Green Kiwi Fruit Extract Ameliorates Aspartame Toxicity on the Lung of Adult Male Abino Rat (Histological and Immunohistochemical Study)

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ABSTRACT

Background: Aspartame (ASP) is a widely used artificial sweetener. Aspartame-containing foodstuff and drinks are usually consumed by diabetic patients and for weight loss. Kiwi fruits have antioxidant and anti-inflammatory actions.

Objective: The aim of the present study was to evaluate the effect of aspartame and the possible role of kiwi fruit on the structure of the lung in adult male albino rats.

Materials and Methods: Forty adult male albino rats were used in three groups; Group I served as control, Group II received aspartame orally in a dose of 40 mg/kg dissolved in distilled water daily for six weeks, Group III received kiwi fruit extract orally in a dose of 37 g/kg body weight three times weekly together with aspartame 40 mg/kg for six weeks. Body weight, serum tumor necrosis factor-α (TNF-α) and nitric oxide (NO) and tissue malondialdehyde (MDA) and superoxide dismutase (SOD) were measured. Lungs were dissected out for staining with H&E, Masson trichrome, alpha-smooth muscle actin (α-SMA), and caspase 3.

Results: Aspartame caused elevated level of serum TNF-α and NO and tissue MDA and SOD and significant histopathological alterations in the lung tissue. Aspartame and Kiwi group showed relatively normal levels of TNF-α, NO, MDA, and SOD and less alteration in the histological and immunohistochemical structure.

Conclusion: Lowering the consumption of aspartame is highly recommended due to the proved toxic effect on the lung and regular intake of kiwi is also advised.

Key Words: Apoptosis, aspartame, kiwi fruit extract, lung, oxidative stress.

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INTRODUCTION

Artificial sweetener is a food additive that duplicates the effect of sugar in taste, but has less food energy. The benefits of substituting artificial sweeteners include lower calorie intake, lower incidence of dental caries, better glycemic control and weight loss[1].

Aspartame is one of the most commonly used artificial sweeteners nowadays. It was approved for use in dry applications by Food and Drug Administration (FDA) in 1981 followed by approval for use in carbonated soft drinks in 1983. Finally, it is approved for its use as a general sweetener in 1996. It is used in many food products such as desserts, yoghurts, vitamins, medicines, chewing gum, and diet beverages[2].

Aspartame is a methyl ester of dipeptide of aspartic acid and phenylalanine. It is digested by esterases and peptidases into aspartic acid, phenylalanine, and methanol[3]. Many researchers reported toxic effects of aspartame on various organs especially its neurotoxic effects on the brain function[4].

There is a growing interest to find non-toxic, safe and inexpensive natural antioxidants especially those derived from plants. Natural antioxidants are usually considered safe by most consumers[5]. Regular intake of the naturally occurring antioxidants is needed to scavenge the harmful effects of reactive oxygen species (ROS). Kiwi fruit (Actinidia delicosa) is a popular fruit in Egypt. It is rich in many essential nutrient substances[6, 7].

The antioxidant capacity of kiwifruit constituents was confirmed using various in vitro chemical assays that measured its ability to remove or delay the formation of reactive oxygen species (ROS). Kiwi fruit juice is an effective inhibitor of lipid oxidation and a potent eliminator of hydrogen peroxide (H₂O₂)[8, 9].

Kiwi fruits intake improves the gastrointestinal motility, lowers the levels of blood lipid, and treats the dermatological diseases. The reported antioxidant and anti-inflammatory properties of kiwi fruits explain their ability to prevent cardiovascular diseases, degenerative diseases and cancer[10].
Alpha-SMA is an actin isoform that plays an important role in fibrogenesis. Alpha-SMA can be found in smooth muscle cells, myofibroblasts, and blood vessels[13]. Caspase-3 is a cysteine protease that participates in the process of apoptotic cell death. The activated caspase-3 abrogates the effect of substrates that protect cellular integrity, such as the DNA-repair enzyme, thereby inducing apoptotic cell death[12]. Therefore in our study, we analyzed the expressions of alpha-SMA and caspase-3.

Among all artificial sweeteners, aspartame has been the most controversial because of its potential toxicity and carcinogenicity, even at the acceptable daily intake in humans[13]. Therefore, we performed this study to investigate the effects of aspartame on the lung tissue in rats and to evaluate the possible protective effects of kiwi fruit extract against aspartame-induced lung damage.

