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Nephrotoxic Impacts of Aspartame and Saccharin in Wistar Rat Model: Biochemical, Molecular and Histopathological Approach

Impactos Nefrotóxicos del Aspartamo y la Sacarina en un Modelo de Rata Wistar: Enfoque Bioquímico, Molecular e Histopatológico

Adil Aldhahrani[1]; Mohamed M. Ahmed[2]; Maram H. Abduljabbar[3]; Razaz A. Felemban[4,5]; Alaa A. H. Alorabi[6] & Mohamed E. Alkafafy[7]

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SUMMARY: The present study was designed to resolve the dispute about the nephrotoxicity of the non-nutritive sweeteners (NNS), mainly aspartame and saccharin. Twenty-five, 7-week old, male Wistar rats were divided into a control group (n=5) and 4 experimental groups (n=5). The first and second experimental groups received daily doses of 250 mg aspartame/ Kg BW (Asp. L) and of 1000 mg aspartame/ Kg BW (Asp. H), respectively. The third and fourth experimental groups received daily doses of 25 mg saccharin / Kg BW (Sacch. L) and of 100 mg saccharin/ Kg BW (Sacch. H), respectively. The experimental groups received the corresponding sweetener dissolved in water by oral route for 56 consecutive days. The biochemical assays indicated that both NNS exerted a dose-dependent significant increase of serum urea and creatinine, compared to the control group. The NNS-treated groups displayed histological changes reflecting dose-dependent nephrotoxic effects. These included glomerular and tubular disorders, and outstanding interstitial haemorrhages and fibroplasia as well. The gene expression levels of the key oncogene (*h*-Ras) and the tumour suppressor gene (*P27*) were also estimated, displaying a significant overexpression of the first and a significant downregulation of the second. Taken together, the biochemical and histological alterations, the overexpression of the key oncogene (*h*-Ras) and the downregulation of the tumour suppressor gene (*P27*) in all NNS-treated rat groups may indicate a potential risk of carcinogenesis, especially on long-term exposure.

KEY WORDS: Aspartame; Saccharin; Rat; Kidney; Nephrotoxicity; *h-Ras*; *P27*.

INTRODUCTION

Artificial sweeteners (AS) are commonly known as sugar substitutes and have significantly substituted sucrose in foods providing synchronized enhanced flavour and reduced calorie intake (Whitehouse *et al*., 2008; Schwarz *et al*., 2024). The most common members of AS are non-nutritive sweeteners (NNS) including aspartame and saccharin (Schwarz *et al*., 2024).

Soluble saccharin (ortho-benzoylsulfimide sodium salt) is the most widely known benzisothiazole derivative and the most commercially available NNS (Ager *et al*., 1998). The ingested saccharine passes through the digestive tract without being digested (Mahmood & Al-Juboori, 2020). Thus, it is not absorbed or metabolized, but rapidly excreted unchanged by the active renal tubular secretion (Goldstein *et al*., 1978). On the other hand, aspartame, L-α-aspartyl-L-phenylalaninel-methyl ester, is also a widely used NNS (Soffritti *et al*., 2006). It is a dipeptide methyl ester, built up of a phenylalanine molecule, an aspartate molecule and a methyl group esterified to the carbonic acid group of the phenylalanine (Monte, 1984). Aspartame is hydrolysed into phenylalanine, aspartic acid and methanol (Monte, 1984).

[1] Clinical Laboratory Sciences Department, Turabah University College, Taif University, Saudi Arabia.
[2] Department of Biochemistry, College of Veterinary Medicine, University of Sadat City, Egypt.
[3] Department of Pharmacology and Toxicology, College of Pharmacy, Taif University, Taif, Saudi Arabia.
[4] Department of Basic Medical Sciences, College of Medicine, King Saud Bin Abdulaziz University for Health Sciences, Jeddah, Saudi Arabia.
[5] King Abdullah International Medical Research Centre, Jeddah, Saudi Arabia.
[6] Self-dependent Scholar, Taif, Saudi Arabia.
[7] Department of Biotechnology, College of Science, Taif University, Taif, Saudi Arabia.
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Methanol is further metabolized into formaldehyde, which exerts oxidative stress on the kidney causing renal injuries (Ardalan *et al*., 2017). Thus, aspartame may be designated as a nephrotoxin (Finamor *et al*., 2014; Ardalan *et al*., 2017).

Although, NNS are widely used to sweeten several products (jams, canned fruit, soft drinks, candies, chewing gum, cosmetic products, and medications); they represent debated safety and health issues (Whitehouse *et al*., 2008; Ardalan *et al*., 2017). Yet, many recent experimental studies reported potential toxic impacts of NNS, including aspartame and saccharine (Ardalan *et al*., 2017; Shaher *et al*., 2023; Schwarz *et al*., 2024). Among the most affected organs are the liver (Alkafafy *et al*., 2015; Azeez *et al*., 2019; Schwarz *et al*., 2024) and the kidney (Martins & Azoubel, 2007; Bahr & Zaki, 2014; Finamor *et al*., 2014; Waggas *et al*., 2015; Alwaleedi, 2016; Amin *et al*., 2016; Ardalan *et al*., 2017; Al-Eisa *et al*., 2018; Azeez *et al*., 2019; Shaher *et al*., 2023; Schwarz *et al*., 2024).

