Advances in Environmental and Life Sciences 6(1) (2024) 48- 53

Contents lists available at Egyptian Knowledge Bank

Advances in Environmental and Life Sciences

journal homepage: https://aels.journals.ekb.eg

The Ameliorative Effect of Lycopene Against Sodium Fluoride Induced Renal Injury in Male Albino Rats (Wistar albino)

Lamees Ahmed Elkady[a], Samir Mohamed El Rayes[a, *], Aida Hamed Soliman[a], Obeid Shanab[b], Waleed F Khalil[c], Wael Mahmoud Aboulthana[d], Kholoud Khaled Emam [a,**]

[a] *Department of Chemistry, Faculty of Sciences, Suez Canal University, 41522, Ismailia, Egypt*
[b] *Department of Biochemistry, Faculty of Veterinary Medicine, South Valley University, 83523, Qena, Egypt*
[c] *Department of Veterinary Pharmacology, Faculty of Veterinary Medicine, Suez Canal University, 41522, Ismailia, Egypt*
[d] *Department of Biochemistry, Biotechnology Research Institute, National Research Centre, 33 El Bohouth St. (Former El Tahrir St.), P.O. 12622, Dokki, Giza, Egypt*

Abstract

The current study aims to investigate the preventive efficacy of lycopene (LP) against sodium fluoride (NaF)-induced renal damage in rats. Four equal groups (6 rats/group) of albino rats with initial average weight 120 ± 10g were assigned as follows: G1 rats served as controls, whereas G2 rats received LP orally (10 mg/kg B.W per day); G3 rats were administered NaF (100 mg/L) in drinking water, and G4 rats were given NaF along with LP. After four weeks of the experimental period, blood samples were collected to estimate urea and creatinine levels in sera. Kidney tissues were also collected to investigate the following parameters: superoxide dismutase (SOD), glutathione reduced content (GSH), malondialdehyde (MDA) and tumor necrosis factor-α (TNF-α) in tissue homogenate. The present data declared that NaF reduced renal antioxidants SOD and GSH while elevating the levels of urea and creatinine, MDA, as well as TNF-α. Administration of LP to NaF-treated rats restored the levels of renal antioxidants and ameliorated the elevated levels of renal biomarkers, MDA, and TNF-α. The study demonstrated that LP supplementation provided strong antioxidant and anti-inflammatory protection against nephrotoxicity caused by NaF.

Keywords: Fluorosis (NaF), Oxidative stress, Lycopene, antioxidants, Tumor necrosis factorα

1. Introduction

Fluoride is a common industrial pollutant and an abundant natural material that may be found in almost any type of environmental matrix. Sodium fluoride (NaF) is one of the well-studied fluorinated molecules that is widely used in dental hygienic products, dental treatments to prevent municipal water fluoridation systems and caries, as well as in insecticides and glass manufacturing [1]. Ingestion of NaF has been linked with benefits and disadvantages on bone and tooth development. Inadequate fluoride intake reduces the incidence of tooth caries and stimulates bone growth [2].

Excessive consumption of fluoride can result in fluorosis, a disorder that can cause dental discoloration as well as skeletal abnormalities including deformities, osteosclerosis, and osteoporosis. High fluoride intake has also been linked to toxicities in the nervous system, gastrointestinal system, and urinary tract. Fluorine has been shown in previous studies to cause genotoxicity, cytotoxicity, immunotoxicity, oxidative stress which resulted in cell death in the liver, kidneys, thymuses, and spleens [1, 3]. Research has provided evidence that fluoride can cause toxicity by interacting with bi-

* Corresponding author.
** Samir_elrayes@yahoo.com*
(Samir Mohamed El Rayes),
***kholoud_khaled@science.suez.edu.eg*
(KholoudKhaled Emam)

doi 10.21608/AELS.2024.293052.1058
Received: 29 May 2024, Revised:1 July 2024
Accepted:7 July 2024, Published: 1 October 2024

ological systems, resulting in oxidative stress that produces reactive oxygen species (ROS). These ROS may disrupt the activity of certain enzymes, ultimately resulting in membrane damage during lipid peroxidation, mitochondrial membrane depolarization, and apoptosis [4].

