

Original Article	A Histological Study of the Effect of Aspartame Versus Sucralose on the Spleen of Adult Male Albino Rats <i>Shereen Adel Saad</i> <i>Anatomy department, Faculty of Medicine, Ain Shams University</i>
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ABSTRACT

Introduction: Aspartame (ASP) is one of the most widely artificial sweeteners consumed worldwide. Some studies declared that administration of ASP even at the Food and Drug Administration permitted level causes oxidative stress by altering the oxidant /antioxidant balance in immune organs of the rats. On the other hand, sucralose is another artificial high-potency sweetening compound. It has been branded under the name Splenda. It is found in many food and beverage products. Studies observed that sucralose administration caused reduction of weight of the spleen and histopathological changes in the thymus of rats.

Aim of the work was to study the histological and ultrastructural changes of the spleen of adult male albino rats, upon exposure to ASP and sucralose.

Materials and Methods: thirty male albino rats were divided into three groups Group I control group: ten adult rats received 1 ml distilled water daily for 3 months. Group II (Aspartame group): ten adult rats administered aspartame (40 mg/kg) daily dissolved in 1 ml distilled water daily for 3 months. Group III (sucralose group): ten rats received sucralose 15 mg/kg daily dissolved in 1 ml distilled water daily for 3 months. At the end of the experiment, spleen specimens were dissected and processed for Both light microscope and ultra-structural examination.

Results: Histological examination of Hx. & E sections of the spleen of ASP group revealed loss of architecture of the spleen accompanied by partial disappearance of demarcation between white and red pulps. Lymphoid follicles showed depletion of lymphocytes. Examination of the ultrathin sections showed degenerated lymphocytes in the white pulp, with highly condensed pyknotic nuclei. In addition, cytoplasm revealed the presence of vacuoles. On the other hand, examination of Hx. & E of the spleen of sucralose group revealed complete loss of architecture of spleen with disappearance of demarcation between red and white pulps. Marked areas of depletion of lymphocytes in both red and white pulps and elongated and thickened fibrous tissue strands were observed. Examination of the ultrathin sections showed extensive red pulp with degenerated white pulp. Degenerated lymphocytes with irregular indented nuclei with widening of peri-nuclear space. In addition degenerated cytoplasm, many vacuoles and esinophil infiltration.

Conclusion: The present study clearly points that both ASP and sucralose can influence the structure of the spleen within the acceptable daily consumption. Whereas sucralose had more degenerative effects on the spleen.

Key Words: Aspartame - Sucralose - Spleen

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INTRODUCTION

Artificial sweeteners have increasingly become an area of controversy in the world of food and nutrition in the last few years (Ahmed Saad 2014).

Aspartame (ASP) (L- aspartyl- L-phenylalanine methyl ester) is one of the most widely artificial

sweeteners consumed worldwide, most commonly found in low calorie beverages, desserts and table top sweeteners added to tea or coffee (Butchko and Stargel, 2001; Oyama et al., 2002). Metabolism of ASP provides approximately 4 kcal/g of energy. However, this energy is negligible as the high intensity sweetening power of ASP approximately

200 sweeter than sucrose by weight which means that little is needed to be added to foods to achieve sweetness (Gougeon *et al.*, 2004; Magnuson *et al.*, 2007).

The established acceptable daily intake of ASP in humans is maintained at 40 mg/kg of body weight (European Food Safety Authority (EFSA), 2006). Earlier studies have investigated that ASP induced hepato-toxic (Iman, 2011) and nephro-toxic effects in rats (Hoda and Mona, 2014). Moreover, ASP was reported to be a multi-potential carcinogenic agent inducing a dose-related, significant increase in lymphomas and leukemias in females (Morando *et al.*, 2007). Other studies declared that administration of ASP even at the Food and Drug Administration permitted level causes oxidative stress by altering the oxidant /antioxidant balance in immune organs of the rats (Choudhary and Devi, 2014).

Sucralose is another artificial high-potency sweetening compound. It has been branded under the name Splenda (Whitehouse *et al.*, 2008). It is a chlorinated disaccharide (1,6-dichloro-1,6-dideoxy- β -D-fructofuranosyl-4-chloro-4-deoxy- α -D-galactopyranoside) (Federal Register, 1998). Sucralose is found in many food and beverage products, used because it is a no-calorie sweetener (Food & drug administration, 2006). In addition sucralose is used as a replacement for, or in combination with, other artificial or natural sweeteners such as aspartame, acesulfame potassium or high-fructose corn syrup. The commercial success of sucralose-based products stems from its favorable comparison to other low-calorie sweeteners in terms of taste, stability and safety. It is used in products such as candy, breakfast bars and soft drinks and canned fruits where in water and sucralose take (Ford, *et al.*, 2011). Sucralose is approximately 600 times sweeter than sucrose by weight (Schiffman *et al.*, 2008). The established acceptable daily intake of sucralose is mentioned as 15mg / kg of body weight (Rahmani and Ranjbar, 2014).

Sasaki *et al.* (2002) declared that ingested sucralose induces DNA damage in gastrointestinal organs. In addition, research on rats and rabbits had shown that sucralose causes many problems like enlarged liver and spleen, decreased red blood cells, reduced

growth rate and extension in the pregnancy period and aborted pregnancy (Bown, 2003). Moreover, Rodero *et al.* (2009) & Rahiman and Pool (2013) declared that exposure to sucralose induces a reduced humoral response that may be associated with adverse effects on the immune system. Goldsmith and Meckel (2001) observed that sucralose administration caused reduction of weight of the spleen and histopathological changes in the thymus of rats. Bown (2003) added that sucralose administration causes atrophy of lymph follicles in spleen and thymus and shrunken thymus glands (up to 40%).