Many of the studies on the safety of NNS have been conducted by manufacturers of these sweeteners and are commonly unavailable to consumers (Naik *et al*., 2018). However, some independent research studies have associated NNS with health disorders (Naik *et al*., 2018) such as nephrotoxicity (Bahr & Zaki, 2014; Finamor *et al*., 2014; Waggas *et al*., 2015; Alwaleedi, 2016; Amin *et al*., 2016; Al-Eisa *et al*., 2018; Azeez *et al*., 2019) and cancers (Soffritti *et al*., 2006, 2007, 2010). Nevertheless, a massive disagreement about this issue still exists and the role of sweeteners in cancer vulnerability has been extensively disputed (Bosetti *et al*., 2009). Unquestionably, aspartame has been the most debatable NNS because of its probable noxiousness (Whitehouse *et al*., 2008) and carcinogenicity (Soffritti *et al*., 2006, 2007, 2010), even at a dosage level approximating the ADI for humans (Whitehouse *et al*., 2008). Likewise, experimental studies on saccharin offer both positive and negative outcomes, including the possibility to convince cancer in rats, dogs, and humans (Whitehouse *et al*., 2008). On the other hand, human-based study indicates that the consumption of AS was not associated with the risk of cancer (Bosetti *et al*., 2009).

Aspartame convinces outstanding modifications in the expression of key oncogene (*h-Ras*) in mice liver and kidney (Gombos *et al*., 2007). Also, Sodium saccharin induces point mutation in the *K-ras* oncogene in the human RSA cells *in vitro* (Suzuki & Suzuki, 1993). Moreover, saccharin convinces remarkable DNA damage than does aspartame (Bandyopadhyay *et al*., 2008). It is worth mentioning that DNA binding properties of aspartame (Kashanian *et al*., 2013) and saccharin (Icsel & Yilmaz, 2014) support the concept of their genotoxicity.

This echoing dispute among autonomous scientific publications and those funded by NNS producers about the safety of NNS had encouraged us to scheme this research proposal endeavouring to unveil some of the mysterious facets of this matter.

MATERIAL AND METHOD

Artificial sweeteners. The AS investigated in the current study: sodium saccharin (Cat. No. AL3367) and aspartame (Cat. No. AL5181-ASPA) were purchased from Alpha Chemika, Mumbai, India.

Animals and experimental design. A total of 25 male Wistar albino rats (7-week age and average body weight of 101± 4.8 g) were purchased from the animal house of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Rats were adapted for a week before the start of the trial. Rats were kept at animal house under standard environmental conditions (ambient temperature of 22 °C, good ventilation, 12 h light/dark cycle, fed a standard pellet diet, and water *ad libitum*). The animal experiments were applied according to the protocols and regulations of animal care and approved by Ethical Committee of Taif University for the project TU-DSPP-2024-154. Rats were allocated into five groups: a control group and four experimental groups (5 rats per each), and housed in cages of polycarbonate. The first and the third experimental groups received daily doses equivalent to the acceptable daily intake (ADI) of 250 mg aspartame and 25 mg saccharin/kg BW, respectively. The second and the fourth experimental groups received daily doses equivalent to 4-fold ADI of 1000 mg aspartame and 100 mg saccharin/kg BW, respectively. Rats in different treated groups received the corresponding sweetener dissolved in water by oral route for 56 consecutive days. Calculation of the experimental ADI was according to those reported by Butchko & Kotsonis (1991) for human ADI and corrected for rats according to Fernstrom (1989).

Sampling. By the end of the trial, rats of all groups were sacrificed, serum and organs were collected. The serum was collected after the centrifugation of blood at 4,000 rpm for 8 min. Serum samples were collected and stored at -80 °C until used for biochemical analyses. Kidneys tissue specimens were collected for histological and gene expression assessments. Histological specimens were kept in 10 % neutral buffered formalin (NBF) and those for molecular analyses were kept in RNA-Later (Sigma-Aldrich, St. Louis, MO, USA) at -80 °C until use.

Biochemical assays. For assessment of the kidney function, serum urea and creatinine were estimated. The biochemical markers (serum urea and creatinine) were determined using

the kits purchased from Biodiagnostics company, Biodiagnostic, Dokki, Giza, Egypt and assayed as described in the instruction manual of each kits.

Analysis of genes expression

RNA extraction and cDNA synthesis. Total RNA was extracted from 100 mg of each tissue sample using QIAzollysis reagent (QIAGEN Inc., Valencia, CA) according to the manufacturer's instructions and as detailed previously (Ahmed *et al*., 2014). Integrity of the prepared RNA was checked by electrophoresis. While, RNA concentration and purity were determined spectrophotometrically at 260 nm and 280 nm. For cDNA synthesis, two µg RNA were reverse transcribed with oligo-dT primer and Moloney murine leukaemia virus (M-MuLV) reverse transcriptase (SibEnzyme Ltd. AK, Novosibirsk, Russia) as previous described (Ahmed *et al*., 2014). The resultant cDNA was preserved at -20°C until used.

Semi-quantitative PCR. The used primers were designed using Oligo-4 computer program (Molecular Biology Insights, Inc., Cascade, CO, USA) and nucleotide sequence published in Genbank (Table I) and synthesized by Macrogen (Macrogen Company, GAsa-dong, Geumcheon-gu., Korea). PCR was conducted in a final volume of 25 µl consisting of 1 µl cDNA, 1 µl of 10 picomolar of each primer (forward and reverse) and 12.5 µl PCR master mix (Promega Corporation, Madison, WI, USA), the volume was brought up to 25 µl using sterilized, deionized water. PCR was carried out using a PeX 0.5 thermal Cycler with the cycle sequence at 94 °C for 5 min one cycle, followed by 25 cycles each of which consisted of denaturing at 94 °C for one min, annealing at the specific temperature corresponding to each primer (Table I) and extension at 72 °C for 1 min with additional final extension for one cycle at 72 °C for 5 min. As a reference, expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was detected by using specific primers (Table I). PCR products were electrophoresed on 1 % agarose A (Bio Basic Inc. Konrad Cres, Markham, Ontario, Canada) gel in 1.0 X- TAE (Tris-Acetate-EDTA) buffer (Sigma–Aldrich, St. Louis, MO, USA) at 100 volts for 30 min. The gel was stained with ethidium bromide and visualized under UV light then photographed using UVP gel documentation system (UVP, Upland, CA, USA). The intensities of the bands were quantified densitometrically using Image J program version1.47 (http://rsb.info.nih.gov/ij/).