Using natural antioxidants as supplements is one approach to addressing NaF toxicities. Lycopene (LP), a bright red carotenoid present in tomatoes, other red vegetables, and fruits such as red carrots, grapefruits, watermelons, and papaya, serves as an example of these naturally occurring supplements [5]. It is widely recognized for its and anti-inflammatory and antioxidant properties, as well as its ability to impact crucial metabolic processes within the body [6]. LP is a natural antioxidant that eliminates free radicals. In addition, LP has been discovered to be therapeutic in mitigating the detrimental oxidative damage inflicted on tissues by environmental toxins [5, 7] Furthermore, LP possesses the capability to recover peroxyl radicals, thereby impeding the progression of lipid peroxidation [8]. Consequently, the current study sought to investigate the ameliorative efficacy of lycopene on NaF-induced renal injury in male albino rats.

2. Materials and Methods

2.1. Chemicals

Sodium fluoride (NaF) and lycopene (LP) were purchased from Sigma-Aldrich chemicals (St. Louis, MO, USA). Kits used for estimation of liver enzymes including serum urea and serum creatinine were obtained from Biodiagnostic Company, Giza, Egypt. ELISA kits used for the estimation of inflammatory markers (TNF-α) were purchased from Life Span Biosciences Inc., USA and oxidative markers (MDA, SOD and GSH) were purchased from Elabscience Biotechnology Company, Beijing, China. All other used chemicals were of analytical degree.

2.2. Experimental albino rats

Twenty-four adult male Wistar albino rats in good health, weighing 120 ± 10 g, were acquired and raised at the Faculty of Veterinary Medicine's animal house at Suez Canal University (SCU), Egypt. They spent seven days as an acclimation period in laboratory conditions in four cages (6 rats/ cage). After being housed rats were given a regular diet and unlimited access to water. Under standard environmental conditions, the animals were housed at 26°C (±1°C). The studied animals were treated and handled consistent with ethical guidelines explained by Faculty of Science, Suez Canal University.

2.3. Design of experiment

Rats were randomly divided into 4 equal groups once acclimated (6 rats/group). Group 1 (G1) served as a control while group 2 (G2) rats received 10 mg/kg B.W. of LP orally every day via stomach tube, in compliance with Abdel-Rahman, et al. [9]. Group 3 (G3) rats were given 100 mg/L of NaF in drinking water to induce toxicity, as reported by Zhao, et al. [10]. In group 4 (G4), the rats were treated with LP and NaF. LP was administered five hours before drinking NaF, and the experimental period lasted four weeks. After four weeks of the experimental period, blood samples were collected from the retro-orbital venous plexus; then the serum was obtained by centrifuging blood at 3000 ×g for 10 minutes and stored at -20 °C for biochemical bioassay. All animals were sacrificed by decapitation under isoflurane inhalation anesthesia. After that, renal tissues were collected and cleansed with cold and physiological saline and known weight of renal tissue was then homogenized in physiological saline (4 mL) and centrifuged at 5000 rpm for 20 minutes at 4°C. Finally, the supernatant was used to assess oxidative status and inflammatory markers.

2.4. Urea and creatinine levels

The concentrations of urea (mg/dL) and creatinine (mg/dL) were determined in the obtained sera using spectrophotometry, following the kit manufacturer's instructions and the described methods by Fawcett and Scott [11] and Bowers and Wong [12].

2.5. Malondialdehyde and antioxidants

The levels of MDA (nmol/g) [13], and the antioxidants; SOD (IU/g) [14], and GSH (mg/g) [15] were

analyzed in the supernatant of homogenate renal tissues, according to the manufacturer's kit protocol.

2.6. Tumor necrosis factor

The level of TNF-α [16] was estimated in renal tissue homogenates and was expressed as Pg/g tissue, according to the manufacturer kit.

2.7. Statistical analysis

The data was presented as mean ± SE (n = 6) and analyzed using one-way ANOVA followed by the Duncan multiple tests in SPSS version 20 (SPSS Inc., Chicago). A probability value less than 0.05 was considered statistically significant.