MATERIALS AND METHODS

2.1. Experimental animals:

Forty adult male Wistar albino rats aged 2-3 months and weighing between 250-280 g were used as experimental animals in the present investigation. They were obtained from the animal house of Faculty of Medicine, Zagazig University, Egypt. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in metallic cages with well-aerated covers with a 12 h light/dark cycles. Each cage contains 3-4 rats. They were housed at normal atmospheric temperature (25 ± 5°C) as well as under good ventilation and received water and standard balanced diet. All animal procedures were following the recommendations the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

2.2. Chemicals:

Aspartame in a powder form was purchased from Sigma Chemical Com (Cairo, Egypt).

2.3. Preparation of kiwifruit extract:

Fresh ripe Hayward green kiwi fruits (Actinidia deliciosa) were purchased from a local market in Zagazig city. The fruits were identified and the kiwifruit extract was prepared in the Faculty of Agriculture, Zagazig University, Egypt. Kiwifruit extract was prepared according to the method of Al-Kawaz and AL Mashhady. Kiwi extract (37 g/kg body weight) was given orally using an orogastric tube three times weekly (day after day) for six weeks. The dose selected for the present work is equivalent to 2 kiwi fruits/day for an average human weighing 60 kg. This dose is based on the study of Collins et al., where it was found to be effective in protecting tissues against oxidative damage.

2.4. Experimental Design:

Animals were divided into three groups designed as the following:

- **Group I (Control group):** included 20 rats, further subdivided into two equal subgroups (ten rats each):
  - **Subgroup Ia (Negative control):** they received distilled water and normal food.
  - **Subgroup Ib: (Positive control; Kiwi fruit extract group):** they received Kiwi fruit extract orally using an orogastric tube three times weekly for six weeks.

- **Group II (Aspartame–treated group):** included 10 rats administered aspartame at a dose level of (40 mg/kg) dissolved in 3ml distilled water at room temperature daily by orogastric tube for six weeks. The dose was adjusted according to Mourad and Noor.

- **Group III (Co-administration group):** included 10 rats administered Kiwi fruit extract orally using an orogastric tube three times weekly together with aspartame daily by orogastric tube for six weeks.

2.5. General observations in rats:

During the experimental period, clinical signs and general appearances, which included the amount of food and water consumed, were checked daily. Mortalities of the rats were recorded as it occurred.

2.6. Body weights

The body weight of each animal was assessed before and at the end of the experiment.

2.7. Sampling:

2.7.1. Collection of blood samples

At the end of the experiment period, (24 hours from the last dose), blood samples were collected using capillary tubes from the retro-orbital venous plexus and centrifuged for 10 min at 5000 rpm to obtain clear serum which was stored at −20 °C for different analysis.

2.7.1. Collection of tissue samples for biochemical and histological analysis

Rats from each group were anesthetized by ether inhalation. Five rats from each group were sacrificed. Lungs were rapidly dissected out, washed with saline and cut into small pieces for determination of malondialdehyde (MDA) and superoxide dismutase (SOD) activity. Those parts of lung tissues were washed with ice-cold saline, blot-dried, suspended in phosphate buffer (pH 6) at 5-times then processed in a Potter-Elvehjem homogenizer. The raw homogenate was then frozen at 85°C until used in the various assays.

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Green Kiwi Fruit Extract Ameliorates Aspartame Toxicity on the Lung of Adult Male Abino Rat. Mousa et al.
The remaining five rats from each group were used for evaluation of lung histological changes. These rats were anesthetized with ether and the thorax was opened where lungs were fixed in situ via intra-tracheal instillation of 10% formalin saline solution. Samples from lower right lobes were carefully dissected, fixed in 10% formalin, and then dehydrated with ascending grades of ethanol (70, 80, 90, 95 and 100%). Dehydration was then followed by clearing the samples in 2 changes of xylene. Samples were then impregnated with 2 changes of molten paraffin wax, then embedded and blocked out to paraffin blocks. Transverse sections of the lung sections (4 μm thick) were cut using a microtome and mounted on a glass slide to investigate the histological and immunohistochemical results through the light microscopic examination.

2.8. Histological study:

The sections were stained with:

1-Hematoxylin and eosin stains (H&E): For studying the general histological structure of the lung.

2-Masson trichrome stain: for collagen fibers in the pulmonary stroma.

2. 9. Immunohistochemical study:

2.9.1. Immunohistochemical detection of caspase-3

This was carried out using the streptavidin-biotin complex immunoperoxidase system. Serial sections of paraffin-embedded specimens were deparaffinized on charged slides. The sections were incubated in 0.1% hydrogen peroxide for 30 min to block the endogenous peroxidase. The sections were then incubated with 1.5% non-immunized goat serum for 30 min at room temperature and then incubated with diluted primary antibody (1:500 dilutions).

