Original Article

Effect of Nano-curcumin versus Black Seed Oil on Renal Cortical Changes Induced by Aspartame in Adult Male Albino Rat. Histological and Immunohistochemical Study

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ABSTRACT

Introduction: Aspartame (ASP) is a widely used an artificial sweetener. However, it is considered a risky compound causing many health problems on prolonged use. Curcumin and black seed oil (BSO) are two of the most important natural antioxidants used in alternative medicine. Recently, curcumin nano-formulations showed better bioavailability and solubility, hence delivering more therapeutic concentrations of curcumin, enhancing its efficacy.

Aim of the Work: This study was carried out to investigate the effect of nano-curcumin versus BSO on aspartame-induced cortical histological changes in adult male albino rat.

Materials and Methods: Forty-five rats were divided into 4 groups; 1 control and 3 experimental groups; Aspartame group: received aspartame orally (200 mg/ kg/day) for 6 weeks, nano-curcumin and BSO groups: received aspartame as the previous group concomitantly with nano-curcumin (200 mg/kg/day) and BSO (0.2 ml/kg/day) respectively orally for 6 weeks. Serum levels of urea and creatinine (Cr) and renal tissue level of superoxide dismutase (SOD) were measured. Renal sections were stained with H&E, Periodic Acid Schiff's Reaction (PAS) and immunohistochemical stains for Caspase-3, Cox-2 and Ki67. Additionally, morphometric measurements and statistical analysis were done.

Results: The aspartame group showed obvious histological degenerative changes in renal corpuscles and tubules with a significant decrease in the glomerular area, mean area% of PAS and mean number of Ki-67 immuno-positive cells with significant increase in the area% of COX-2 and caspase-3 immunoreaction. Whereas, nano-curcumin and BSO groups showed apparently normal structure of the renal cortex and amelioration of the biochemical and morphometric parameters detected in the aspartame group.

Conclusion: Both nano-curcumin and BSO proved a protective effect on the renal cortex against the adverse changes caused by aspartame and their efficiency was nearly similar. These findings can be considered clinically after extra human-based studies to define the suitable patient and to adjust the required dose.

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Key Words: Aspartame, BSO, nano-curcumin, rat, renal cortex.

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INTRODUCTION

Aspartame, is a widely used artificial sweetener all over the world. It is used by millions of people who need to restrict their sugar intake in diet as it represents a low-calorie option. In addition, it has a sweeter flavor than sugar by about 200 times and does not cause tooth decay[1]. On the other hand, it is considered as a very harmful compound raising a lot of controversy concerning its safety[2].

Health problems as headache, dizziness and nephrotoxicity all were related to aspartame use. Metabolites resulting from aspartame break down in the gastrointestinal tract are mainly responsible for reactive oxygen species (ROS) release causing cytotoxicity and oxidative stress that impair renal functions on prolonged use[3]. In addition, numerous studies have confirmed that aspartame intake at high doses can be carcinogenic increasing the possibility of developing cancers as lymphoma and cancers of urinary tract and nervous system[4,5].

Curcumin and black seed are two of the most important alternative herbal medicines. Curcumin is a natural polyphenol derived from Curcuma longa plant[6]. It has a wide variety of medicinal properties, including anti-inflammatory, anti-oxidation, anti-microbial and anti-tumor effects in addition to multi-organ protective effects[7]. On the other hand, poor water solubility and bioavailability are considered the major disadvantages of curcumin as a therapeutic herbal agent. So, it was important to develop new methods to solve this problem. Curcumin nano-formulations development is one of the best methods that can improve curcumin bioavailability and solubility, hence delivering therapeutic amounts of curcumin and increasing its effectiveness[8].

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Black seed or Nigella sativa, one of the members of the family Ranunculaceae; has been used all over the world as a food additive and therapeutic agent for different diseases, like bronchial asthma, allergies, hypertension, obesity, gastrointestinal troubles and cancer in addition to enhancing the immunity of the body[9]. Most of the pharmacological effects of black seed oil (BSO) are related to its active constituent; thymoquinone (TQ); acting as anti-inflammation and antioxidation substance[10]. Additionally, it was found that BSO has a cyto-protective effect protecting kidney tissue against ROS, hence preventing renal dysfunction and damage[11,12].

Current research was done to investigate the effect of nano-curcumin versus black seed oil on aspartame-induced cortical histological changes in male adult albino rat.

MATERIALS AND METHODS

Animals

Forty-five albino male adult rats (of average body weight 180-200 gram) were kept in Kasr El -Aini Animal House, Faculty of Medicine, Cairo University and treated in agreement with the standards approved by the institution's Animal Use Committee (approval number CU III F 55 23). They were kept in appropriate well-ventilated wire cages at room temperature and provided with free access to food and water.

Drugs

- Aspartame (Diet Sweet): was purchased from Seif Pharmacy (Cairo, Egypt) as tablets 20 mg.
- Nano-curcumin: was provided by Nano-Tech, Giza, Egypt as a powder of yellow color.
- Black Seed oil: It was bought as a bottle containing 250 ml of virgin cold compressed oil (Organic Nation Company).

Experimental design

Group I (control): fifteen rats were divided into 3 subgroups (each with 5 rats):

- Subgroup Ia: corresponding to group II, 1 ml of distilled water was given to rats orally once/day for 6 weeks
- Subgroup Ib: corresponding to group III, rats received two ml distilled water, as a single oral dose/day for 6 weeks.
- Subgroup Ic: corresponding to group III, 1.04 ml of distilled water was given to animals once per day orally, for 6 weeks.

Other 30 rats were equally divided into 3 experimental groups:

Group II (ASP): animals were given ASP (200 mg/kg/day), crushed and prepared in 1 ml distilled water and given orally once per day for 6 weeks[13].

Group III (Nano-curcumin): rats were given ASP as group II concomitantly with nano-curcumin (200 mg/kg/day), dissolved in 1ml of distilled water and given as a single oral dose/day for 6 weeks[14].

Group IV (BSO): rats were given ASP as group II concomitantly with BSO (0.2 ml/kg/day) orally once daily for 6 weeks[15].

Experimental procedure

Biochemical investigation

At the end of experiment (six weeks), 3 ml of blood were collected from each rat via tail vein (just before sacrifice) for testing serum levels of urea and creatinine (Cr). Also, level of superoxide dismutase (SOD) was measured in the renal tissue. These investigations were carried out in Biochemistry Department laboratory, Faculty of Medicine, Cairo University.

Histological studies

Rats were sacrificed at the Department of Medical Histology and Cell Biology, Faculty of Medicine, Cairo University by IP injection of phenobarbital at a dose of 60 mg/kg[16] and the kidney was dissected. From each rat, the right kidney was used for biochemical analysis of tissue SOD level and the left one was quickly fixed in formaldehyde 10% and processed to form paraffin blocks. Serial sections of 6 µm thickness were cut and subjected to the following stains:

a. Hematoxylin and Eosin[17].
b. PAS[18].
c. Immunohistochemical staining[19] for:
    1. Caspase-3 antibody, is a rabbit polyclonal antibody (Thermo Fisher scientific Laboratories, MA, USA). Its positive reaction appears as brown cytoplasmic deposits.
    2. Cox-2 antibody is a rabbit polyclonal antibody (NeoMarkers, Lab Vision Corporation, CA, USA). Its positive reaction appears as brown cytoplasmic deposits.
    3. Ki67 antibody, is a rabbit monoclonal antibody (Thermo Fisher scientific Laboratories, MA, USA). Its positive reaction appears as brown nuclear deposits.