Although, some studies declared that aspartame and sucralose have deleterious effects on immune system, yet histological details of these effects were lacking in the available literature. Hence the aim of this work was to study the histological and ultrastructural changes of the spleen of adult male albino rats, upon exposure to ASP and sucralose. .

MATERIAL AND METHODS

Animals

Thirty adult male albino rats weighing 180 - 220 gm (4- 6 months) were obtained from The Animal House of The Bilharzial Research Unit, Ain Shams University. The animals were housed in conventional wire-mesh cages in a room temperature. Rats were fed on standard rat diet and allowed free water access. Animals were allowed to acclimatize to experimental conditions by housing them for 10 days prior to the experiment.

Drugs

ASP was purchased from Amryia pharmaceutical company (20 mg / tablet). Each tablet Was dissolved in 2.5 ml of distilled water and each rat received 1 ml containing 8 mg of ASP (EFSA, 2006).

Sucralose was purchased from Al Madina pharmaceutical company as powder. Every 3 mg of sucralose was dissolved in 1 ml distilled water and each rat received 1 ml containing 3 mg of sucralose. Both were given orally by gastric intubation to the rats for 3 months (Rahmani and Ranjbar 2014).

Experimental protocol

Animals were divided into three groups, ten rats each:

Group I (control group): Ten adult rats received 1 ml distilled water daily for 3 months.

Group II (Aspartame group): Ten adult rats administered aspartame (40 mg/kg) daily dissolved in 1 ml distilled water daily for 3 months (EFSA, 2006).

Group III (Sucralose group): Ten rats received sucralose (15 mg/kg) daily dissolved in 1 ml distilled water daily for 3 months (Rahmani and Ranjbar 2014).

At the end of the experiment, all rats were anaesthetized using ether inhalation. The spleen was dissected. Specimens for the light microscopic examination were fixed in 10% neutral-buffered formalin, dehydrated, and embedded in paraffin blocks. 5µm-thick sections were subsequently cut and stained with Hx. & E (Drury & Wallington, 1980) and (Bancroft & Gamble, 2002).

Other specimens 1mm³ in size were fixed in 4% glutaraldehyde and processed for electron microscopic examination. Semithin sections were stained with Toluidine blue and examined by light microscope. The ultrathin sections were stained with Uranyl Acetate and Lead Citrate, examined and photographed under Philips 201-transmission electron microscope (Graham & Orenstein, 2007).

RESULTS

Control group (group I):

Histological examination of Hx. & E sections of the control spleen revealed that spleen had two distinct zones the white pulp and the red pulp. Clear demarcation was evident between both pulps. The spleen was surrounded by thin connective tissue capsule, few septa were seen extending into the parenchyma of the spleen (Fig. 1). The white pulp consisted of lymphoid tissue, which was subdivided into the periarteriolar lymphatic sheath (PALS), rounded lymphoid follicles and the peripheral pale marginal zone. PALS was composed of

small dark lymphocytes surrounding central arteriole (Figs. 1, 2). In semithin sections lymphatic follicles were seen as aggregations of lymphocytes of variable sizes. Lymphocytes appeared as dense basophilic cells with scanty cytoplasm and large nucleus. Few pale stained cells were observed among the lymphocytes (Figs. 3, 4). Both the PALS and follicles were separated from the red pulp by the marginal zone which appeared as a thin layer of less densely populated lymphocytes (Fig. 2). The red pulp appeared as irregular cords of blood cells intermingled with few lymphocytes and separated by venous sinusoids (Figs. 2, 4). Elongated fibers were observed extending between cells of both red and white pulps (Fig. 3).

Examination of the ultrathin sections of the control spleen clarified that lymphocytes appeared variable in size, with high nuclear cytoplasmic ratio. The nuclei were large, oval or rounded, heterochromatic with irregular rim of dense chromatin beneath the nuclear membrane and prominent nucleoli. The scanty cytoplasm contained few ill defined organelles (Fig. 5). Other lymphocytes appeared relatively large in size with prominent cytoplasm which contained numerous ribosomes and mitochondria variable in size and shape (Fig. 6).

Aspartame group (group II): Histological examination of Hx. & E sections of the spleen of ASP group revealed loss of architecture of the spleen accompanied by partial disappearance of demarcation between white and red pulps. Loss of the three distinct zones of the white pulp (PALS, lymphatic follicle and marginal zone) was observed (Fig. 7). Areas of depletion were noticed in both white and red pulps (Figs. 7, 8). Lymphoid follicles showed depletion of lymphocytes (Fig 9) other follicles appeared irregular and fused together and formed confluent mass of lymphocytes (Fig. 8). In addition the capsule appeared thickened in some sections (Fig. 9) and separated in others (Fig.7). Fibrous tissue strands were observed between white and red pulps (Fig.9).

Semithin sections showed degenerated PALS, with irregular clusters of lymphocytes and multiple small vacuoles around the central arteriole (Fig. 10). Some lymphocytes had

pyknotic nuclei (Fig. 11). Plasma cells, with cart wheel appearance and few bilobed cells were observed. Some large oval giant cells with elongated nucleus or binucleated and pale cytoplasm were noticed (Fig. 11). Thickened fibers were observed (Figs. 10, 11).

Examination of the ultrathin sections of the spleen of ASP group showed degenerated lymphocytes in white pulp, with highly condensed pyknotic nuclei (Fig. 12), other lymphocytes appeared with irregular and indented nuclei (Fig. 13). Cytoplasm showed aggregations of mitochondria (Figs. 12, 13) and focal areas of degeneration (Fig. 13). In addition, cytoplasm revealed the presence of vacuoles. Most of the vacuoles were small in size, scattered (Figs. 12, 13) and few were large and adherent (Fig. 13).

