the cellular antioxidant SOD activity was significantly elevated by D-Gal or γ -irradiation exposure. This somewhat contradictory outcome is still not entirely clear, but this outcome can be explained in part by MDA stimulation at a sublethal concentration that induces adaptive response and increases cell tolerance primarily through SOD induction through transcriptional activation of the signaling pathway linked to nuclear factor erythroid 2–factor-2 (Nrf-2) signaling pathway, thereby attempting to protect hepatocyte against forthcoming oxidative stress (Pahl and Baeuerle 1994). The aforementioned findings can be elucidated by the existence of a better equilibrium between free radical scavenging enzymes and the development of ROS in young tissue, which is very necessary for oxidative stress cell resistance. The findings may highlight the significance of the animal's starting age in relation to D-Gal-mediated depletion of antioxidants due to oxidative stress (Xu et al. 2009). The previous findings

contradicted those of Liu et al. (Liu et al. 2013), who suggested a significant decrease in SOD activity which is a characteristic of various ages at the start of the D-galactose treatment.

No substantial improvement in GSH or NO levels was noted it was predicted that D-Gal would decrease GSH levels and increase the concentration of NO. An unchanged GSH level was followed by D-Gal-treated rats, leading to a satisfactory explanation that either GSH's response to increased lipid peroxidation is restricted or a lack of improvement in the GSH level is a cause for elevated levels of oxidative stress. The absence of adequate changes in GSH levels under conditions of increased ROS can impair the antioxidant system's capability, leading to increased oxidative stress (Hadzi-Petrushev et al. 2015). In addition, oxidative stress stimulation at a sublethal concentration, which induces adaptive response and increases cell tolerance primarily via GSH induction through transcriptional Nrf-2 signaling pathway activation, protects the hepatocytes from potential oxidative stress (Pahl and Baeuerle 1994). Furthermore, Wei and Lee (Wei and Lee 2002) suggested that no major changes in GSH or NO associated with D-Gal in the community of young rats could be found in the light of compulsory attention to developmental changes and the impact of aging on the antioxidant enzyme. Moreover, the aforementioned findings can be explained by the existence of low development of ROS and a better balance between free radical scavenging enzymes in young tissue, which is very important for the oxidative stress resistance of cells. The findings may highlight the significance of the animal's starting age to D-Gal-mediated depletion of antioxidants due to oxidative stress (Xu et al. 2009). The previous results were in contradiction with the findings of Liu et al. (Liu et al. 2013) who indicated that a significant decrease in GSH levels exists, which is a characteristic of various ages at the start of D-Gal treatment.

Nonsignificant increases in NO levels were observed in the D-Gal-treated group in the present study, which could be interpreted as resulting from increased NO oxidation by ROS. Consequently, nitrotyrosine generation in combination with tyrosyl radical or peroxynitrite formation in combination with superoxide ion reduces the availability of NO levels (Ferrer-Sueta et al. 2018; Reiter et al. 2000).

Cu is considered as a cofactor of many enzymes, but free ionic copper is cytotoxic because it mediates the development of the extremely reactive hydroxyl radical through the Fenton reaction. Therefore, it is believed to cause premature senescence and oxidative stress.

After γ -irradiation, the reduction of hepatic copper may be related to the excess of its usage by cuproenzymes which reduce the oxygen to water or hydrogen peroxide (Kotb et al. 1990) or due to de novo syntheses of Cu-SODs and catalase that inhibit the development of superoxide ion, H_2O_2 , and hydroxyl radicals (Fee and Valentine 1977). Nada et al. (Nada et al. 2012) suggested that the decrease in copper levels is linked to the radiolytic loss of cofactors of essential metalloelements that record the 20% loss in rats post irradiation of both Cu- and Zn-dependent SODs.

Aging is well known to induce Fe accumulation in many tissues in vivo, which is associated with the pathology of many age-related diseases (Xu et al. 2008; Zecca et al. 2001). These alterations may be as a

result of iron homeostasis deregulation at the cellular level. But, the mechanism needs to be further investigated. This was confirmed by the previous result of Killilea et al. (Killilea et al. 2003) who stated that the total iron content in the fibroblasts was presented to rise exponentially during cellular senescence, getting tenfold greater levels of Fe compared to young cells. Moreover, low-dose exposure to H_2O_2 lead to early fibroblast cell senescence and enhanced iron accumulation related to senescence. This accumulation may also associate to the amplified amounts of oxidative stress and the decline of cellular function that describe senescent cells.