For immunostaining, sections were boiled in 10Mm of citrate buffer with a pH of 6 (catalogue number AP 9003) for 10 minutes to unmask the antigens. Then the sections were allowed to cool in ambient air for 20 minutes. The primary antibody was then added to the sections and left on them for an hour. Immunostaining was carried out using the Ultravision detection system (Cat. No. TP - 015- HD). After that, the sections were counterstained by Mayer's hematoxylin (Cat. No. TA- 125-MH). The used reagents were supplied by Lab Vision Thermo Scientific (Fremont, California, USA).

Morphometric study

The following parameters were measured:

1. Glomerular area in H and E sections.
2. Area % of PAS +ve reactivity.
3. Area % of Caspase-3 positive immune-reactivity.
4. Area % of COX-2 immunopositivity.
5. Number of Ki67 immunopositive cells.

Image analysis was carried out by Leica Qwin 500 C" image analyzer computer system (Cambridge, England) present in Department of Medical Histology and Cell Biology, Faculty of Medicine, Cairo University. All measurements were done in ten non overlapping fields (x400) from different sections of each group.

Statistical-analysis

Biochemical results and morphometric measurements were represented as mean ± standard deviation (SD). They were analyzed using one-way analysis of variance (ANOVA) test, followed by Tukey post hoc test. The findings were considered significant when *P value* was less than 0.05. Calculations were done by statistical package for social sciences (SPSS) software (version 21, IBM, Armonk, New York, USA)[20].

RESULTS

Biochemical Results

Serological Results

Serum level of urea in mg/dl (Histogram 1) & creatinine in mg/dl (Histogram 2).

The highest values for serum urea as well as serum creatinine levels achieved in group II recorded a significant rise versus control group. While group III and IV recorded a significant decline versus group II, but non-significant with control.

SOD renal homogenate level (Histogram 3)

The mean values for SOD in group II was significantly lowered versus control group. Its increased values in group III and IV recorded a significant increase versus group II.

Histological Results

H and E sections: (plates 1,2)

Control group showed a typical histological renal cortical architecture; representing Malpighian renal corpuscle comprising glomeruli with regular intact Bowman's capsule separated from each other by capsular space. Proximal convoluted tubules (PCTs) appeared with small lumen, its lining displayed tall cuboidal cells exhibiting rounded nuclei with dark eosinophilic cytoplasm and distal convoluted tubules (DCTs) revealed larger lumen with a lining of cube shaped cells possessing rounded nuclei and lighter cytoplasm. (plate 1A,B). Conversely, sections of group II (ASP group), revealed many destructive alterations in the renal corpuscles and tubules. Many corpuscles exhibited shrunken glomeruli with dilated Bowman`s capsule. Tubules were displayed dilatation of their lumen, vacuolated cytoplasm with pyknotic nuclei. Separated epithelial cells containing disintegrated nuclei and eosinophilic casts were visible in certain tubules (plate 1C,D).

In sections of group III (plate 2A,B) and group IV (plate 2C,D) respectively, seemingly typical histological organization of renal corpuscles with preserved glomeruli and tubules were observed apart from presence of few cells with pyknotic nuclei in some tubules. In addition to some tubular vacuolated cytoplasm in group IV.

PAS sections: (plate 3)

Group I illustrated PAS +ve reaction in apical brush borders of PCTs as well as basal laminae of the renal tubules, Bowman's capsule's parietal (outer) layer and glomerular capillaries (plate 3A). Meanwhile, group II showed partially or completely disrupted brush borders, as well as interrupted basal laminae of most renal tubules, glomeruli and Bowman's capsule parietal layer (plate 3B). On the other hand, group III (plate.3C) and IV (plate 3D) revealed +ve reaction with intact brush border in most tubules. Basement membrane of many tubules, glomeruli and Bowman′s capsule parietal layer also appeared regular.

Immunostaining with Caspase-3: (plate 4)

Regarding caspase-3 immuno-reactivity, it was undetectable in control group (plate 4A). Whereas, sections from group II showed widespread strong cytoplasmic immune-reactivity for caspase-3 in cells of renal tubules (plate 4B). In groups III (plate 4C) and IV (plate 4D) moderate cytoplasmic immune-reactivity was detected in the renal tubules' cells.

Immunostaining with Cox-2: (plate 5)

The examined sections from the control group exhibited negative COX-2 immunoreaction (plate 5A). While group II displayed a strong Cox-2 expression in the cytoplasm of the cells lining the renal tubules and glomeruli (plate 5B). However, group III (plate 5C) as well as group IV (plate 5D) revealed faint cytoplasmic expression at the cells of renal tubules and glomeruli.

Immunostaining with ki67: (plate 6)

Weak Ki-67 nuclear immune-reactivity was detected in the control group at some tubular cells (plate 6A). Additionally, group II revealed very weak localized immune-reactivity (plate 6B). However, group III (plate 6C) and group IV (plate 6D) exposed intense widespread Ki-67 immuno-expression in the nuclei of numerous tubular lining cells.

Morphometric Results

Glomerular area in H&E sections: (Histogram 4)

Lowest value for mean glomerular area was recorded

in group II shown statistically significant difference from other groups. Meanwhile, Groups III and IV did not differ significantly from group I.

Area % of PAS +ve reaction: (Histogram 5)

The reported data for area % of PAS reached the lowest value in group II recorded a statistically significant decrease compared to control group. These values were elevated in groups III and IV displayed significant statistically rise to group II, but not significant with the control.

Area percent of Caspase-3 & COX-2 +ve immunoreactivity: (Histograms 6,7 respectively)

Mean area % of Caspase-3 as well as Cox-2 +ve immune-expression was elevated in sections related to all groups recording a significant raise versus control sections. However, the values of groups III and IV demonstrated a substantial drop contrasting to group II.

Mean number of ki67 immunopositive cells (Histogram 8)

Mean number of Ki-67 immuno-reactive cells was lowered significantly in group II versus the control group, with significant increase documented in groups III and IV regarded groups I and II.

