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Role of Spirulina Extract in Ameliorating Histological and Ultrastructural Changes Induced by Sodium Benzoate in the Renal Cortex of Adult Male Albino Rats

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Abstract:

Background: Spirulina is a blue green algae used as a food supplement. Sodium benzoate is used as a food preservative. **Aim of the work:** To assess the role of spirulina extract in ameliorating histological and ultrastructural changes induced by sodium benzoate in the kidney of adult male albino rats**. Materials and methods:** Thirty two adult male albino rats divided into four groups (8 rats each):

Group I (control): received 1ml of distilled water daily.

Group II (Spirulina): received 1ml of prepared spirulina solution 500 mg/kg/day orally.

Group III (Sodium benzoate): received 600 mg/kg body weight sodium benzoate daily dissolved in distilled water orally.

Group IV (Sodium Benzoate+ Spirulina group): received both sodium benzoate and spirulina in the same dose and manner as group II and group III .

All rats were given treatment orally once daily for 28 consecutive days then renal specimens were processed for light and electron microscopic study. **Results:** Group III showed significant increase in body weight compared with group I and group II. Group II and group IV showed non significant decrease in body weight compared to group III. Histopathological changes of renal cortex were noticed in group III. Group IV revealed improvement in the renal cortical tissue. **Conclusion:** Sodium benzoate increases the body weight and has hazardous effects on the histology of the renal cortex. While, Spirulina extract can ameliorate histopathological changes induced by sodium benzoate in renal cortex. Thus, Spirulina can be used as a Therapeutic agent to treat the renal cortex from sodium benzoate induced injury.

Key words:

Sodium benzoate, Spirulina, renal cortex, histological, ultrastructural

1. Introduction:

Sodium benzoate (NAB) has antifungal and antibacterial properties. Therefore it is known as a preservative in food, medical products and cosmetics. It is a salt derived from benzoic acid. It has no taste or smell and is water soluble. Its use prevents proliferation of bacteria, yeast and mold [1,2].

Despite its worldwide use in beverages, salads, vinegar, jams, and sauces, it has multiple hazardous effects on many organs. NAB was also known to be useful in treatment of acute hyperammonemia and multiple sclerosis. Moreover, it was used in cases of hepatic encephalopathy [3].

It has been set that the highest safe dose of sodium benzoate in food is one g/kg. However, its safety in human is controversial. NAB increases oxidative stress and has adverse effects on proliferation of neurons, reproductive system and liver cells. High doses of NAB harms pancreas leading to inhibition of insulin secretion and diabetes mellitus [4].

Spirulina (SP) is a blue green algae. It is a food supplement for both human and animal. It grows in fresh water in warm countries. It provides minerals, essential fatty, amino acids, carotenoids and vitamins. It has anti-inflammatory, antitumor, hepatoprotective, radio protective, antimicrobial, strengthening immune system. It has also metallo-protective and antioxidant properties [5,6].

In addition, SP provides C phycocyanin that has antioxidant properties with a high capacity to reduce free radicals such as superoxide and hydroxyl radicals. It protects against reactive oxygen species due to suppressive effect on lipid peroxidation chain reaction [7].

Objectives of the research:

To assess the role of spirulina extract in ameliorating histological and ultrastructural changes induced by sodium benzoate in the kidney of adult male albino rats

2. Materials And Methods:

Thirty two adult male albino rats weighed (180-220 grams each) were involved in this experiment. They were collected from animal house of Faculty of Medicine, Tanta University, Egypt, and were fed on the standard laboratory diet ad libitum. They were all kept under the same environmental

conditions. This animal experiment was approved by the local ethical committee of the Faculty of Medicine, Tanta University, Egypt (Approval number: (36264PR1202\4\25).

Chemicals:

Sodium benzoate was provided from Misr Company for Pharmaceuticals, Qalyubia Governorate, Egypt.

Spirulina powder was purchased as a fine dark blue-green powder from Imtenan, Cairo, Egypt. Dissolved spirulina solution was prepared at a concentration of 100 mg/1ml by dissolving 1000 mg spirulina powder in 10 ml in distilled water [8].

Experimental design:

Rats were divided into four groups (8 rats each) as follows:

Group I (control):

Each one received 1ml of distilled water daily (the diluting vehicle for both spirulina and sodium benzoate solution) for 28 consecutive days then sacrificed at the end of the study.

Group II (Spirulina group):

Each one received 1ml of prepared spirulina solution 500 mg/kg/day orally for consecutive 28 days then sacrificed [8].

Group III (Sodium Benzoate group):

Each one received 600 mg/kg body weight sodium benzoate once daily dissolved in distilled water orally for 28 consecutive days then sacrificed [3].

Group IV (Sodium Benzoate+ Spirulina group):

Each one of them received both sodium benzoate and spirulina in the same dose and manner as group II and group III for consecutive 28 days.

After scarification of the rats from different subgroups, specimens of the kidney were taken and subjected to histological examination.

At the end, sacrificed rats were safely collected in a special package according to safety and health precaution measures to be incinerated later.

The duration of the study was 28 days.

I) Statistical Analysis:

Regarding the weight, it was measured at the start and at the end of the experiment. The collected data were analyzed statistically. The mean, the standard deviation (S.D) and the (P) value were calculated using Statistical Package for the Social Sciences version 20. Differences were considered as ** Highly significant if P value ≤ 0.001 and significant if P-value <0.05 *.

II) Sample Preparation and Examination

The specimens of kidney of different subgroups were extracted. The right one was fixed in 10% formol saline for light

microscopic examination. The left one was fixed in 3% glutaraldehyde in 0.1 phosphate buffer solution for transmission electron microscopic examination [9]. Hematoxylin and Eosin Staining was used for light microscopic examination of renal cortical tissue [10].

3. Results:

I) Statistical study:

Body weight measurements :

The initial body weight of rats from all groups ranged from 180 to 220 gm. Final body weight statistical analysis at time of scarification showed a significant increase in body weight in rats from group III (NAB group) compared to the control group. Non significant difference was noticed between control group and the other groups (group II&IV). A significant increase in the body weight was noticed in group III in comparison with group II. Non significant difference was seen between group II and group IV. Non significant decrease in weight was noticed in group IV in comparison with group III.

Table 1 Final body weight (gm) from all groups.

Groups	**Final body weight (g)**		**ANOVA**	
	Range	**Mean±SD**	**F**	**P Value**
Group I (control)	**290-305**	**297.75±4.652**	**3.888936**	**<0.05**
Group II (SP group)	**293-305**	**298.62±4.033**		
Group III (NAB group)	**280-345**	**315.75±22.78**		
Group IV (NAB & SP group)	**293-305**	**300.87±5.33**		
T test:				
P Value	Groups I and II 0.693	Groups I and III 0.046*	Groups I and IV 0.232	
	Groups II and III 0.055*	Groups II and IV 0.357	Groups III and IV 0.093	

ANOVA, Analysis of variance. P>0.05, no significant difference.

