SEBHA UNIVERSITY JOURNAL OF PURE & APPLIED SCIENCES VOL.23 NO. 2 2024
DOI: 10.51984/JOPAS.V23I2.3157

مجلة جامعة سبها للعلوم البحتة والتطبيقية
Sebha University Journal of Pure & Applied Sciences

Journal homepage: www.sebhau.edu.ly/journal/jopas

The Effects of Ubiquinone on the Antioxidant System in Male Rats Exposed to Saccharin-Induced the Hepatic Toxicity

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Keywords:
Saccharin
Coenzyme Q10
Oxidative Stress
Antioxidant
The Hepatic Toxicity and Male Rats

ABSTRACT

Saccharin (Sac) is a widely used artificial sweetener with significant applications in the food industry, pharmaceutical formulations, and tobacco products. Despite its popularity, saccharin has drawn attention due to its potential carcinogenic effects and associations with various health risks, including renal impairment, hepatic dysfunction, obesity, and diabetes. This study aimed to investigate the protective effects of Ubiquinone, or coenzyme Q10 (COQ10), on liver toxicity induced by saccharin, focusing on oxidative stress and antioxidant markers. In this experiment, rats were divided into six groups of ten. The control group received no treatment, while the second group was administered COQ10 at a dosage of 20 mg per kilogram of body weight. The third and fourth groups were given saccharin at 1/10 and 1/20 of the lethal dose 50 (LD50), respectively. The fifth and sixth groups received saccharin at the same dosages as the third and fourth groups, but with additional COQ10 supplementation. All treatments were administered orally for 30 days, after which liver tissues were collected to assess oxidative stress and antioxidant markers. The results revealed that saccharin significantly increased oxidative stress in the liver, as evidenced by elevated levels of malondialdehyde (MDA) and oxidized glutathione (GSSG). Additionally, saccharin-treated groups exhibited a marked decrease in antioxidant markers, including reduced glutathione (GSH) and superoxide dismutase (SOD). However, the groups that received COQ10 alongside saccharin showed significant improvement, with oxidative stress and antioxidant levels nearly returning to those observed in the control group. These findings suggest that saccharin consumption promotes the generation of reactive oxygen species and contributes to liver damage, characterized by necrotic hepatocytes, sinusoidal dilatation, and inflammatory infiltration. The protective effects of COQ10 in mitigating saccharin-induced oxidative stress highlight its potential as a therapeutic agent for preventing liver damage associated with saccharin intake.

تأثير ألانزيم المساعد Q 10 على نظام مضادات الأكسدة في ذكور الجردان المعرضة للسمية الكبدية الناجمة عن السكرين

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الكلمات المفتاحية
السكرين
الكوانزيم Q 10
مضادات الأكسدة
الإجهاد التأكسدي
السمية الكبدية وذكور الجردان.

الملخص

السكرين (ساك) هو بديل للسكر، وكثيرا ما يستخدم إما في صناعة المواد الغذائية، أو في التركيبات الصيدلانية وحتى في منتجات التبغ. تعتبر كيمياء السكرين مثيرة للاهتمام بسبب الاشتباه في كونه مادة مسرطنة وإمكانية استخدامه كترياق للتسمم بالمعادن. يزيد استهلاك السكرين على المدى الطويل من خطر الإصابة بالسمنة والسكري واختلال وظائف الكبد والقصور الكلوي. في الدراسة الحالية، استكشفنا تأثيرات الإنزيم المساعد الكو انزيم 10 ضد السكرين بجرعات مختلفة على السمية الكبدية (الإجهاد التأكسدي وعلامات مضادات الأكسدة). الطرق: تم تقسيم 60 فأراً إلى 6 مجموعات (10 فئران لكل مجموعة)، المجموعة الأولى السيطرة، الثانية تعامل ب الكو انزيم 10 20 ملغم / كغم وزن الجسم، الثالثة تعامل بالسكرين LD5010/1 ، الرابعة تعامل بالسكرين LD5020/1 ، الخامس تعامل مع السكرين LD50 10/1 بالإضافة إلى الكو انزيم 10 والسادس تعامل مع السكرين LD5 20/1

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Article History : Received 04 May 2024 - Received in revised form 24 August 2024 - Accepted 28 September 2024