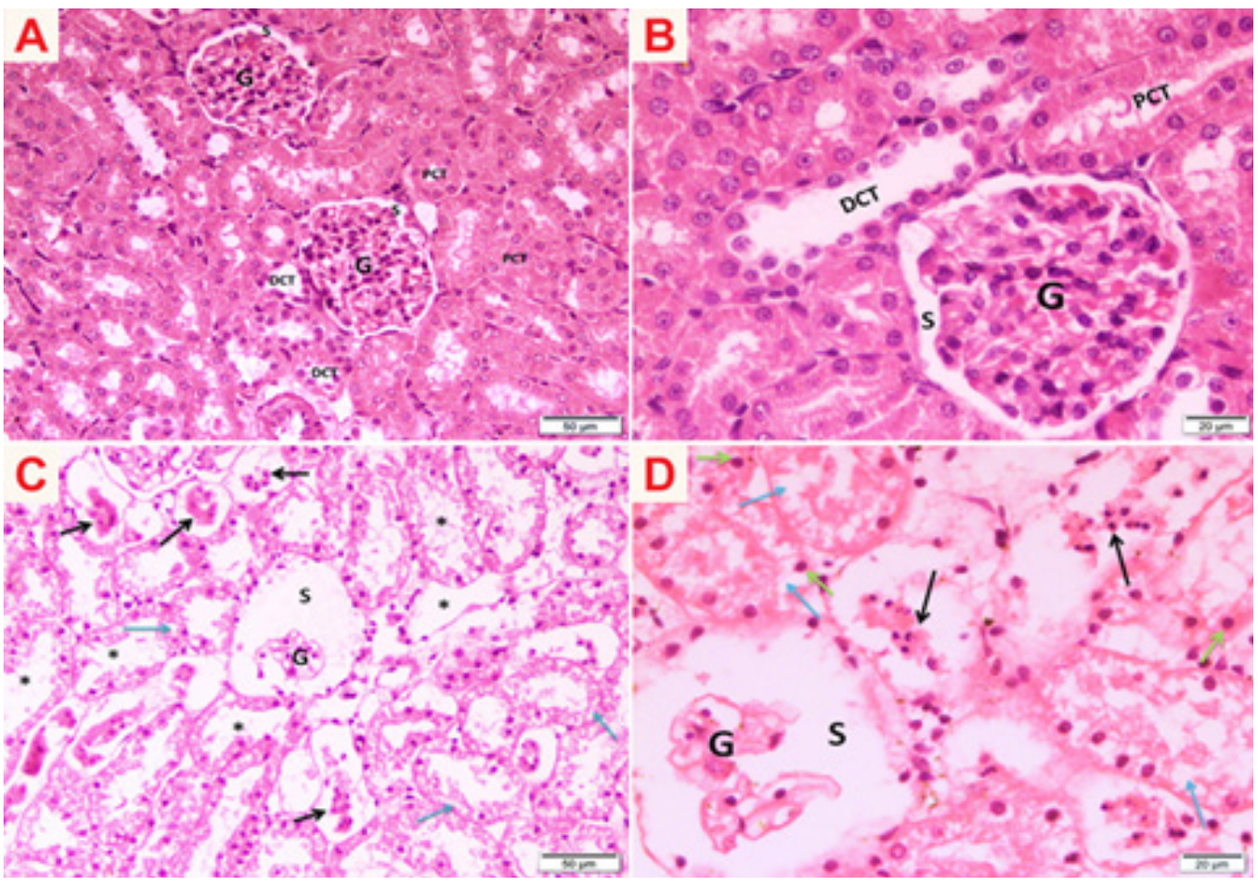


Plate 1: Photomicrographs of H and E:
A&B) control group: displaying Malpighian renal corpuscles exhibiting glomeruli (G) encircled with Bowman's space (S). PCTs (PCT) reveal small lumen with a lining made of tall cuboidal cells having basal rounded nuclei and dark eosinophilic cytoplasm. DCTs (DCT) have a larger lumen and is lined by cubical cells with central rounded nuclei and paler cytoplasm.
C&D) group II: illustrating glomeruli (G) displaying shrinkage associated with dilated Bowman`s space (S). Renal tubules exhibited dilated lumina (astrix), vacuolated cytoplasm (blue arrows) with pyknotic nuclei (green arrows). Some tubules demonstrated epithelial cellular sloughs with disintegrated nuclei and eosinophilic casts in their lumina (black arrows).

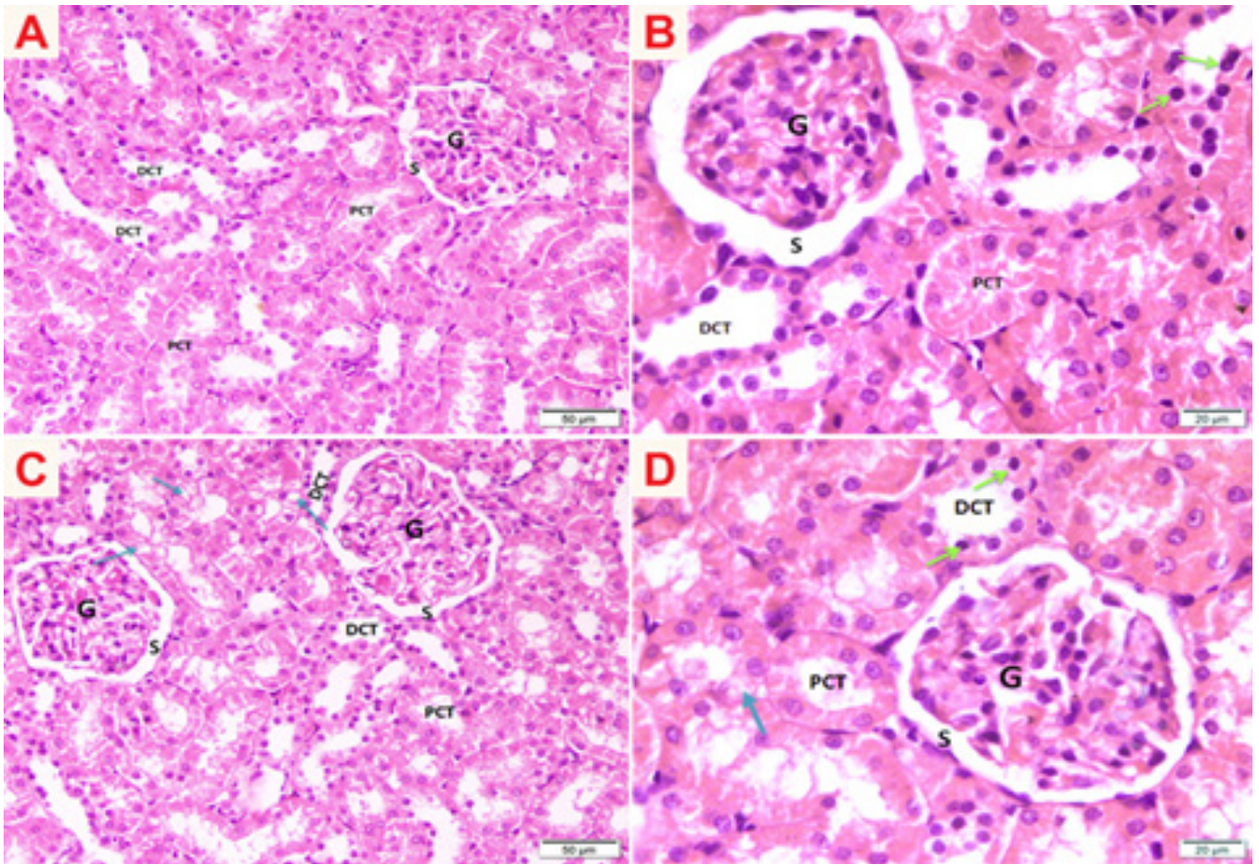


Plate 2: Photomicrographs of H and E:
A&B) group III: revealed apparently normal renal corpuscles with well-formed glomeruli (G) encircled by seemingly normal Bowman's space (S). PCT and DCT appeared almost normal with intact lining. However, few pyknotic nuclei (green arrows) are noted in some tubules.
C&D) group IV: showing deceptively normal corpuscles and glomeruli (G) with obviously normal Bowman's space (S). Majority of PCT and DCT appeared typical. Note pyknotic nuclei (green arrows) and vacuolated cytoplasm (blue arrows) in few cells of some tubules.
(H&E: A,C,E&G x200; B,D,F&H x400).