Primary antibody for caspase 3: A rabbit polyclonal antibody of IgG type was carried out for localization of caspase 3 (GTX 110543). The sections were then washed 3-times with PBS (pH 7.4) for 30 min and then incubated with biotinylated goat anti-mouse immunoglobulin serum for 60 min. After being gently washed with PBS, the sections were incubated with avidin-biotin-peroxidase complex. Ultimately, sites for peroxidase binding were detected using DAB (3, 30-diaminobenzidine) substrate. Tissue sections were then counterstained with hematoxylin and subjected to light microscopy analyses and morphometric measures. The kits were purchased from Sigma–Aldrich.

2.9.2. Immunohistochemical detection of Alpha-smooth muscle actin:

Thick sections (4 μm) were cut from paraffin-embedded blocks, and then deparaffinized in xylene and rehydrated in descending grades series of alcohol, respectively. The sections were boiled in 10 mmol/l citrate buffer (pH 6.0) for 20 min for antigen retrieval and then were left to cool at room temperature. An overnight incubation at room temperature with anti α SMA. Clone1A4 (mouse monoclonal antibody) (Dako, Glostrup, Denmark) with a dilution of 1:50 followed by using the detection kit (Dako) to determine α SMA expression using an appropriate substrate/chromogen (diaminobenzidine reagent) followed by Mayer’s hematoxylin as a counterstain. Then tissue sections were subjected to light microscopy analysis and morphometric analysis.

2.10. Serological assays

2.10.1. Serum TNF-α assay

Serum TNF-α was assayed by using ELISA kit purchased from R&D Systems (Minneapolis, MN, USA) according to the protocol provided by the manufacturer.

2.10.2. Serum NO assay

Serum nitrate concentration as a stable end product of nitric oxide was estimated by the Griess reaction after quantitative conversion of nitrate to nitrite by nitrate reductase using R&D system Gmbtt, Germany.

2.11. Determination of MDA levels and SOD activity in lung tissue

2.11.1. Determination of Malondialdehyde level

MDA (a biomarker of oxidative stress) was measured colorimetrically in lung homogenates using a commercially available kit (Biodiagnostic, Cairo, Egypt).

2.11.2. Determination of SOD activity

The SOD activity was measured in the lung homogenates using a commercial chemical colorimetric assay kit (Biodiagnostic, Giza, Egypt).

2.12. Morphometric analysis:

The thickness of the inter-alveolar septum, area percentage of collagen fibers, and the area percentage of immune reaction to caspase 3 and α SMA were measured within 9 fields for each rat at a total magnification X 400 using image analysis software Fiji image J (National Institute of Health; NIH, Bethesda, MD, USA). The kits were purchased from Sigma–Aldrich (St. Louis, MO).

2.13. Statistical analysis:

Data were presented as mean ± SD. Statistical comparison between groups was determined by one-way analysis of variance (ANOVA) followed by Tukey test for multiple comparison using version 5 Graph Pad Prism (Graph pad Software, Inc., CA, USA). p-value<0.05 is considered significant.
Green Kiwi Fruit Extract Ameliorates Aspartame Toxicity on the Lung of Adult Male Abino Rat. Mousa et al.

2.14. Scaling of histological findings:
Grading of histological and immunohistochemical findings was performed using the data obtained from the morphometric analysis and histological examination.

RESULTS

3.1. General observations in rats:
Rats of aspartame-treated group showed decreased appetite and motor activity. The appetite and activity is relatively returned to normal level in the co-administration group. No mortalities were recorded.

3.2. Body Weight
The final body weight of aspartame-treated group showed a significant decrease in body weight in comparison to negative control group and kiwi fruit extract group. The co-administration group showed a marked increase in body weight which was non-significant with the first two groups (Tables 1&2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup Ia</th>
<th>Subgroup Ib</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>268.63 ± 19.04</td>
<td>263.3 ± 22</td>
<td>266.1 ± 17.9</td>
<td>267.5 ± 18.8</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>328.8 ± 19.96</td>
<td>323.3 ± 24.1</td>
<td>287.2 ± 41.31</td>
<td>311.97 ± 31.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum level of TNF-α (pg/ml)</td>
<td>270.5 ± 77.8</td>
<td>266.88 ± 45.7</td>
<td>436.6 ± 33</td>
<td>299 ± 34.7</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Table 3: Statistical analysis of initial body weight (g) and final body weight (g) of the experimental rat groups using one-way ANOVA test:

