Histopathological Effects of Sodium Saccharin Toxicity on Liver and Kidney of Rats in Duhok City-Iraq

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Abstract

The most frequently used synthetic sweetener that is used by patients who have high blood glucose and in food administration is sodium saccharin. The danger of sodium saccharin is still in debate, according to the outcomes of some previous research. The aim of this study was to observe the changes that happen due to sodium saccharin's effects on the renal and hepatic organs of laboratory albino rats by histotechnique. Forty experimental rats were used in this study; they were 3–4 months old, and their weight was between 250 and 325 gram. They were separated into four groups (each group contained 10 rats); the control group was the first one (water and food ad libitum). The second, third and fourth groups have been administered by three different measures of sodium saccharin, which were started at 2.5, 5, and 10 mg/kg respectively. The oral gavages were used to give treatments to rats once daily for 120 days; at the end of the experimentation, for histopathological technique, the organs were collected. Depending on the dose, the pathological lesions were different in the hepatic and renal tissues as an outcome of therapeutic effects with sodium saccharin compared to the normal group. The ending results demonstrate that the toxic effects of sodium saccharin in rats taken at 10 mg/kg are higher than those of animals in group 2 treated at 5 mg/kg, and the effects appear lower in group 1 when administered at 2.5 mg/kg of substance. This indicates that sodium saccharin requires an elevated dose to generate pathological changes. The conclusion of this research was that sodium saccharin toxicity became higher for the liver and kidneys by raising its dose.

Keywords: Sodium saccharin, Histopathology, Hepatotoxicity, Nephrotoxicity, Rats.

Introduction

The consciousness concerning the dangers of too much consumption of refined sugars increased, which then motivated the community to alternate them with synthetic sweeteners. Over the period, the attention of consumers to synthetic sweeteners has increased because of their low calories, significance in diabetes and obesity, and organization to keep away from the effects of sucrose [1–2]. Very small amounts of synthetic sweeteners are required for sweetening foods because they are effectively sweeter than sucrose. Artificial sweeteners are widely used in squeeze fruits, carbonated drinks, parched products, dairy products, and drink mixtures with powder in high quantities as low-calorie products. Even though many sweeteners have been permitted by food and drug organizations, most of the sweeteners that are used are artificial, and it has been reported that too much consumption leads to undesirable health effects, either in animal models or humans [3].

Several of the synthetic sweeteners with low calories that food productions commonly use include saccharin, aspartame, and acesulfame-K to replace sucrose [4]. The most frequently known derivative of benzisothiazole and the main marketable non-nutritive sweetener offered is sodium saccharin or soluble saccharin [5]. Saccharin is the oldest of the non-nutritive synthetic sweeteners and is distinguished by being a non-caloric, influential, non-natural sweetener as it is three to five hundred...
grades sweeter than sucrose, but it has a somewhat bitter taste [6].

Saccharin is an extremely constant complex with reverence to hotness and time so that it can be used in warm drinks and in food manufactures that require a rise in temperature for preparation like preserved vegetables, condensed sugar jams, and bakery products [7]. There are a variety of saccharin's structures, like sodium saccharin, potassium saccharin, calcium saccharin, and acid saccharin. The most frequently used is sodium saccharin because it is very delicious [8]. The standard eating of saccharin per day is 2.5 mg/kg of life weight [9]. When saccharin is ingested, it truly goes throughout the gastro-intestinal tract of a human without being digested. The trial revealed that saccharin is not absorbed via the intestine or metabolized [10–11].

The absorption degree of saccharin happens quickly and depends on food ingestion. Once it is eliminated from the food, it takes three days to totally clear the tissue [24]. Continuing saccharin eating raises the risk of fatness and diabetes, in addition to hepatic and renal impairment. Also increase the risk of brain carcinogenesis [12]. Saccharin influences and changes biochemical indicators in the liver and kidney organs at both higher and lower doses [13].