* P≤0.05, significant difference. **P≤0.001, highly significant difference.

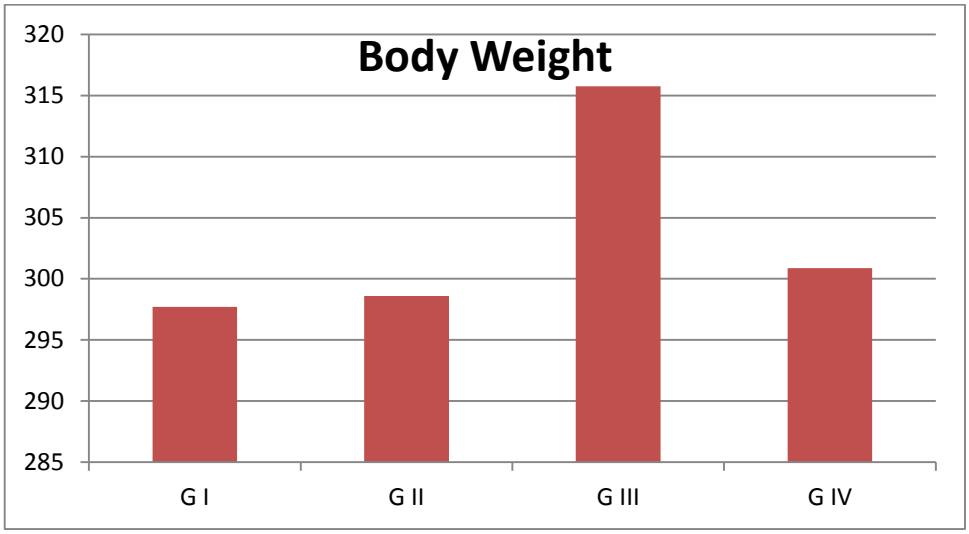

Fig. (1): A histogram showing final body weight of rats from all groups at scarification time.

Hematoxylin and Eosin stained sections of the renal cortex:

Examination of Photomicrographs of cross sections of the renal cortex of both group I (control) and group II revealed the normal structure of the renal cortex and showed the normal renal corpuscles with regular continuous Bowman's capsule. Cells of proximal tubules had eosinophilic cytoplasm, rounded basal nuclei and intact brush border. Distal tubules had clear wide lumin. Cells of distal tubules had clear wide luminal surface and basally placed nuclei. Peritubular capillaries were observed Fig. 2(A & B).

While group III (NAB group) showed renal corpuscles with wide capsular space, discontinuity of Bowman's capsule and adhesion of the glomerulus with parietal layer of Bowman's capsule. Intertubular hemorrhage was noticed. Vacuolated tubules and congested peritubular capillaries were also seen. Proximal tubules cells contained vacuolated cytoplasm with destructed brush border. Some cells had dark pyknotic nuclei. Cells of distal tubules showed vacuolated cytoplasm Fig.3 (A, B, C & D).

Regarding group IV (NAB & SP group), examination of sections of renal cortex showed improvement in the histological features compared to NAB group and revealed renal corpuscles either with regular continuous Bowman's capsule or with point of adhesion with parietal layer of Bowman's capsule. Peritubular capillaries were observed as that of the control group. Some capillaries appeared congested. Proximal tubules had cells with eosinophilic cytoplasm, basal basophilic nuclei and intact brush border. However, some cells had vacuolated cytoplasm. Cells of distal tubules had clear wide luminal surface and basally placed nuclei.

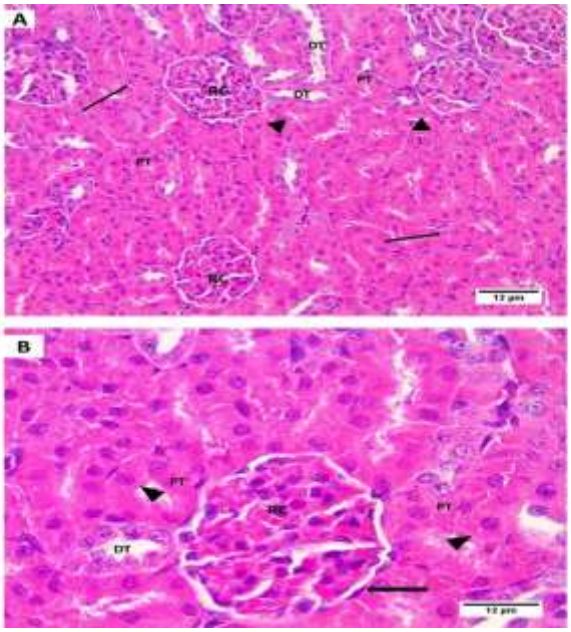

Fig. (2): Renal cortex Hx. & E

Photomicrographs of cross sections of the renal cortex of group I (control) (A&B) showing: (A): Renal corpuscles (Rc) are seen. Cells of proximal tubules (PT) have an eosinophilic cytoplasm, rounded basal nuclei and striated luminal border (arrow heads). Distal tubules (DT) have clear wide lumin. Peritubular capillaries are observed (arrows). (A: Hx&E x400). (B): Renal corpuscle (Rc) with regular continuous Bowman's capsule (arrow). Proximal tubules (PT) cells appear containing eosinophilic cytoplasm, basal basophilic nuclei and intact brush border (arrow heads). Cells of distal tubules (DT) have clear wide luminal surface and basally placed nuclei. Peritubular capillaries are observed (B: Hx&E x1000).