3. Results

3.1. Levels of urea and creatinine

The data presented in **Table 1** revealed that NaF-intoxicated albino rats exhibited a significant ($P \leq 0.05$) increase in creatinine and urea levels compared to the control group. Adding LP to the NaF group significantly ($P \leq 0.05$) lowered urea and creatinine levels compared to the NaF group. No significant differences were observed among the control groups.

3.2. Malondialdehyde and antioxidants

The present data indicated that drinking NaF significantly (P≤0.05) increased MDA levels (**Figure 1**), whereas SOD and GSH levels (**Figures. 2 and 3)** were significantly (P≤0.05) decreased as compared to the control groups. Conversely, LP co-administration resulted in significant (P≤0.05) ameliorating in MDA and antioxidant levels compared to the NaF group and comparable to control groups. No detectable changes between control groups.

3.3. Tumour necrotic factor

As depicted in Figure 4, drinking NaF significantly (P≤0.05) raised TNF-α level in the sera of the NaF group when compared to other groups. Administration of LP to the NaF group significantly (P≤0.05) lowered TNF-α levels than levels of the NaF group. There were no significant differences between the control groups.

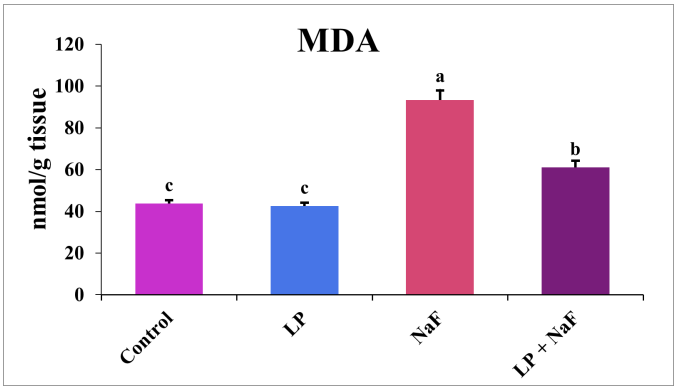

Figure 1: The protective effect of lycopene on MDA levels raised by NaF in renal tissues of male albino rats. Values were expressed as mean± SE (6 rats/group) with different letters (a, b &c) indicating significance between groups at P≤0.05.

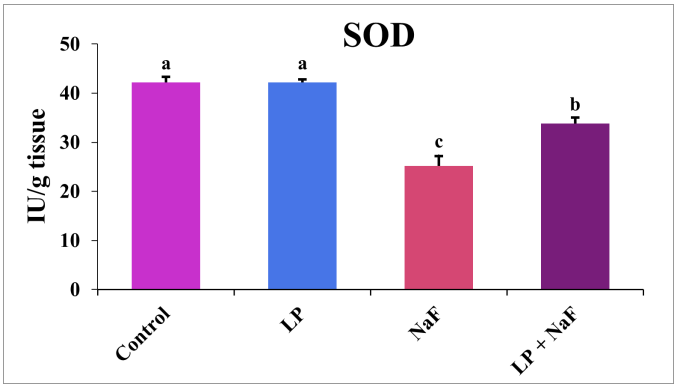

Figure 2: The ameliorative effect of lycopene on SOD activities on renal tissues of male albino rats influenced by NaF. Values were expressed as mean± SE (6 rats/group) with different letters (a, b &c) indicating significance between groups at P≤0.05.