Histological examination. Small specimens from the kidney were fixed in 10 % NBF for 24 h, then washed under running tap water and preserved in 70 % ethanol. The samples were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in Paraplast Plus® (Sigma–Aldrich, St. Louis, MO, USA) and sectioned at 5 µm thickness. Tissue sections were mounted on glass slides. Sections were stained with hematoxylin and eosin for studying the general histology (Bancroft *et al*., 1996), and picrosirius red (PSR) for detection of newly formed collagen (Junqueira *et al*., 1979).

Photomicrography. Photomicrographs were captured with a digital camera (Leica EC3, Leica Microsystems Ltd., Heerbrugg, Switzerland), mounted on Leica DM LB light microscope (Leica Microsystems, Wetzlar, Germany).

Statistical analysis. Statistical analysis for the obtained results was performed using analysis of variance (ANOVA) followed by the least significant difference (LSD) test for the multiple comparisons among the groups by using SPSS software (SPSS version 13.0, IBM, Chicago, IL, USA) with $P < 0.05$ regarded as statistically significant. Results were expressed as means ± standard errors of means (SEM).

RESULTS

Biochemical findings. The current findings that reflect the impacts of AS on the serum markers (creatinine and urea) of the kidney function were displayed in histograms (Fig. 1). Application of aspartame and saccharin raised the serum levels of urea and creatinine at both low and high doses, relative to the control. The serum urea levels were significantly high in both rat groups of Asp. L (49.75±2.55 mg/dl) and Sacch. L (54.26±2.04 mg/dl), relative to the control (28.49±1.69 mg/dl). In case of the high doses, the serum urea levels were rather higher in both rat groups of

Table I. Primers and PCR conditions used for the tested genes.

Product size	Annealing temperature & Cycles number	Primer Sequence (5` -3`)	Gene, accession number
430 bP	55°C, *25* cycles	F-CCT CTG GAA AGC TGT GGC GT R-TTG GAG GCC ATG TAG GCC AT	**GAPDH (M17701)**
542 bP	56°C, 30 cycles	F-AGC CCC TGT AGA AGC GAT GA R-CAT CAG GCG GGT TCA GTT TC	**ha-*Ras* (NM_001130441)**
238 bP	58°C, 30 cycles	F-GAG GGC AGA TAC GAG TGG CAG R-CTG GAC ACT GCT CCG CTA ACC	**P27 (D86924)**

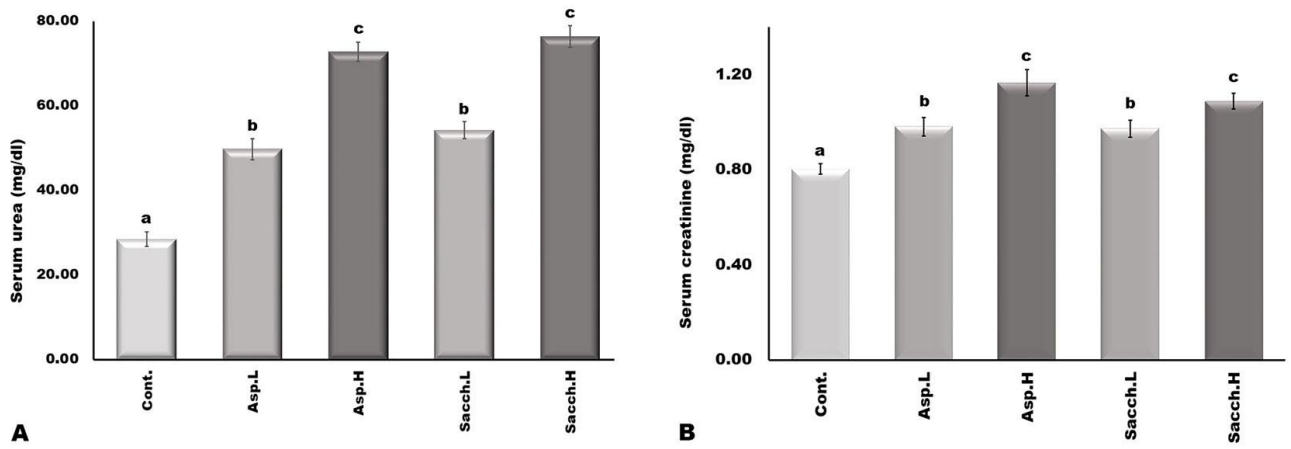

Fig. 1. Histogram showing the effect of artificial sweeteners intake on the serum levels of urea (A) and creatinine (B). Asp.L, one-fold ADI of aspartame; Asp.H, four-fold ADI of aspartame; Sacch.L, one-fold ADI of saccharin; Sacch.H, four-fold ADI of saccharin.; Cont., control. Columns carrying different letters are significantly different at $P < 0.05$.