Sucralose group (group III):

Histological examination of Hx. & E sections of the spleen of sucralose group revealed complete loss of architecture of spleen with disappearance of demarcation between red and white pulps. Marked areas of depletion of lymphocytes in both the red and the white pulps were observed (Figs. 14, 15). Elongated and thickened fibrous tissue strands were extending throughout the parenchyma (Fig. 15). Thickening of the capsule and

trabeculae was observed in some sections (Fig. 14) while irregular capsule was noticed in other sections (Fig. 15).

In semithin sections, there was marked depletion of lymphocytes. Most of the lymphocytes were small in size and few were large. Some lymphocytes appeared with small dense nuclei (Figs. 16, 17), other lymphocytes appeared, with pyknotic nuclei and rarified rim of cytoplasm (Fig. 18). Marked congestion was observed in the relatively abundant red pulp (Fig. 17). Small rounded vacuoles were seen (Fig. 17). Some neutrophils (Fig. 16), plasma cells with characteristic cart wheel appearance, fibroblast and bilobed esinophils were observed (Fig. 18).

Examination of the ultrathin sections of the spleen of sucralose group showed extensive red pulp with degenerated white pulp (Figs. 19, 20). Degenerated lymphocytes with irregular, indented nuclei (Figs. 21, 22) or pyknotic nuclei with widened perinuclear space (Figs. 20) were observed. Other lymphocytes showed degenerated cytoplasm (Fig. 21), aggregated groups of mitochondria (Figs. 19, 21). Moreover, vacuoles of different sizes were observed either small (Figs 19, 22), or large or joining each other (Fig. 21). Esinophil infiltration was detected (Fig. 22).

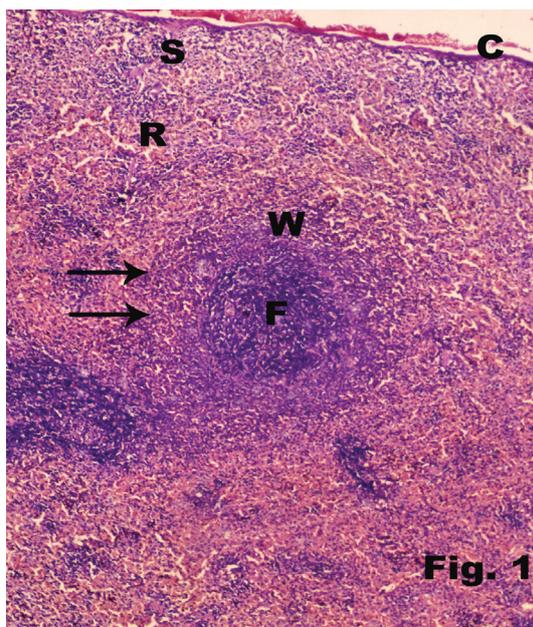


Fig. 1: A photomicrograph of a section of the spleen of group I (control) showing white pulp (W) and red pulp (R). Note: clear demarcation between red and white pulps (↑), thin connective tissue capsule (C) with few septa (S) and rounded lymphatic follicle (F).
Hx.&E.; X200

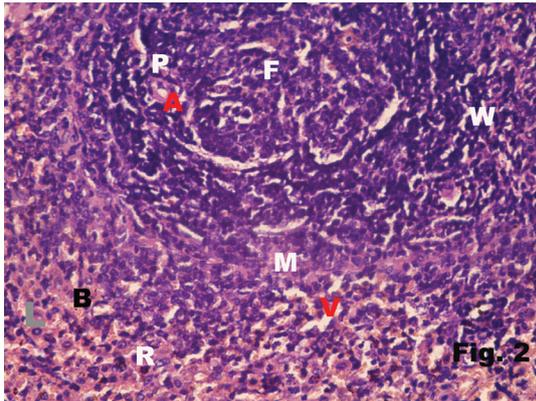


Fig. 2: A photomicrograph of a section of the spleen of group I (control) showing white pulp (W) and red pulp (R). The white pulp consists of periarteriolar sheath (P), rounded lymphoid follicle (F) and peripheral pale marginal zone (M). Red pulp consists of irregular cords of blood cells (B) intermingled with few lymphocytes (L) and separated by venous sinusoids (V). Note a central arteriole (A). Hx.&E.; X400

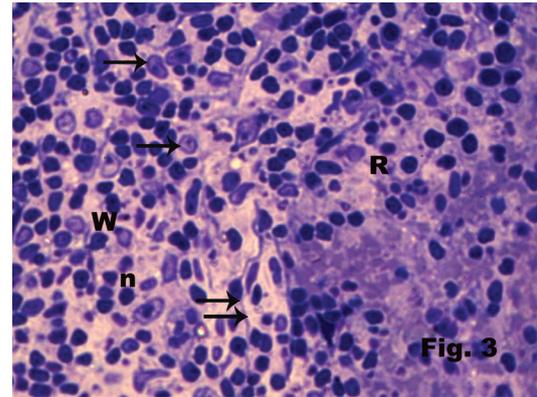


Fig. 3: A Semithin section of the spleen of group I (control) showing white pulp (W) and red pulp (R). Lymphocytes appear as dense basophilic cells with large nucleus (n) and scanty cytoplasm. Few pale staining cells are seen (↑). Note the elongated fibers (↑↑). Toluidine blue; X 1000

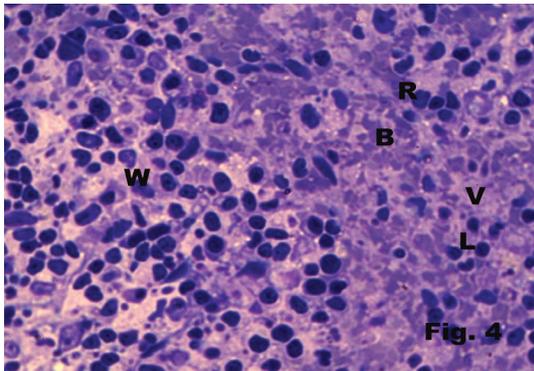


Fig. 4: A Semithin section of the spleen of group I (control) showing white pulp (W) and red pulp (R). Red pulp consists of irregular cords of blood cells (B) intermingled with few lymphocytes (L) and separated by venous sinusoids (V). Toluidine blue; X 1000