Ingestion of the different doses of sodium saccharin appeared to have obvious bad effects and extensively changed numerous biomarkers in clinical symptoms, including fatness, food eating, hematology, urinalysis, biochemistry of blood, necropsies, and histological examinations, as revealed by some researchers [14–15]. In addition, saccharin may lead to eczema and urticaria in several citizens. Due to the avid concentration of saccharin in the placenta, it is limited to consumption in pregnant women [16]. The majority of studies concluded that long-term use of saccharin is the main reason for hepatic and kidney toxicity; therefore, the purpose of this study was to verify the pathological lesions induced by variant doses of sodium saccharin administration on the kidney and liver.

**Material and Methods**

**Ethical approve**: Ethically the work was done according to the Animal Ethics Committee of the College of Veterinary Medicine - University of Duhok (Ethical code No. DR1996919CV, approved on the 11th of June, 2019).

**Laboratory animals**
The animals used in this experiment were forty mature wistar male rats (three to four months old) weighing between 250g and 325g. The rats were obtained in the College of Veterinary Medicine from the animal house at Duhok University. The animals were maintained in a circulated air condition at standard house temperature (22 ±2) °C and were normally exposed to dark and light cycles. In addition, water and food were given to them ad libitum. However, the guidelines for laboratory animal care were used for rat handling and curing [17]. The current study was permitted by the animal ethics committee of the College of Veterinary Medicine, University of Duhok.

**Chemicals**
Sodium saccharin was acquired from the German company of Alfa Aesar Thermo Fisher Scientific.

**Experimental Design**
The rats were classified into four groups (10 rats for each one). The control group received just food and distilled water, while the experimental groups, which were groups 1, 2, and 3, were administered sodium saccharin dissolved in water at three different doses that were started from 2.5, 5, and 10 mg/kg of rats weight, in that order. The treatments were selected depending on ADI (5 mg/kg). The entire doses were administered orally via oral gavages, a single dose daily for 120 days.

**Histopathological Analysis:**
After 120 days of experimentation, diethyl ether was used for euthanizing laboratory animals through inhalation [18]. Samples of both liver and kidney from different groups of experiments were fixed in 10% neutralized buffered formalin, then the tissues were dehydrated in an ascending concentration of alcohol, and for clearing, the samples were were placed in xylene. After that, a pure white paraffin wax was used for embedding tissues for paraffin block preparation at a melting point of 54–56 °C. The paraffin blocks were cut at 4-5 μm with a rotary microtome (Leica, Germany) for the preparation of sections. Finally, the staining of the sections was applied by using hematoxylin and eosin (H&E) stains [19]. The prepared slides were evaluated under a field microscope and pictures taken using a digital computerized camera canon (Leica, Germany). The histopathological changes were studied and interpreted.

**Results**
The histopathological result revealed by staining tissue sections with H&E hematoxylin and eosin in the hepatic tissues of normal animals was that the central veins (CV) were normal in the lobular patterns from which branch out the liver cords, which were then divided by blood sinusoids. Underlining the margin of the lobule of the liver, portal tracts appeared. As a result of the meeting of three constant structures, including portal vein branches, liver arteries, and bile ducts, the portal tract (PT) was created. Hepatic cells were big and polyhedral in nature, and the cytoplasm contained mild eosinophilic granules. The nuclei were large and...
varied in size, with prominent nucleoli as well as a few cells that were binucleated (Figure: 1A).

However, the results from the liver rats who received three different dosages of sodium saccharin showed clear histological alteration according to doses; the severity of lesions increased with increasing dose; there were mild degenerative changes of liver cells with pyknosis of nuclei as well as central vein congestion and sinusoids (2A). While (2B) showed mild inflammatory cell infiltration in the peri-portal with congestion of the portal vein, On the other hand (Figure: 4A), there appeared disarrangement of parenchymal tissue with dilatation and congestion of both central veins and liver sinusoids, as well as increased Kupffer cell numbers.

In addition to the presence of some droplets of fatty changes with enlargement of sinusoids (Figure: 4B), The degenerative changes of the liver were more clearly observed in those rats that received large doses of saccharin (10 mg/kg) compared to the other two previous groups, which received a lower dose, representing cellular swelling, vacuolar cytoplasm, and necrosis. The nuclei of hepatic cells showed severe changes like pyknotic, karyolytic, and karyorrhexis, and this observation was dose-dependent, while the blood sinusoids were occluded with blood and congested, while kupffer cell infiltration was increased (Figure: 6A). The distribution of degenerative changes was revealed as a focal area or entire degeneration with scatter or focal infiltration of mononuclear inflammatory cells in the liver lobule (Figure: 6B).