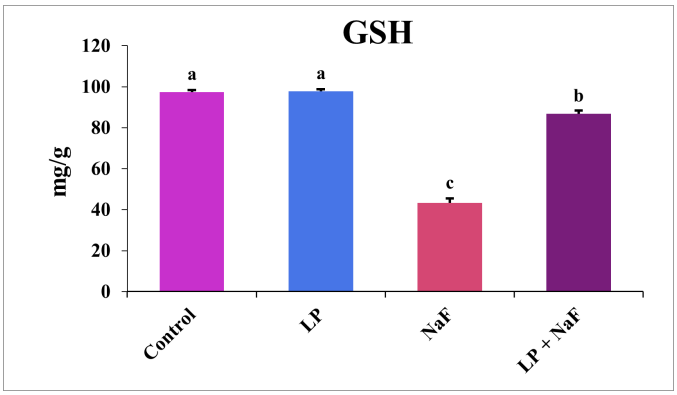

Figure 3: The ameliorative effect of lycopene on GSH contents on renal tissues of male albino rats influenced by NaF. Values were expressed as mean± SE (6 rats/group) with different letters (a, b &c) indicating significance between groups at P≤0.05.

Table 1: Effect of lycopene (LP) onurea and creatinine levels in sera of male rats influenced by NaF

Parameters	Control	LP (10 mg/kg B.W.)	NaF (100 mg/L)	LP (10 mg/kg B.W.) + NaF (100 mg/L)
Urea (mg/dL)	35.42 ± 0.20^{c}	35.51 ± 0.12^{c}	86.82 ± 0.47^{a}	39.73 ± 0.15^{b}
Creatinine (mg/dL)	1.01 ± 0.25^{c}	1.03 ± 0.18^{c}	2.95 ± 0.29^{a}	1.36 ± 0.16^{b}

Each value representsthe mean ± SE (n =6), means in the same row with different superscript letters(a, b&c) are significantly different at P≤0.05.

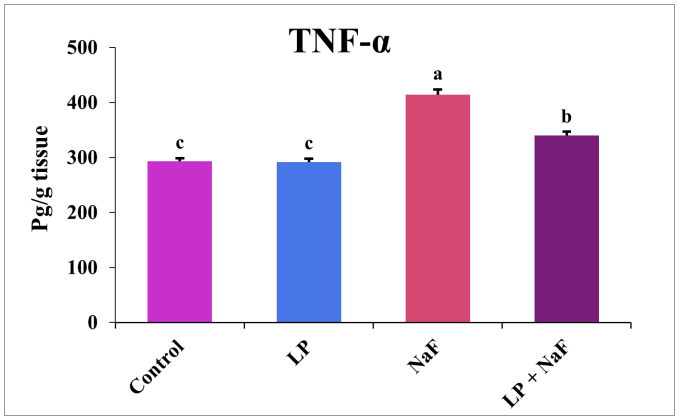

Figure 4: The protective effect of lycopene onTNF-α levels induced by NaF in renal tissues of male albino rats. Values were expressed as mean± SE (6 rats/group) with different letters (a, b &c) indicating significance between groups at P≤0.05.

4. Discussion

The kidney is an essential and multifunctional organ that is crucial to the body's ability to maintain homeostasis and extract waste [2]. It is extremely susceptible to fluoride-induced toxicity since it is the primary location of fluoride accumulation and excretion [1, 4].Renal dysfunctions are now more common than ever because of the rise in the prevalence of diseases like hypertension and diabetes as well as increased exposure to environmental pollutants [1, 3]. The present research investigates the possible protective role of LP on oxidative damage and inflammation caused by NaF, which consequently induces renal toxicity.

The serum urea and creatinine levels are considered to be good markers of renal health and function; however, creatinine measurement is more accurate because urea levels can also be affected by a number of other renal issues, such as dehydration [4].

According to present data, intoxication with NaF elevated serum creatinine and urea levels. The obtained results were in accordance with previous studies of Khan, et al. [1], Sharma, et al. [2] and Adelakun, et al. [4] who reported that administration of NaF (5 mg/kg B.W) via gastric gavage for 30 days significantly elevated the levels of creatinine and blood urea in serum of rats. The elevated levels of urea and creatinine in serum were attributable to severe renal damage resulting from the breakdown of glomerular filtration that leads to various degrees of tubular reabsorption, indicating a potent renal toxic activity [2, 4].

