Renal Histopathology and Functional Disorders Following Chronic Exposure to Nicotine and Sodium Nitrite in Male White Rats

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Abstract: The kidneys are a metabolic organ, they are exposed to toxins that are excreted in urine, which can lead to tissue damage. The kidneys are subjected to harmful elements, either directly from the environment or through food additives and preservatives, which disrupt kidney functions as a result of tissue destruction. Two of the most hazardous chemicals, nicotine and sodium nitrite, have been evaluated in the current investigation. **Objectives:** The current study aims to assess the histological and biochemical changes in the kidneys of male rats resulting from simultaneous treatment with nicotine and sodium nitrite. **Methods**: Twenty-four male Fischer rats (Male F-344/NHsd rats) were used in four groups. The control group received 0.5mg/Kg daily subcutaneous injections of saline solution for 45 days. The second group received an injection of 0.8 mg/kg of nicotine daily. The third group received an injection of 0.5 mg/kg of sodium nitrite daily. The fourth group received daily injections of nicotine and sodium nitrite at doses of 0.8 mg/kg and 0.5 mg/kg, respectively, for 45days. Blood samples were taken to measure indicators of kidney function, such as creatinine, urea, uric acid, and electrolytes. Kidney samples were collected from all animals for histological examinations. **Results:** The levels of urea, creatinine, uric acid, sodium, potassium, and chloride were significantly elevated at a confidence level of ($P< 0.001$) compared to the control group. The rats treated with either nicotine or sodium nitrite exhibited various tissue changes, including necrosis, congestion, degeneration, hypertrophy of renal cells, cell death. **Conclusion:** The simultaneous treatment with sodium nitrite and nicotine led to changes in histological characteristics and metabolic markers. These included pathological physiological changes resulting from stimulation by either nicotine or sodium nitrite. Additionally, caused further pathological abnormalities in the kidneys. To reduce the risks associated with exposure to these chemicals and minimize their harmful effects on the kidneys and other organs, it is recommended to limit the use of preserved foods and frozen meats, as well as to quit smoking.

Keywords: *Nicotine, Sodium nitrite, Oxidative stress, free radical, Renal necrosis.*

1. Introduction

Statistics indicate that 6 million people worldwide die each year due to smoking [1]. Smoking affects the physiology of the human body, including the respiratory, cardiovascular, and renal systems. The primary harmful component in cigarettes and tobacco is nicotine, which can be inhaled through active or passive smoking. Nicotine is extracted from the leaves of the *Nicotiana Rustica* plant, which belongs to the Solanaceae family. It is a potent stimulant and a highly toxic organic compound that contains nitrogen and alkaloids commonly found in tobacco [2]. Nicotine is one of the chemical constituents in tobacco and cigarette smoke that causes numerous health issues and is responsible for its addictive properties[3]. It is one of hundreds of substances present in cigarette smoke [4]. Interestingly, nicotine has been used as an insecticide, as well as a drug for treating spasms, diuretic purposes, bacterial infections, antimicrobial applications, anesthesia, antiepileptic treatments, and antifungal activities[5,6.] However, it has detrimental effects on the heart, liver, reproductive system, lungs, and kidneys, and numerous studies have consistently demonstrated its potential to cause cancer [7]. Currently, there is a variety of nicotine products available, such as patches, gum, and electronic cigarettes[8 9]. Exposure to nicotine is a reality for smokers, and non-smokers are also at risk from passive exposure. The duration and quantity of exposure to smoke determine the level of risk. Numerous studies have shown that nicotine produces an excess of free radicals, which in turn stimulate oxidative stress, tissue damage, and a range of other diseases within the body[10]. These free radicals interact with cellular components, altering their structure and function, which can lead to various metabolic disorders and diseases that affect biological activities[11]. Despite the harmful effects associated with consuming canned goods, demand for them continues to rise. Most food products contain preservatives such as sorbates, nitrites, sulfites, and benzoates. Small amounts of nitrite salts are added to meats, poultry, and fish as preservatives. This practice has been in place for many years [12].Nitrite significantly inhibits the growth of botulinum toxin, enhances the color and flavor of processed meats, slows the onset of spoilage during storage, prevents the development of off-flavors, and preserves the flavors of spices [13]. Nitrates and nitrites are the building blocks for the synthesis of N-nitroso compounds, a family of genotoxic substances that includes nitrosamines and nitramines. Further research is essential to determine the role of N-nitroso compounds in adverse pregnancy outcomes, as these chemicals have been shown to cause congenital malformations in animal models [14] .In addition to the

