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Research Article

Physiological and biochemical evaluation of Vitamin H7 in Rabbits stressed by Triton

Abeer Ali Kadhim*
Department of Biology, College of Sciences, Mosul University, Mosul, Iraq
Amina Jasim Al-Hayani
Department of Basic Nursing Sciences, College of Nursing, Nineveh University, Mosul, Iraq
Muntaha Mahmoud Al-Kattan
Department of Biology, College of Sciences, Mosul University, Mosul, Iraq

*Corresponding author. Email: abesbio84@uomosul.edu.iq

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Abstract
Vitamin H7 (biotin) is essential for fatty acid synthesis and glucose metabolism. Under stress conditions, such as those induced by Triton (a nonionic surfactant that increases oxidative stress markers and induces hyperlipidemia), the body may require more biotin to manage oxidative stress and maintain metabolic balance. Studying its effects in Triton-stressed rabbits can reveal its potential protective benefits. The aim was to understand how Triton affects the vital functions of rabbits and whether Vitamin H7 plays a role in protecting these functions from negative impacts. Forty male rabbits were divided into four groups and treated daily for eight weeks. The control received no treatment. The Triton group was given water with Triton 300 mg/kg. The Vitamin H7 group received 200 µg/kg, and the Triton and Vitamin H7 group received both treatments. The treatment continued for eight consecutive weeks. The Triton group showed the highest activity of aryl esterase and bilirubin, as well as the lowest activity of superoxide dismutase (SOD) and acetylcholinesterase (Ach), while the Vitamin H7 group showed low activity of aryl esterase and Total Bilirubin, Along with high superoxide dismutase (SOD) activity. The Triton with Vitamin H7 group showed mitigation of the negative effect of triton and improvement in total Bilirubin levels. Triton induces toxic effects, such as oxidative stress, and impacts cell membranes, leading to an increase in aryl esterase activity and a decrease in Ach and SOD. In contrast, Vitamin H7 helps mitigate these toxic effects by supporting cellular metabolism and improving enzyme function, reducing total bilirubin levels, and enhancing liver function.

Keywords: Bilirubin, Enzymes, Oxidative stress, Triton , Vitamin H7.

INTRODUCTION

Commonly referred to as biotin, Vitamin H7 is a type of water vitamin that falls under the B vitamin category in terms of classification within the vitamin family hierarchy (Godswill *et al.,* 2020). Biotin plays a role in various biological processes by facilitating the conversion of macronutrients, such as carbohydrates and proteins, into usable energy sources (Ofoedu *et al.,* 2021). In its capacity as a coenzyme for carboxylase enzymes, crucial for bodily functions such as gluconeogenesis (the formation of glucose without carbohydrate sources), the synthesis of fatty acids, and the metabolism of amino acids, biotin plays a pivotal role in maintaining overall health and well-being (Penberthy *et al*., 2020). Biotin plays a crucial role in metabolism, and it also helps maintain healthy skin and hair, while strengthening your nails. A lack of biotin levels can cause skin irritations or hair loss (Rodríguez-Cano *et al.,* 2020). Feeling down or tired are all signs of a possible deficiency in this vital nutrient. While not common, a biotin shortage can occur when you consume large amounts of raw egg whites, which contain avidin that blocks biotin absorption, or during times of stress or chronic illnesses (Kannan *et al*., 2024).

Chemical stress caused by substances like Triton poses a significant obstacle to living beings, as it profoundly affects their physical and chemical operations (Haro-Reyes et al., 2022). Triton is a surfactant compound widely used in experiments to study the effects of chemical stress on the human body. Tritons' distinguishing feature lies in its capacity to harm cell mem-

branes, resulting in disruptions to metabolic levels of oxidative stress and inflammation within cells (Inverarity, 2020). When a living being encounters a substance such as Triton in its environment, its body responds by triggering physiological responses to manage the stress (Boraschi *et al.,* 2020). These reactions involve a rise in the generation of radicals that harm cells, proteins, and DNA, leading to disturbances in the crucial roles of organs like the liver, kidney, and heart. These alterations may lead to a decline in the system's effectiveness and present symptoms associated with cellular and tissue injuries (Gwozdzinski *et al*., 2021). Additionally, Triton may affect the equilibrium and metabolism of organisms, thereby diminishing their capacity to withstand and respond to pressure. So, Triton acts as an instrument for researching the fundamental processes of chemical stress impacts and how these impacts can result in either enduring or sudden illnesses (Soncini and Klein, 2023). Grasping these processes may help in creating measures to prevent or treat the harm inflicted by harmful elements (Nemček and Hagarová, 2021). The aim of this research was to examine how Vitamin H7 can protect rabbits from stress induced by Triton exposure. The research involved monitoring variations in Aryl esterase levels, Superoxide dismutase activity, Acetylcholinesterase activity, and total Bilirubin concentration that may arise due to stress, and how biotin can help alleviate these changes.