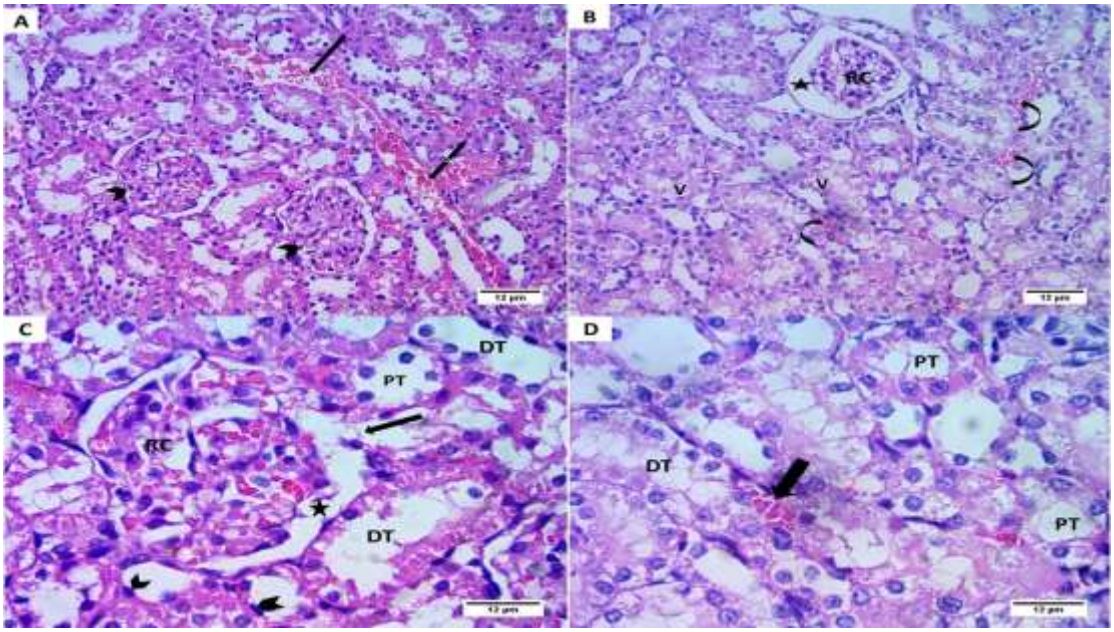

Fig. (3): Renal cortex Hx. & E

Photomicrographs of cross sections of the renal cortex of group III (NAB group) (A,B,C &D) showing: (A): Renal corpuscle with adhesion of the glomerulus with parietal layer of Bowman's capsule (arrow head). Intertubular hemorrhage is noticed (arrows). (B): Renal corpuscle with wide capsular space (star). Vacuolated tubules (V) and congested peritubular capillaries (curved arrows) are also seen (A&B: Hx & E x 400). (C): Renal corpuscle with widening of capsular space (star) and discontinuity of Bowman's capsule (arrow). Proximal tubules (PT) cells contains vacuolated cytoplasm with destructed brush border. Some cells have dark pyknotic nuclei (arrow heads). Cells of distal tubules (DT) show vacuolated cytoplasm. (D): Proximal tubules (PT) cells contain vacuolated cytoplasm with destructed brush border. Cells of distal tubules (DT) show vacuolated cytoplasm. Congested peritubular capillaries (arrow) are also seen (C&D: Hx & E x 1000).

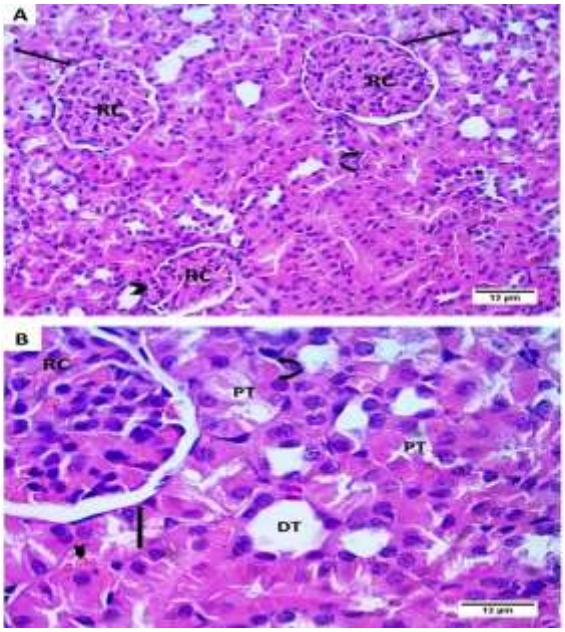

Fig. (4): Renal cortex Hx. & E

Photomicrographs of cross sections of the renal cortex of group VI (NAB & SP group) (A&B) showing: (A): Renal corpuscles (Rc) either with regular continuous Bowman's capsule (arrow) or with point of adhesion (arrow head) with the parietal layer of Bowman's capsule. Peritubular capillaries are observed (B: Hx&E x400). (B): Renal corpuscles (Rc) with regular continuous Bowman's capsule (arrow). Proximal tubules (PT) have cells with eosinophilic cytoplasm, basal basophilic nuclei and intact brush border. However, some cells have vacuolated cytoplasm. Cells of distal tubules (DT) have clear wide luminal surface and basally placed nuclei. Peritubular capillaries are observed (curved arrow). Some capillaries appear congested (arrow head) (Hx & E x1000).

Electron microscopic examination of the renal cortex:

Examination of the ultrastructural sections of the renal cortex of control group and group II showed epithelial cells of proximal convoluted tubules containing a large rounded euchromatic nucleus. Apical microvilli, basement membrane and multiple mitochondria were noticed. Epithelial cells of distal convoluted tubules were seen resting on the basement membrane and contained large well defined euchromatic nuclei and multiple longtudinal mitochondria. Podocyte bodies with interdigitating regular spaced feet processes separated by filtration slits were also seen. Endothelial cells lining the glomerular capillaries were separated by fenestrations. The glomerular basement membrane appeared with outer and inner electrolucent layers with an intermediate electron dense layer Fig. 5, 6 (A&B).

While, group III (NAB group) showed epithelial cells of proximal convoluted tubules with destructed apical part and destructed brush border and containing nuclei with condensed chromatin & indented nuclear membrane. Areas of rarified cytoplasm, vacuoles and swollen mitochondria with destructed cristae were also noticed. Epithelial cells of distal convoluted tubule showed shrunken nuclei with condensed chromatin resting on a thickened basement membrane. The cytoplasm contained vacuoles and swollen mitochondria with destructed cristae. Distorted podocytes and disturbed, swollen, effaced, fused and irregularly spaced Foot processes were seen. Also, thickened glomerular basement membrane with loss of the trilaminar manner of it was seen Fig. 7 (A,B & C) and Fig. 8 (A, B).

Regarding group IV (NAB & SP), revealed epithelial cells of proximal convoluted tubule containing normal rounded euchromatic nuclei and multiple mitochondria. The apical part shows the intact brush border. Some vacuoles was seen in the cytoplasm. Epithelial cells of distal convoluted tubule was seen resting on the thin basement membrane and contained euchromatic nuclei and longtudinal mitochondria. Podocytes with euchromatic nucleus were seen. Nearly regular spaced Interdigitating feet processes were seen separated by filtration slits. Some foot processes appear effaced. The glomerular basement membrane appeared with outer, inner electrolucent layers and an intermediate electron dense layer Fig. 9, 10 (A & B).