Asp. H (72.75±2.29 mg/dl) and Sacch. H (76.33±2.60 mg/dl), relative to the control and to their corresponding of low doses (Fig. 1A). Likewise, the serum creatinine levels were significantly high in both rat groups of Asp. L (0.98±0.039 mg/dl) and Sacch. L (0.97±0.035 mg/dl), relative to the control (0.80±0.023 mg/dl). In case of the high doses, the serum creatinine levels were rather higher in both rat groups of Asp. H (1.17±0.055 mg/dl) and Sacch. H (1.09±0.034 mg/dl), relative to the control and to their corresponding of low doses (Fig. 1B).

Molecular findings. The expression of the mRNA of both *h-Ras* and P27 was examined in the kidney from the control and all the experimental groups after receiving the aspartame and saccharin at different dose rates for 56 consecutive days. The expression of *h-Ras* was significantly higher in all treated groups compared to the control group and in a dose-dependent way (Fig. 2A). On the other hand, P27 mRNA expression was significantly downregulated in all treated groups compared to the control group and this downregulation was also dose-dependent (Fig. 2B).

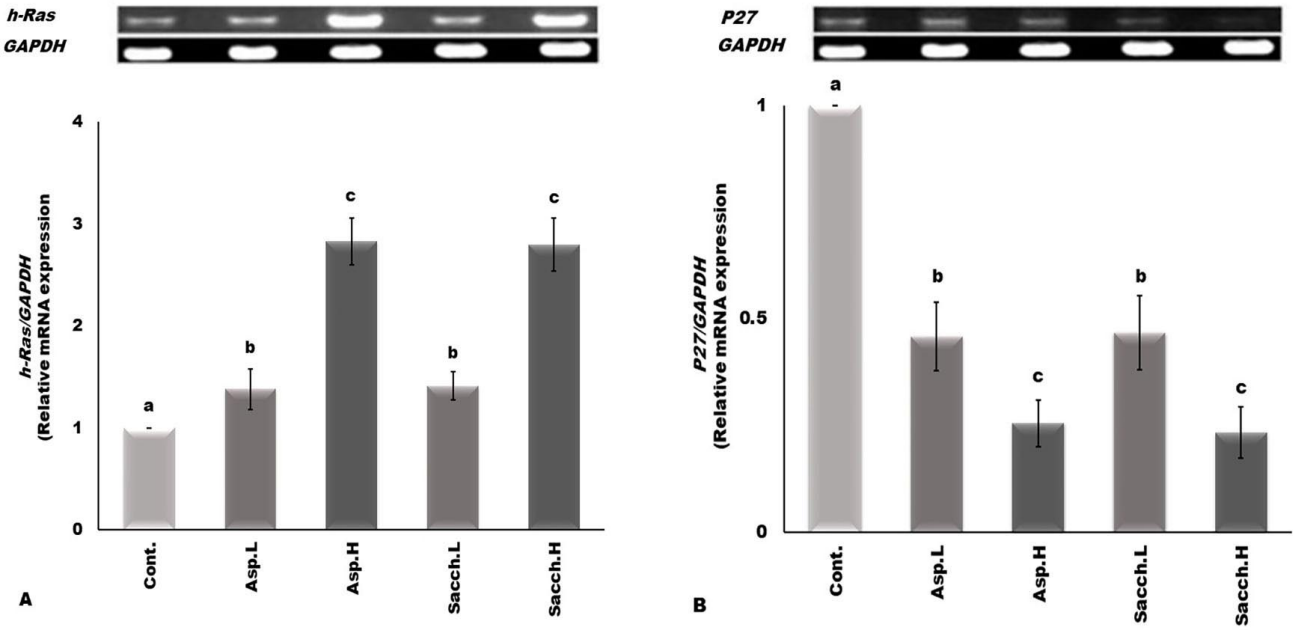

Fig. 2. Effects of artificial sweeteners intake on *h-Ras* (A) and P27 (B) mRNA expression in renal tissue of rat. Notes: Representative blots and results of densitometric analyses of at least five independent experiments are shown. Values are means ± SE. Asp.L, one-fold ADI of aspartame; Asp.H, four-fold ADI of aspartame; Sacch.L, one-fold ADI of saccharin; Sacch.H, four-fold ADI of saccharin.; Cont., control. Columns carrying different letters are significantly different at $P < 0.05$.

Histological findings. Hematoxyline and eosine stained renal sections from control rats displayed the normal renal architecture of the outer renal cortex and the inner renal medulla (Fig. 3a,b). The renal cortex displayed the renal corpuscles and the associated proximal and distal convoluted tubules (Fig. 3a). On the other hand, the renal sections of the renal medulla stained with Hematoxyline and eosine (Fig. 3b) and PSR (Fig. 3c) presented profiles of thin tubules and the collecting ducts. The PSR-stained sections, more or less fail to demonstrate newly formed collagen fibres within the renal interstitium in control rat kidney (Fig. 3c).

Renal sections from rats received aspartame or saccharine showed obvious histological changes, which were more pronounced in animals receiving higher doses of both NNS. The histopathological changes including glomerular, tubular and interstitial findings (Figs. 4 and 5). The glomerular findings ranging from dilatation of capsular space, hyperplasia, narrowing of capsular space and finally glomerular atrophy. The tubular changes involving proximal and distal tubules. The histopathological changes ranged from cloudy swelling, vacuolar and hydropic degeneration, necrosis and finally desquamations of the tubular epithelium. The most common outstanding intertubular findings were interstitial congestion and haemorrhages (Figs. 4 and 5).

Compared to the renal sections from the control rats (Fig. 3c), the sections stained with PSR for demonstrating collagen deposition (pink coloured), displayed a remarkable interstitial fibrosis in all treated groups receiving aspartame (Figs. 4c,f) and saccharin (Figs. 5b,e). The collagen deposition was more pronounced in the kidneys from rat groups received the higher doses of both sweeteners (Figs. 4f and 5e).