endogenous production that occurs within the body, humans are exposed to N-nitroso compounds through their diet, tobacco smoke, and other external sources[15]. Foods that may contain pre-formed nitrosamines due to cooking or preservation techniques include processed meats, beer, smoked fish, cigarettes, and other environmental sources[16 17]. The hazard of $NaNO_2$ lies in their addition to food, as they react with the amines present in the meal in the stomach, forming free radicals such as nitrosamines. This was pointed out by Abidin, and Alshaib [18 .]Antioxidants.have linked sodium nitrite to negative health effects, as it increases oxidative stress, which can cause tissue death in multiple organs, including the kidneys. The reactive nitrogen species generated from nitrite exposure can result in tissue damage, dysregulation of inflammatory responses, hepatic toxicity, and nephrotoxicity, among other harmful effects [19 20]. The kidneys function as metabolizing organs that release toxins into the urine exposing them to exogenous or endogenous toxicants that cause damage and tissue destruction. Chronic renal diseases can be caused by the primary or secondary excretion of certain poisons and substances [21]. The kidneys are negatively impacted by nicotine. It may worsen renal problems by raising albumin discharge and proteinuria. Furthermore, some studies have shown that smoking is a significant risk factor for the development of chronic kidney disease, diabetic nephropathy, and necrotic or apoptotic death of renal cells [22]. Overuse of meat preservatives, such as sodium nitrite, has been shown to pose a serious risk to human health and function. Few researches have been done on the effects of the co-administration of sodium nitrite and nicotine on the kidneys of male albino rats. Numerous studies have examined the effects of each substance alone on the kidneys. The objective of the current investigation was to assess the effects of co-administration of nicotine and sodium nitrite on the kidney of male albino rats in terms of histological and biochemical alterations.

2. Materials and methods

This experiment was conducted at the Al-Zawiya Medical Research Center in Libya. 24 male F-344/NHsd Fischer rats (14-15 weeks, 200 ± 25gm). During the experimental period, white rats were housed in plastic cages, with 6 animals randomly assigned to each group. They were provided with daily food and fresh tap water available and libitum. The animals were left for a week for acclimation before starting the experiment. The animals were divided into 4 groups randomly as follows; control group rats were injected subcutaneously with saline solution (0.9% Normal saline) at a dose equal to (0.5mg/Kg) for 45 days. The remaining treated groups were handled as follows: Group 2, in addition to the standard diet, was injected daily with nicotine subcutaneously at a dose of 0.8 mg/kg body weight daily. Group 3; The rats were injected subcutaneously with sodium nitrite at a dose of 0.5 mg/kg daily. Group 4 was injected with both sodium nitrite and nicotine, receiving 0.5 mg/kg of sodium nitrite, followed by reinjection of 0.8 mg/kg of nicotine one hour later daily. Nicotine or sodium nitrite continued to be given to the rats daily for 45 days.

Biochemical Measurements

After the animals were sacrificed, blood samples were collected immediately following cardiac puncture into blood tubes and placed in a centrifuge at 3000 RPM for 15 minutes to harvest the serum. Kidney function markers (urea, creatinine, and uric acid) were measured using biochemical analysis tools. Additionally, electrolytes such as sodium (Na^+), potassium (K^+), and chloride (Cl^-)were determined and compared.