MATERIALS AND METHODS

Study Location

The study was conducted at the College of Science, University of Mosul, from March 1, 2024, to May 1, 2024.

Chemicals

The chemical Triton was obtained from Sigma-Aldrich (USA), while Vitamin H7 was acquired from EMA Pharm (Egypt).

Biochemical estimations

Kits of Aryl Esterase Enzyme and Superoxide Dismutase Enzyme were obtained from Company: Randox Laboratories, Crumlin, County Antrim, Northern Ireland, United Kingdom; Kits of Acetylcholinesterase Enzyme from Company Biocon, Bengaluru, Karnataka, India and Kits of Total Bilirubin- from Company Biolabo Reagents, Maizy, Aisne, Hauts-de-France, France.

Animals

Male New Zealand white rabbits *Oryctolagus cuniculus* aged 8-9 months weighing 1250-1500 grams were used. The rabbits were purchased from local markets and housed in specially designed aluminium cages under appropriate conditions: a temperature of 24-27°C, 14 hours of light per day, and good ventilation. Water and feed were provided ad libitum, with the feed containing 16.5% protein as specified by the National Research Council.

Design of Experiment

Forty male rabbits were at random divided into 4 groups, with 10 rabbits in each group. After an acclimation period, the rabbits were treated daily for 8 weeks:
Control Group: No treatment was administered.
Triton Group: This group was administered Triton in water at a dose of 300 mg/kg body weight (Abdul-Rahman, 1995).
Vitamin H7 Group: This group received Vitamin H7 at 200 µg/kg B.W
Triton and Vitamin H7 Group: This group received Triton in water at a dose of 300 mg/kg body weight, along with Vitamin H7 at a dose of 200 µg/kg body weight (B.W.).
After an overnight fast, the animals were anaesthetized with ether, and blood samples were collected from each rabbit into prepared tubes. The tubes were left for a period for the blood to clot. Then, the tubes were placed in a centrifuge to separate the serum and transferred to Abendorff tubes, which were stored in deep freezing at -20 °C until the biochemical experiment was conducted.

Animal ethical approval

All necessary ethical approvals for conducting this study were obtained from the Biology Department, Science College, and University of Mosul. Animals were handled humanely, with all procedures in place to minimize pain and distress, as approved by the research vide no. UM.SCI.BIO.2023.054.

Statistical analysis

The results were statistically analyzed using a simple factorial experiment design with a completely randomized design (CRD). Using SAS statistical software, Duncan's multiple range test was used to determine substantial differences, with a signification level of ($P \leq 0.05$).

RESULTS AND DISCUSSION

Table 1 illustrates the effect of Vitamin H7 and Triton on the activity of the aryl esterase enzyme in the blood serum of male rabbits after 8 weeks of treatment. Triton Group showed a significant increase in aryl esterase activity, with a value of 9.66±0.00011 IU/ml.The Vitamin H7 Group received Vitamin H7 at 200 mg/kg in drinking water. This group showed the lowest enzyme activity among the groups, with a value of 5.16±0.00016 IU/ml.
Triton with the Vitamin H7 Group received Triton at 300 mg/kg, along with Vitamin H7 at a concentration of 200

mg/kg. This group showing enzyme activity of 5.27±0.00013 IU/ml. The results indicate substantial differences (P ≤ 0.05) between the different groups. The Triton group exhibited the highest enzyme activity, while the Vitamin H7 group showed the lowest activity. The Triton with Vitamin H7 group had enzyme activity intermediate between the control group and the group treated with Triton alone.