بالإضافة إلى الكو انزيم 10 .تم إعطاء Sac والكو انزيم 10 عن طريق الفم لمدة 30 يومًا. ثم تم إجراء اختبارات الكبد (الإجهاد التأكسدي وعلامات مضادات الأكسدة). النتائج: أظهرت البيانات المكتشفة أن هناك زيادة في الحالة التأكسدية للكبد تظهر من خلال زيادة مستويات المانولدالهيد والجلوتاثيون المؤكسد في المجموعات المعالجة بالكيس. أيضًا، هناك انخفاض في علامات مضادات الأكسدة الجلوتاثيون وتخفيض الأكسيد الفائق. من ناحية أخرى أظهرت مجموعات العلاج الأخرى بـ الكو انزيم 10 تحسناً ملحوظاً بالمقارنة مع مجموعات السكرين وشفاء تقريباً مقارنة مع مجموعة السيطرة. الاستنتاج: خلصت البيانات التي تم الحصول عليها إلى أن السكرين غير آمن لإدراجه في النظام الغذائي، حيث يؤدي استهلاكه إلى زيادة أنواع الأكسجين التفاعلية، وتدهور خلايا الكبد النخرية وتوسع الجيوب الأنفية وكذلك الارتشاح الخلوي الالتهابي. يعادي الكو انزيم 10 التأثيرات الضارة للسكرين. نقترح أن يكون الكو انزيم 10 بمثابة استراتيجية علاجية تستهدف السمية الكبدية عن طريق ألاجهاد التأكسدي.

1. Introduction:

The use of synthetic, non-nutritive sweeteners has gained popularity as an alternative to nutritive sweeteners, particularly for reducing total calorie and carbohydrate intake. These artificial sweeteners (ASNs) are often recommended for glucose management and weight reduction, especially among patients with diabetes [1]. In 2015, the World Health Organization (WHO) advised that individuals of all ages should limit their sugar intake to less than 10% of their total daily caloric intake [2]. In response to consumer demand for healthier and lower-sugar options, food manufacturers have increasingly adopted non-sugar sweeteners [3]. These sweeteners mimic the taste of sucrose without contributing to caloric intake or undergoing significant metabolic processing.

Among the artificial sweeteners approved by the US Food and Drug Administration are saccharin (Sac), aspartame, sucralose, acesulfame potassium, and neotame [4]. Saccharin, commonly known as Sac, is a non-nutritive, non-caloric artificial sweetener. Chemically known as osulfabenzamide or 2,3-dihydro-3-oxobenzisosulfonazole, saccharin has been associated with potential carcinogenicity and a risk of diabetes in certain regions of the world [5]. Referred to as E954 in food additive codes, saccharin and its derivatives—saccharin sodium, saccharin acid, and calcium saccharin—are sulfonamide compounds that are 300–500 times sweeter than sucrose [6]. Saccharin is valued for its stability, resistance to pH changes, and heat tolerance, making it a common choice in products such as hot beverages, reduced-sugar jams, baked goods, and canned vegetables. It is also valued for its long shelf life and cost-effectiveness.

Saccharin passes through the gastrointestinal tract without undergoing significant metabolism in animals and humans; however, it has been observed to stimulate insulin release in rats [7]. After administration, 10–40% of the saccharin dose is typically recovered from feces, while 66–84% is excreted primarily through urine. Radioactive traces of saccharin have been detected in tissues such as the liver, heart, pancreas, adrenals, testes, and thymus up to three days after dosing, suggesting that saccharin may infiltrate tissues and exert specific biological effects [8].

Coenzyme Q10 (COQ10), also known as ubiquinone-10, is an endogenous compound found within the mitochondrial membranes of living organisms. It plays a crucial role in the electron transport chain, facilitating ATP production through oxidative phosphorylation and acting as a potent antioxidant [9]. In various animal studies, COQ10 has been shown to reduce oxidative stress markers and inflammatory responses [10]. As a fat-soluble antioxidant synthesized within the mitochondria of energy-demanding tissues such as the kidneys, heart, liver, and muscles, COQ10 is essential for maintaining cellular health [11]. It functions in cellular metabolism as an electron transporter. Additionally, it protects membranes and lipoproteins from oxidative damage, including protein and lipid peroxidation [12]. While COQ10 is abundant in foods such as meat, fish, nuts, and certain oils, it is found in much lower concentrations in cereals, vegetables, fruits, and dairy products. COQ10 deficiency is a growing concern in various clinical conditions, particularly those related to aging and cellular dysfunction [13].