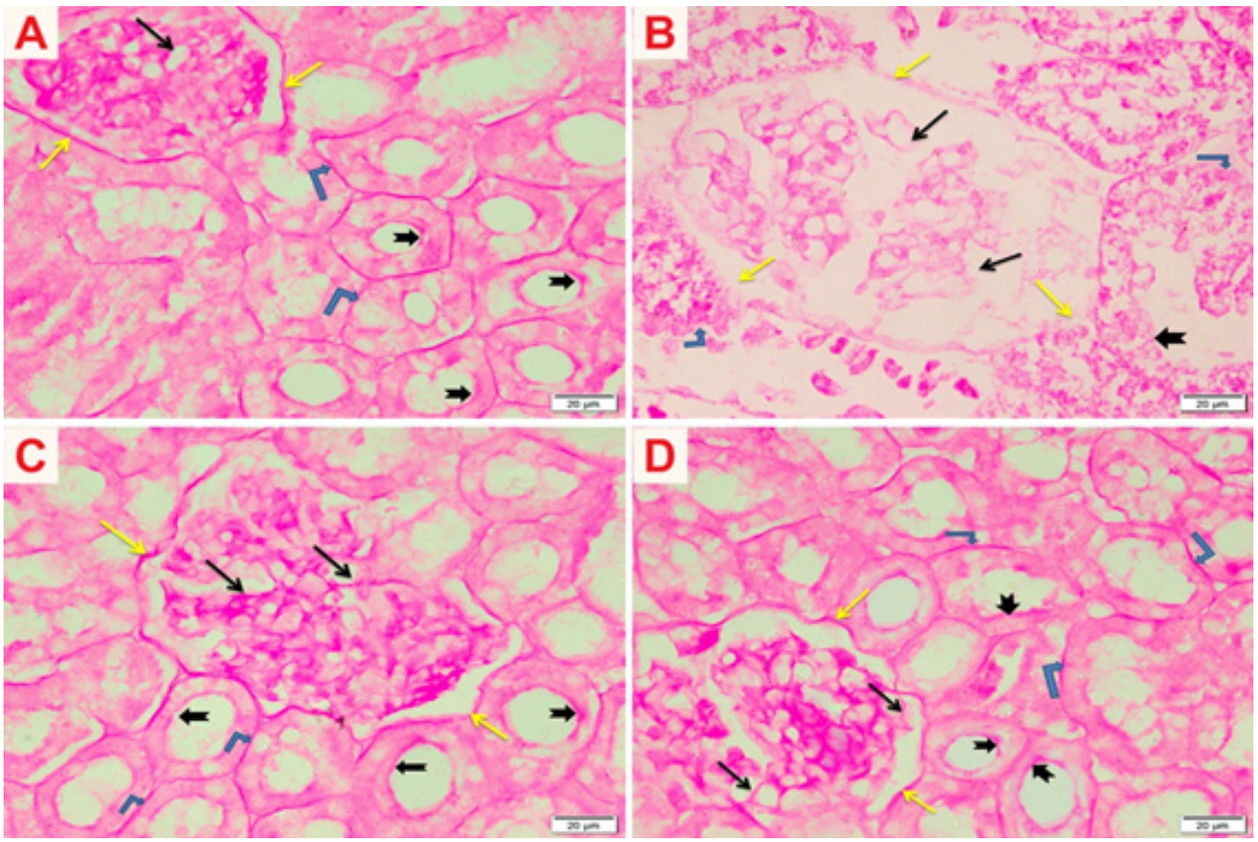


Plate 3: Photomicrographs of PAS:
A) Control group: representing +ve reaction of PAS located in brush borders of PCTs (notched arrows), basal laminae of renal tubules (blue arrows), glomeruli (black arrow) and parietal layer of Bowman's capsule (yellow arrows).
B) group II: viewing areas of partially or completely disrupted brush borders (notched arrows). Interrupted basal laminae of most tubules (blue arrows), glomerular capillaries (black arrow) and parietal layer of Bowman's capsule (yellow arrows) were also noticed.
C) group III & D) group IV: displaying +ve reaction in intact brush borders (notched arrows) and basal laminae of many tubules (blue arrows) as well as glomerular capillaries (black arrows) and parietal layer of Bowman's capsule (yellow arrows).
(PAS, x400).