The normal kidney structure with light microscopic examination demonstrated a normal microscopic picture; the renal corpuscles structure revealed normal with rounded glomeruli and standard undamaged Bowman's capsule. The Bowman's capsule consisted of two covers of epithelial cells: the visceral layer, which is the inner layer, and the outer parietal layer, while the space between the two layers is the Bowman's space (Figure: 1B).

Kidney sections in the tissue of animals treated with 2.5 mg/kg saccharin showed degeneration and necrosis in renal convoluted tubules and proliferation of mesenchymal cells of the glomeruli (Figure: 3A); there was also marked damage and desquamation of the epithelial lining of the renal tubules, accompanied by the presence of bleeding between renal tissue and atrophy of the glomeruli (Figure: 3B). The same microscopic features appeared in rats treated with 2.5 mg/kg, which were also repeated in the sections that were treated with 5 mg/kg, but may be more severe than previous ones, as shown in (Figure: 5A-B).

In the group treated with sodium saccharin, the consequence of the drug intake was obvious on the renal organ, and the dose-dependent effects clearly appeared, which showed severe destruction in the kidney section, resulted in necrosis, and most cells lining renal tubules were breakdown. Besides the strengthening of nuclear chromatin in others, some of the renal glomeruli were shrinkage and disappeared, and there was a presence of hemorrhage with a narrowing of the glomerular hollow space. The congestion and destruction of the walls of the kidney tubules resulted in damage to focal areas of the renal organ, leaving only the remains of some cells, and this observation of renal tissues was dose-dependent (Figures: 7 A-B).
Fig. 2. Histopathological examination of rat liver treated with 2.5mg/kg showed mild degenerative changes of hepatocytes with nuclear pyknosis (blue arrow), congestion of central vein and sinusoids (red arrow) showed in section (a), while (b) shows mild infiltration of inflammatory cells in the peri portal (black arrow) with congestion of portal vein (blue arrow). H&E 20x.
Fig. 3. Histopathological examination of rat kidney were treated with 2.5mg/kg showed proliferation of mesangial cells of glomeruli (black arrow) with degenerative changes and lose of brush borders of renal tubules (blue arrow) in part (a), and swelling of cells lining of renal tubules with narrowing of their lumens (yellow arrow), increase of the bowman’s space (blue arrow) with atrophy of some glomeruli tuft (white arrow) as well as presence of hemorrhage between tubules (green arrow).

Fig. 4. Histopathological sections of rat liver were treated with 5 mg/kg showed hemorrhage and congestion of central veins (red arrow), mild infiltration of inflammatory cells between hepatic cells (black arrow) in part (a) with degeneration and presence of some droplets of fatty changes in hepatocytes (yellow arrow) as well as enlargement with hemorrhage of sinusoids (blue arrow) in part (b). H&E. 20x
Fig. 5. Histopathological sections of rat kidney were treated with 5 mg/kg showed degeneration and necrosis of renal tubules (black arrow), hemorrhage between renal tubules (red arrow) with narrowing of the bowman’s space (white arrow) in part (a), severe necrosis of most of renal tubules with losing of their lumens (black arrow) and infiltration of inflammatory cells and hemorrhage (blue arrow) in part (b). H&E 20x

Fig. 6. Histopathological changes of rat liver were treated with 10 mg/kg showed focal area of inflammatory cells (blue arrow) with severe necrosis of hepatic cells (red arrow) and increased the number of kupffer cells (green arrow) in the part (a), severe degeneration and fatty changes presence inside of hepatocytes with nuclear changes (black arrow) as well as severe peri portal infiltration of inflammatory cells (yellow arrow) in part (b). H&E 20X
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Fig. 7. Histopathological changes of rat kidney were treated with 10 mg/kg showed severe degeneration and necrosis in most of glomeruli and lose of glomerular space (blue arrow) with severe necrosis of tubules (red arrow) in part (a), severe infiltration of inflammatory cells (blue arrow) in part(b).H&E 20x