Histological examination

Water and food were withdrawn from the experimental animals 24 hours before the end of the experiment. The animals were then euthanized, and the kidney weights of each rat were recorded. Kidney samples were placed in a 10% formalin solution until further use. The samples were subsequently processed in a tissue processor for 15.5 hours through the following stages: 70% ethanol for 6.5 hours, followed by a series of increasing concentrations of ethanol (80%, 90%, and 95%) for 1 hour each, and finally 3 hours in 100% ethanol. The tissues were then passed through three changes of xylene, each for 1 hour. Finally, they were immersed in paraffin wax at a melting point of 56ºC. The paraffin blocks were kept until sectioning. Paraffin sections (4.5 µm) were prepared, stained with H&E, and examined under a light microscope.

RESULTS

Biochemical parameters

The effects of co-exposure to sodium nitrite and nicotine on urea, creatinine, and uric acid concentrations significantly increased compared to the control group. The changes in urea, creatinine, and uric acid concentrations were almost the same as observed with individual exposure (Table 1). There was an increase in urea levels in all of the exposed groups but was more prominent in the co-exposed group than controls.

Table (1): Effects of simultaneous sodium nitrite and nicotine on serum urea, creatinine, and uric acid levels of male albino rats

Parameters (mg/dl)	**GROUPS**			
	Control	sodium nitrite	Nicotine	**Nicotine + sodium nitrite**
	Mean±SD	Mean±SD	Mean±SD	**Mean±SD**
Urea	29.8 ±1.9	40± 2.5	31.3±2.06	**73.8.± 1.7*** **
Creatinine	0.4 ± 0.06	0.7± 0.07	0.6 ± 0.05	**0.85±0.06*** **
Uric acid	2.6 ± 0.19	3.8 ± 0.2	3.08±0.7	**5.7±0.6*** **

***p< 0.001 Statistically very high significant difference, as compared to the control group**.**

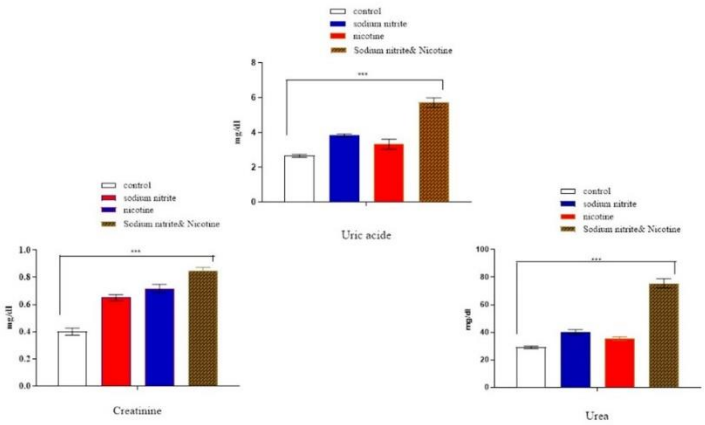

Fig (1): Dose response of effects of simultaneous of sodium nitrite and nicotine in serum kidney function urea, creatinine and uric acid in male albino rats. Group of rats were injected with 0.5mg/kg body weight with sodium nitrite at time Ø hour, an hour latter injected with 0.8 mg/kg body weight nicotine daily for 45 days. The bar chart showing significantly increase of urea, creatinine and uric acid concentration in simultaneous of sodium nitrite and nicotine treated animal compared to the control group on the one hand and with compared to the sodium and nicotine group individually. Data are expressed as mean±SD in each group (n=6) statistical analysis was collocated with Dunnett's multiple comparison tests, indicated with an asterisk ($P < 0.05$).

Serum levels of sodium, potassium, and chloride were high in all treated groups as compared to controls. Co-exposure of nicotine and sodium nitrite demonstrated a significant increase in the levels of electrolytes, especially sodium values (Table 2).

Table (2): Effects of simultaneous sodium nitrite and nicotine on serum sodium, potassium, and chloride levels of male albino rats.