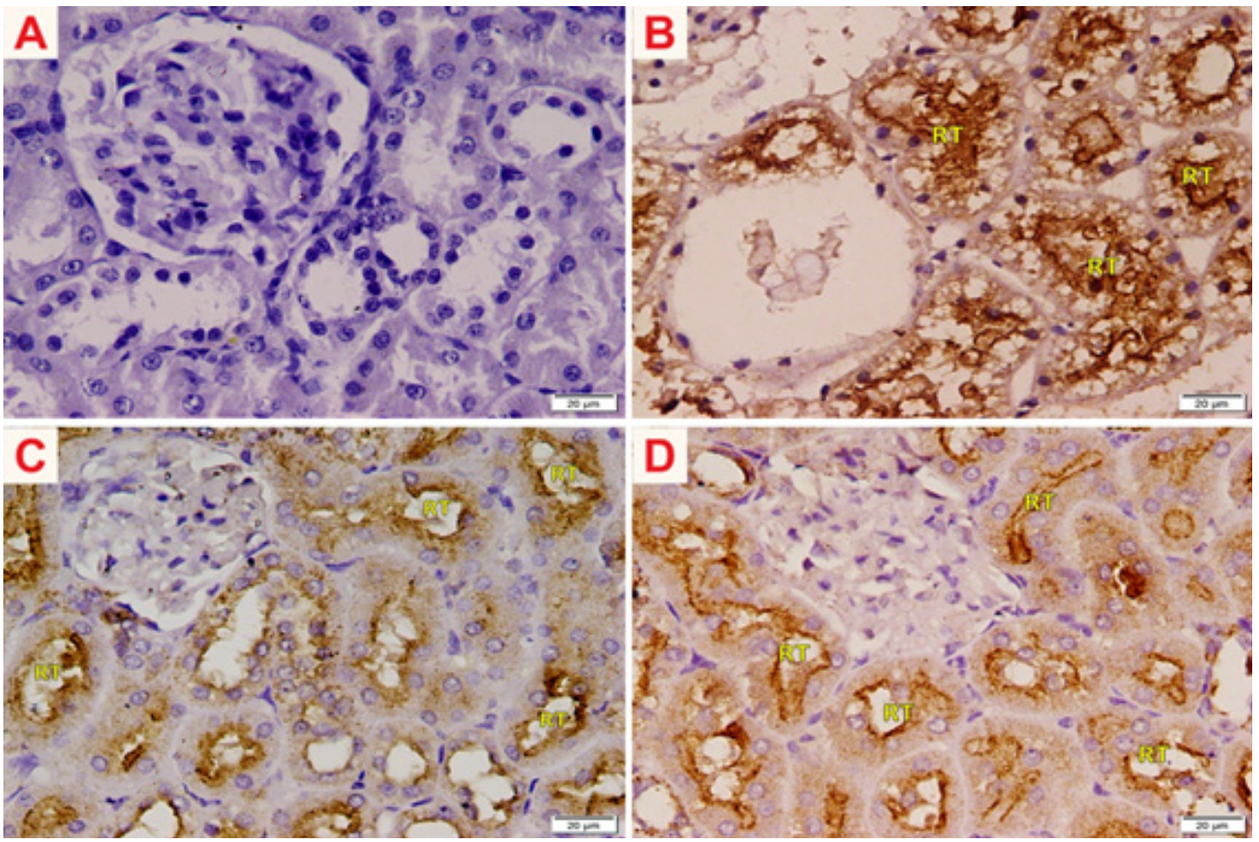


Plate 4: Photomicrographs of Caspase-3:
A) Control group: showing -ve caspase-3 reaction.
B) group II: revealing widespread strong cytoplasmic immunoreactivity in cells of renal tubules (RT).
C) group III & D) group IV: viewing moderate cytoplasmic caspase-3 immunoreactivity (RT).
(caspase-3, × 400).

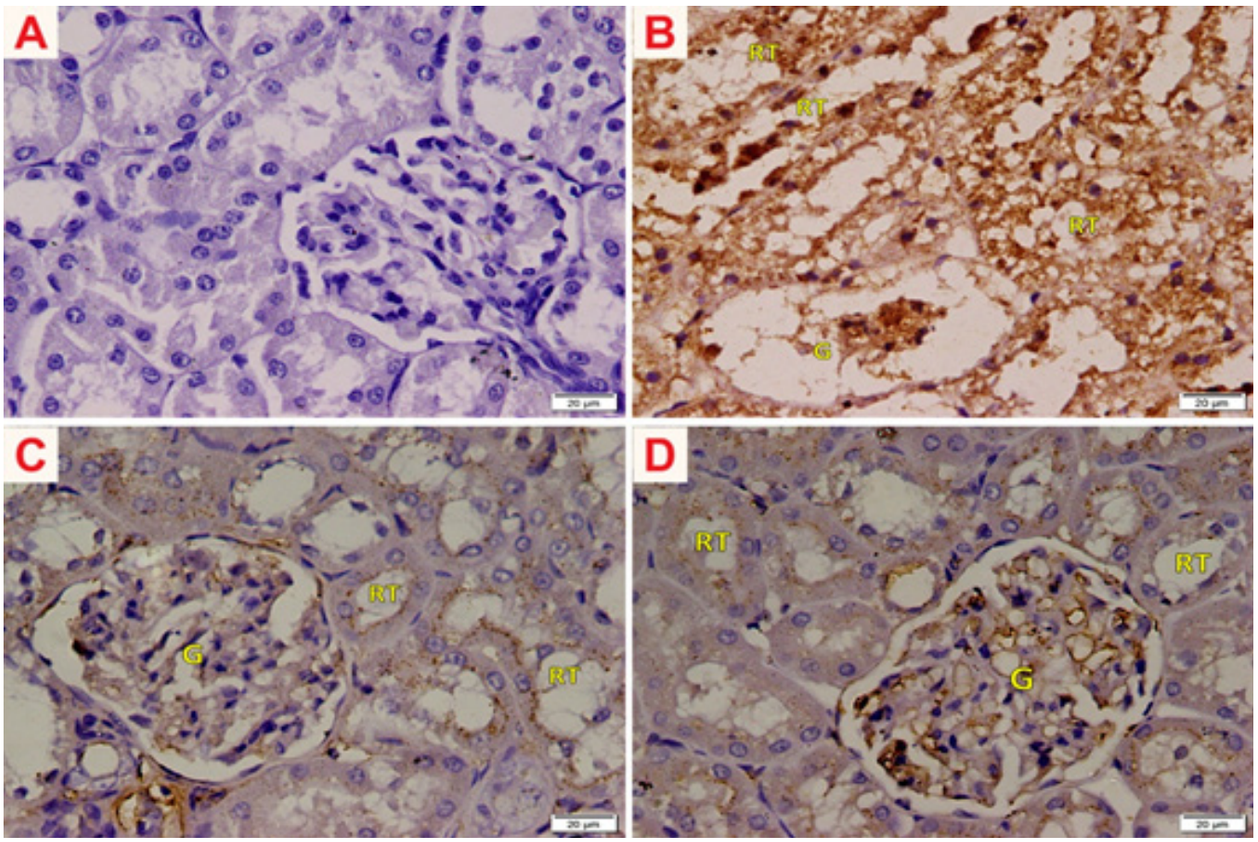


Plate 5: Photomicrographs of COX-2:
A) Control group: showing negative COX-2 immunoreaction.
B) group II: exhibiting strong COX-2 reaction in cytoplasm of renal tubular cells (RT) and glomeruli (G).
C) group III & D) group IV: illustrating faint cytoplasmic expression at cells of renal tubules (RT) and glomeruli (G).
(COX-2, × 400).