This study aimed to evaluate the protective effects of COQ10 as an antioxidant against saccharin-induced liver toxicity.

2. Materials and Methods:

2.1 Animal Subjects:

Throughout the experiment, Wistar male rats weighing between 150 and 180 g were used. The rats were housed in a laboratory room for one week prior to testing, following their acquisition from the animal facility of the National Organization for Drug Control and Research in Dokki, Cairo, Egypt. The animals were kept on a 12-hour light/dark cycle and provided with standard laboratory pellets and unrestricted access to water. The study adhered to the ethical guidelines set by the Canadian Council on Animal Care, and the Ethics Committee of the National Research Centre in Egypt approved all animal-related activities (Registration Number 17/004). The operating document for this committee is the NIH Guide to the Care and Use of Laboratory Animals.

2.2 Drugs and Chemicals:

Saccharin (Sac), a white crystalline powder, was acquired from Comell Lab, located in Al-Maadi, Cairo City, Egypt. The saccharin was dissolved in distilled water according to the procedure outlined in [14]. Coenzyme Q10 (COQ10) was obtained from the Arab Company for Pharmaceuticals and Medicinal Plants (Mepaco-Medifood, Enshas El Raml, Sharkeia, Egypt) and was subsequently dissolved in maize oil as described in the study by M. O. H. (Reg. No. 895/2012) [15].

2.3Treatments:

To evaluate the effects of treatments, six groups of ten rats each were randomly assigned as follows. The rats received daily oral gavage for 30 days with the specified treatments:

- **Group 1 (G1):** Normal control group, received distilled water at a dose of 1 ml/100 g body weight (bw).
- **Group 2 (G2):** Received coenzyme Q10 (COQ10) at a dose of 20 mg/kg body weight (bw).
- **Group 3 (G3):** Received 1/10 of the saccharin LD50 dose (2 g/kg bw).
- **Group 4 (G4):** Received 1/20 of the saccharin LD50 dose (1 g/kg bw).
- **Group 5 (G5):** Received 1/10 of the saccharin LD50 dose (2 g/kg bw) in combination with COQ10 (20 mg/kg bw).
- **Group 6 (G6):** Received 1/20 of the saccharin LD50 dose (1 g/kg bw) in combination with COQ10 (20 mg/kg bw).

2.4 Liver Homogenate Preparation and Biochemical Assay:

Decapitation was used to kill the rats after the liver was properly removed. The next step was to separate the liver tissues, count them rapidly to avoid drying out, and then store them at a temperature of -80°C. For 5 minutes, the homogenate was spun at 4,000 rpm in a cooling centrifuge (2k15; Sigma/Laborzentrifugen). The succeeding supernatant was used to evaluate the **oxidized and reduced glutathione** (GSH, GSSG) according to [16] and oxidative stress parameter malondialdehyde (MDA) according to [17] by HPLC. Superoxide dismutase (SOD) activity was assayed in the liver tissue by the method of [18] by spectrophotometer.

2.5 Statistical Analysis:

Data are expressed as mean ± standard error (SE). The results presented are the averages of eight animals per group. A one-way analysis of variance (ANOVA) was conducted to determine overall

differences between groups. Post hoc analysis using Duncan's multiple range test was performed to identify specific group differences. Statistical analyses were conducted using SPSS software, version 17, compatible with Windows. Statistical significance was set at $p < 0.05$.

3. Results

3.1 Markers of Oxidative Stress in Hepatic Tissue

3.1.1 Malondialdehyde (MDA) Content of Liver Tissue

The liver malondialdehyde (MDA) content for different experimental groups is presented in Figure 1. The COQ10 treatment group showed no significant change in liver MDA content compared to the control group. In contrast, liver MDA levels were significantly elevated in the groups treated with both low and high doses of saccharin (1/10 and 1/20 of LD50) after one month of treatment.

Additionally, in the groups receiving saccharin at 1/10 LD50 and 1/20 LD50 in combination with COQ10, there was a notable reduction in liver MDA content compared to the saccharin-only treatment groups at corresponding doses. However, MDA levels in these combination groups did not return to normal levels.