On the contrary, co-administering LP to rats intoxicated with NaF resulted in a downregulation of creatinine and urea levels, suggesting that the LP has nephroprotective properties against NaF intoxication. This reduction may be attributed to LP's therapeutic value and antioxidant activities [7]. This finding was in accordance with Albrahim and Robert [5], Khan, et al. [17], and Salem and Salem [18] who demonstrated that administration of LP (4 mg/kg B.W.) by gavage once every other day for two months produced a significant reduction in creatinine and urea levels when compared to cadmium intoxicated rats.

Oxidative stress is caused by the excessive generation of ROS, such as hydrogen peroxide, superoxide, and hydroxyl radicals, due to the imbalance between free radicals and antioxidants in the body [2]. The present data explored that NaF caused oxidative stress in renal tissue, evidenced by the elevation of MDA levels, along with a reduction of SOD activities and GSH contents in the NaF group. Lipid peroxidation in renal cellular membranes may be the cause of the observed increase in renal oxidative stress, resulting in damage. These were in agreement Albrahim and

Robert [5] and Adelakun, et al. [4]. In addition, previous studies revealed that ingestion of NaF caused a decrement in the antioxidant enzyme levels and an increment of MDA levels because NaF induces a huge production of ROS and free radicals that damage many biomolecules and cause a wide range of molecular and cellular effects, including cytotoxicity [1, 2].

Lycopene supplementation ameliorated the NaF impacts in renal tissues by improving the content of GSH and SOD activity and lowering MDA levels. It is supposed that LP has antioxidant action and can neutralize oxygen and peroxyl radicals leading to a low generation of MDA level [18, 19]. These findings agreed with the previous studies of Abdel-Rahman, et al. [9] and Elsayed, et al. [19] who reported that oral administration of LP (10 mg/kg B.W./d) for 30 days ameliorated the cisplatin impacts in renal tissues by increasing the content of GSH and SOD activity and decreasing MDA levels.

In this investigation, NaF intoxication increased TNF-α levels, suggesting that it stimulated inflammatory reactions via the creation of cytokines in renal tissues. This elevation activates the pathway of p38-MAPK, resulting in various pathological alterations in the kidney. Furthermore, it causes the renal tissue to produce chemokines and inflammatory cytokines [20]. The generation of inflammatory responses is linked to the activation of the p38-MAPK pathway, which is thought to be a major mediator of the harmful effects of NaF exposure on the kidneys [1, 20].

The TNF-α can attract immune cells to the sites of tissue injury, therefore, it is considered a "master regulator" of the inflammatory response [3].Additionally, increased TNF-α levels activate inducible nitric oxide (NO) synthase, resulting in the production of peroxynitrite, a hazardous nitrogen species [4]. As a result of triggering nitrative stress and inflammatory reactions in the treated rats, the elevated renal levels of NO in rats given NaF alone may potentially accelerate renal damage [3, 4]. Supplementation with LP improved TNF-α levels, thus it could be attributed to the strong antioxidant capacity of LP. This protective effect was in accordance with Albrahim and Robert [5].

5. Conclusion

The present study concluded that LP has a protective effect against renal oxidative damage and inflammation caused by the consumption of NaF. This protective role might be achieved by reducing MDA and TNF-α levels, along with increasing SOD activities and GSH contents. As a result, LP could lower oxidative stress and inflammation in renal tissue.

6. Abbreviations

Body weight: B.W; Day: d; Gram: gm; kilogram: kg; lycopene: LP; Malondialdehyde: MDA; Reactive oxygen species: ROS; Reduced glutathione: GSH; Sodium fluoride: NaF; Statistical Package for Social Sciences: SPSS; Superoxide dismutase: SOD; Tumor necrosis factor-α: TNF-α. **NO: Nitric oxide**

References

[1] M. U. Khan, P. Basist, S. Gaurav, N. R. Zahiruddin, S. Penumallu, Ahmad, Ameliorative effect of traditional polyherbal formulation on TNF-α, IL-1β and Caspase-3 expression in kidneys of wistar rats against sodium fluoride induced oxidative stress, J Ethnopharmacol 318 (2024) 116900–116900.

[2] P. Sharma, P. K. Verma, S. Sood, M. Singh, D. Verma, Impact of Chronic Sodium Fluoride Toxicity on Antioxidant Capacity, Biochemical Parameters, and Histomorphology in Cardiac, Hepatic, and Renal Tissues of Wistar Rats, Biol Trace Elem Res 201 (1) (2023) 229–241.