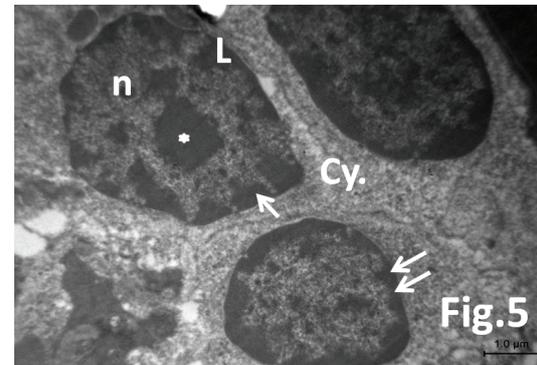


Fig. 5: An electron micrograph of a section of the spleen of group I (control) showing lymphocytes (L). The nuclei (n) appear heterochromatic with irregular rim of dense chromatin (↑) and prominent nucleoli (*). Note scanty cytoplasm (cy.). Uranyl acetate and lead citrate X 8000

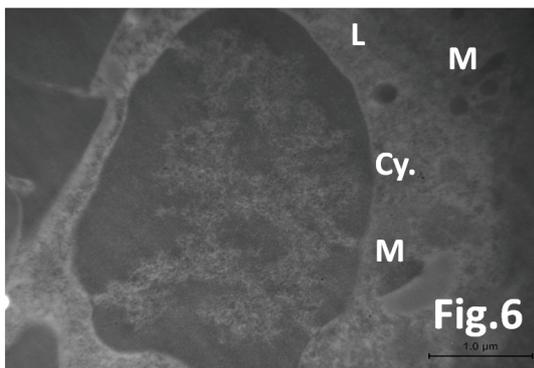


Fig. 6: An electron micrograph of a section of the spleen of group I (control) albino rat showing relatively large lymphocyte (L) with prominent cytoplasm (cy.) containing numerous ribosomes and mitochondria (M) variable in size and shape. Uranyl acetate and lead citrate X 15,000

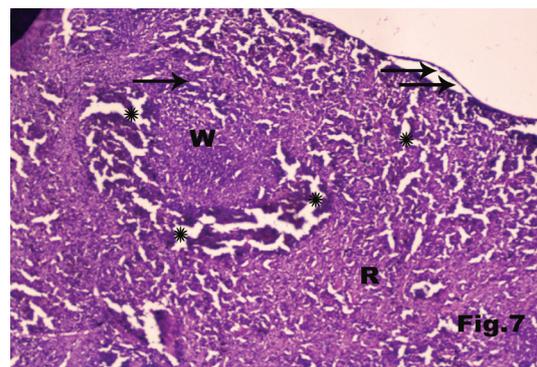


Fig. 7: A photomicrograph of a section of the spleen of group II (ASP) showing partial disappearance of the demarcation (↑) between white (W) and red (R) pulps. Areas of depletion (*) in both white and Red pulps and loss of the three distinct zones of white pulp are observed. Separation of the capsule in some regions (↑↑). Hx.&E.; X200

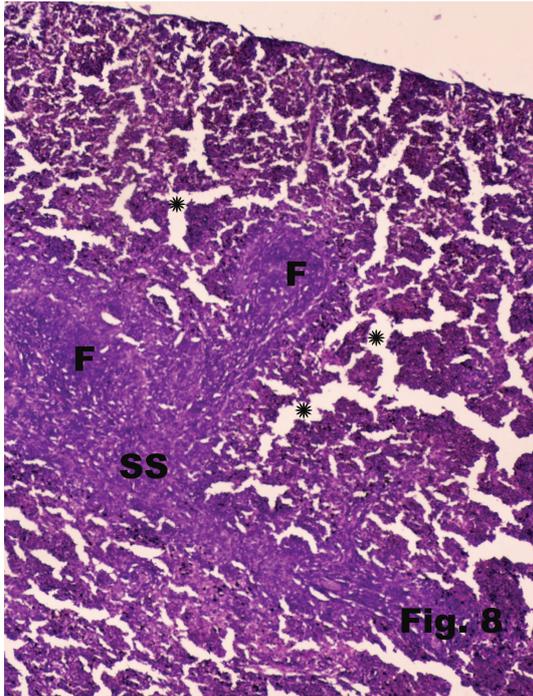


Fig. 8: A photomicrograph of a section of the spleen of group II (ASP) showing irregular lymphoid follicles (F) fused together forming a confluent mass of lymphocytes (SS). Areas of depletion (*) in the red pulp are noticed. Hx.&E.; X400

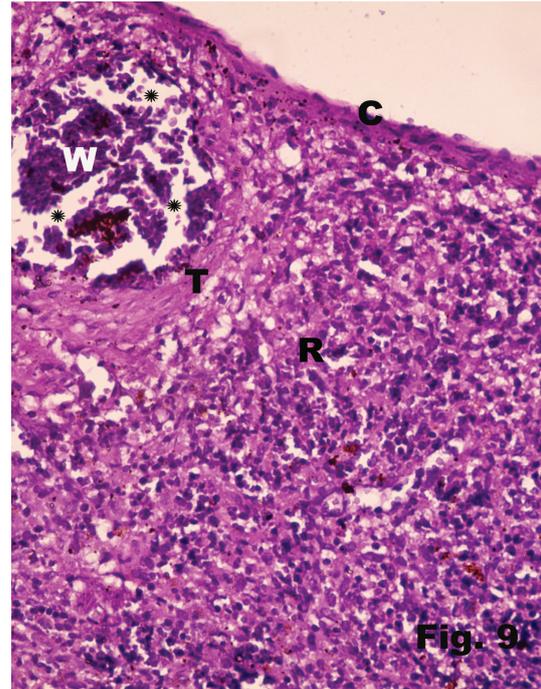


Fig. 9: A photomicrograph of a section of the spleen of group II (ASP) showing thickening of the capsule (C), lymphocytic depletion (*) in the lymphoid follicle. Note fibrous tissue strands (T) between white (W) and red (R) pulps. Hx.&E.; X400