Parameters (mmol/L)	**GROUPS**			
	Control	sodium nitrite	Nicotine	**Nicotine + sodium nitrite**
	Mean ± SD	Mean ± SD	Mean ± SD	**Mean ± SD**
Sodium	144±2.6	164.5±3.3	147±2.3	**190±1.9****
Potassium	6.0±0.5	6.5±0.8	6.3±0.5	**7.9±0.7***
Chlorides	101±1.6	105±2.7	103±1.2	**111±1.7***

*p< 0.05 Statistically significant difference, as compared to the control group.

** p< 0.01 Statistically high significant difference, as compared to the control group.

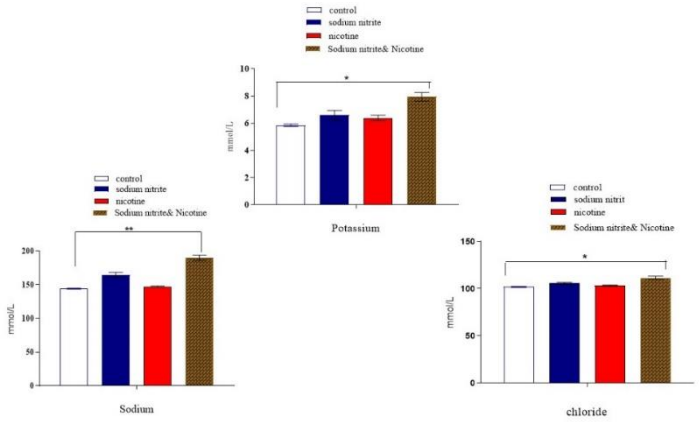

Fig (2): Dose response of effects of simultaneous of sodium nitrite and nicotine in serum kidney function Sodium, potassium and chloride in male albino rats. Group of rats were injected with 0.5mg/kg body weight with sodium nitrite at time Ø hour, an hour latter injected with 0.8 mg/kg body weight nicotine daily for 45 days. The bar chart showing increase of Sodium, potassium and chloride levels in simultaneous of sodium nitrite and nicotine treated animal compared to the control group on the one hand and with compared to the sodium and nicotine group individually. Data are expressed as mean±SD in each group (n=6) statistical analysis was collocated with Dunnett's multiple comparison tests, indicated with an asterisk ($P < 0.05$).

Histological results

In this study, changes in kidney histology were examined in three key zones. Namely, the renal corpuscles zone, the cortical renal tubules, and the medullary renal tubule. Sodium nitrite-treated kidney tissue showed shrinkage of glomerular tuft, and congestion of glomerular capillaries. The nicotine-treated group showed degeneration and congestion of the glomerular and Bowman's capsules. All histopathological changes are shown in Fig (1).

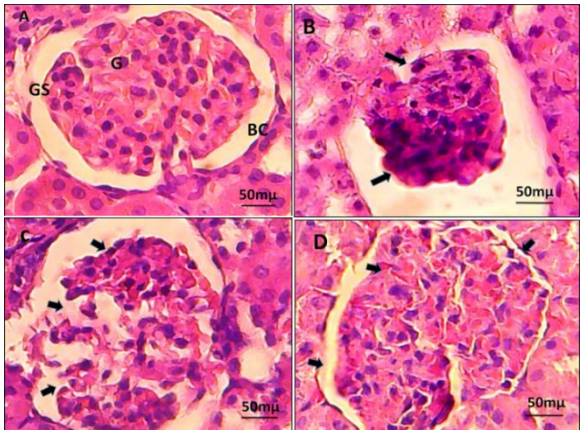

Fig (3): Micrograph of the kidney (cortical zone) collected from control and treated rats