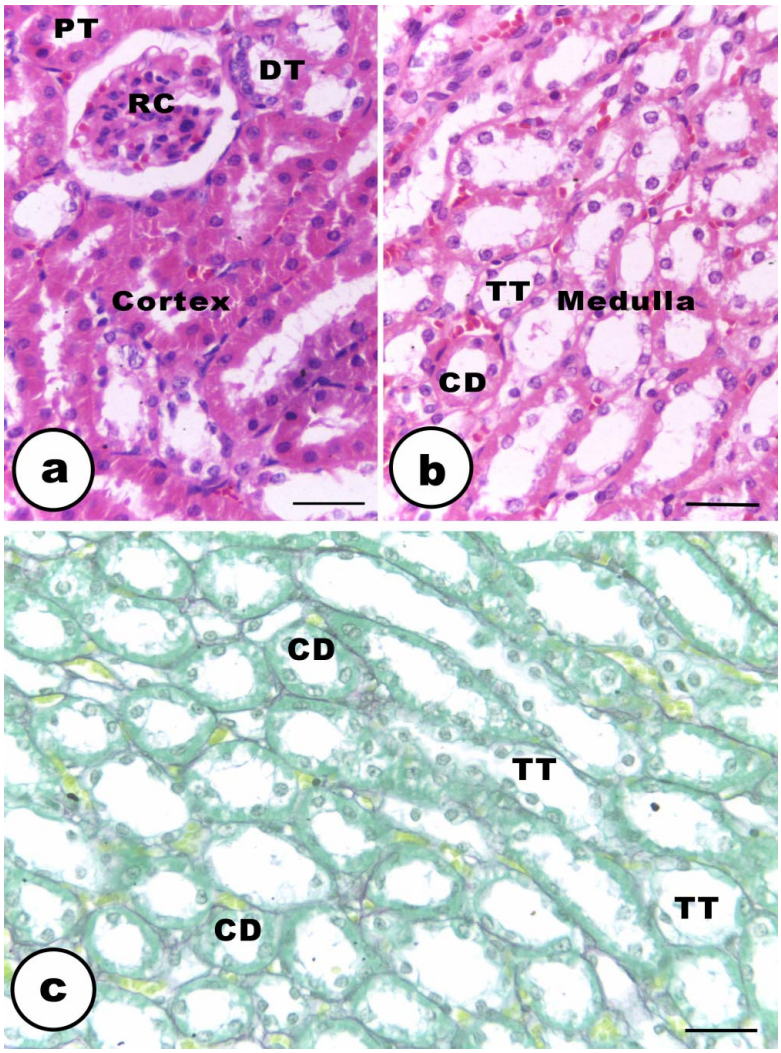

Fig. 3. Renal sections from control rat group: H & E stained sections of renal cortex (a) showing renal corpuscle (RC), proximal (PT) and distal (DT) convoluted tubules; H & E stained sections (b) and PSR-stained sections (c) of renal medulla displaying thin tubules (TT) and collecting ducts (CD). Scale bar = 30 µm.

DISCUSSION

The nephrotoxic effects of aspartame are mainly ascribed to its metabolic by-products. As a dipeptide methyl ester, aspartame is hydrolysed by the chymotrypsin of the pancreatic juice in the duodenum into aspartic acid, phenylalanine, and methanol (Burgert *et al*., 1991). Methanol is oxidized in the tissues by alcohol dehydrogenase to a highly reactive intermediate product: micromolar formaldehyde (Liesivuori & Savolainen, 1991). The *in vitro* studies on micromolar formaldehyde demonstrated its cytotoxic potentials (Nakao *et al*., 2003). This cytotoxicity was denoted by increased numbers of shrivelled cells. On the other hand, the ingested saccharine passes through the digestive tract without being digested (Mahmood & Al-Juboori, 2020). Thus, it is not absorbed or metabolized, but rapidly excreted unchanged via renal route, by the active tubular secretion (Goldstein *et al*., 1978). A positive relationship between oxidative stress and saccharin consumption was reported, and thus, saccharin may be a potential oxidative stress initiator (Azeez *et al*., 2019). This may agree with previous studies reported that sodium saccharin exhibits oxidative cytotoxic and tumor-promoting effects (Yu *et al*., 1992; Mahmood & Al-Juboori, 2020).