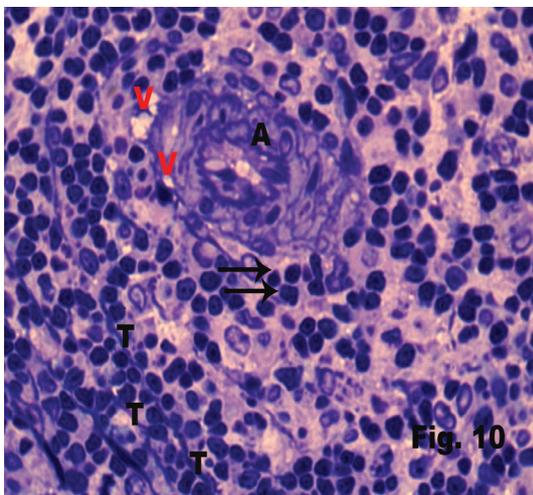


Fig. 10: A Semithin section of the spleen of group II (ASP) showing degenerated PALS with irregular clusters of lymphocytes (↑↑) and multiple small vacuoles (v) around the central arteriole (A). Thickened fibers (T) are seen. Toluidine blue; X 1000

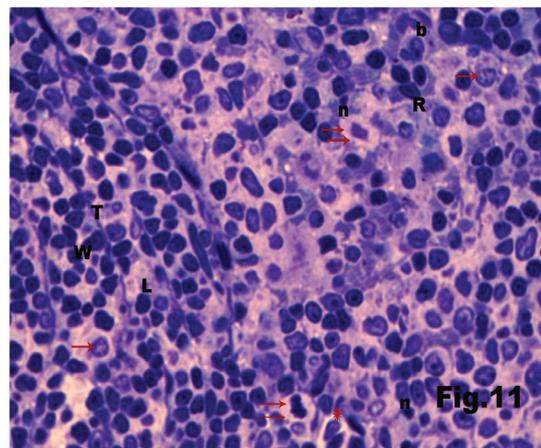


Fig. 11: A Semithin section of the spleen of group II (ASP) showing both white pulp (W) and red pulp (R). White pulp consists of lymphocytes (L) of variable size. Some lymphocytes appear with pyknotic nuclei (n). Note cart wheel plasma cells (↑), few bilobed cells (b) and large pale oval cells with elongated nucleus (↑↑) or binucleated (*). Note thickened fibers (T). Toluidine blue; X 1000

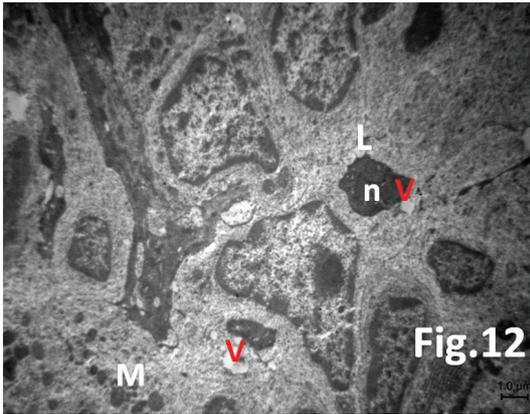


Fig. 12: An electron micrograph of a section of the spleen of group II (ASP) showing degenerated lymphocytes (L) with highly condensed pyknotic nuclei (n). Groups of mitochondria (M) and small scattered vacuoles (v) are observed.

Uranyl acetate and lead citrate X 4000

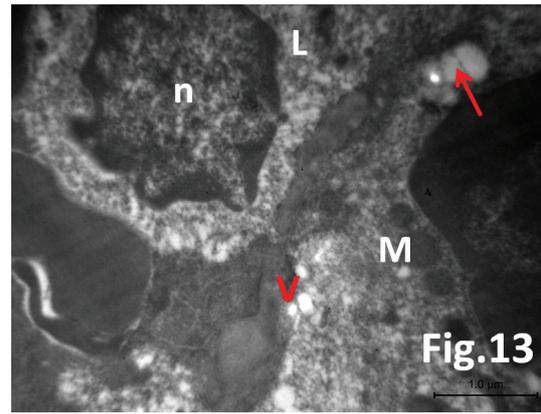


Fig. 13 : An electron micrograph of a section of the spleen of group II (ASP) showing degenerated lymphocytes (L) having irregular nuclei (n). Note groups of mitochondria (M) and small scattered vacuoles (v) or large adherent ones (↑).

Uranyl acetate and lead citrate X 15,000

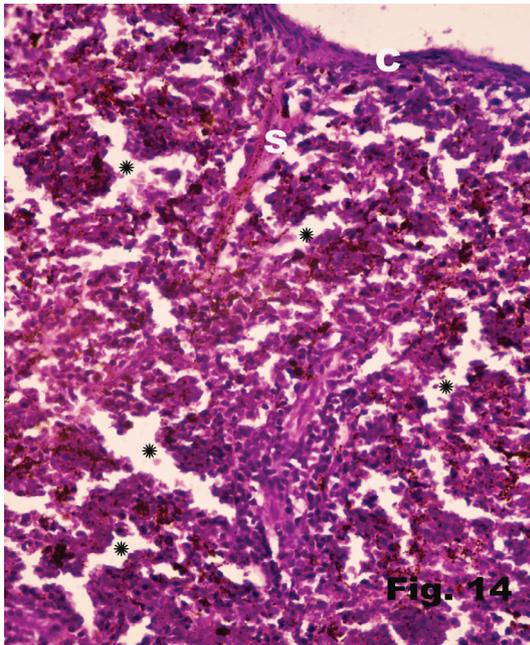


Fig. 14: A photomicrograph of a section of the spleen of group III (sucralose) showing complete loss of architecture of the spleen and marked areas of depletion (*) with complete loss of demarcation between the white and red pulps. Note thickening of capsule (C) and septa (S).