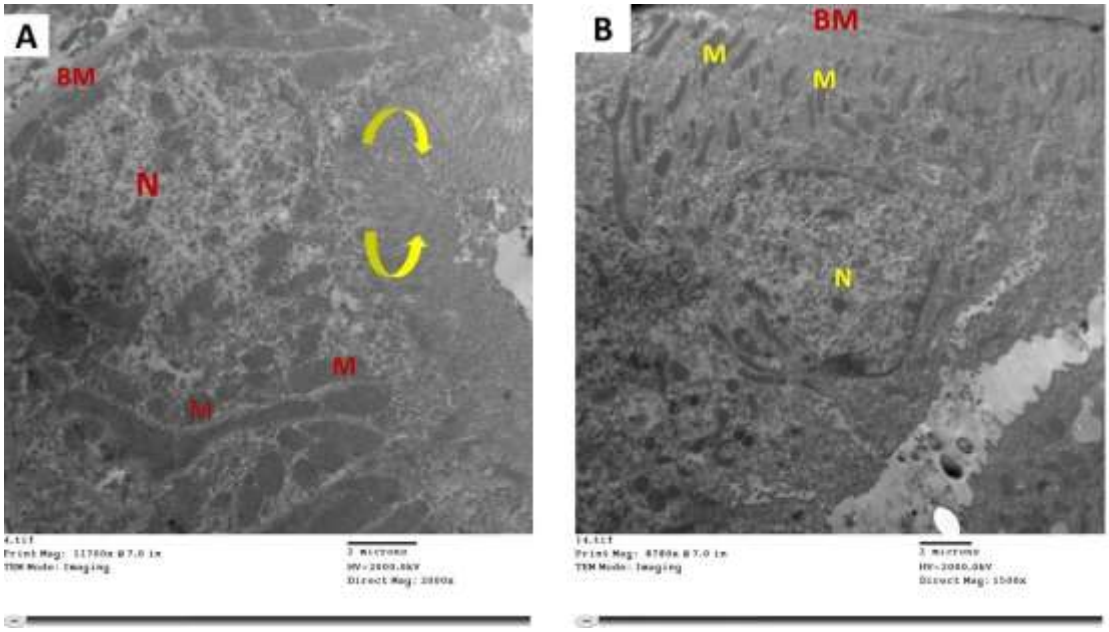

Fig. (5): An electron micrograph of a section of renal cortex from Group I (Control group) (A&B) showing: (A): Epithelial cell of proximal convoluted tubule containing a large rounded euchromatic nucleus (N). Multiple mitochondria (M) appear within cytoplasm. Apical microvilli (curved arrows) and basement membrane (BM) are seen. (B): Epithelial cell of distal convoluted tubule resting on the basement membrane (BM) containing large well defined euchromatic nucleus (N). The cytoplasm shows shows multiple longtudinal mitochondria (M).

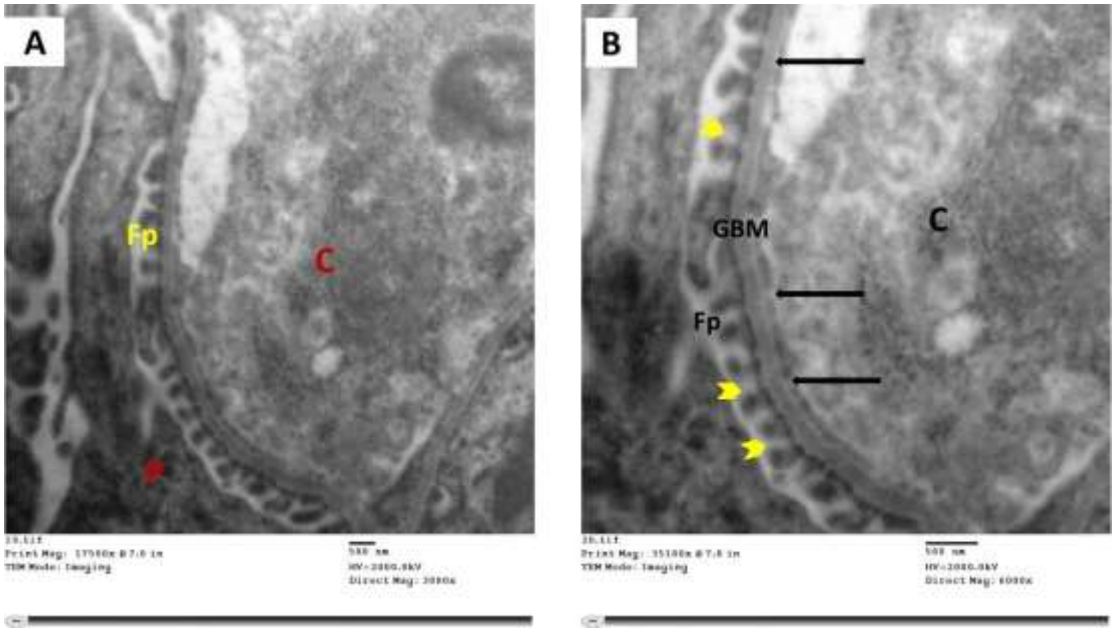

Fig. (6): An electron micrograph of a section of renal cortex from Group I (Control group) (A&B) showing: (A): A portion of renal glomerulus showing glomerular capillary (C). Part of podocyte body (P) and interdigitating foot processes (Fp) are seen. (B): A higher magnification of the previous photo showing endothelial cells lining the

capillary (C) separated by fenestrations (arrows). The glomerular basement membrane (GBM) appears with outer and inner electrolucent layers with an intermediate electron dense layer. Nearly regular spaced feet processes (Fp) are seen separated by filtration slits (arrow heads).