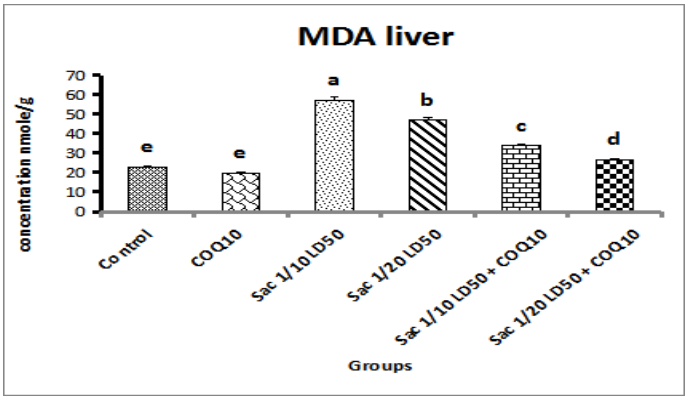

Figure 1: Showing liver malondialdehyde (MDA), (nmole/g) of different treated groups. Values with different superscripted letters are significantly different (P<0.05).

3.1.2 Oxidized Glutathione (GSSG) Content in Liver Tissue

The liver oxidized glutathione (GSSG) content for different experimental groups is detailed in Figure 2. There was no significant difference in liver GSSG content between the COQ10 treatment group and the control group. In contrast, liver GSSG levels were significantly elevated in the groups treated with saccharin at both doses (1/10 and 1/20 of LD50) after one month of treatment ($p < 0.05$) compared to the control group and the groups receiving saccharin combined with COQ10 (Sac 1/10 LD50 + COQ10 and Sac 1/20 LD50 + COQ10).

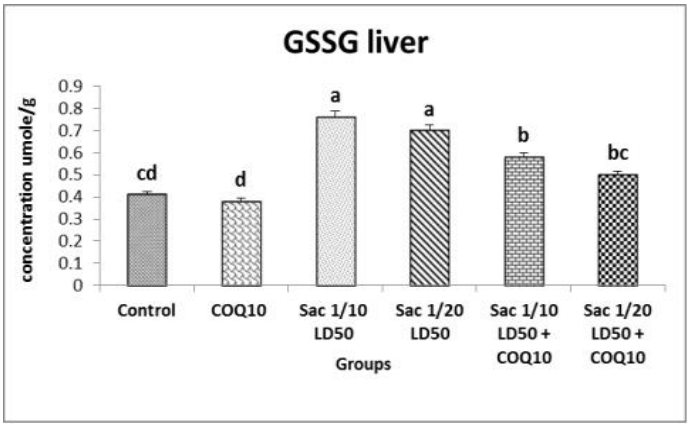

Figure 2: Showing liver oxidized glutathione (GSSG) content and (umole/g tissue) of different treated groups. Values with different superscripted letters are significantly different (P<0.05).

3.2 Antioxidant Parameters in Liver Tissue

3.2.1 Glutathione (GSH) Content in Liver Tissue

The results of liver GSH content are shown and Figure 3. The data showed that the COQ10 group's liver GSH content did not significantly differ from that of the control group. In contrast, there was marked increase in liver GSH content for a high saccharin dosage treated group (1/10 of LD50) after a month of the treatment.

There was a statistically significant difference ($p<0.05$) between the Sac 1/20 LD50 and Sac 1/10 LD50 groups in terms of liver glutathione (GSH) levels. When comparing the groups treated with Sac 1/10 LD50 and Sac 1/20 LD50 alone to those treated with Sac 1/10 LD50 + COQ10 and Sac 1/20 LD50 + COQ10, it is clear that the latter two combinations significantly raise the liver GSH concentration. The Sac 1/10 LD50 + COQ10 group did not vary significantly from the control group ($P > 0.05$) at the same time.

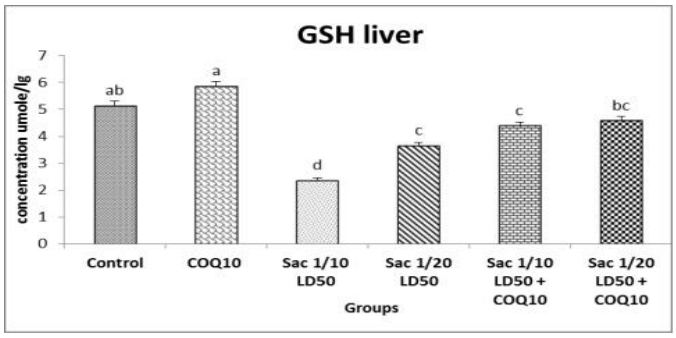

Figure 3: The liver glutathione (GSH) concentration and (umole/g tissue) of various treatment groups. Values with different superscripted letters are significantly different (P< 0.05).