D-galactose induced a significant increase of iron in the liver. These findings are in complete agreement with Beregovskaia et al. (Beregovskaia et al. 1988) and Nada et al. (Nada et al. 2008) who revealed that Fe level elevation could be correlated with bone marrow's inability to use the Fe available in the diet and released from damaged red cells due to oxidative stress caused by D-Gal. The increased level of iron may be due to proteolytic modification of oxidative stress-induced ferritin (García-Fernández et al. 2005) and transferrin (Trinder et al. 2000). Fe overload is associated with liver damage, characterized by a large accumulation of iron in hepatic parenchymal cells, resulting in fibrosis and ultimately hepatic cirrhosis (Pietrangelo 2016). Increased iron levels by D-Gal agree with Xu et al. (Xu et al. 2012) who suggested that Fe accumulation is generally accepted as an essential characteristic of the aging process, particularly in postmitotic tissue. Consequently, both heme-Fe and heme biosynthesis decrease significantly with aging (Mancuso et al. 2013).

Zn is an important cofactor that maintains the activity of several proteins, such as metallothionein and Cu/Zn SOD that play a role in combating oxidative stress. Zn is also a cofactor for proteins involved in mediating the reaction and repair of DNA damage, such as the p53 tumor suppressor protein (Theocharis et al. 2004). Suboptimal Zn intake can therefore encourage oxidative stress generation and single- or double-strand DNA breaks comparable to DNA damage caused by radiation (Blount et al. 1997). Thus, precise regulation of cytosolic Zn buffering is important to preserve the redox state of the cell because both increased and decreased Zn levels induce oxidative stress (Maret 2006).

The results of the current study showed a significant decrease of Zn in the liver by D-Gal or γ -irradiation which could be due to molecular process disruption that allows optimum Zn levels to be preserved within the cells (Colvin et al. 2010). In addition, increased Zn efflux transporter ZnT-1 levels in the hepatic cell membrane may lead to excess Zn efflux from the liver, resulting in lower levels of zinc in the hepatic cell cytosol (Sekler et al. 2007). Moreover, lower Zn levels in the liver may be mediated by the incorporation of zinc in superoxide dismutase with antioxidant function because the balance between oxidative stress and performing antioxidative defense preserves proteostasis and cellular function and also affects the rates of aging and telomere shortening. Furthermore, the induction of superoxide dismutase plays a significant role in cell protection against an excessive amount of ROS and acted against oxidative stress, thus constituting the main system of protection against oxidative stress induced by D-Gal or γ -irradiation (Andrews 2000).

Oxidative damage caused by either D-Gal or γ -irradiation may be due to a decline in Zn levels because increasing evidence points to Zn as a positive regulator of autophagy. In vitro studies have consistently shown that both basal and induced autophagy is important for Zn (Liuzzi and Yoo 2013). This was confirmed by the fact that high doses of Zn were shown to induce autophagy in MCF-7 breast cancer cells in culture medium (20–200 μ M; (Hwang et al. 2010), astrocytes (Park et al. 2011), and also in human hepatoma cells (Liuzzi and Yoo 2013). In addition, Zn depletion caused by treatment with TPEN or Chelex-100 cell-permeable Zn chelators has been able to suppress autophagy (Liuzzi et al. 2014). Moreover, a possible Zn function of Zn was suggested as a modulator of inflammation associated with the senescence-associated secretory phenotype (Malavolta et al. 2015).

Zn deficiency was observed during aging and increases the suspicion that a possible relationship between Zn and cellular senescence-related hepatic injury may exist. This was explained by the study of Rudolf and Rudolf (Rudolf and Rudolf 2015) who found that colon cancer cells cultivated in a low Zn environment for 6 weeks resulted in morphological changes and typical markers of senescence, while Zn supplementation induced increased ROS formation, causing senescence in vascular smooth muscle cells. Zn also declined the antioxidative ability of the cell by downregulating catalase expression (Patrushev et al. 2012). Additionally, Carrì et al. (Carrì et al. 2003) stated that an imbalanced intracellular Zn homeostasis mediates both oxidative damage and neuronal cell death in neurodegenerative diseases. So, oxidative damage and hepatic cellular senescence may be stimulated by unbuffered controlling mechanisms of intracellular/ cytosolic zinc.