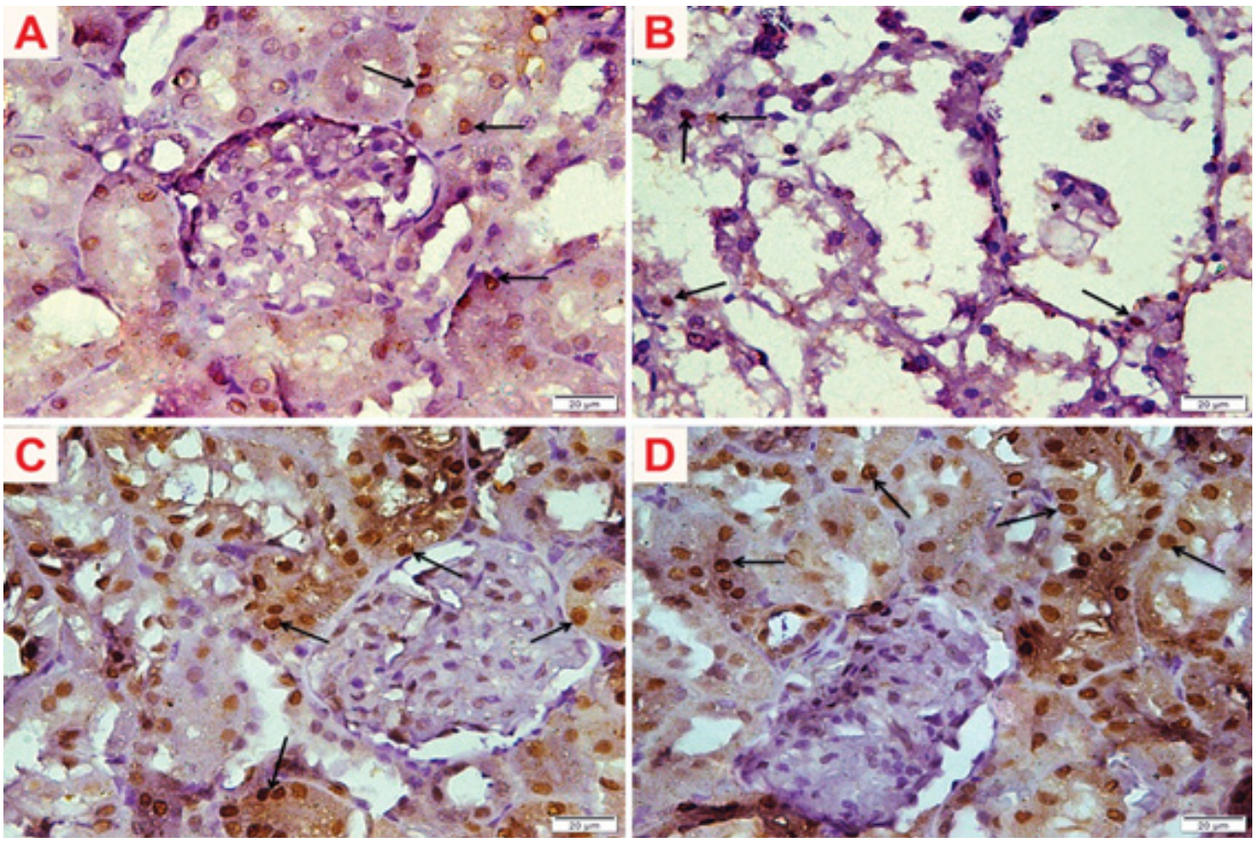
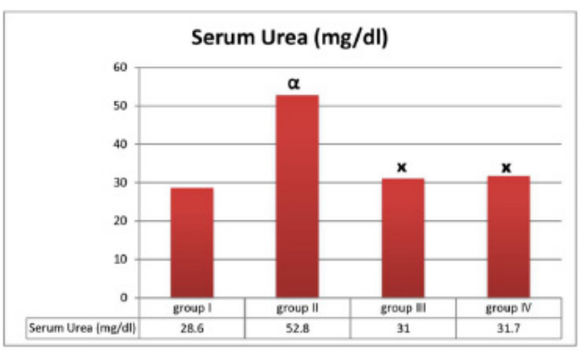
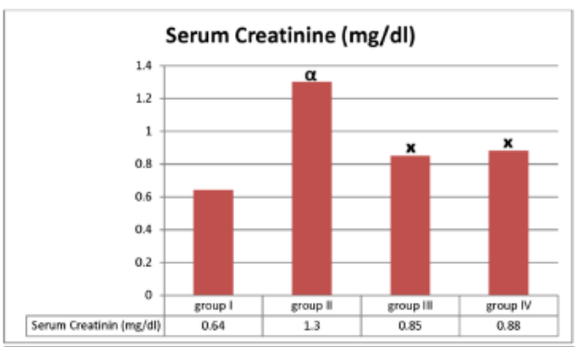


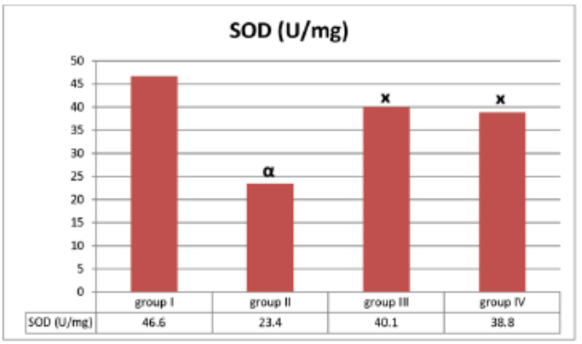
Plate 6: Photomicrographs of Ki-67:
A) Control group: showing weak Ki-67 immunoreaction in some tubular cells (black arrows).
B) group II: revealing very weak localized immune-reactivity (black arrows).
C) group III & D) group IV: displaying intense widespread Ki-67 nuclear immune-reactivity in many tubular cells (black arrows).
(Ki-67, × 400).



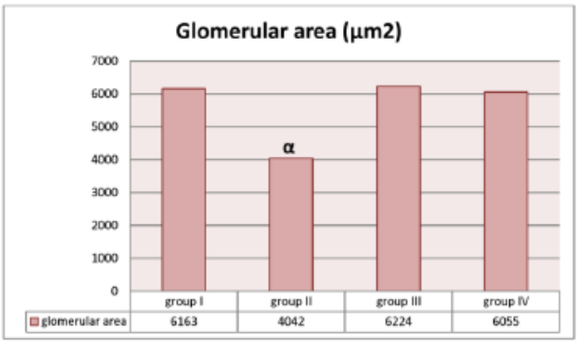
Histogram 1: Mean values of Serum Urea in the studied groups.



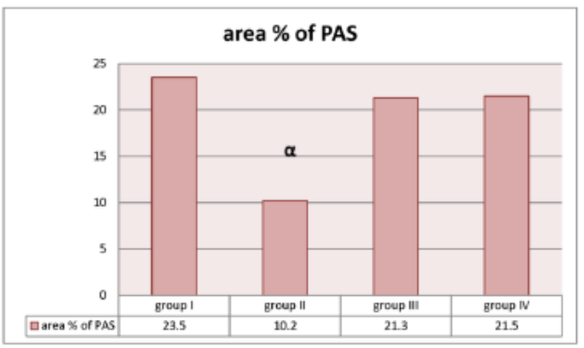
Histogram 2: Mean values of Serum Creatinine in the studied groups
α Significant difference compared to group I.
x Significant difference compared to group II.
p value < 0.05 is statically Significant.



Histogram 3: Mean values of SOD in the studied groups
α Significant difference compared to group I.
x Significant difference compared to group II.
p value < 0.05 is statically Significant.



Histogram 4: Mean values of Glomerular area (in micrometers) in the studied groups
α Significant difference compared to group I.
p value < 0.05 is statically Significant.



Histogram 5: Mean values of area % of PAS reaction in the studied groups
α Significant difference compared to group I.
x Significant difference compared to group II.
p value < 0.05 is statically Significant.