3.2.2 Hepatic Superoxide Dismutase (SOD) Activity

The results of hepatic superoxide dismutase (SOD) activity for the experimental groups are shown in Figure 4. The data reveal a significant increase in liver SOD activity in the COQ10 treatment group compared to the control group. This indicates that COQ10 administration effectively enhanced SOD activity, suggesting an improvement in the antioxidant defense system of the liver.

In contrast, a notable decrease in liver superoxide dismutase (SOD) levels was observed in the groups treated with saccharin at varying doses (1/10 and 1/20 of LD50) during a one-month treatment period ($P<0.05$). Meanwhile, the groups treated with Sac 1/10 LD50 + COQ10 and Sac 1/20 LD50 + COQ10 showed a significant improvement in liver SOD activity compared to the groups treated with Sac 1/10 LD50 and Sac 1/20 LD50. However, the activity did not approach the normal level at a significant level of 0.05.

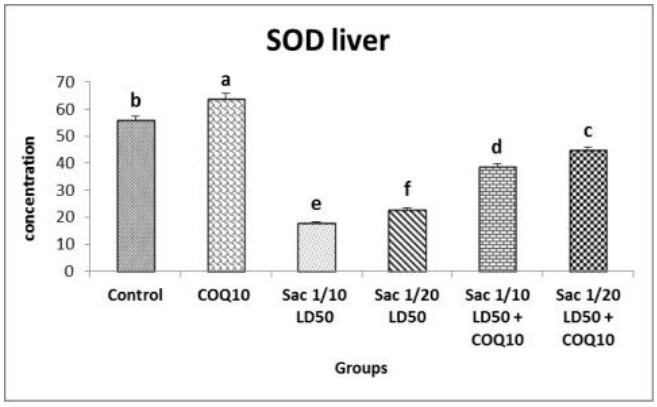

Figure 4: The liver superoxidase (SOD) activity. Values with different superscripted letters are significantly different (P< 0.05).

4. Discussion:

Oxidative stress refers to a state of imbalance when the presence of oxidants outweighs that of antioxidants, leading to possible harm to cells or their constituents. Residual oxygen species (ROS) are molecules that contain oxygen and demonstrate chemical reactivity. Increased quantities of ROS lead to oxidative damage. However, ROS are produced as a typical consequence of metabolic processes, and even little quantities of ROS formation have substantial impacts on cellular signaling and regulatory pathways [19]. The production of ROS by cells is a regular and controlled phenomenon. However, an increase in ROS levels may have negative consequences, potentially resulting in oxidative stress and disease. The occurrence of oxidative stress might arise from a disparity between the generation of radical species and the efficacy of antioxidant defense

mechanisms. The aforementioned disparity has the potential to cause harm to cellular macromolecules, including proteins, DNA, and lipids [20].

The current investigation demonstrates that there was a critical increment in liver tissue MDA and GSSG contents and a decrease antioxidant parameters GSH and SOD in Sac-treated gatherings at various levels (G3 and G4) in comparison to the group under supervision. Interestingly, these results were contract in the rats given COQ10 G2, G5 and G6. This is consistent with the current investigation [21] which demonstrated that the administration of aspartame and saccharin resulted in elevated plasma MDA and GSSG levels, maybe due to an increase in oxidative stress. The oxidative stress induced by a high dose of saccharin may be attributed to hepatic cell inflammation. This assertion is substantiated by the findings of [22], who observed that rats administered with saccharin exhibited portal infiltration characterized by the presence of mononuclear inflammatory cells, primarily macrophages and lymphocytes. Hence, inflammatory responses result in the oxidation of lipids, oxidative or nitrosative stress, and an abundance of ROS and DNA-reactive aldehydes [23].