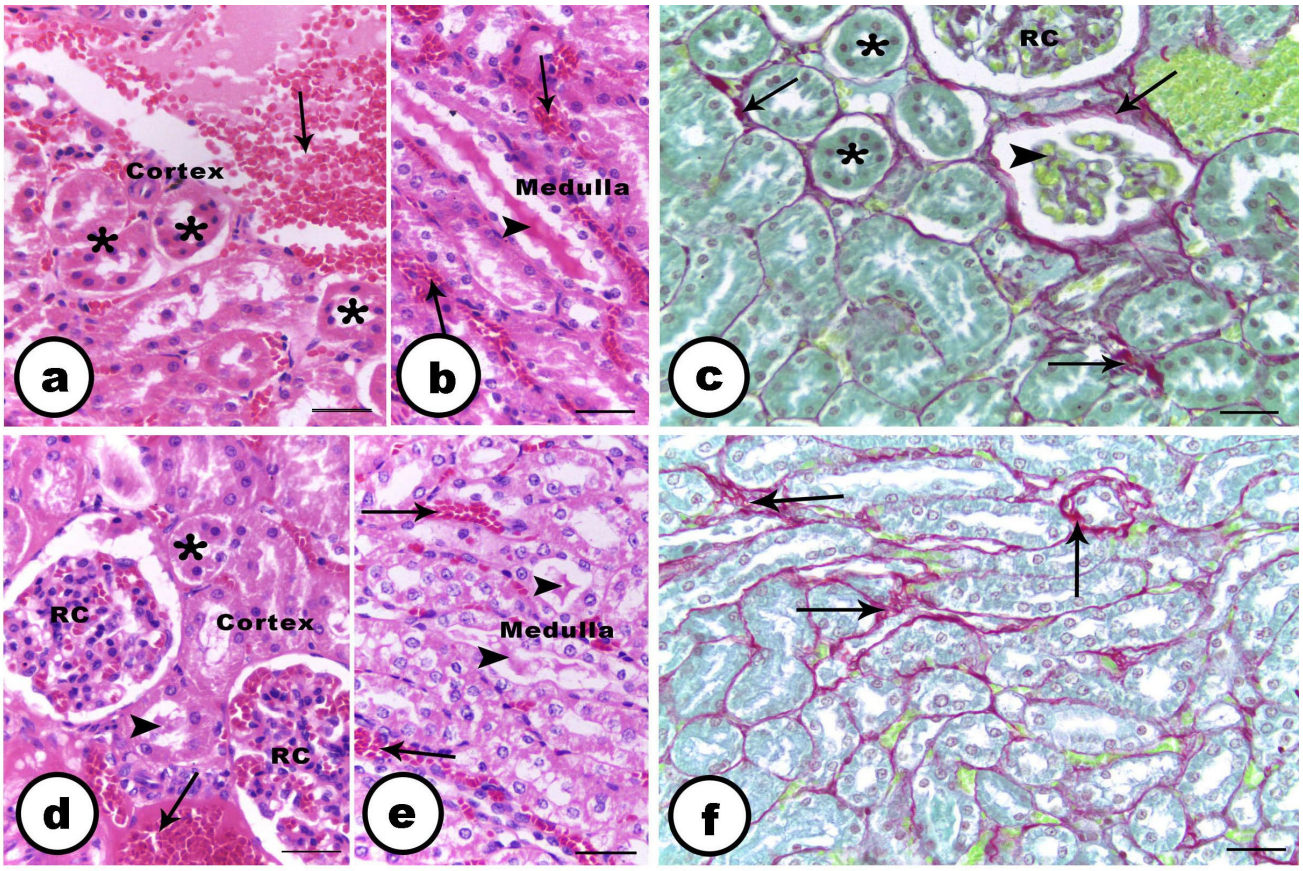

Fig. 4. Effect of aspartame intake on the histological architecture of the kidney: Sections of kidney from rat group treated with low dose of aspartame (Asp.L): H & E stained sections of renal cortex (a) showing proximal convoluted tubules lined with epithelial cells with deeply acidophilic cytoplasm and pyknotic nuclei (asterisks) and interstitial haemorrhage (arrow); H & E stained sections of renal medulla (b) displaying intra-tubular desquamations (arrowheads) and interstitial haemorrhage (arrow); and PSR-stained sections of renal cortex (c) exhibiting degenerating renal tubules (asterisks), atrophying (arrowhead) renal corpuscle (RC), depositions of newly formed collagen fibres (arrows) around the renal corpuscles and renal tubules. Sections of kidney from rat group treated with high dose of aspartame (Asp.H): H & E stained sections of renal cortex (d) showing degenerating renal corpuscles (RC), proximal convoluted tubules with pyknotic nuclei (asterisks) and intra-tubular desquamations (arrowhead) and interstitial haemorrhage (arrow); H & E stained sections of renal medulla (e) displaying intra-tubular desquamations (arrowheads) and interstitial haemorrhage (arrow); and PSR-stained sections of renal medulla (f) exhibiting peritubular deposition of newly formed collagen fibres (arrows). Scale bar = 30 µm.

The current biochemical findings of elevated serum biomarkers (urea and creatinine) of the kidney functions indicate that both aspartame and saccharin induced a dose-dependent nephrotoxic effects. Our findings of aspartame-induced renal disorders, go in line with the findings of previous experimental studies on rats (Bahr & Zaki, 2014; Finamor *et al*., 2014; Waggas *et al*., 2015; Alwaleedi, 2016; Amin *et al*., 2016; Al-Eisa *et al*., 2018). The elevation in creatinine and urea may be ascribed to diminished glomerular filtration rate and retention of urea and creatinine in the blood. This notion may be supported by the histopathological changes involved both the glomerular and the tubular elements in male (Al-Eisa *et al*., 2018) and female (Waggas *et al*., 2015; Othman & Bin-Jumah, 2019) rats. It is worth noting that the use of aspartame leads to nephrotoxicity, as indicated from morphometric alterations (glomerular and tubular) in the rat fetal kidney (Martins & Azoubel, 2007). Contradicting to these findings, Torigoe *et al*. (2024), reported that there were no differences either in serum biomarkers (creatinine and urea) levels, or in renal histology between the control and aspartame-treated mice groups.