DISCUSSION

As physical wellness is gradually gaining curiosity, people have looked for nutritional options low in calorie content and not promoting weight gain including aspartame[21,22,23].

The present work was designed for exploring the effect of nano-curcumin versus BSO on aspartame-induced cortical histological changes in the adult male albino rat model.

In this research, control rats (group I) revealed a typical histological renal cortical construction. Serological analysis moved in synchrony with the morphometric outcomes displaying typical kidney physiological system as assessed by serum urea and Cr levels.

Statistically significant elevation of urea and creatinine levels in serum was reported in group II. This was coping with other authors who declared that aspartame usage significantly raised levels of urea and creatinine in serum of rats treated with it versus those of control rats[24].

The mean value for SOD in group II showed a significant decline versus control group. This finding was matching with previous results, it stated that treatment with aspartame caused significant deterioration in SOD function in a number of organs including kidney[25]. It was explained that the decrease in SOD in ASP treated groups as a primary defense that prevents the oxidative damage by ROS to macromolecules[26].

Sections of group II, revealed many signs of break down in Malpighian corpuscles and convoluted tubules going with morphometric findings which showed the lowest value for mean glomerular area in group II as compared to other groups.

These results coincide with previous study which informed that kidney section from rats received ASP showed damage of the glomeruli in conjunction with the renal tubules with cellular death[27]. Similarly, in another study, rats given formaldehyde, that is a byproduct of aspartame, revealed deteriorated glomeruli, vacuolization and widening in DCTs[28].

The truth that ASP produces methanol as one of its breakdown radicles helps in explaining its damaging effect on kidney, as methanol is converted to formaldehyde, then to formic acid, releasing oxidative stress promotors which damages cells by causing lipid peroxidation and cell membrane disintegration[29,30].

Bowman's capsule parietal layer, glomerular capillaries and renal tubules basement membrane were all partially or entirely damaged in the current study's evaluation of PAS-stained sections of group II. Morphometric results added more support to the previous findings illustrating a significant decrease in area% of PAS compared to the control provided an additional evidence for this. A previous study linked similar findings to brush border microvilli breakdown[31].

The start and rise of apoptotic conveys inside the diseased cells is intimately associated with a series of caspase complexes controlling apoptotic process[32]. Group II in the present work displayed diffuse strong cytoplasmic immune-reactivity for caspase-3 in the tubular lining cells. Supporting this finding morphometrically, a significant rise in the Caspase-3 area percentage was recorded versus

the control group. This apoptotic change could be due to oxidative stress caused by long-term aspartame use[33].

Ki-67 is a protein found in the nuclei, linked with cellular proliferation. It is related to the transcription of rRNA. Inactivation of Ki-67 antigen causes a decrease in the synthesis of rRNA[34].

Examination of Ki-67 in group II revealed very weak nuclear immune-reactivity. This was supported by morphometric analysis, which revealed a marked decline in Ki-67 immuno-reactivity values relative to the control group.

A recent work agrees with this, revealing that D-galactose (D-gal) which produces oxidative stress-cell damage as aspartame the distribution of Ki67 showed a significant reduction compared with the control rats[35]. Contrarily, it was documented that Ki-67 immunopositivity was significantly increased after administration of aspartame[36].

The primary enzyme required for transforming arachidonic acid into prostaglandins is called cyclo-oxygenase (COX). There are three known subtypes; COX type-1, COX type-2, and COX type-3, which share a lot in common structurally but differ greatly in terms of expression pattern and function[37]. COX-2 is an enzyme involved with damage triggers such inflammation, infection and tissue injury[38].

Group II manifested a strong immunopositivity for COX-2 in the cytoplasm of the renal tubular and the glomerular cells that was ensured morphometrically via a significant increase in area% of COX-2 immunopositivity versus the control rats. This findings were previously clarified as this elevation was correlated with free radicals and inflammation caused by ASP[39].

Opposing to group II, group III recorded a considerable decline in serum urea and Cr in experimental rats which received nano-curcumin. This agrees with previous research which reported that ingestion of the nano-curcumin highly reduced urea and Cr records in serum indicating its preventive and curative effects on the renal tissue[6].

Concerning SOD level in the current work, group III exhibited a significant ascent in its value relative to group II. This agrees with previous work which reported that the monosodium glutamate intoxicated rats ameliorated by curcumin co-administration expressed a highly increased SOD activity in the renal tissue[40].

Regarding group III in this study, the renal histological analysis revealed a normal histological appearance of Malpighian corpuscles with well-formed glomeruli and tubules. However, pyknotic nuclei were noted in few cells of some tubules. These findings go parallel with the morphometric analysis which showed a non-significant difference versus the control rats. Such outcomes were compatible with previous results which stated that the rats treated with nano-curcumin displayed a typical kidney histology comparable to that of the control rats[6]. Also, previous authors noted a well-formed brush border on the apices of the tubular lining cells in nano-curcumin treated animals[41].

Group III PAS slices illustrated intact brush border in most tubules. Bowman's capsule outer layer, basal laminae of the glomeruli and many renal tubules all appeared normal and regular. These results were enforced morphometrically as PAS reaction area% recorded a significant rise contrasting to group II with inconsiderable deviation relative to group I. A further support was reached from another author who noted similar observations detected in curcumin treated rats[42].

In the current research, group III showed a moderate cytoplasmic immunopositivity for caspase-3 in the cells lining kidney tubules. This was illustrated by morphometric records revealing a significantly decreased area percent of caspase-3 +ve reaction versus group II.

Similar findings were previously coping with that, it was declared that curcumin inhibited apoptosis via up-regulating Bcl-2 protein that oppose apoptosis and down-regulating Bax and caspase-3 proteins that trigger apoptosis[43]. Additionally, comparable results were obtained by[44].

Examination of Ki-67 in group III showed intense widespread Ki-67 immuno-expression in the nuclei of numerous tubular lining cells. This goes parallel with the morphometric analysis displaying a notably raised area% of Ki-67 +ve reaction relative to groups I and II.

A comparable discovery was made by other authors who claimed that Ki-67 in curcumin- treated groups showed a similar staining to the control[45].

Examination of Cox-2 in group III revealed faint cytoplasmic expression at the cells of the renal tubules and glomeruli. Morphometric results enforced the latter finding revealing a substantial descent of area% of Cox-2 immunopositivity relative to group II. Similar outcomes were recorded by[46,47].

In this study, concerning Group IV (rats receiving BSO), urea and Cr levels declined significantly versus that belonging to group II. This resembles the conclusions of other authors who reported that these values were dramatically reduced and mostly were normalized after BSO administration to animals[11]. Moreover, SOD level in the same group compared to that related to group II demonstrated a significant increase. Such finding showed matching with results of[48].

Renal sections of group IV illustrated the standard histological configuration of Malpighian corpuscles, glomeruli and tubules, although minor lining cells displayed intensely stained, shrunken nuclei and cytoplasmic vacuolation in some tubules. These findings go with the morphometric evaluation which exhibited inconsiderable deviation from the control.