Furthermore, [24] observed an increase in MDA levels because of lipid peroxidation due to the impact of ROS on the lipids of cellular membranes. Malondialdehyde is produced because of lipid peroxidation generated by large doses of saccharin. The hepatic cells may be harmed by free radicals, leading to an elevation in the release of liver enzymes into the blood. This phenomenon was found in rats treated with saccharin, especially those that were given a high dosage of 500 mg/kg body weight.The elevated lipid profile in the saccharin group may not be the only cause of the increased oxidative markers (MAD, GSSG, and NO), as liver function abnormalities may also be involved [25].

In relation to the hepatic antioxidant system, the present study [26] demonstrated a notable suppression of the protective mechanism of antioxidants when saccharin was administered. This was evident through a decrease in SOD activity, as well as a corresponding reduction in GSH content. These effects effectively prevented cell death caused by harmful free radicals. Consequently, the concentrations of these substances in the tissue mixture fell as GSH was used to eliminate ROS and transformed into oxidized GSSG. Conversely, however, lipid peroxidation, which is a result of ROS acting on cellular membrane lipids, raised MDA levels.

MDA is the most effective product for assessing lipid peroxidation. A study [27] showed that COQ10 may reduce MDA levels both in living organisms and in laboratory settings, hence inhibiting lipid peroxidation. The addition of COQ10 has the potential to decrease levels of MDA via many routes. To begin with, reduced levels of ROS are associated with decreased levels of MDA. COQ10, an integral constituent of the mitochondrial respiratory chain, has inhibitory effects on the production of endogenous ROS inside the mitochondria. In addition, COQ10 has a role in controlling lipid metabolism and could hinder lipid oxidation via many pathways associated with gene expression and cellular activities [28].

In contrast, [29] who observed that COQ10 therapy may reduce the increase in proinflammatory state because in the current investigation, elevated levels of the antioxidant enzymes GSH and SOD were found in the rat liver. Monosodium glutamate (MSG) reduced the tissue activity of SOD and CAT enzymes and raised bladder MDA levels. Similarly, [30] showed that COQ10 enhanced the activity of superoxide dismutase (SOD) in the liver homogenates of diabetic rats, maybe due to a reduction in oxidative stress. COQ10 inhibits the generation of superoxide anion via two potential mechanisms: (1) COQ10 exerts its inhibitory effect on cytoplasmic Nicotinamide adenine dinucleotide (NADH) levels by an indirect method, either by enhancing electron transport systems or by facilitating the retrieval of β-tocopherol, therefore directly generating superoxide. (2) The COQ10 in the cell membrane accumulates electrons [31].

In the same line, by lowering the levels of MDA and raising overall antioxidant activity, taking a COQ10 supplement significantly relieved this oxidative state. However, in contrast [32] suggested that treating high levels of free radicals with COQ10 causes lipid peroxidation in diabetes and that COQ10 interacts with free oxygen radicals to stop membrane lipid peroxidation. In addition, [33] they demonstrated that supplementing with COQ10 can help lower lipid peroxidation.

CAT and SOD, the two primary antioxidant enzymes, are responsible for regulating redox signaling inside cells and shielding cells from oxidative damage.

Conclusion:

COQ10 protects from Sac-induced oxidative damage. The reliable protective mechanisms provided by the studied COQ10 may include the induction of antioxidants and/or the scavenging of free radicals produced after in vivo Saccharin consumption. Our suggested COQ10 has antioxidant capacity to treat oxidative stress-induced liver damage.

Abbreviations and Acronyms:

Saccharin (Sac)
Coenzyme Q10 (COQ10)
Malondaldehyde (MDA)
Oxidized Glutathione (GSSG)
Glutathione (GSH)
Super Oxide Demotes (SOD)
World Health Organization (WHO)
Standard Error (SE)
Residual Oxygen Species (ROS)
Catalyse (CAT)
Adenosine Triphosphate (ATP).

References

[1] Azeez, O. H.; Alkass, S. Y. and Persike, D. S.; (2019): Medicina Article Long-Term Saccharin Consumption and Increased Risk Of Obesity, Diabetes, Hepatic Dysfunction, And Renal Impairment In Rats. Medicina (Kaunas)., 9:55(10): 681-186.

[2] Dunford, E. K.; Coyle, D. H.; Yu Louie, J. C.; Anneliese, K. R.; Pettigrew, B. S. and Jones, A.l.; (2022): Changes in the Presence of Nonnutritive Sweeteners, Sugar Alcohols, and Free Sugars in Australian Foods. Journal of the Academy of Nutrition and Dietetics., 122(5): 991-999.