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subgroup Ia</td>
<td>0.981**</td>
<td>0.032*</td>
</tr>
<tr>
<td>Subgroup Ib</td>
<td>0.981**</td>
<td>0.076**</td>
</tr>
<tr>
<td>Group II</td>
<td>0.032*</td>
<td>0.076**</td>
</tr>
<tr>
<td>Group III</td>
<td>0.648**</td>
<td>0.857**</td>
</tr>
</tbody>
</table>

ns: non-significant
*: significant

3.3. Serological results:

3.3.1. Serum TNF-α
Serum TNF-α in the aspartame-treated group (436.6± 33) was significantly higher than that of the negative control rats (270.5± 77.8) and that of kiwi fruit extract group rats (266.88 ± 45.7). Co-administration of kiwi fruit extract along with aspartame markedly decreased the level of serum TNF-α (299± 34.7) in comparison with corresponding values in the rats of aspartame-treated group (Tables 3&4, Figure 1).

Fig 1: A bar chart showing the comparison between mean values of Serum vel of TNF-α (pg/ml) among the studied groups.
3.3.2. Serum NO levels

Serum NO levels in the aspartame–treated group (5.64 ± 0.57) was significantly higher than that of the negative control rats (1.67 ± 0.24) and that of Kiwi fruit extract group rats (1.62 ± 0.26). Co-administration of Kiwi fruit extract along with Aspartame markedly decreased the level of serum NO to 1.84 ± 0.5 (Table 5&6, Figure 2).

Table 5. Statistical analysis of the serum NO levels by one-way ANOVA test:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Subgroup Ia</th>
<th>Subgroup Ib</th>
<th>Group II</th>
<th>Group III</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum NO levels</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>p</td>
</tr>
<tr>
<td>(μmol/g)</td>
<td>±SD</td>
<td>±SD</td>
<td>±SD</td>
<td>±SD</td>
<td>±SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.67</td>
<td>1.62</td>
<td>5.64</td>
<td>1.84</td>
<td>&lt;0.001**</td>
<td></td>
</tr>
</tbody>
</table>

*ns: non-significant
**: highly significant

Table 6: Tukey’s test for comparative analysis of the serum NO levels among the groups

<table>
<thead>
<tr>
<th>Subgroup Ia</th>
<th>Subgroup Ib</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.998**</td>
<td>0.998**</td>
<td>0.998**</td>
<td>0.998**</td>
</tr>
<tr>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

3.4. Superoxide dismutase (SOD) activity and Malondialdehyde level in lung tissue:

3.4.1. Superoxide dismutase (SOD) activity:

Tissue SOD activity in the aspartame–treated group (2.06 ± 0.63) was significantly lower than that of the negative control rats (3.59 ± 0.53) and that of kiwi fruit extract group rats (3.6 ± 0.39). Co-administration of kiwi fruit extract along with aspartame markedly increased the tissue SOD activity to 3.03 ± 0.596 (Table 7&8, Figure 3).

Table 7 Statistical analysis of the Superoxide dismutase (SOD) activity in the lung tissue by one-way ANOVA test:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Subgroup Ia</th>
<th>Subgroup Ib</th>
<th>Group II</th>
<th>Group III</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/g)</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>±SD</td>
<td>±SD</td>
<td>±SD</td>
<td>±SD</td>
<td>±SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.59</td>
<td>3.6</td>
<td>2.06</td>
<td>3.03</td>
<td>&lt;0.001**</td>
<td></td>
</tr>
</tbody>
</table>

Table 8: Tukey’s test for comparative analysis of the superoxide dismutase (SOD) activity in the lung tissue among the groups

<table>
<thead>
<tr>
<th>Subgroup Ia</th>
<th>Subgroup Ib</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>1**</td>
<td>1**</td>
<td>1**</td>
<td>1**</td>
</tr>
<tr>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

*ns: non-significant
**: highly significant

Fig. 2: A bar chart showing the comparison between mean values of serum NO levels among the studied groups.

Fig. 3: A bar chart showing the comparison between mean values of SOD activity (U/g) in lung tissue among the studied groups.
3.4.2. MDA levels in the lung tissue:

Tissue MDA levels in the aspartame–treated group (1.623 ± 0.35) was significantly higher than that of the negative control rats (0.98 ± 0.17) and that of kiwi fruit extract group rats (0.94 ± 0.18). Co-administration of kiwi fruit extract along with aspartame markedly decreased the tissue MDA levels to 1.036 ± 0.25 (Table 9&10, Figure 4).