Hx.&E.; X400

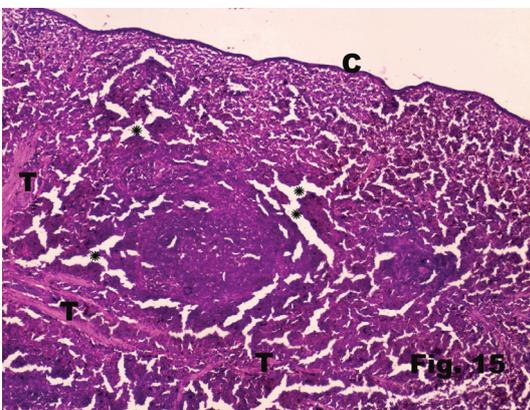


Fig. 15: A photomicrograph of a section of the spleen of group III (sucralose) showing loss of architecture of spleen. Irregular capsule (C) and elongated thickened fibrous strands (T) are observed. Note areas of depletion (*).

Hx.&E.; X200

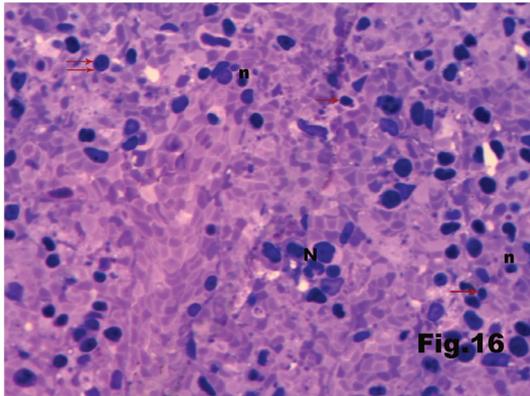


Fig. 16: A Semithin section of the spleen of group III (sucralose) showing marked depletion of lymphocytes. Most of lymphocytes are small (↑), few are large (↑↑). Some lymphocytes appear with small dense nuclei (n). Some neutrophils (N) are observed.
Toluidine blue; X 1000

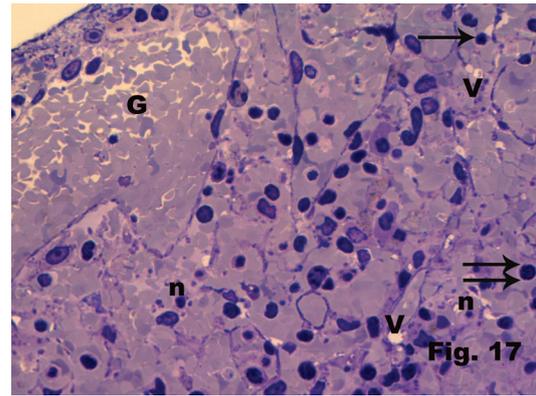


Fig. 17: A Semithin section of the spleen of group III (sucralose) showing severe congestion (G). Most of lymphocytes are small (↑) and few are large (↑↑). Some lymphocytes appear with small dense nuclei (n). Small rounded vacuoles (v) are observed.
Toluidine blue; X 1000

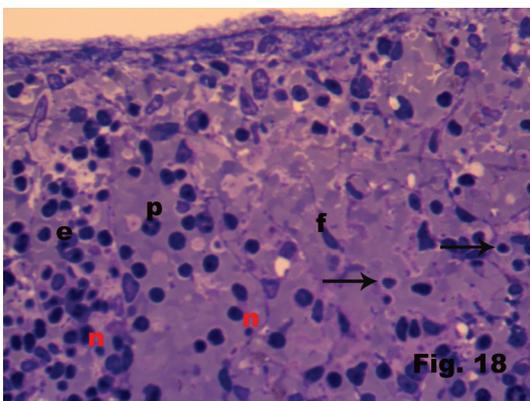


Fig. 18: A Semithin section of the spleen of group III (sucralose) showing some lymphocytes with pyknotic nuclei (n), rarified cytoplasmic rim (↑). Fibroblasts (f), cart wheel plasma cells (p) and bilobed eosinophils (e) are seen.
Toluidine blue; X 1000

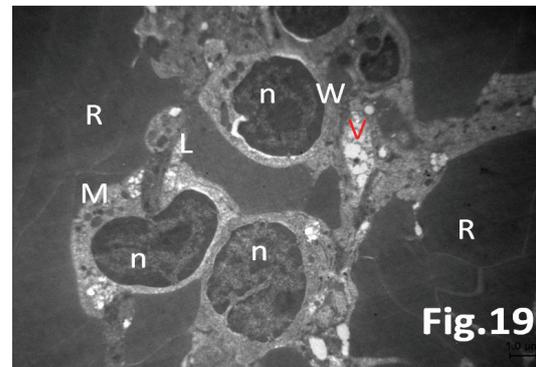


Fig. 19: An electron micrograph of a section of the spleen of group III (sucralose) showing extensive red pulp (R) and degenerated white pulp (W). Degenerated lymphocytes with slightly irregular nuclei (n), groups of mitochondria (M) and vacuoles (v) of variable sizes are seen.
Uranyl acetate and lead citrate X 4000