Likewise, our findings of saccharin-induced nephrotoxicity, go in line with those of previous studies reported a dose-dependent nephrotoxic effect of saccharine sodium on rat kidney (Amin & Almuzafar, 2015; Amin *et al*., 2016; Azeez *et al*., 2019; Nabi *et al*., 2024). Similar to our findings, the consumption of saccharin over a long period of time impairs the kidney function, as indicated from a significant elevation in the serum creatinine level in the

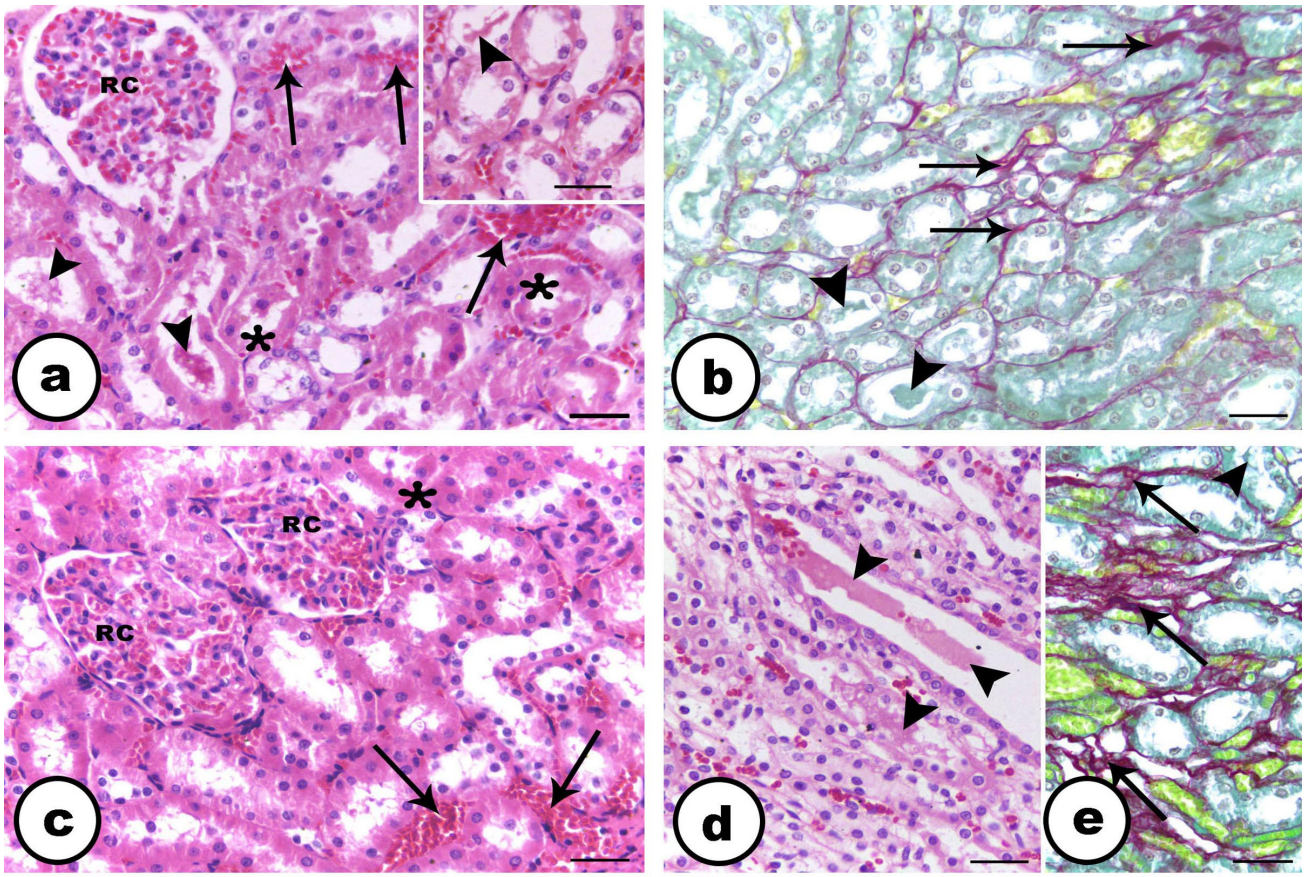

Fig. 5. Effects of saccharine intake on the histological architecture of the kidney: Sections of kidney from rat group treated with low dose of saccharin (Sacch.L): H & E stained sections of renal cortex (a) showing hyperplastic renal corpuscle (RC), intra-tubular desquamations (arrowheads), degenerating proximal convoluted tubules (asterisks) and interstitial haemorrhage (arrow), and renal medulla (inset) displaying intra-tubular desquamations (arrowheads); PSR-stained sections of renal medulla (b) presenting intra-tubular desquamations (arrowheads) and interstitial haemorrhage (arrow). Sections of kidney from rat group treated with high dose of saccharin (Sacch.H): H & E stained sections of renal cortex (c) showing glomerular hyperplasia and sever narrowing of the capsular space of the renal corpuscles (RC), degenerating renal tubules (asterisks) and interstitial haemorrhage (arrow); H & E stained sections of renal medulla (d) displaying intra-tubular desquamations (arrowheads) and interstitial haemorrhage; and PSR-stained sections of renal medulla (e) exhibiting peritubular deposition of newly formed collagen fibres (arrows) and intra-tubular desquamations (arrowhead). Scale bar = 30 μm.

saccharin-treated groups compared to the control group (Amin *et al*., 2016; Azeez *et al*., 2019). Also, Nabi *et al*. (2024), mentioned that these effects included hyperplastic glomerular changes, and degenerative tubular alterations. These histopathological alterations were severe enough to reach glomerular atrophy, desquamation of tubular epithelium, and interstitial haemorrhages. Additionally, Jacquillet *et al*. (2018), reported that there were no major effects of saccharin on the glomerular filtration rate or urine flow in the kidney in an in vivo experiment of acute saccharin infusion in male Wister rats. They also proposed that the renal dysfunction is associated only with the long-term intake of NNS (Jacquillet *et al*., 2018).