Table 9 Statistical analysis of the malondialdehyde (MDA) levels in the lung tissue by one-way ANOVA test:

<table>
<thead>
<tr>
<th>Group Parameter</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA levels (mmol/g)</td>
<td>0.98 ± 0.17</td>
<td>0.94 ± 0.18</td>
<td>1.623 ± 0.35</td>
<td>1.036 ± 0.25</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Table 10: Tukey’s test for comparative analysis of the malondialdehyde (MDA) levels in the lung tissue among the groups

<table>
<thead>
<tr>
<th>Subgroup Parameter</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subgroup Ia</td>
<td>0.984**</td>
<td>0.984**</td>
<td>0.964**</td>
<td>&lt;0.001**</td>
<td></td>
</tr>
<tr>
<td>Subgroup Ib</td>
<td>0.984**</td>
<td>0.984**</td>
<td>0.837**</td>
<td>&lt;0.001**</td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>0.964**</td>
<td>0.837**</td>
<td>&lt;0.001**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ns: non-significant
*: significant
**: highly significant

3.5. Histological results:

The negative control group and the kiwi extract group showed nearly the same histological and immunohistochemical results.

3.5.1. Hematoxylin and eosin (H&E):

Hematoxylin and eosin (H&E) stained lung sections from rats of the negative control and kiwi fruit extract groups (Fig.5 A) showed normal alveoli with thin walls and inter-alveolar septae. Sections from the lung of rats of the aspartame-treated group (Fig.5 B) showed widespread diffusion of inflammatory reactions throughout the lung with marked mononuclear cell infiltration, vascular congestion, and area of hemorrhage. There were thick inter-alveolar septa with collapsed alveoli. The co-administration group (Fig.5 C) showed improved histological features compared to that of rats of the aspartame-treated group. There was mild congestion of blood vessels. The thickness of inter-alveolar septum returned to almost the normal level. There were a few areas of inflammatory cells infiltration. Statistical analysis of the thickness of inter-alveolar septum is shown in Tables 11&12.s

Table 11 Statistical analysis of the thickness of the inter-alveolar septum (µm) by one-way ANOVA test:

<table>
<thead>
<tr>
<th>Group Parameter</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness of the inter-alveolar septum (µm)</td>
<td>5.3 ± 1.7</td>
<td>5.2 ± 1.3</td>
<td>21.2 ± 5.8</td>
<td>7.6 ± 1.2</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Table 12: Tukey’s test for comparative analysis of the thickness of the inter-alveolar septum (µm) among the groups

<table>
<thead>
<tr>
<th>Subgroup Parameter</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subgroup Ia</td>
<td>0.369**</td>
<td>0.369**</td>
<td>0.369**</td>
<td>&lt;0.001**</td>
<td></td>
</tr>
<tr>
<td>Subgroup Ib</td>
<td>0.322**</td>
<td>0.322**</td>
<td>0.322**</td>
<td>&lt;0.001**</td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>0.369**</td>
<td>0.322**</td>
<td>&lt;0.001**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ns: non-significant
**: highly significant
3.5.2. Masson Trichome stain:

Staining for collagen fibers in lung sections using Masson trichome stain (Fig. 6 A) indicated that control and Kiwi fruit extract rats show a minimal amount of collagen fibers in the inter-alveolar septae. In the aspartame-treated group (Fig. 6 B), there was excessive deposition of collagen fibers especially detected in the peribronchial, perivascular and inter-alveolar septae. The co-administration group (Fig. 6 C) showed a significant decrease in the collagen fibers (Tables 13 & 14).

Table 13 Statistical analysis of the area percentage of collagen fibers by one-way ANOVA test:

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>Subgroup</th>
<th>Group II</th>
<th>Group III</th>
<th>Parameter</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ia</td>
<td>Ib</td>
<td></td>
<td></td>
<td>Area percentage of collagen fibers</td>
<td>5.3 ± 1.7</td>
<td>5.2 ± 1.3</td>
<td>21.2 ± 5.8</td>
<td>7.6 ± 1.2</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Table 14: Tukey’s test for comparative analysis among the groups

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Group Ia</th>
<th>Subgroup</th>
<th>Group Ib</th>
<th>Group II</th>
<th>Group III</th>
<th>Parameter</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>0.879**</td>
<td>0.879**</td>
<td>0.879**</td>
<td>0.322**</td>
<td>0.08 ± 0.16</td>
<td>Area percentage of immune reaction to Alpha-smooth muscle actin</td>
<td>0.9 ± 0.86</td>
<td>9.9 ± 5.2</td>
<td>5.2 ± 1.76</td>
<td>&lt;0.001**</td>
<td></td>
</tr>
<tr>
<td>Ib</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ns</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ns: non-significant
**: highly significant