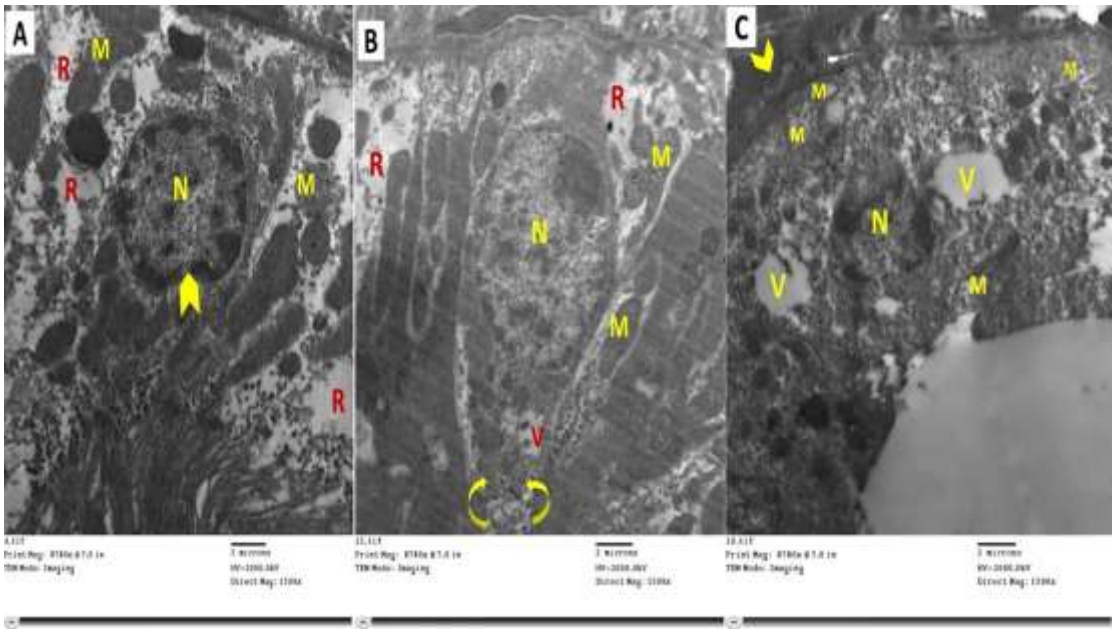

Fig. (7): An electron micrograph of a section of renal cortex from Group III (NAB group) (A,B&C) showing: (A): Epithelial cell of proximal convoluted tubule containing a nucleus (N) with condensed chromatin & indented nuclear membrane (arrow head). The cytoplasm shows areas of rarified cytoplasm (R) and mitochondria with destructed cristae (M). (B): Another epithelial cell of proximal convoluted tubule with destructed apical part and destructed brush border (curved arrows). The cytoplasm shows vacuoles (V), areas of rarified cytoplasm (R) and swollen mitochondria (M) with destructed cristae. (C): Epithelial cell of distal convoluted tubule showing shrunken nucleus (N) with condensed chromatin, resting on a thickened basement membrane (arrow head). The cytoplasm contains vacuoles (V) and swollen mitochondria with destructed cristae (M).

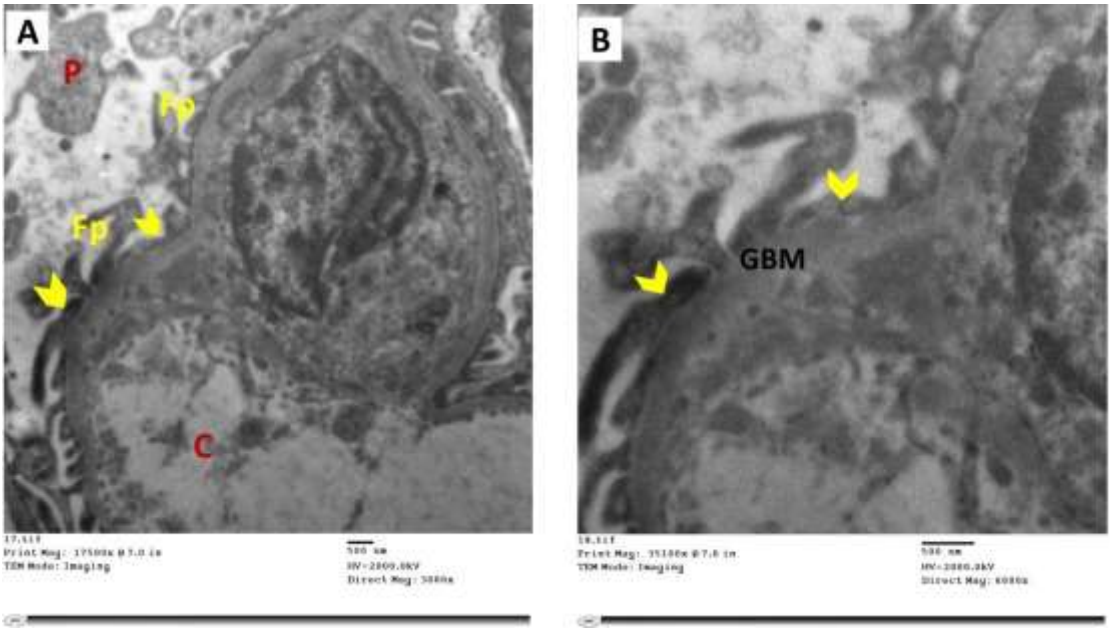

Fig. (8): An electron micrograph of a section of renal cortex from Group III (NAB group) (A&B) showing: (A): Distorted podocyte (P) and a capillary are seen. Foot processes (Fp) are disturbed, swollen, effaced, fused and irregularly spaced (arrow heads). A higher magnification of the previous photo showing thickening of the glomerular basement membrane (GBM) with loss of the trilaminar manner of it. Effacement of foot processes is noticed (arrow heads).

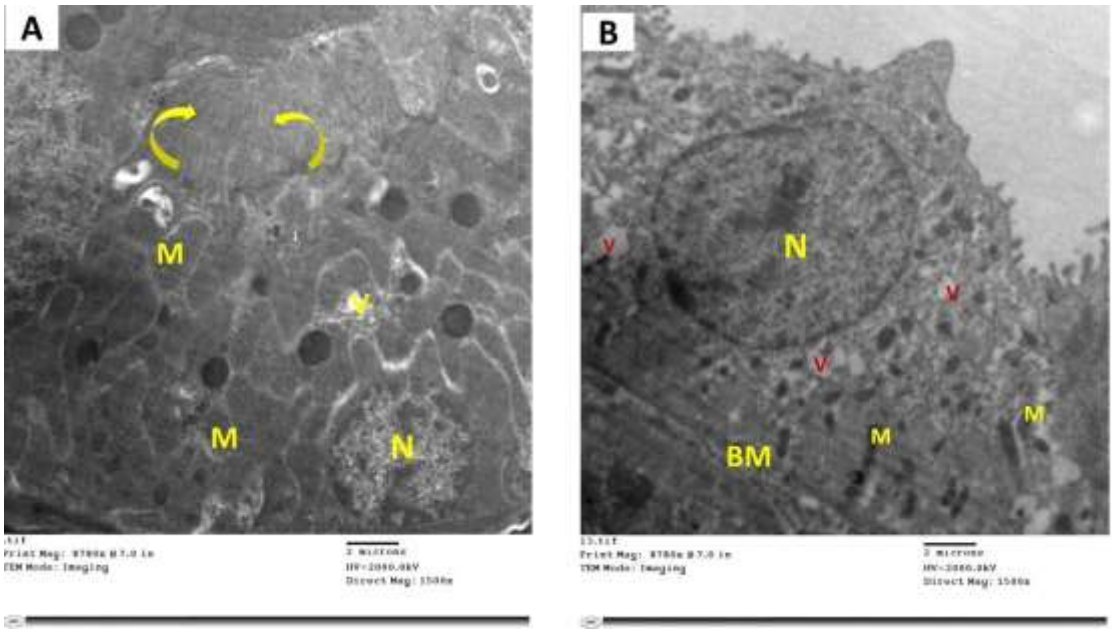

Fig. (9): An electron micrograph of a section of renal cortex from Group IV (NAB & SP group) (A&B) showing: (A): Epithelial cell of proximal convoluted tubule containing normal rounded euchromatic nucleus (N) and multiple mitochondria (M). The apical part shows the intact brush border (curved arrows). Vacuole is seen in the cytoplasm (V). (B): Epithelial cell of distal convoluted tubule resting on the thin