A; control group section showing a normal renal structure with rounded renal corpuscles (RC) formed by the Glomerulus (G) and the bowman capsule (BC). **B**; treated group section by (sodium nitrite) shows shrink glomerular tuft and congestion (thin arrows). **C;** a section of nicotine treated group shows degeneration and congestion of the glomerular and Bowman's capsule (thin arrows). **D**; renal corpuscles section harvested from the group that was treated with sodium nitrite & nicotine, showing marked congestion of glomerulus tuft (head arrows) with hemorrhage and degeneration of endothelial lining the Bowman capsule (head arrows) (H&E x 400). Regarding to cortical collecting tubule, the sodium nitrite-treated group showed degeneration in the renal tubules and focal necrosis. Nicotine-treated kidney tissue showed tubular necrosis and infiltration. Co-exposure to sodium nitrite and nicotine showed shrinkage of glomeruli, marked necrotic change, and infiltration with some inflammatory cells

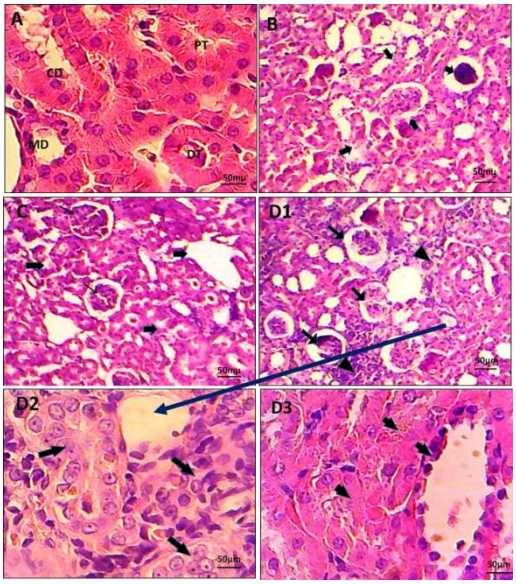

Fig (4): Micrographs for cortical renal tubules, harvested from control and treated groups. A; section from the control group showing normal proximal convoluted tubule (PCT) with a narrow lumen, lined with high cuboidal cells and homogeneous acidophilic cytoplasm, distal convoluted tubules (DCT) with wide lumen and lined with low cuboidal cells. B; A section of sodium nitrite treated group shows degeneration changes in the renal tubules and focal necrosis (small arrows). C; treated group section by nicotine showing tubular necrosis, infiltration, and periglomerular edema (thick arrows). D1, D2, and D3 sections of sodium nitrite& nicotine treated group. D1; showing shrinkage of glomeruli, marked necrotic change and infiltrated with some inflammatory cells (head narrow), periglomerular edema, and degeneration of epithelial cells lining tubules (thin arrows). D2; loss of brush border of PCT and patchy cloudy swelling with cells being necrotic with karyolitic nuclei (thin arrows). D3; tubular necrosis and cell debris seen in the lumen (head arrows). (H&E 400 x). Severe histopathological alterations, including necrosis, inflammatory cell infiltration, and congestion of renal tubules (loop of Henle and the collecting tubules) were noted in individual as well as co-exposure groups.

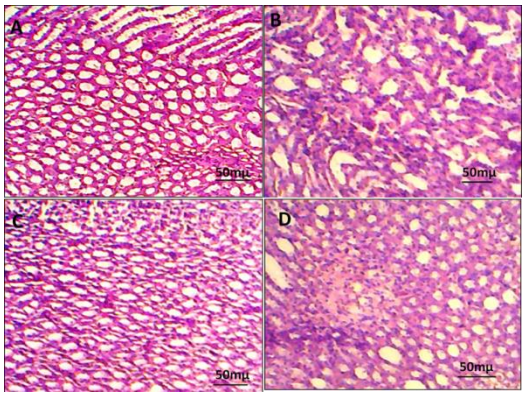

Fig (3): Micrographs of parenchymal renal tubules harvested from control and treated rat groups. **A**; section from the control group shows a normal histological structure of longitudinal and transverse renal parenchymal tubules. **B**; treated group section by (sodium nitrite) showing inflammatory cells, infiltration with necrosis in the parenchymal portion. **C**; section of nicotine treated group shows congestion and infiltration. **D**; section of simultaneous of two agents (sodium nitrite & nicotine) showing acute infiltration with inflammatory cells with focal necrosis in parenchymal tubules (arrows). (H&E 400x).