These outcomes support the previous conclusions which reported that the kidney tissues of group receiving BSO showed nearly restored normal tissues[11]. These findings are similar to results obtained by previous authors who reported that rats treated with black seed oil showed a normal appearance of the tubules, glomeruli and tubule-interstitial cells[49].

Comparable results were previously obtained and explained by that BSO suppressed necrosis in the tubular cells thanks to limiting the oxidative stress series hence prevention of break down signs in the kidney tissue[50].

In the current work, the majority of renal tubules in PAS sections of group IV displayed intact brush borders and regular parietal layer of Bowman's capsule, basal laminae of many tubules, and glomerular capillaries. These outcomes were translated morphometrically by a significantly raised area% of PAS reaction in this group relative to group II with non-significant deviation from the control group. A past study came in line with this observation and reported that PAS section of nigella Sativa extract was comparable to that of the control[51].

Similar observations were reported by previous authors who claimed that PAS sections of rats given nigella sativa showed a non-interrupted basal laminae and brush borders on the tubules[52].

The cells of the renal tubules in group IV had moderate Caspase-3 cytoplasmic immunopositivity. This was reinforced morphometrically by a considerable descent in Caspase-3 immunopositivity versus group II.

This was reported formerly, that kidney in Nigella sativa group revealed a considerable reduction in the cellular apoptosis[53]. Similarly, in a prior study, it was found that rats treated with Nigella sativa displayed a substantial reduction in caspase-3 gene expression[9].

Numerous tubular cells in group IV that were examined with Ki-67 displayed a widespread intense nuclear staining confirmed by a notable rise in area% of Ki-67 immunopositivity relative to groups I and II.

This copes with a previous research which found that the active component of BSO named "thymoquinone" significantly conserved cellular proliferation in the rats relative to the control ones[54]. Contrarily, it was assigned that thymoquinone dramatically reduced Ki-67 gene expression in tumor tissues compared to the control[55].

Group IV showed faint Cox-2 cytoplasmic expression at the cells of renal tubules and glomeruli. This goes parallel with morphometric analysis which displayed a substantial decline of Cox-2 immunopositivity versus group II.

This was in agreement with previous work which reported that BSO group revealed weak to mild positive immune-reactivity for COX-2 in the renal tubules and renal corpuscles. Such finding is most probably linked to inflammation suppressing properties of TQ, which inhibits COX-2 in the kidney and lowers formation of inflammatory prostaglandins[56].

This finding is similar to that obtained by other authors who stated that thymoquinone negatively affects inflammation promoting molecules including nitric oxide, TNF-, interleukin 1,6, and COX-2 via blocking cascades of IRAK-associated AP-1/NF-B[57].

CONCLUSION

We can conclude that both nano-curcumin and BSO succeeded in protecting the cortical tissue from the adverse effects induced by aspartame and their efficiency was nearly similar, it can be explained by their anti-apoptotic, anti-oxidant and anti-inflammatory actions. However, further experimental and clinical studies are recommended for better confirmation of their action on humans.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثيرالجزيئات المتناهية الصغر للكركمين مقابل زيت الحبة السوداء علي تغيرات القشرة الكلوية المحدثة بالأسبارتام في ذكر الجرذ الأبيض البالغ. دراسة هستولوجية وهستوكيميائية مناعية

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المقدمة: الأسبارتام هو مُحلي صناعي يستخدم على نطاق واسع. على الرغم من ذلك فهو يعتبر مركب خطير حيث أنه يسبب العديد من المشاكل الصحية عند استخدامه لفترات طويلة. ويعتبر الكركمين وزيت الحبة السوداء إثنان من أهم مضادات الأكسدة الطبيعية المستخدمة في الطب البديل. حديثا أظهرت الصياغات النانوية للكركمين توافر بيولوجي وذوبان افضل له ومن ثم وصول تركيزات علاجية أكتر من الكركمين والتي تحسن فعاليته.

الهدف من العمل: تم عمل هذه الدراسة لفحص تأثير الجزيئات المتناهية الصغر للكركمين مقابل زيت الحبة السوداء علي التغيرات الهستولوجية للقشرة الكلوية المحدثة بالأسبارتام في ذكر الجرذ البالغ.

المواد والأساليب: تم تقسيم ٤٥ فأر إلى أربع مجموعات. المجموعة الضابطة وثلاث مجموعات تجريبية وهى: مجموعة الأسبارتام: وقد أخذت الأسبارتام فمويا بجرعة ٢٠٠ ملليجرام لكل كيلوجرام فى اليوم لمدة ٦ أسابيع. مجموعتا الجزيئات المتناهية الصغر للكركمين وزيت الحبة السوداء: وقد أخذتا الأسبارتام كما في المجموعة السابقة بالتزامن مع الجزيئات المتناهية الصغر للكركمين بجرعة ٢٠٠ملليجرام لكل كيلوجرام فى اليوم وجرعة ٢.٠ مللي لكل كيلو جرام في اليوم على التوالي فمويا لمدة ٦ أسابيع. تم قياس مستوي اليوريا والكرياتينين في مصل الدم ومستوي أنسجة الكلي لفوق أكسيد الدسميوتاز. كما تم صباغة قطاعات الكلي بالهيماتوكسيلين والإيوسين وصبغة بيرأيوديك شيف الحامضي والصبغ الهستوكيميائي المناعي ضد كاسباس-٣ وإنزيم الأكسدة الحلقى-٢ و ك أى٦٧بالإضافة إلى ذلك تم عمل القياسات المورفومترية والتحليل الإحصائي.

النتائج: أظهرت مجموعة الأسبارتام تغيرات إنحلالية واضحة في الأنابيب والكبيبات الكلوية، ووجد انخفاض ذو دلالة إحصائية كبيرة فى منطقة الكبيبات ومتوسط نسبة مساحة تفاعل بيرأيوديك شيف الحامضي بالإضافة إلى متوسط عدد الخلايا الموجبة للصبغة المناعية ك أى٦٧ علاوة على وجود زيادة ذات دلالة إحصائية كبيرة في متوسط النسبة المئوية لمساحة التفاعل الهستوكيميائى المناعى لإنزيم الأكسدة الحلقى-٢و كاسباس-٣. بينما أظهرت مجموعتا الجزيئات متناهية الصغر للكركمين وزيت الحبة السوداء مظهرا طبيعيا لنسيج قشرة الكلي، وتحسنت أيضا نتائج الكيمياء الحيوية والقياسات المورفومترية غير الطبيعية التي ظهرت في مجموعة الأسبارتام.

الخلاصة: أثبت كل من الجزيئات متناهية الصغر للكركمين وزيت الحبة السوداء تأثيرا وقائيا ملحوظًا على البنية النسيجية لقشرة الكلى ضد التغيرات الضارة التي يسببها الأسبارتام وكانت فاعليتهما متماثلة تقريبا. و عليه يمكن أخذ هذه النتائج في الاعتبار إكلينيكيا و لكن بعد إجراء دراسات إضافية على الإنسان لتحديد المريض المناسب وضبط الجرعة المطلوبة.