Contradicting to our findings, Enuwosa *et al*. (2021), via an *in vitro* study on model of primary human glomerular microvascular endothelial cells, assumed that aspartame and saccharin exhibit protective effects on the glomerular microvasculature against the barrier disruption induced by the vascular endothelial growth factor. However, they failed to identify the definite relevant mechanism, and hypothesized an unknown alternative signalling pathway (Enuwosa *et al*., 2021)

The intake of aspartame for a long period of time may result in a dose-dependent increased production of free radicals and oxidative stress in renal tissues (Mourad, 2011; Ardalan *et al*., 2017), as well as injury of the renal tissues (Ardalan *et al*., 2017; Shaher *et al*., 2023). The oxidative stress involves either elevated level of pro-oxidants such as reactive oxygen species (ROS), or reduced level of antioxidants promoting dysfunction and degradation of the cells (Halliwell

& Gutteridge, 2007). Additionally, the generation of free radicals induces breakdowns in the DNA (Lin *et al*., 2006). Thus, both cytological and histological alterations may be a result of direct effect of ROS, or via indirect impact of ROS on the DNA and initiation of apoptosis and other degenerative changes (Al-Eisa *et al*., 2018). Moreover, previous studies on HeLa cells reported that low doses of aspartame modify the mRNA expression of apoptotic genes and downregulate the expression of the tumor suppressor gene p53 (Pandurangan *et al*., 2015; Shaher *et al*., 2023). Thus, aspartame could be a carcinogen in male and female rodents (Soffritti *et al*., 2010). Also, Sodium saccharin possesses carcinogenic effects in rats (Ellwein & Cohen, 1990).

The present molecular outcomes denoted by upregulation of *h-Ras* and downregulation of P27 are in agreement with that reported in mice (Soffritti *et al*., 2006), in rats (Alkafafy *et al*., 2015), and in human RSA cells *in vitro* (Suzuki & Suzuki 1993). Aspartame convinces outstanding modifications in the expression of key oncogene (*h-Ras*) in mice (Gombos *et al*., 2007) and in rats (Alkafafy *et al*., 2015). Also, sodium saccharin induces point mutation in the *K-ras* oncogene in the human RSA cells *in vitro* (Suzuki & Suzuki, 1993), and significantly modifies the expression of key oncogene (*h-Ras*) in rat (Alkafafy *et al*., 2015). On the other hand, aspartame and saccharine have been reported to downregulate the expression of the tumor suppressor gene P27 in rats (Alkafafy *et al*., 2015). Moreover, saccharin convinces remarkable DNA damage than does aspartame (Bandyopadhyay *et al*., 2008). It is worth mentioning that DNA binding properties of aspartame (Kashanian *et al*., 2013) and saccharin (Icsel & Yilmaz, 2014) support the concept of their genotoxicity. This notion has been recently supported by an in silico study (Arulanandam *et al*., 2025). However, in disagreement with these findings Otabe *et al*. (2019), reported that NNS have no potential genotoxicity or mutagenicity.

CONCLUSION

Taken together the remarkable biochemical (elevated serum creatinine and urea), and the histological changes in kidney sections from all NNS-treated groups reflected the nephrotoxic effect of aspartame and saccharin. It is worth noting that the severity of toxic effect was dose-dependent. Moreover, the upregulation of the key onchogen (*h-Ras*) and the downregulation of the tumour suppressor gene (P27) in all treated rat groups may indicate a potential risk of kidney carcinogenesis, particularly on long-term exposure.

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RESUMEN: El presente estudio se diseñó para resolver la controversia sobre la nefrotoxicidad de los edulcorantes no nutritivos (ENN), principalmente aspartamo y sacarina. Veinticinco ratas Wistar macho de 7 semanas de edad se dividieron en un grupo control (n=5) y cuatro grupos experimentales (n=5). El primer y segundo grupo experimental recibieron dosis diarias de 250 mg de aspartamo/kg de peso corporal (Asp. L) y de 1000 mg de aspartamo/kg de peso corporal (Asp. H), respectivamente. El tercer y cuarto grupo experimental recibieron dosis diarias de 25 mg de sacarina/kg de peso corporal (Sacch. L) y de 100 mg de sacarina/kg de peso corporal (Sacch. H), respectivamente. Los grupos experimentales recibieron el edulcorante correspondiente disuelto en agua por vía oral durante 56 días consecutivos. Los ensayos bioquímicos indicaron que ambos NNS ejercieron un aumento significativo dependiente de la dosis de urea y creatinina séricas, en comparación con el grupo control. Los grupos tratados con NNS mostraron cambios histológicos que reflejaban efectos nefrotóxicos dependientes de la dosis. Estos incluyeron trastornos glomerulares y tubulares, y también hemorragias intersticiales destacadas y fibroplasia. También se estimaron los niveles de expresión génica del oncogén clave (*h-Ras*) y del gen supresor de tumores (P27), mostrando una sobreexpresión significativa del primero y una significativa disminución de la expresión del segundo. En conjunto, las alteraciones bioquímicas e histológicas, la sobreexpresión del oncogén clave (*h-Ras*) y la disminución de la expresión del gen supresor de tumores (P27) en todos los grupos de ratas tratadas con NNS podrían indicar un riesgo potencial de carcinogénesis, especialmente con la exposición a largo plazo.

PALABRAS CLAVE: Aspartamo; Sacarina; Rata; Riñón; Nefrotoxicidad; *h-Ras*; P27.

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Corresponding author:
Mohamed Alkafafy
Department of Biotechnology
College of Science
Taif University
Taif, 21944
SAUDI ARABIA

E-mail: m.kafafy@tu.edu.sa