TABLE 1. Shows the main lesions in both liver and kidney with the effects of three different doses of sodium saccharin.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Organs</th>
<th>Main findings</th>
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<tbody>
<tr>
<td>2.5 mg/kg</td>
<td>liver</td>
<td>Mild degeneration, congestion and infiltration of inflammatory cells.</td>
</tr>
<tr>
<td></td>
<td>kidney</td>
<td>Mesangial cells proliferation, degeneration of tubules, atrophy and hemorrhage.</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>liver</td>
<td>Hemorrhage, congestion and some droplets of fatty changes.</td>
</tr>
<tr>
<td></td>
<td>kidney</td>
<td>Degeneration and moderate to severe necrosis of tubule, hemorrhage between tubules and increase of inflammatory cells.</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>liver</td>
<td>Severe necrosis of hepatocytes, focal area of inflammatory cells, severe degeneration and fatty changes with nuclear changes and peri portal infiltration of inflammatory cells.</td>
</tr>
<tr>
<td></td>
<td>kidney</td>
<td>Severe degeneration and necrosis in most of glomeruli, severe necrosis of tubules with severe infiltration of inflammatory cells.</td>
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Discussion

Nowadays, synthetic sweeteners are generally used. After people improve their feelings about common healthiness, the topic of adding sweeteners to foodstuffs will acquire a hazardous and efficient aspect. It is exactly not viable to receive a diet that is entirely free of sweeteners and other food supplies. The toxicity of sweeteners has expanded, and many researchers are concerned about their toxic consequences. Some research has exposed that people who use artificial sweeteners may lead to some dangerous behaviours. This, as reported in this research, may be caused by harm to the kidneys and liver from eating large amounts of food over an extended period of time [20]. There are some risks to individuals due to the intake of sweeteners, like toxicity in the liver and kidney, multiple arterial sclerosis, respiratory problems, tumors, hypersensitivity, and dysfunction of the immune system [21].

There are many types of artificial sweeteners that have been accepted by the FDA, like saccharin, acesulfame, aspartame, neotame, and sucralose. While the commonly used form is sodium saccharin due to its greater deliciousness [13], however, because of its deliciousness, large amounts of sweet food and beverages will be consumed which, as these studies show, can cause damage to the cell membrane through peroxidation and ultimately lead to cell death. In order to know more about the toxic effects of the synthetic sweeteners, the current work was intended to provide improved information concerning saccharin toxic power, mainly the mechanisms leading to liver toxicity and kidney dysfunction. The histopathological effects of saccharin on the hepatic and kidney sections of laboratory animals cured with various amounts of sodium saccharin were evaluated after 120 days.

In this research, the treated rats with saccharin at three different doses that were mentioned previously
for 120 days showed high cellular damage in the liver and kidney according to the dose. In addition, saccharin is not absorbed or metabolized throughout the human and animal digestive systems, so it is excreted through the kidney without any changes. Depending on this cause, the FDA judged that saccharin is not dangerous [11–22]. These are disagreements with this current research, while Alkaafay et al., (2015) showed in their study that sodium saccharin may provoke stress on the hepatic cells due to oxidation via decreasing the activity of the catalase enzyme and declining the concentration of total antioxidants in plasma [23]. It was confirmed that saccharin destructively influences both liver and kidney sections and changes biochemical markers, either at high or low doses, in rats [24]. The findings of this research are also in line with those of [13–25], who showed that the administration of 10 mg/kg of animal weight and an elevated dose of 500 mg/kg body weight of saccharin revealed a major raise in the serum markers of liver function as well as the activity of liver enzymes like ALT, AST, and ALP. This modification was recommended as an ordinary sign of liver damage, so the lesions that appeared in the liver might be due to a lower antioxidant concentration in plasma, which is caused by saccharin and then elevates the free radical level, which interacts with cellular membranes, or could lead to hepatic parenchymal damage as well as result in aminotransferase activity [26]. The early indication of liver toxicity is the rise of aminotransferase enzyme, which is measured as a signal of tissue destruction [24]. Since it was revealed before, sodium saccharin can affect liver enzymes, so its consumption led to hepatic damage. Consequently, the levels of total protein and albumin might also be affected as an outcome of liver cell damage with loss of function [27–28].