3.6. Immunohistochemical results

3.6.1. Alpha smooth muscle actin

Sections stained with Alpha-smooth muscle actin showed that control (Fig. 7 A) rats showed a very weak cytoplasmic reaction. In the aspartame-treated group (Fig. 7 B), the reaction was strongly positive. In the co-administration group (Fig. 7 C), the reaction significantly reduced, but still higher than the control group (Tables 15 & 16).
Green Kiwi Fruit Extract Ameliorates Aspartame Toxicity on the Lung of Adult Male Abino Rat. Mousa et al.

3.6.2. Caspase 3

Sections stained using caspase 3 indicated that control (Fig. 8 A) rats show very weak cytoplasmic reaction. In the aspartame-treated group (Fig. 8 B), the was strongly positive cytoplasmic reaction. In the co-administration group (Fig. 8 C), the reaction significantly reduced, but still higher than the control group (Tables 17&18).

Table 17 Statistical analysis of the area percentage of immune reaction to caspase 3 in the lung tissue by one-way ANOVA test:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subgroup Ia</th>
<th>Subgroup Ib</th>
<th>Group II</th>
<th>Group III</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ±SD</td>
<td>1.02 ± 0.31</td>
<td>1.016 ± 0.36</td>
<td>13.99 ± 3.41</td>
<td>6.6 ± 1.8</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Table 18: Tukey’s test for comparative analysis of the area percentage of immune reaction to caspase 3 in the lung tissue among the groups

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Ib</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Group II</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Group III</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

3.7. Grading of histological findings:

Grading of histological and immunohistochemical findings was demonstrated in the following table (table 10):

Table 10. showing the grading of histopathological lesions

<table>
<thead>
<tr>
<th>Group</th>
<th>Negative Control Group</th>
<th>Kiwi fruit extract Group</th>
<th>Aspartame-treated Group</th>
<th>Co-administration Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickened inter-alveolar septum</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Areas of congestion and hemorrhage</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Inflammatory cells infiltration</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Increased apoptosis</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Excessive deposition of collagen fibers</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

DISCUSSION

Today's interest in fitness and physical health has increased and so many low-calorie, non-weight-bearing dietary alternatives have been sought, including aspartame\[27, 28\].

In the present study, a significant decrease in the body weight in the aspartame-treated group was detected. Many researchers explained the mechanisms by which aspartame can reduce weight. Rogers et al.[29] mentioned that aspartame induced satiety leading to weight loss. Hall et al.[30] attributed the satiating effect of aspartame to the rising circulating levels of phenylalanine causing suppression of food intake in humans and animals and increased cholecystokinin secretion that delay gastric emptying. Also, Beck et al.[31] suggested that aspartame decreased effects of neuropeptide Y on lipid metabolism. Neuropeptide Y is the peptide that promotes weight gain and fat deposition.
Antioxidants and free radical scavenging systems exist in the cells to protect them against the damaging effects of free radicals\[32]. The present study showed that the oral administration of aspartame resulted in a significant decrease in the level of SOD and a significant elevation in MDA in the lung tissue and a significant increase in the serum nitric oxide (NO) levels as (markers of oxidative stress) compared to the control group. This was in agreement with Mourad and Noor\[16] who mentioned that oral administration of 40 mg/kg of aspartame at the periods of 2, 4 and 6 weeks induced oxidative stress in the liver and kidney of male albino rats.

Tumor necrosis factor- alpha (TNFα) is one of the serum cytokines which represent the chemical messengers between immune cells, playing important roles in the immune response\[46]. The results of this study showed a significant increase in the serum level of TNFα in aspartame treated group compared to the control group. These results were in agreement with Gul et al.,\[33\] who reported a significantly increased level of TNFα in mice treated with aspartame. Also, Ashok et al.,\[34\] mentioned that the alteration observed in the serum levels of cytokines after aspartame administration indicates that its action not only at the cellular level but also affects cytokine expressions.

In our study, histological examination of the lung obtained from aspartame-treated group showed apparent histological changes in the form of numerous alveoli with collapsed alveolar walls, congestion of the blood vessels and appearance of inflammatory cells in the inter-alveolar septa. These results agreed with El Haliem and Mohamed\[15\] who mentioned that the effect of aspartame was evident on the hepatic tissue, where tissue degradation and change in structure of liver cells showed signs of cellular death. In addition, there was a marked dilatation and congestion of blood vessels. Invasion of inflammatory cells was observed in some areas around the central vein.