[3] C. Caglayan, F. M. Kandemir, E. Darendelioğlu, S. Küçükler, A. Ayna, Hesperidin protects liver and kidney against sodium fluoride-induced toxicity through anti-apoptotic and anti-autophagic mechanisms, Life Sci 281 (2021) 119730–119730.

[4] S. A. Adelakun, B. Ogunlade, O. P. Fidelis, O. A. Adedotun, Cyperus esculentus suppresses hepato-renal oxidative stress, inflammation, and caspase-3 activation following chronic exposure to sodium fluoride in rats' model, Phytomedicine Plus 2 (1) 100163–100163.

[5] T. Albrahim, A. A. Robert, Lycopene Effects on Metabolic Syndrome and Kidney Injury in Rats Fed a High-Fat Diet: An Experimental Study, ACS Omega 7 (35) (2022) 30930–30938.

[6] T. Albrahim, M. A. Alonazi, Lycopene corrects metabolic syndrome and liver injury induced by high fat diet in obese rats through antioxidant, anti-inflammatory, antifibrotic pathways, Biomedicine & Pharmacotherapy 141 111831–111831.

[7] T. Albrahim, Lycopene Modulates Oxidative Stress and Inflammation in Hypercholesterolemic Rats, Pharmaceuticals (Basel) 15 (11) (2022).
[8] J. Dong, W. Li, L. M. Cheng, G. G. Wang, Lycopene attenuates LPS-induced liver injury by inactivation of NF-κB/COX-2 signaling, Int J Clin Exp Pathol 12 (3) (2019) 817–825.
[9] H. G. Abdel-Rahman, H. M. A. Abdelrazek, D. W. Zeidan, R. M. Mohamed, A. M. Abdelazim, Lycopene: Hepatoprotective and Antioxidant Effects toward Bisphenol A-Induced Toxicity in Female Wistar Rats, Oxid Med Cell Longev 2018 (2018) 5167524–5167524.
[10] S. Zhao, Systematic impacts of fluoride exposure on the metabolomics of rats, Ecotoxicol Environ Saf 242 (2022) 113888–113888.
[11] J. K. Fawcett, J. E. Scott, A rapid and precise method for the determination of urea, J Clin Pathol 13 (2) (1960) 156–165.
[12] L. D. Bowers, E. T. Wong, Kinetic serum creatinine assays. II. A critical evaluation and review, Clin Chem 26 (5) (1980) 555–61.
[13] H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal Biochem 95 (2) (1979) 351–359.
[14] M. Nishikimi, N. Appaji, K. Yagi, The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen, Biochem Biophys Res Commun 46 (2) (1972) 849–54.
[15] E. Beutler, O. Duron, B. M. Kelly, Improved method for the determination of blood glutathione, J Lab Clin Med 61 (1963) 882–890.
[16] H. Engelmann, D. Novick, D. Wallach, Two tumor necrosis factor-binding proteins purified from human urine. Evidence for immunological cross-reactivity with cell surface tumor necrosis factor receptors, J Biol Chem 265 (3) (1990) 1531–1537.
[17] N. I. Khan, S. Noori, T. Mahboob, Efficacy of lycopene on modulation of renal antioxidant enzymes, ACE and ACE gene expression in hyperlipidaemic rats, J Renin Angiotensin Aldosterone Syst 17 (3) (2016).
[18] N. A. Salem, E. A. Salem, Hepatorenal and testicular protective effects of lycopene against cadmium induced toxicity in male rats, J Nephrol Ther 6 (5) (2016) 1–7.
[19] A. Elsayed, Synergistic protective effects of lycopene and N-acetylcysteine against cisplatin-induced hepatorenal toxicity in rats, Sci Rep 11 (1) (2021) 13979–13979.
[20] L. S. Araújo, Renal expression of cytokines and chemokines in diabetic nephropathy, BMC Nephrol 21 (1) (2020) 308–308.