DISCUSSION

The kidney is a major site of chemical excretion, which results in its propensity to exhibit nicotine as one of the environmental contaminants, producing severe organ damage in animals and humans. Studies have shown that the kidney is one of the primary targets in nicotine-associated toxicity, and is therefore also susceptible to injury. [23]The kidney is a major site of chemical excretion and plays an important role in mediating toxicity of numerous chemicals.[24]. During this process, the kidney may exhibit chemically-induced toxicological effects more than other organs. The present study has investigated the potentially toxic effects of sodium nitrite and nicotine on the kidneys of adult rats. This study showed an increase in serum urea, creatinine, and uric acid in the rats treated with sodium nitrite serum, suggesting an impairment of kidney functions. These effects could also be attributed to the changes in the threshold of tubular re-absorption, renal blood flow, and glomerular filtration [25]. The

damage produced by sodium nitrite in the permeability of the glomerular capillaries is increased leading to increased levels of excreted kidney function such as urea, creatinine, and uric acid. The lesions produced in the kidney tubules will eventually cause dysfunction in the transport mechanism to and from the renal epithelium [26]. Many studies have noticed that Guinea pigs that received sodium nitrite orally, daily for 35 days had significantly increased serum urea, uric acid, and creatinine concentrations suggesting an impairment of kidney function. Furthermore, similar results have been reported on the effect of toxicity of sodium nitrite on the serum urea, uric acid, and creatinine concentrations] 25 27 28]. In the current study, a light microscopic examination of the kidneys of treated rats with sodium nitrate showed inflammatory cells, and infiltration with necrosis in a parenchymal portion of kidney parenchyma compared to the control group. The changes were observed such as glomerular tuft shrinkage, congestion, and degeneration in the renal tubules. Focal necrosis and dissolution could occur as a result of nitric oxide (NO) formation, which causes vascular smooth muscle relaxation at high levels. This might induce dilatations of their lumen, increase their blood flow, and finally lead to fibrosis [29]. These results are consistent with those obtained by Galaly &Ibrahim (2012) who noticed that the kidneys of rats treated with sodium nitrite, showed mild congestion and degenerative changes, after 10 weeks. The zones of congestion, degenerative changes, and accumulation of lymphocytes around renal blood vessels have been detected in the kidneys of rats treated with sodium nitrite [30]. Nicotine-induced oxidative damage in kidney rats. The present study showed a significant increase in serum urea, uric acid, and creatinine concentration in all treated groups compared with the control group. Many studies have reported that the breakdown of membrane phospholipids and lipid peroxidation is due to the generation of free radicals, which leads to changes in the membrane structure, fluidity, transport, and antigenic properties. [31]. These results were approved by Azab and Albasha (2015), who reported that the serum urea, creatinine, and uric acids were significantly increased in Guinea pigs treated with nicotine [32] . They have indicated that treatment of Guinea pigs with nicotine caused a significant increase in serum sodium and potassium ions concentrations compared with the control group. The present result of the simultaneous effects of sodium nitrite and nicotine on kidney tissues showed glomeruli shrinkage, increased cellular proliferation, infiltration of contents of capillaries with obliteration of their lumen, and perhaps hyalinization. The renal tubules also showed swelling with cells being necrotic with karyolitic nuclei and loss of brush border of PCT. Besides, cell debris was noticed in the lumen, and focal necrosis in the parenchymal tubules. Renal tubular epithelial cells were particularly sensitive to ischemia and toxins. Several factors predisposed the tubules to toxic injury including vast charge surface for tubular reabsorption, their higher metabolic rate, their oxygen consumption requirement, and their vulnerable enzyme systems. The exposure of renal cells to chemicals induced 3 major processes: glomerular filtration, tubular secretion, and tubular reabsorption. The relative contribution of each process to the total renal elimination depends highly on the chemical properties. Interactions that reduce the uptake of xenobiotics into proximal tubular cells will lead to elevated concentrations of free chemicals in the systemic circulation. This can markedly increase the magnitude of the chemical effects and raise the risk for chemical-induced toxicity. The effect of the administered chemical agent on the structure and function of the kidneys are dependent on the functional state of the liver too. It was suggested that the events related to the kidneys and liver are dependent on independent etiologies. Some chemicals systemically applied to rats were reported to be distributed in a higher concentration in the liver compared to the kidneys. It can be concluded that the treatment of rats with nicotine or sodium nitrite caused serious pathophysiological changes in the kidneys. Simultaneous nicotine and sodium nitrite administration caused even more pathophysiological changes in the kidney. Therefore, food preservatives, frozen meat, and smoking should be reduced to minimize the risk of exposure to these chemicals and reduce their harmful effects on the kidneys and other organs.