In addition, this current study showed that continuous eating of saccharin may also lead to kidney injury, where there is bleeding, narrowing in the Bowman’s space, and general destruction in some areas of renal tissues due to congestion of renal tubules due to inhibition of antioxidant activity and free radical initiation as a result of declining cellular antioxidant enzymes [29–30], and this is confirmed by [24], who showed that saccharin destructively affects liver and kidney tissues together and changes biochemical markers at elevated and low doses in rats. However, Turley and Dietschy (2003) stated in their study that chronic saccharin consumption may cause an increase in creatinine and urea levels in the blood significantly at all amounts, which might result from disorders in renal tasks, especially due to a decrease in the filtration rate of glomeruli [31], as well as the fact that stress caused by oxidation plays an essential role in the destruction of hepatic and renal cells [32]. This can lead to injury to the cell membrane through the peroxidation of unsaturated fatty acids in phospholipids in the cell membrane, although another cause of cell death may be due to cell swelling.

**Conclusion**

The results of the current research recommend that the consumption of saccharin for a long period of time leads to an enhanced risk of fatness and hyperglycemia, and histopathological interpretation of affected tissues confirms that it has high cellular toxic effects on the liver and kidney and that the dose is important. The cellular toxic effect was evaluated experimentally on both the liver and kidney, which were observed to have severe hemorrhage, degenerative changes, and necrosis. Therefore, it is advised by this data to do immunohistochemical to assess apoptosis.

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**Author's contributions:** In this work, each author contributes equally.

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التأثيرات النسيجية لسكرين الصوديوم السمية على الكبد والكلى لدى الجرذان في مدينة دهوك - العراق

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الخلاصة
ملح الصوديوم سكرين هو المُحكم الصناعي الأكثر استخدامًا من قبل مرضى ارتفاع السكر في الدم وفي معظم الأغذية. لا يزال خطر الصوديوم سكرين موضع نقاش وفقًا لنتائج بعض الأبحاث السابقة. هدفت هذه الدراسة إلى الكشف عن التغييرات التي تحدث بسبب تأثيرات الصوديوم سكرين على الكلي والكبد لدى الجرذان باستخدام تقنيات الأنسجة.

تم استخدام 40 من الجرذان تجريبياً في هذه الدراسة، تراوحت أعمارها بين 3-4 أشهر وكان وزنهم يتراوح بين 250-325 غم. تم تقسيمهم إلى أربع مجموعات (كل مجموعة تحتوي على 10 جرذان) كنت مجموعتي الأولى والثانية والثالثة والرابعة تحتوي على مجموعات مختلفة من الصوديوم سكرين، بدأ من 2.5 و 5 و 10 ملغم / كغم من وزن الجسم. تم استخدام أنابيب المعدة للاستعمال في إعطاء العلاجات للجرذان مرة واحدة يوميًا لمدة 120 يومًا في نهاية التجربة تم جمع الأعضاء لأغراض تقنيات الأنسجة المرضية. اكتشفت الأدلة المرضية في أنسجة الكبد والكلى بفضل الرؤيا على الجرعات النتائج التأثيرات العلاجية مع الصوديوم سكرين مقارنة بالمجموعات الأخرى. تظهر النتائج النهائية أن التأثيرات السامة للصوديوم سكرين لدى الجرذان يرتفع مع زيادة جرعة الصوديوم سكرين في المجموعة الثانية بواقع 2.5 ملغم / كجم من الجرذان. وهذا يشير إلى أن الصوديوم سكرين يكون له تأثير سام يزيد تأثيرات غير مرضية. خلص هذا البحث إلى أن ممكناً أن يسبب الصوديوم سكرين تأثيرات سامة على الكبد والكلى.

الكلمات الدالة: الصوديوم سكرين، السمية المرضية، السمية الكلوية، سمية الكبد، جرذان.