Table 1 illustrates the effect of Vitamin H7 and Triton on the activity of the superoxide dismutase (SOD) in the blood serum of male rabbits after 8 weeks of treatment. The Triton group (300 mg/kg) showed the lowest SOD enzyme activity, with a value of 0.001 IU/ml. In contrast, the Vitamin H7 group, which received Vitamin H7 at 200 mg/kg, exhibited the highest SOD enzyme activity, with a value of 0.005 IU/ml.

Table 1 illustrates the effect of Vitamin H7 and Triton on the activity of the acetyl cholinesterase in the blood serum of male rabbits after 8 weeks of treatment. The Triton group, which received Triton at 300 mg/kg, exhibited a significant reduction in acetylcholinesterase activity, with a value of 0.13±0.0007 IU/mL. The Vitamin H7 with Triton group, which received Triton at 300 mg/kg along with Vitamin H7 at 200 mg/kg, exhibited enzyme activity similar to that of the control group, with a value of 0.30±0.0009 IU/ml.

The results showed substantial differences (P ≤ 0.05) between the different groups. The Triton group displayed the lowest enzyme activity, while the Vitamin H7 group alone showed the highest activity. This reflects the inhibitory effect of Triton on enzyme activity, while Vitamin H7 enhances this activity, whether administered alone or in combination with Triton.

Table 1 displays the effect of Vitamin H7 and Triton on the total Bilirubin concentration in the blood serum of male rabbits after 8 weeks of treatment. The Triton group, which received Triton at 300 mg/kg, showed an increase in total Bilirubin concentration with a value of 0.32±0.003. Vitamin H7, at 200 mg/kg, exhibited the lowest total Bilirubin concentration among the groups, with a value of 0.20±0.001 mg/100 ml. The Vitamin H7 with Triton group showed a total Bilirubin concentration of 0.21±0.001 mg/100 ml.

The results indicate substantial differences (P ≤ 0.05) between the groups. The Vitamin H7 group alone displayed the lowest Total Bilirubin concentration, reflecting an effective reduction in Bilirubin levels. Meanwhile, the Vitamin H7 with Triton group had a lower Total Bilirubin concentration than the Vitamin H7 alone group.

The latest research has shown the impacts of Triton on physical and chemical factors, while also highlighting how Vitamin H7 can effectively enhance these factors (Mohammadi *et al.,* 2023). The findings show that Triton negatively affects the activity of arylesterase and superoxide dismutase (SOD) showing how chemical stress impacts these enzymes involved in defending against damage in the body (Atamanalp *et al.,* 2021). Aryl esterase works to break down compounds containing aromatic esters, thereby protecting cells from oxidative stress damage. When exposed to stressors, such as Triton enzyme activity, the level of harm from stress may change, indicating a shift in the level of harm. Upon exposure to toxins or chemicals, the level of arylesterase may increase as a response to this exposure, as the body attempts to eliminate these toxins or reduce their effects by increasing arylesterase activity (Zhanassova *et al.,* 2021; Rafeeq *et al.,* 2022).

The chemical compound Triton exhibits surfactant properties that can disrupt membranes, leading to increased membrane permeability (Gooran *et al.,* 2024). Additionally, this disruption could lead to enzymes like arylesterase leaking into the bloodstream, which then lowers their effectiveness in the serum (Fathi and Mardani, 2024). Triton also affects the signalling pathways involved in the formation and control of arylesterase activity. It is suggested that Tritons' impact on these signalling pathways may affect how enzymes interact with their reaction sites (Lampitella *et al.,*

Table 1. Effect of treatment with vitamin H7 and triton on the activity of (aryl esterase enzyme, super oxide dismutase enzyme, Acetylcholinesterase enzyme and concentration of total Bilirubin) in the blood serum of male rabbits after 8 weeks of treatment.

Groups	Treatments	Aryl Esterase Enzyme (IU/ml)	Superoxide dismutase enzyme (IU/ml)	Acetyl cholinesterase Enzyme (IU/ml)	Total Bilirubin (mg/100 ml of blood)
1	Control	6.74 ± 0.00014 B	0.003±0.0008 B	0.30±0.0009 B	0.25±0.003 B
2	Triton 300 mg/kg	9.66 ± 0.00011A	0.001±0.0000 C	0.13±0.0007 C	0.32 ±0.003A
3	Vitamin H7 200 mg/kg	5.16 ±0.00016 D	0.005±0.0008 A	0.31±0.0007 A	0.20±0.001 D
4	Triton 300 mg/kg with Vitamin H7 200 mg/kg	5.27± 0.00013C	0.003±0.0000 B	0.30±0.0009 B	0.21±0.001 C