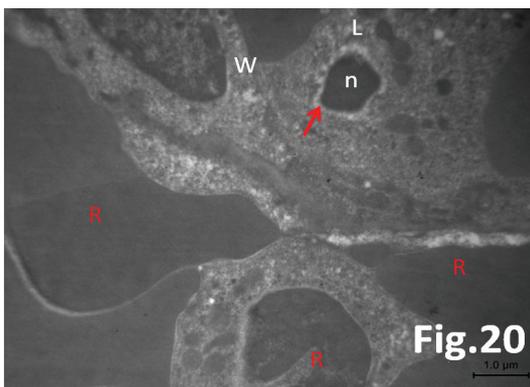


Fig. 20: An electron micrograph of a section of the spleen of group III (sucralose) showing extensive red pulp (R) and degenerated white pulp (W). Lymphocytes (L) with highly condensed pyknotic nuclei (n) and widened perinuclear space (↑).
Uranyl acetate and lead citrate X 8000

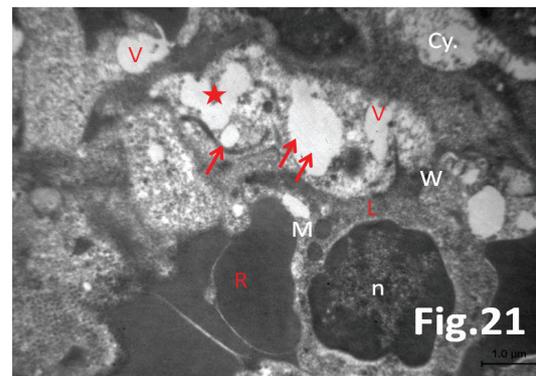


Fig. 21: An electron micrograph of a section of the spleen of group III (sucralose) showing red pulp (R) and white pulp (W). Degenerated lymphocytes (L) with irregular nuclei (n) and degenerated cytoplasm (Cy.), containing either small vacuoles (↑) or large (↑↑) and few are joined together (*). Note groups of mitochondria (M).
Uranyl acetate and lead citrate X 8000

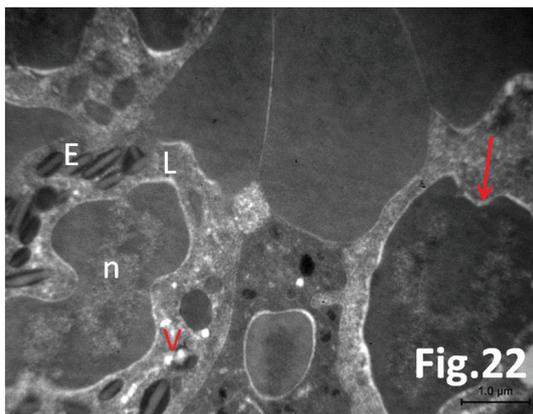


Fig. 22: An electron micrograph of a section of the spleen of group III (sucralose) showing degenerated lymphocytes (L) with irregular indented nuclei (n) and widened perinuclear space (↑). Small vacuoles (V) are seen. Note the eosinophil infiltration (E).

Uranyl acetate and lead citrate X 10,000

DISCUSSION

The present work investigated the effect of ASP and sucralose on the structure of the spleen. Both exhibited deleterious effects on lymphoid tissue of the spleen. However, sucralose had more degenerative effects, in addition to its effect on the red pulp and vascularity of the spleen. ASP caused loss of architecture of spleen with signs of degeneration and disruption of the white pulp, which was confirmed by electron microscopic examination. *Choudhary & Devi (2014)* declared that after 90 days of aspartame treatment, rats showed degenerated white pulp of the spleen. *Abdel Fattah (2012)* concluded that consumption of aspartame induced histopathological changes in bone marrow of mother rats and their offsprings during gestation period and after delivery. *Morando et al. (2007)* added that ASP caused significant increase in lymphomas and leukemias in females. Aspartame metabolites methanol and formaldehyde may be the causative factors behind degenerative changes observed (*Choudhary & Devi 2014*). This was supported with *Humphries et al., (2008)* who declared that methanol is not metabolized within erythrocytes and rapidly enter portal circulation to be oxidized in the liver into formaldehyde and diketopiprazine (a carcinogen). Authors added that formaldehyde accumulate in the tissues and alter both mitochondrial DNA and nucleic DNA. Damaged DNA causes inadequate function of the cell.

In the present study some lymphoid follicles showed depletion of lymphocytes, this could be explained by *Choudhary & Devi (2014)* who declared that ASP has a strong effect on the cells involved in immunity. The authors added that administration of ASP causes oxidative stress by altering the oxidant – antioxidant balance in immune organs of rats. Also, Aspartame can increase the excess free radicals which inactivate the scavenging system. Free radicals cause oxidative stress which results in immune suppression.

The current work revealed many cytoplasmic vacuoles of variable sizes. ASP administration was proved to cause vacuolation of cells in different organs such as submandibular gland (*Mohammed et al., 2015*) and liver (*Abdallah 2002*). *Mohammed et al. (2015)* explained the presence of vacuoles to be a cellular defense mechanism against toxic substances, in which these substances were aggregated in the vacuoles, thus preventing their interference with cellular metabolism.

In the present study assorted cells were recognized, including plasma cells, bilobed cells and large giant pale cells with elongated nuclei, other cells were binucleated. *Most et al. (1997)* reported that after aspartame administration, white blood cells migrated to the site of lesion, coalased with macrophages to form giant cells. The presence of such assorted cells denotes the inflammation of the spleen.