Many researches explained the mechanism of aspartame induced toxic effects. They mentioned that the metabolism of aspartame in the gastrointestinal tract leads to the formation of three main components; aspartic acid, phenylalanine, and methanol. Methanol is primarily metabolized by oxidation to formaldehyde and then to formate; this leads to the formation of superoxide anion and hydrogen peroxide. Also, aspartic acid induces the formation of free radicals\[36, 37\]. Therefore, the histopathological changes observed in aspartame treated group may attribute to accumulation of free radicals and increased oxidative damage to cellular proteins.

Masson trichrome stained sections in aspartame treated group revealed increased amount of collagen fibers around blood vessels and bronchioles and also in the inter-alveolar septa. Mourad\[38\] attributed the increase in collagen and ground substance formation in aspartame treated animals to the increased lipid peroxidation which has an oxidative damaging effect on proteins and nucleic acids. This opinion is in agreement with Khidr et al.,\[39\] who observed a significant elevation in lipid peroxidase level in rat tissues after 28 days treatment with aspartame.

Recently, it has been shown that the proliferating cells in the alveolar interstitium contain α-smooth muscle actin (α- SMA) as do myofibroblasts in wound healing and other fibroactive diseases\[39\]. In this study, the immunohistochemical examination of α- SMA showed a significantly increased expression in lung tissues of aspartame treated group compared to the control group. Several histochemical studies have shown that the increased numbers of myofibroblasts, characterized by α- SMA expression, in the lung represent the cell type most responsible for the increase in lung type 1 collagen expression. Thus, these cells appear to play an important role in the pathogenesis of pulmonary fibrosis, and their presence may contribute to the increased extracellular matrix deposition and contractility of lung tissue\[40\].

Apoptosis is a physiological process that is considered as an important mode of programmed cell death. The caspase cascade system plays a vital role in the induction, transduction, and amplification of intracellular apoptotic signals. Caspase-3 is a key factor in apoptosis\[41\].

In our study, there was a significantly increased expression of activated caspase 3 in aspartame treated animals when compared to control group. This agreed with Ashok and Sheeladevi\[22\] who concluded that aspartame exposure causes increased production of free radicals playing a significant role in the apoptotic death. Free radicals can also attack DNA strand to induce breaks and base modifications that can lead to point mutation\[43\].

Our results revealed that kiwi fruit extract co-administration resulted in a significant alleviation of aspartame-induced pulmonary changes as evidenced by the improvement of the biochemical indices and structural profiles. Similar results were recorded by Amer et al.,\[7\] who reported that KFE ameliorated gastric and hepatic lesions induced by indomethacin. Also, administration of kiwi fruit juice to mice induced a marked decrease in urinary oxidative stress markers and exerted antioxidant effects in a dose-dependent manner\[44\].

As the pulmonary fibrosis induced by aspartame in group II resulted from the increased oxidant burden together with deficiency of antioxidants. A rationale therapeutic approach is to reverse the imbalance between oxidants and antioxidants in the lung by enhancing the antioxidant system. Therefore, kiwi fruit could protect against aspartame induced lung fibrosis via its antioxidant activity\[27\].

 jumlah
The ameliorative effects of kiwi fruit extract on the aspartame-induced lung injury may be attributed to the combination of several different mechanisms including antioxidant activity, anti-inflammatory properties, and direct scavenging of free radicals resulting in a reduction of oxidative stress and lipid peroxidation. It also inhibits lipid peroxidation by metal chelating activity\[45\].

The antioxidant effect of kiwi fruit has been linked to the additive and synergistic effects of its bioactive substances such as polyphenols, flavonoids, carotenoids, and vitamin C\[46\]. Kiwi fruit contains a higher source of potentially antioxidant polyphenol content than in other fruits. Polyphenols could enhance the synthesis of endogenous antioxidant enzymes, such as superoxide dismutase and catalase\[47\], while their potential anti-inflammatory property attributed to inhibition of inducible nitric oxide synthase and expressions of cyclooxygenase 2 enzyme\[48\].

It also contains isoflavones and flavonoids which have an important function as anti-carcinogenic, neuroprotective, and cardioprotective activities. Carotenoids, flavonoids, and vitamin C are also known for their strong antioxidant activities\[49\].

Apart from vitamin C and polyphenols, the antioxidant capacity of kiwi fruit is also related to the presence of an antioxidant peptide called iskaper, a 39 residue peptide\[50\]. The iskaper peptide was also able to control the release and expression of proinflammatory cytokines TNF-α, ICAM-1 and COX-2 in a mixed in vivo-in vitro study\[51\].