Reference

1. Samet, J. M. (2013). Tobacco smoking: the leading cause of preventable disease worldwide Surgery Clinics, 23(2), 103–112.

2. Mitra, S. P. (2016). Chemistry of cigarette smoking--a review.

3. Tiwari, R. K., Sharma, V., Pandey, R. K., & Shukla, S. S. (2020). Nicotine addiction: Neurobiology and mechanism. Journal of Pharmacopuncture, 23(1), 1.
4. Cooper, R. G. (2006). "Effect of tobacco smoking on renal function." Indian J Med Res 124(3): 261-268.

5. Lakhan, S. E. and A. Kirchgessner (2011). "Anti-inflammatory effects of nicotine in obesity and ulcerative colitis." Journal of translational medicine 9(1): 129.

6. Salehi, B., Zakaria, Z. A., Gyawali, R., Ibrahim, S. A., Rajkovic, J., Shinwari, Z. K., Khan, T., Sharifi-Rad, J., Ozleyen, A., Turkdonmez, E., & others. (2019). Piper species: A comprehensive review on their phytochemistry, biological activities and applications. Molecules, 24(7), 1364.

7. Mahurpawar, M., & others. (2015). Effects of heavy metals on human health. Int J Res Granthaalayah, 530(516), 1–7.

8. Dani, J. A., D. Ji and F.-M. Zhou (2001). "Synaptic plasticity and nicotine addiction." Neuron 31(3): 349-352.

9. Walker, N., Parag, V., Verbiest, M., Laking, G., Laugesen, M. and Bullen, C., 2020. Nicotine patches used in combination with e-cigarettes (with and without nicotine) for smoking cessation: a pragmatic, randomized trial. The Lancet Respiratory Medicine, 8(1), pp.54-64.

10. Malińska, D., Wi\keckowski, M. R., Michalska, B., Drabik, K., Prill, M., Patalas-Krawczyk, P., Walczak, J., Szymański, J., Mathis, C., der Toorn, M., & others. (2019). Mitochondria as a possible target for nicotine action. Journal of Bioenergetics and Biomembranes, 51, 259–276.

11. Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. Indian Journal of Clinical Biochemistry, 30, 11–26.

12. Salama, M. F., A. Abbas, M. M. Darweish, A. A. El-Hawwary and M. M. Al-Gayyar (2013). "Hepatoprotective effects of cod liver oil against sodium nitrite toxicity in rats." Pharmaceutical biology 51(11): 1435-1443.

13. Vaclavik, V. A. and E. W. Christian (2014). Food Preservation. Essentials of food science, Springer: 323-342.

14. Griesenbeck, J. S., M. D. Steck, J. C. Huber, J. R. Sharkey, A. A. Rene and J. D. Brender (2009). "Development of estimates of dietary nitrates, nitrites, and nitrosamines for use with the Short Willet Food Frequency Questionnaire." Nutrition journal 8(1): 16.