basement membrane (BM) containing large euchromatic nucleus (N) and longtudinal mitochondria (M). Some vacuoles (V) are seen.

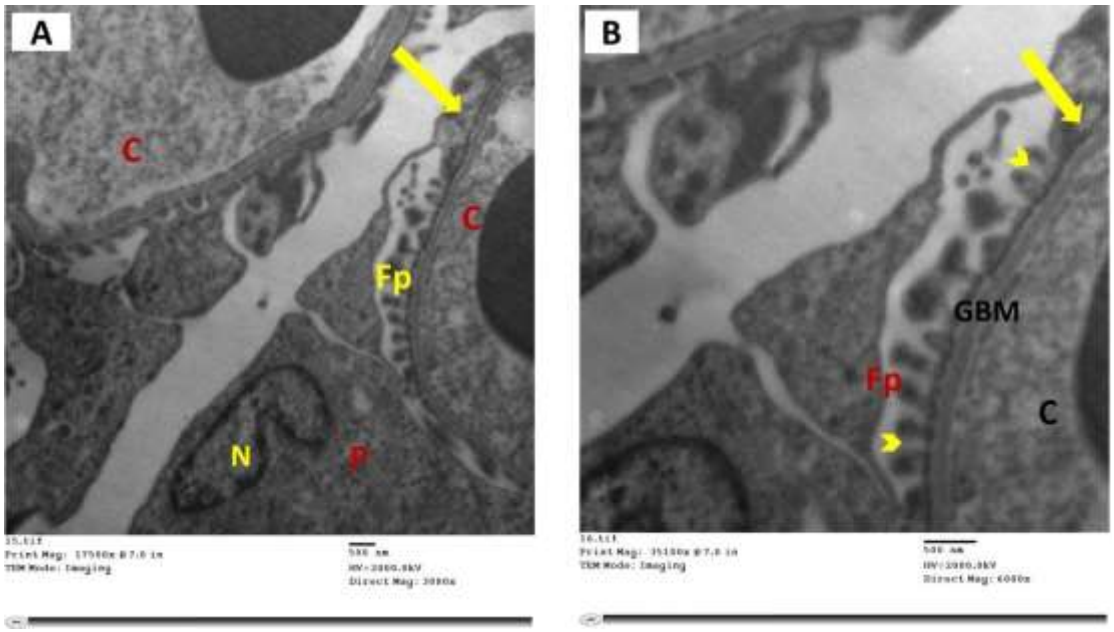

Fig. (10): An electron micrograph of a section of renal cortex from Group IV (NAB & SP group) (A&B) showing: (A):Parts of two glomerular capillaries (C). Part of podocyte (P) with euchromatic nucleus (N) is seen. Interdigitating foot processes (Fp) are seen. Some foot processes appear effaced (arrow). (B): A higher magnification of the previous photo showing the glomerular basement membrane (GBM) with outer, inner electrolucent layers and an intermediate electron dense layer. Nearly regular spaced feet processes (Fp) are seen separated by filtration slits (arrow heads). While some feet processes appear effaced (arrow).

4. Discussion:

Kidney diseases are classified into chronic and acute diseases. Chronic kidney disease is characterized by the gradual loss of kidney function. It may result from hypertension, diabetes and obesity. The worldwide rise in kidney disease is a result of lifestyle and food habits. NAB is used worldwide as a food preservative that was reported to be harmful to different body organs [2,11].

Spirulina is one of the most common herbal medicine for treatment of many diseases. It has many pharmacological properties as anti-inflammatory, anti-cancer, antidiabetic, anti-microbial, anti-histaminic, anti-infertility, hypotensive, neuroprotective, hepatoprotective, and anticarcinogenic properties[12].

It is called “the food of the future” due

to its excellent components and high energy. It has been used in healthy foods, animal feed, and biological products since the 1980. It can be supplied as spirulina pills, capsules, pastries, blocks, and spirulina containing chocolate bars [13].

Regarding final body weight statistical analysis at time of scarification. A significant increase in body weight was seen in group III (NAB group) compared to the control group. Non significant difference was noticed between control group and the other groups. A significant increase in the body weight was noticed in group III NAB group in comparison with group II (SP group). Non significant difference between group II and group IV was found. Non significant decrease was noticed in rats from group IV (NAB & SP group) in comparison with group III NAB group. These results coincided with (**Lee et al., 2024**) [14] who found that sodium benzoate treatment was associated with obesity due to increased fat accumulation.

In contrast with our results, some researchers explained that sodium benzoate decreased the body weight and stated that this was caused by deterioration of health status [15].

Furthermore, previous studies reported non significant difference in body weight between sodium benzoate treated rats and the control group [16].

Light and electron microscopic examination of sections of renal cortex of group II (SP group) showed the normal features similar to control group. These results coincided with other workers who noticed that spirulina did not change the normal structure of kidney [17,18].

On the other side, some researchers noticed that intake of large doses of Spirulina induced histopathological alteration and impaired function of the kidney in a dose dependent manner [19].

In this experiment, light microscopic picture of the renal cortex of group III (NAB treated) revealed obvious changes indicating impaired renal function. Hx & E sections demonstrated some renal corpuscles with adhesion of the glomerulus with Bowman's capsule. While, other renal corpuscles showed wide capsular space and discontinuity of Bowman's capsule. Proximal tubules cells contained dark pyknotic nuclei, vacuolated cytoplasm with destructed brush border and congested peritubular capillaries. Distal tubule cells contained vacuolated cytoplasm. Intertubular hemorrhage was also noticed. These

findings were in accordance to other workers who suggested that sodium benzoate caused nephrotoxicity due to increased levels of oxidative free radicals. [20].