CONCLUSION

The present study showed that administration of aspartame to albino rats exerted harmful effects on the lung as manifested by histological and immunohistochemical examination. Therefore, it is recommended to restrict or avoid the use of aspartame as a sustainable source of sweetness in routine life, and if its use is necessary, decrease the dose. Also, our study revealed that kiwi fruit has significant protective effects against aspartame induced pulmonary damage. So, kiwi fruit is recommended as a nutritional supplement with antioxidant activity.

ABBREVIATIONS

TASP: Aspartame  
FDA: Food and Drug Administration  
GLP-1: glucagon-like peptide-1  
GST: glutathione-S-transferase  
H2O2: hydrogen peroxide  
KFE: kiwi fruit extract  
MDA: malondialdehyde  
NO: nitric oxide  

PBS: phosphate buffer saline  
ROS: Reactive oxygen species  
SOD: Superoxide dismutase  
TNF-α: Tumor necrosis factor-α  
α SMA: Alpha-smooth muscle actin.

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CONFLICT OF INTEREST

There are no conflicts of interest.

AUTHOR CONTRIBUTION

All authors shared in designing and performing the experiment. Dr. Hanaa S.E. Mousa wrote the results. All authors participated in revising the final manuscript.

REFERENCES


الملخص العربي

مستخلص فاكهة الكيوي الخضراء يحسن الآثار السمية للأسبارتام على رئة جرذ الذكر البالغ (دراسة هستولوجية و هستوكيميائية مناعية).

هناك سعاد السيد موسى - كريمة فوزي عبد الفضل - محمد زايد محمد حسن - هناء سعيد السيد موسى 1 - كريمة فوزي عبد الفضل 1 - محمد زايد محمد حسن 1 - هناء سعيد السيد موسى.


الخلاصة: الأسبارتام هو محلى صناعي واسع الاستخدام يوجد في العديد من الأطعمة والمشروبات، يستهلك من قبل مرضى السكر وإنقاص الوزن كما أن فاكهة الكيوي لها تأثيرات مضادة للأكسدة ومضادة للالتهابات.

الهدف من الدراسة: تقييم تأثير الأسبارتام والدور المحتمل لفاكهة الكيوي على التركيب الهستولوجي لرئة في ذكور الجرذان البالغة.

المؤسسة والمعدات: تم استخدام أربعين من الذكور البالغين من الجرذان البيضاء في ثلاث مجموعات المجموعة الأولى (المجموعة الظابطة) وشملت على 20 جرذ وقد قسمت بالتساوي إلى مجموعة فرعية ضابطة سالبة، ومجموعة فرعية ضابطة إيجابية، والمجموعة الثانية (مجموعة الأسبارتام) وشملت على 10 جرذان وقد تم إعطاؤها الأسبارتام عن طريق الفم بجرعة 40 مجم / كجم مذاب في الماء المقطر يومياً لمدة سنة سابعة، والمجموعة الثالثة تلقت خلاصة فاكهة الكيوي عن طريق الفم بجرعة 37 مجم / كجم من وزن الجسم ثلاث مرات أسبوعياً مع الأسبارتام 40 مجم / كجم لمدة سنة سابعة، تم قياس وزن الجسم وعمل نخور الورم وأكسيد النيترات في الدم مستويات مالونهايد ونشاط سوبر اكسيد ديسموتاز في نسيج الرئة. أيضاً تم فحص الرئة من جميع المجموعات هستولوجيا باستخدام صبغة الهيماتوكسيلين و الإيوسين و صبغة الماسون و الكاسبار 3 و الإلفا 3 و الإلفا 4 وأكين للعضلات المياسم.

النتائج: تسبب الأسبارتام في ارتفاع مستوى عامل نخر الورم وأكسيد النيترات في الدم وارتفاع مستويات مالونهايد وانخفاض نشاط سوبر أكسيد ديسموتاز في نسيج الرئة وكذلك تسبب في حدوث تغييرات واضحة في نسج الرئة، أما المجموعة الثالثة فاظهرت تحسنًا في مستويات عامل نخر الورم وأكسيد النيترات في الدم ومستوى مالونهايد ونشاط سوبر أكسيد ديسموتاز في نسيج الرئة وأيضا تحسن في التركيب الهستولوجي للرئة.

الخلاصة: يوصى بتقليل استهلاك الأسبارتام بسبب التأثير السام المؤكد على الرئة وينصح أيضًا بتناول الكيوي باانتظام.