[3] Erbaş. O.; Erdoğan, M. A.; Khalilnezhad, A.; Solmaz, V.; Gürkan, F. T.; Yiğittürk, G. H. A.; Eroglu, H. A. and Taskiran, D.; (2018): Evaluation of long-term effects of artificial sweeteners on rat brain: a biochemical, behavioral and histological study. J Biochem Mol Toxicol., 32(6):22053–8.

[4] Moktar, K. A.; Ayesh, M. H. B.; El-Gammal, H. L.; Ahmed-Farid, O. A. and Abou-khzam, B. A. f.; (2021): Effect of β-oxidation stimulant against metabolic syndrome of saccharin in rat: A behavioral, biochemical, and histological study. Journal of Applied Pharmaceutical Science., Vol. 11(01), pp 061-071.

[5] Gümüş, A.B.; Tunçer; A.K.E.; Yıldız, T.A.; Bayram, İ.K. (2022): Effect of saccharin, a non-nutritive sweetener, on insulin and blood glucose levels in healthy young men: A crossover trial. Diabetes Metab Syndr., 16(6):102500.

[6] Krishnasamy, K. (2020), Artificial Sweeteners. Pathology and Microbiology., 10. (1): 5772-5779.

[7] Sünderhauf, A.; Pagel, R.; Künstner, A.; Wagner, A. E.; Rupp, J.; Saleh, M. I.; Derer, S.; Christian, Sina.; (2020): Saccharin Supplementation Inhibits Bacterial Growth and Reduces Experimental Colitis in Mice. Nutrients, 12(4): 1122.

[8] Gong, T.; Wei, Q.; Mao, D.; (2016): Effects of daily exposure to saccharin and sucrose on testicular biologic functions in mice. Biology of Reproduction., 95(6):116 (1–13).

[9] Helal, E. G. E.; Al-Shamrani, A.; Abdelaziz, M. A.; El-Gamal, M. S.; (2019): Comparison between The Effect of Sucralose and Sodium Saccharin on Some Physiological Parameters in Male Albino Rats., The Egyptian Journal of Hospital Medicine., 74 (7): 1552-1559.

[10] Manzar, H.; Abdulhussein, D.; Timothy, E.Y.; Cordeiro, M.F.; (2020): Cellular Consequences of Coenzyme Q10 Deficiency in

Neurodegeneration of the Retina and Brain., Int J Mol Sci., 21(23): 9299.

[11] Magnani, F.; Mattevi, A.; (2019): Structure and mechanisms of ROS generation by NADPH oxidases., Curr. Opin. Struct. Biol., 59:91–97.

[12] Juan, C.A.; la Lastra, J.M.P.; Plou, F.J.; Lebeña, E. P.; (2021): The Chemistry of Reactive Oxygen Species (ROS) Revisited: Outlining Their Role in Biological Macromolecules (DNA, Lipids and Proteins) and Induced Pathologies., Int J Mol Sci., 22(9): 4642.

[13] Camacho, D. J. H.; Bernier, M.; Lluch, G. L.; Navas, P.; (2018): Coenzyme Q10 supplementation in aging and disease. Frontiers in Physiology, 9: 44- 50.

[14] Song, M.; Kim, H.; Lim, Y.; Jang, I.; (2017): Effects of coenzyme Q_{10} on the antioxidant system in SD rats exposed to lipopolysaccharide-induced toxicity. Lab Anim Res. 2017 Mar; 33(1): 24–31.

[15] Alsoufi, M.A.; Aziz, R.A.; Hussein, Z.G.; (2017): Effect of some artificial sweeteners consumption in biochemical parameters of rats. Curr. Res. Microbiol. Biotechnol., 5 (3): 1095-1099.

[16] Jayatilleke, E. and Shaw, S. (1993): A high-performance liquid chromatographic assay for reduced and oxidized glutathione in biological samples. Anal. Biochem., 214(2): 452-457.

[17] Karatepe, M. (2004): Simulatenous determination of ascorbic acid and free malondialdehyde in human serum by HPLC-UV. Chromatographic Line., 12:362-365.

[18] Marklund S. and Marklund G. (1974): Involvement of thesuperoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem.,47(3):469-474.