Moreover, these results coincided with other researchers who noticed that prolonged use of sodium benzoate could lead to many changes in the kidney starting from inflammation and distortion of kidney architecture to impaired kidney function. Peritubular capillaries congestion was seen in NAB treated group and reported by other workers [21].

Cytoplasmic vacuolation was attributed to damage of the cellular organelles. NAB activates apoptotic pathways as a result of oxidative stress and increased concentration of oxidative markers as malondialdehyde. In addition, decreased antioxidants like superoxide dismutase and accumulation of reactive Oxygen species could affect mitochondrial function causing cell destruction and inducing apoptosis [22,23].

Ultrastructural examination of sections of renal cortex from group III (NAB group) confirmed the light microscopic results and showed epithelial cells of proximal convoluted tubule with destructed brush border and containing nuclei with condensed chromatin & indented nuclear membrane. Areas of rarified cytoplasm, vacuoles and swollen mitochondria with destructed cristae were also noticed. Epithelial cells of distal convoluted tubule showed shrunken nuclei with condensed chromatin resting on a thickened basement membrane. The cytoplasm contained vacuoles and swollen mitochondria with destructed cristae. Distorted podocyte and disturbed, swollen, effaced, fused and irregularly spaced Foot processes were seen. Also, thickened glomerular basement membrane with loss of its the trilaminar manner was seen. These results were in accorance to other authors who stated that sodium benzoate treated rats showed glomerular atrophy, renal cell necrosis, vascular congestion, tubular damage, vacuolization of tubular cells [20].

These results coincided with other authors who explained that prolonged sodium benzoate intake caused mitochondrial dysfunction and damage of mitochondria resulting in apoptosis and renal cells necrosis. These changes caused chronic kidney diseased [21]. Mitochondrial dysfunction occurred as a result of reactive oxygen species and renal oxidative stress. Prolonged

sodium benzoate intake impaired renal functions [24,25].

In our experiment, light microscopic sections of renal cortex in group IV (NAB & SP treated) revealed improvement in the histological features compared to NAB group and revealed renal corpuscles either with regular continuous Bowman's capsule or with point of adhesion with parietal layer of Bowman's capsule. Peritubular capillaries were observed as that of the control group. Some capillaries appeared congested. Proximal tubules had cells with eosinophilic cytoplasm, basal basophilic nuclei and intact brush border. However, some cells had vacuolated cytoplasm. Cells of distal tubules had clear wide luminal surface and basally placed nuclei. These results coincided with other workers who stated that Spirulina significantly ameliorates kidney damage thorough its antioxidant & anti-inflammatory activity. It caused improvement of both kidney function markers and histopathology of kidney. Spirulina had protective effect on kidney as a result of its antiapoptotic, anti-inflammatory and antioxidant properties [18,25].

Additionally, spirulina is a rich source of C phycocyanin which is a powerful antioxidant with a high capacity to scavenge free radicals such as superoxide and hydroxyl radicals. It provides significant protection against ROS due to its radical scavenging action and suppressive impact on lipid peroxidation chain reaction [8].

SP is a great source of necessary fatty acids as gamma linoleic acid and alfa linolenic acid that ameliorate tissue regeneration. Meanwhile, it is a rich supply of flavonoids that decreased peroxidation of lipid and enhanced DNA synthesis [26].

Ultrastructural examination of sections of renal cortex from group IV (NAB & Sp group) confirmed the light microscopic results and revealed improvement as compared to NAB group and showed epithelial cells of proximal convoluted tubule containing normal rounded euchromatic nuclei and multiple mitochondria. The apical part shows the intact brush border. Some vacuoles was seen in the cytoplasm. Epithelial cells of distal convoluted tubule was seen resting on the thin basement membrane and contained euchromatic nuclei and longtudinal mitochondria. Podocytes with euchromatic nucleus were seen. Nearly regular spaced interdigitating feet processes were seen separated by filtration slits. Some foot processes

appear effaced. The glomerular basement membrane appeared with outer, inner electrolucent layers and an intermediate electron dense layer. These results were in accordance to other workers who reported that SP had protective and therapeutic effects on ultrastructure of kidney tissue [27].

In contrast with our results, some authors mentioned that people using supplementary products containing spirulina developed renal failure. Furthermore, they suffered from dysphasia, headache, diarrhea and dehydration. In another case study, SP caused diarrhea and erythema in a 14-years old individual after his oral intake of four Spirulina tablets [19,28,29].

5. Conclusion:

This study demonstrates that sodium benzoate increases the body weight and has hazardous effects on the histology of the renal cortex. While, Spirulina extract can ameliorate histopathological changes induced by sodium benzoate in renal cortex. Thus, Spirulina can be used as a Therapeutic agent to treat the renal cortex from sodium benzoate induced injury.

Conflict of interest:

No conflict of interest.

6. References:

1. **Piper, J.D. and Piper, P.W., (2017)**: Benzoate and sorbate salts: a systematic review of the potential hazards of these invaluable preservatives and the expanding spectrum of clinical uses for sodium benzoate. Compr. Rev. Food Sci. Food Saf. 16 (5), 868–880.
2. **Davidson, P.M.; Taylor, T.M.; David, J.R.D (2021).** Antimicrobials in Food, 4th ed.; CRC Press: Boca Raton, FL, USA; ISBN 978-0-367-17878-9.
3. **Asejeje, F. O.; Ajayi, B. O.; Abiola, M. A.; Samuel, O.; Asejeje, G. I.,; Ajiboye, E. O., et al., (2022):** Sodium benzoate induces neurobehavioral deficits and brain oxido□ inflammatory stress in male Wistar rats: Ameliorative role of ascorbic acid. Journal of Biochemical and Molecular Toxicology, 36(5), e23010.
4. **Li, D., Zhang, L., Yang, P., He, Y., Zhou, T., Cheng, X., Jiang, Z., Long, Y., Wan, Q., Yan, P. and Gao, C., (2024)**: Sodium benzoate induces pancreatic inflammation and β cell apoptosis partially via benzoylation. Ecotoxicology and Environmental Safety, 270, p.115877.
5. **Avdagić, N., Ćosović, E., Nakaš-Ićindić, E., Mornjaković, Z., Začiragić, A., and Hadžović-Džuvo,**

A. (2008): Spirulina platensis protects against renal injury in rats with gentamicin-induced acute tubular necrosis. Bosnian journal of basic medical sciences, 8(4), 331.