Values are expressed as mean (±) standard deviation and number of male rabbits per group = 10; Paired letters within a column indicate statistically significant differences between treatments at the probability level (P ≤ 0.05)

2024). Moreover, Triton decreases SOD level, indicating oxidative stress, because Triton has the potential to boost the generation of radicals and reactive oxygen species (ROS) within cells. These free radicals play a role in oxidizing proteins, including enzymes. These enzymes undergo oxidation processes that occur within their structure, resulting in a decrease in their functionality (Chen *et al.,* 2024).

Conversely, Vitamin H7, also referred to as Biotin, exhibits an impact on the efficiency of arylesterase and superoxide dismutase enzymes, which hints at its potential as a safeguard against harm induced by Triton exposure (Coremen *et al.,* 2022). Vitamin H7 plays a role in bolstering the bodys defense mechanism against oxidative stress (Patani *et al.,* 2023). Biotin could potentially elevate the quantities of antioxidant enzymes, such as SOD, that combat free radicals. By diminishing stress, levels of Vitamin H7 may effect in arylesterase activity by reducing oxidative harm inflicted upon it (Olfat *et al.,* 2022). Biotin is vital for carboxylase enzymes involved in metabolism and can help improve cellular membrane health and enzyme function, such as arylesterase activity (Mukherjee *et al.,* 2023). Additionally known as Vitamin H or Biotin (H7), this essential nutrient has shown promise in protecting cells from harm caused by Triton exposure by boosting cell well-being and reducing protein and enzyme damage (Ashok, 2021). Moreover, tissues heal better with biotin support, and other enzymes become more active, which can improve the function of arylesterase (Honeder *et al.,* 2023).

The present study indicated that when Triton and Vitamin H7 were used together, the study group showed better arylesterase and SOD activity levels overall, compared to the control group, which used Triton alone. At how Vitamin H7 could help reduce some of the negative impacts caused by Triton exposure although it wasn't enough to completely bring the activity levels back up. The study showed that the Triton group exhibited higher levels of total Bilirubin compared to the control group, potentially due to liver toxicity from Triton. Affecting its ability to process total Bilirubin properly and impacting liver function negatively, which may lead to disruptions in total Bilirubin metabolism resulting in its accumulation in the bloodstream. This causes a slight elevation in Total Bilirubin levels due to toxic effects on liver cells, which can be attributed to an inflammatory response or direct harm to hepatic cells (Bianco, 2021; Creeden *et al.,* 2021).

There was a drop in total Bilirubin levels among the participants who received Vitamin H7 treatment, which indicates that Vitamin H7 might help the liver eliminate bilirubin or improve its metabolism, leading to lower blood levels of Bilirubin—a potential explanation for the impact of Vitamin H7 (biotin). Total bilirubin could be seen as a way to boost liver function by improving cellular metabolism and aiding in crucial processes for eliminating bilirubin from the body (Lengfeld *et al.,* 2021). The present study found that when Vitamin H7 was given along with Triton in the experimental group, there was a decrease in Total Bilirubin levels compared to the control group, but still higher than the group that received Vitamin H7 without Triton administration. This suggests that while Vitamin H7 can help alleviate some of the harmful effects of Triton on Total Bilirubin levels, it may not entirely negate them, resulting in a partial reduction in Total Bilirubin concentrations. By studying these factors together, we can gain a comprehensive understanding of the physiological impact of Triton-induced stress and the potential role Vitamin H7 plays in combating these effects.

Conclusion

The present study concluded that Triton can induce oxidative stress, disrupt cell membranes, increase the activity of aryl esterase, and decrease the functionality of SOD and Ach in the rabbits. A protective role of Vitamin H7 was observed in counteracting these effects by restoring metabolic processes, reducing total bilirubin levels, and improving the functional capacity of enzymes and the liver. This study is considered one of the few that investigated the protective effect of Vitamin H7 against Triton-induced oxidative stress, highlighting its novel potential in therapeutic applications. The study suggests that Vitamin H7 could be used as a therapeutic and protective agent and recommends further research to determine the optimal dose and administration duration of Vitamin H7.

Conflict of interest

The authors declare that they have no conflict of interest.

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