[19] Olama, N. K.; Taha, M.; Rady, H. Y.; (2018):The potential protective role of coenzyme q10 on the cyclophosphamide induced lung toxicity in adult male albino rats: a histological and ultrastructural study. Int J Sci Rep., (9)4:225-234.

[20] Yang, S.; Lian, G.; (2020): ROS and diseases: role in metabolism and energy supply. Mol Cell Biochem.; 467(1): 1–12.

[21] Juan, C. A.; Pérez, J.M.; Plou, F. J.; Pérez-Lebeña, E.; (2021): The Chemistry of Reactive Oxygen Species (ROS) Revisited: Outlining Their Role in Biological Macromolecules (DNA, Lipids and Proteins) and Induced Pathologies. Int J Mol Sci.; 22(9): 4642.

[22] Amin, K. A.; Al-muzafar, H. M.; Abd-Elsttar, A. H.; (2016): Effect of sweetener and flavoring agent on oxidative indices, liver and kidney function levels in rats. Indian J Exp Biol., 2016; 54(1): 56-63.

[23] Biswas, i.; Das, R.; Banerjee, E.R.; (2017): Role of free radicals in human inflammatory diseases. AIMS Biophysics.; 4, (4): 596-614.

[24] Hasan, H. M.; Alkass, S. Y.; Persike de Oliveira, D. S.; (2023), Impact of Long-Term Cyclamate and Saccharin Consumption on Biochemical Parameters in Healthy Individuals and Type 2 Diabetes Mellitus Patients. Medicina., 59(4), 698.

[24] Popkin, B. M.; Hawkes, C.; (2016): Sweetening of the global diet, particularly beverages: Patterns, trends, and policy responses. Lancet Diabetes Endocrinol., 4, 174–18

[26] Amin, K. A.; AlMuzafar, H. M.; (2015): Alterations in lipid profile, oxidative stress and hepatic function in rat fed with saccharin and methyl-salicylates. Int J Clin Exp Med., 8: 6133-6144.

[27] Hormozi, M.; Mirzaei, R.; Nakhaee, A.; Payandeh, A.; Izadi, S.; Haghighi, J. D.; (2019): Effects of Coenzyme Q10 Supplementation on Oxidative Stress and Antioxidant Enzyme Activity in Glazers with Occupational Cadmium Exposure: a Randomized. Toxicol. Ind. Health., 35 (1), 32–42.

[28] Jing, L.; He, M. T.; Chang, Y.; Mehta, S. L.; He, Q. P.; Zhang, J. Z.; (2015): Coenzyme Q10 Protects Astrocytes from ROS-Induced Damage through Inhibition of Mitochondria-Mediated Cell Death Pathway., Int. J. Biol. Sci., 11 (1), 59–66.

[29] El Agamy, D. F.; Naguib, Y. M.; (2019): COQ10 ameliorates monosodium glutamate-induced alteration in detrusor activity and responsiveness in rats via anti-inflammatory, anti-oxidant and channel inhibiting mechanisms., BMC Urol., 19: 103.

[30] Modi, K.; Santani, R. K.; Goyal, P. A.; (2006): Effect of coenzyme Q10 on catalase activity and other antioxidant parameters in streptozotocin-induced diabetic rats. Biol Trace Elem Res., 109(1):25-34.

[31] Zhang, Y.; Huang, X.; Liu, N.; Liu, M.; Zhu, L.; (2022): Discovering the Potential Value of Coenzyme Q10 in Oxidative Stress: Enlightenment From a Synthesis of Clinical Evidence Based on Various Population. Front Pharmacol., 13: 936233.

[32] Samimi, F.; Baazm, M.; Eftekhar, E.; Rajabi, S.; Taghi, M.; Mashayekhi, F. J.; (2019): Possible antioxidant mechanism of coenzyme Q10 in diabetes: impact on Sirt1/Nrf2 signaling pathways., Res Pharm Sci., 14(6): 524–533.

[33] Huang, K.; Chen, C.; Hao, J.; Huang, J.; Wang, S.; Liu, P.; (2015): Polydatin promotes Nrf2-ARE anti-oxidative pathway through activating Sirt1 to resist AGEs- induced upregulation of fibronetin and transforming growth factor-ß1 in rat glomerular messangial cells. Mol Cell Endocrinol., 399:178–189.