Concerning Zn concentrations in liver tissue, irradiation caused a decrease in liver Zn levels. Similar findings were obtained by Ali et al. (Ali et al. 2012) who suggested that hepatocellular damage and oxidative stress caused by administration of anserine and/or Zn before or after radiation exposure are found to provide safety against γ -irradiation in rats. The results obtained do not coincide with Yukawa et al. (Yukawa et al. 1980) and Smythe et al. (SMYTHE et al. 1982) who observed that γ -irradiation caused an increase in Zn levels in various organs in the entire body. Consequently, Okada (Okada 1970) suggested that the spleen, lymph nodes, and bone marrow of the lymphoid organs are highly radiosensitive. It was clarified that Zn, released by irradiation from these damaged tissues, could accumulate in the liver, increasing its levels.

Reduced levels of Zn by both D-Gal and γ -irradiation may contribute to the zinc's ability to mitigate oxidative damage and promote the process of repair and recovery mediated by its role as important cofactors for various antioxidant enzymes such as Cu and Zn superoxide dismutase. These antioxidant enzymes are necessary to limit lipid peroxidation, help repair the modification of the oxidative base, and help restore the functionality of misfolded proteins that accompany the cellular senescence process induced by D-Gal or γ -irradiation (Marreiro et al. 2017). However, the relationship between the status of trace elements, cellular senescence, and age-related diseases is poorly studied. In addition, limited knowledge exists on the various trace element interactions, especially during cellular senescence. Cellular reactions to changes in patterns of both single trace elements and trace elements and how these

(synergistic) changes affect senescent cells, organs, or the entire organism are less studied. Finally, it is important to focus on the mechanism of cellular signaling driving these changes.

In conclusion, the findings obtained in the current study showed that β OHB has the potential to minimize γ -irradiation exposure or D-Gal injection-induced hematological changes and hepatic cellular senescence in rats. At least partially, this effect can be mediated by enhancing the antioxidant function of G6PD and modulating the alteration of trace elements. These data indicate that β OHB has a protective impact against hepatic cellular senescence-related liver injury and has promising application as an antioxidant and anti-aging medication.

Declarations

Acknowledgments

This study was carried out at the Egyptian Atomic Energy Authority, National Center for Radiation Research and Technology, Cairo, Egypt. The authors acknowledge the members of the trace elements laboratory.

Conflict of interests

No conflict of interest was declared by the authors.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical approval and consent to participate

Rats were cared according to the national ethical guidelines instruction of the IAEC of Faculty of Science, Tanta University, Tanta, Egypt.

Authors Contributions

Prof. Tarek Mohamed, Prof. Asrar Hawas and Dr. Doaa El Gamal contributed to the study conception and design. Material preparation, data collection and analysis were performed by Dr. Mahmoud Habieb and Dr. Marwa Abd El Hameed. The first draft of the manuscript was written by Dr. Mahmoud Habieb and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Consent for publication

“Not applicable” in this section.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due [to preserve the security of this article] but are available from the corresponding author on reasonable request.

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Figures

Figure 1

Effect of DL-β-hydroxybutyrate (72.8 mg/kg) on blood glucose-6-phosphate dehydrogenase activity (G6PD) of rats intoxicated with D-galactose (150 mg/kg bw) or exposed to γ-irradiation (5 Gy).

Figure 2

Effect of DL-β-hydroxybutyrate (72.8 mg/kg) on liver MDA, NO, GSH levels and SOD activity of rats intoxicated with D-galactose (150 mg/kg bw) or exposed to γ-irradiation (5 Gy).

Figure 3

Effect of DL-β-hydroxybutyrate (72.8 mg/kg) on hepatic copper, zinc and iron contents in rats intoxicated with D-galactose (150 mg/kg bw) or exposed to γ-irradiation (5 Gy).