6. **Maddiboyina, B.; Vanamamalai, H.K.; Roy, H.; Ramaiah; Gandhi, S.; et al., (2023)**: Food and drug industry applications of microalgae Spirulina platensis: A review. J Basic Microbiol.; 63(6): 573-583.
7. **Ibrahim M, Almaeen A, Abd El Moneim M, Tammam H, Khalifa A and Nasibe M. (2018)**:Cadmium-induced hematological, renal, and hepatic toxicity: the amelioration by spirulina platensis. The Saudi Journal of Forensic Medicine and Sciences. 1(1): 5-14.
8. **Elkelany, M.M. and Kashef, S.M., (2023)**: Role of spirulina extract in ameliorating histological and immunohistochemical changes induced by sertraline in the testis of adult albino rat. Egyptian Journal of Histology, 46(1), pp.136-149.
9. **Kuo, J. (2007)**: Electron microscopy: Methods and protocols. (Editor: JohnKuo). Second edition, Publisher: Humana Press, Western Australia.2007;67.
10. **Bancroft DR. and Cook HC. (1994):** Manual of histological techniques and their Diagnostic applications. Churchill Livingston. Edinbuegh. London. New York. Tokyo P: 23.
11. **Eckardt, K.U., Delgado, C., Heerspink, H.J., Pecoits-Filho, R., Ricardo, A.C., Stengel, B., Tonelli, M., Cheung, M., Jadoul, M., Winkelmayer, W.C. and Kramer, H., (2023)**: Trends and perspectives for improving quality of chronic kidney disease care: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. Kidney international, 104(5), pp.888-903.
12. **Cho J, Baek S, Cheong S and Kim M. (2020):** Spirulina Enhances Bone Modeling in Growing Male Rats by Regulating Growth-Related Hormones. Nutrients; 12(4): 1187-206.
13. **Matufi F and Choopani A. (2020)**: Spirulina, food of past, present and future. Health Biotechnology and Biopharma. 3(4): 1-20.
14. **Lee, J.D., Lee, J., Vang, J. and Pan, X., (2024)**: Sodium Benzoate Induces Fat Accumulation and Reduces Lifespan via the SKN-1/Nrf2 Signaling Pathway: Evidence from the Caenorhabditis elegans Model. Nutrients, 16(21), p.3753.
15. **Helal EG, Barayan AW, Abdelaziz**

MA, EL-Shenawe NS (2019): Adverse effects of mono sodium glutamate, sodium benzoate and chlorophyllins on some physiological parameters in male albino rats. Egypt J Hospit Med. 74(8):18571864.

16. **Al-Ameen, S.A., Jarjees, E.H. and Tawfeeq, F.K., (2022)**: Effect of sodium benzoate on some biochemical, physiological and histopathological aspects in adult male rats. Iraqi Journal of Veterinary Sciences, 36(2): 267-272.
17. **Gargouri, M., Soussi, A., Akrouti, A., Magné, C. and El Feki, A. (2018)**: Ameliorative effects of Spirulina platensis against lead-induced nephrotoxicity in newborn rats: modulation of oxidative stress and histopathological changes. EXCLI journal, 17, p.215.
18. **Abouzed, T.K., Soliman, M.M., Khatab, S.A., Gouda, W.M., Eldomany, E.B. and Dorghamm, D.A. (2022)**: The protective impacts of Spirulina platensis against cisplatin-induced renal injury through the regulation of oxidative stress, pro-inflammatory cytokines and Bax/Bcl2. Toxicology Research, 11(1), pp.169-178.
19. **EL-Tantawi, H.G. and Abozeid, F.S., (2020)**: Evaluation of the side effects of different doses of spirulina on various organs in rats: Biochemical, histological and histochemical assessment. Egyptian Journal of Histology, 43(2), pp.455-464.
20. **Zeghib, K. and Boutlelis, D.A., (2021):** Food additive (sodium benzoate)-induced damage on renal function and glomerular cells in rats; modulating effect of aqueous extract of Atriplex halimus L. Iranian Journal of Pharmaceutical Research: IJPR, 20(1), p.296.
21. **Sheir, M.A., Almaski, A.M., Almughamisi, M.A., Abduljawad, S.H., Elsebaie, E.M. and Ahmed, R.A. (2025)**: Nephroprotective Effect of Aged Black Garlic Extract as a Functional Flock Medicinal on Sodium Benzoate-Induced Chronic Kidney Disease in Albino Rats. Life, 15(2), p.217.
22. **Akter, D., Islam, M.M., Hossain, M.I., Esrafil, M., Dey, B.P., Bari, L. and Zubair, M.A. (2024)**: Toxicological and histopathological effects of sodium benzoate used in commercially available fruit juice on liver and kidney tissue in mice model. Clinical Nutrition Open Science, 58, pp.302-315.

23. **Zhang, L.; Chen, Z.; Li, X. (2022)**: Sodium Benzoate and Its Role in Renal Inflammation: A Review. Toxicol. Rep., 9, 1012–1023.
24. **Khan, I.S.; Dar, K.B.; Ganie, S.A.; Ali, M.N. (2022)**: Toxicological impact of sodium benzoate on inflammatory cytokines, oxidative stress and biochemical markers in male Wistar rats. Drug Chem. Toxicol, 45, 1345–1354.
25. **Zakaria, M.M., El-Tantawy, F.M.M., Khater, S.M., Derbala, S.A., Farag, V.M.E.M. and Abdel-Aziz, A.A.F., (2019)**: Protective and curative role of Spirulina platensis extracts on cisplatin induce acute kidney injury in rats. Egyptian journal of basic and applied sciences, 6(1), pp.54-67.
26. **Mehdinezhad, N., Aryaeian, N., Vafa, M., Saeedpour, A., Ebrahimi, A., Mobaderi, T., Fahimi, R. and Sajadi Hezaveh, Z., (2021)**: Effect of spirulina and chlorella alone and combined on the healing process of diabetic wounds: an experimental model of diabetic rats. Journal of Diabetes & Metabolic Disorders, 20, pp.161-169.
27. **Khalil, S.R., Salem, H.F., Metwally, M.M., Emad, R.M., Elbohi, K.M. and Ali, S.A., (2020)**: Protective effect of Spirulina platensis against physiological, ultrastructural and cell proliferation damage induced by furan in kidney and liver of rat. Ecotoxicology and Environmental Safety, 192, p.110256.
28. **Marles, R.J., Barrett, M.L., Barnes, J., Chavez, M.L., Gardiner, P., Ko, R., Mahady, G.B., Dog, T.L., Sarma, N.D., Giancaspro, G.I. and Sharaf, M., (2011):.** United States pharmacopeia safety evaluation of Spirulina. Critical reviews in food science and nutrition, 51(7), pp.593-604.
29. **Petrus, M., Culerrier, R., Campistron, M., Barre, A. and Rougé, P., (2009)**: Allergy net. Allergy, 65, pp.924-925.