Following sucralose administration, the present work showed marked areas of lymphocytic depletion, many atrophic lymphocytes with irregular or pyknotic nuclei, widened perinuclear space, rarified rim of cytoplasm and many vacuoles denoting marked degeneration of cells. There is an adequate evidence that sucralose had deleterious effects on the immune parameters in experimental animals (*Grice and Godsmith 2000*). *Finn and Lord (2000)* noticed shrunken thymus gland (up to 40 %) and atrophy of lymph follicles in spleen and thymus following sucralose administration. In addition, the number of lymphocytes appeared diminished in dogs treated with sucralose (*Grice and Godsmith 2000*).

Moreover, *Goldsmith and Meckel (2001)* observed that sucralose administration caused reduction of weight of the spleen and thymus

glands. Such reduction was associated with remarkable histopathological changes whose interpretation varied among pathologists. It was attributed either to direct effect of sucralose, undernutrition or stress (*Scientific Committee on Food, 2000*). Reduction in total white blood cells and lymphocyte count were also observed in 48-week study of sucralose administration (*Federal Register 1998*).

In the present work following sucralose administration marked congestion was noticed with extensive prominent red pulp. Focally dilated blood vessels stuffed with intravascular lymphocytes, were observed in the colon of male rats following sucralose administration. The blood vessels were associated with alterations in gut epithelium including lymphocytic infiltration into the epithelium (*Mohamed et al., 2008*).

In the current work, thickening of spleen capsule and trabeculae with other fibrous strands were observed in some sections of both ASP and sucralose administration. This was explained by *Menezes et al 2005* who reported that all extensive injuries were repaired with collagen fibers or scar irrespective of their cause. Therefore the increased thickness of the capsule and of trabeculae in our experiment may be due to increase in collagen fibers deposition induced by injury resulting from aspartame and sucralose administration. Moreover, the present study showed fibrous tissue strands that were more prominent in spleen subjected to sucralose, which may support the fact that the tissue injury could be more severe in sucralose group.

CONCLUSION

The present study clearly points that both ASP and sucralose can influence the structure of the spleen within the acceptable daily consumption. Whereas the sucralose had more degenerative effects on the spleen. However, artificial sweeteners are relatively new and their uses are being researched and extended every day. Further work is necessary to determine the exact mechanism by which artificial sweeteners can affect immune organs.

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دراسة نسيجية لتأثير الأسبارتام مقابل السكرلوز على طحال ذكور الجرذان البيضاء البالغة

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ملخص البحث

مقدمة: الأسبارتام هو واحد من أكثر المحليات الاصطناعية المستهلكة في جميع أنحاء العالم وقد أعلنت بعض الدراسات ان الاسبارتام في المستوي المسموح به من منظمات الغذاء و الدواء يسبب الإجهاد التأكسدي عن طريق تغيير توازن المواد التي تساعد علي الاكسده والمواد المضادة للأكسدة في الأجهزة المناعية للفئران.

ومن ناحية اخري السكرلوز محلي اصطناعي اخر عالي الفاعلية وقد وصف تحت اسم سبلندا و هو يوجد في العديد من المنتجات الغذائية والمشروبات. لاحظت الدراسات أن تعاطي السكرلوز يسبب خفض الوزن الطحال و تغييرات في أنسجة الغدة الصعترية في الفئران.

الهدف من البحث: هو دراسة التغيرات النسيجية والتركيبية في الطحال في ذكور الجرذان البيضاء البالغة، عند تعاطي الاسبارتام و السكرلوز. الطرق و المواد المستخدمة: تم تقسيم ثلاثين ذكور الجرذان البيضاء إلى ثلاث مجموعات:

المجموعة الأولى: مجموعة ضابطة : تكونت من عشرة فئران اعطيت 1 مل ماء مقطر يوميا عن طريق الفم لمدة 3 أشهر.

المجموعة الثانية: (مجموعة الأسبارتام): تكونت من عشرة فئران اعطيت الأسبارتام (40 ملغ / كلغ) في 1 مل ماء مقطر يوميا عن طريق الفم لمدة 3 اشهر.

المجموعة الثالثة: (مجموعة السكرلوز): تكونت من عشرة فئران اعطيت السكرلوز (15 ملغ / كلغ) في 1 مل ماء مقطر يوميا عن طريق الفم لمدة 3 أشهر. وفي نهاية التجربة، تم تشريح عينات الطحال وتجهيزها للفحص المجهرى بالميكروسكوب الضوئي و الالكتروني .

النتائج: تسبب الاسبارتام في فقدان البنية الداخليه للطحال، يرافقه اختفاء جزئي لترسيم الحدود بين اللب الأبيض والأحمر. وأظهرت النتائج استنزاف الخلايا الليمفاوية في اللب الابيض وأظهر الفحص الالكتروني ضمور الخلايا الليمفاوية في اللب الأبيض، مع نوى تغلظية مكثفه للغاية. وبالإضافة إلى ذلك، لوحظ وجود فجوات في السيتوبلازم. من ناحية أخرى، اظهرت مجموعة السكرلوز فقدان كامل لبنية الطحال مع اختفاء ترسيم الحدود بين اللب الأحمر والأبيض. وقد لوحظت مناطق واسعه تتسم باستنزاف الخلايا الليمفاوية في كل من اللب الأحمر والأبيض وقد ظهرت حزم سميكة من النسيج الليفى. وأظهر الفحص الالكتروني اتساع اللب الأحمر مع تدهور اللب الأبيض و ظهور الخلايا الليمفاوية الضامره ،مع نوي غير منتظم وبالإضافة إلى ذلك لوحظ ضمور في السيتوبلازم، واتساع مساحة محيط بالنواة، العديد من الفجوات و ظهور خلايا (esinophil) .

والخلاصة: إن الدراسة تشير بوضوح أن كلا من الاسبارتام و السكرلوز يمكن أن يؤثر على هيكل الطحال بمعدل الاستهلاك اليومي المقبول في حين كان للسكرلوز المزيد من الآثار التنكسية في الطحال.