16. Jakszyn, P. and C. A. González (2006). "Nitrosamine and related food intake and gastric and oesophageal cancer risk: a systematic review of the epidemiological evidence." World journal of gastroenterology: WJG 12(27): 4296.

17. Abushofa, F.A., Azab, A.E. and Alkadrawy, S., 2019. Hepatic pathophysiological changes induced by nicotine and/or sodium nitrite injection in male albino rats. East African Scholars J Med Sci, 2(4), pp.184-196.

18. Abdeen, A., A. EL-Shayeb, H. Hassan and S. El-Agamy (2015). "The protective role of garlic oil against the histopathological and histochemical changes induced by sodium nitrite toxicity in kidneys of albino rats." J Jazan Univ Appl Sci Branch 4: 57-67.

19.Elsyade, R. and Khaled, D., 2023. The Possible Protective Role of Quercetin on Nicotine Induced Liver and Kidney Damage of Neonates Albino Rats: Histological and Immunohistochemical Study. Egyptian Journal of Histology, 46(2), pp.495-505.
20. De, G. S. B., P. Fritsch and C. Cazottes (1983). "Effects of dietary nitrite and nitrate on experimentally-induced inflammation in the rat." International journal of tissue reactions 5(2): 173-180.
21. Martinez, M. C., & Andriantsitohaina, R. (2009). Reactive nitrogen species: molecular mechanisms and potential significance in health and disease. Antioxidants \& Redox Signaling, 11(3), 669–702.
22. Sebastian, M. (2009). Renal toxicity. In Handbook of Toxicology of Chemical Warfare Agents (pp. 561–574).

23 Kataria, A., Trasande, L., & Trachtman, H. (2015). The effects of environmental chemicals on renal function. Nature Reviews Nephrology, 11(10), 610–625.

24. Schnellmann, R. G., & others. (2001). Toxic responses of the kidney. Casarett and Doull's Toxicology the Basic Science of Poisons, New York: The McGraw-Hill.

25. Zurovsky, Y. and C. Haber (1995). "Antioxidants attenuate endotoxin-gentamicin induced acute renal failure in rats." Scandinavian journal of urology and nephrology 29(2): 147-154.

26. Fommei, E., S. Ghione, L. Palla, F. Mosca, M. Ferrari, C. Palombo, S. Giaconi, P. Gazzetti and L. Donato (1987). "Renal scintigraphic captopril test in the diagnosis of renovascular hypertension." Hypertension 10(2): 212-220.

27. Hassan, H. A., S. M. El-Agmy, R. L. Gaur, A. Fernando, M. H. Raj and A. Ouhtit (2009). "In vivo evidence of hepato-and reno-protective effect of garlic oil against sodium nitrite-induced oxidative stress." International journal of biological sciences 5(3): 249.

28. Aboulgasem, G. J., A. Azab and M. M. Almaky (2015). "Sodium nitrite induced biochemical alterations in the blood serum and its amelioration by aqueous extract of Libyan propolis in Guinea pigs." International Journal of Science and Research 4: 1040-1048.

29. Isenberg, J. S., Hyodo, F., Matsumoto, K.-I., Romeo, M. J., Abu-Asab, M., Tsokos, M., Kuppusamy, P., Wink, D. A., Krishna, M. C., & Roberts, D. D. (2007). Thrombospondin-1 limits ischemic tissue survival by inhibiting nitric oxide--mediated vascular smooth muscle relaxation. Blood, 109(5), 1945–1952.

30. Ismail, M. F., D. A. Ali, A. Fernando, M. E. Abdraboh, R. L. Gaur, W. M. Ibrahim, M. H. Raj and A. Ouhtit (2009). "Chemoprevention of rat liver toxicity and carcinogenesis by Spirulina." International journal of biological sciences 5(4): 377.

31, Azab, A. E., & Albasha, M. O. (2019). Hepatorenal Protective Effects of Pomegranate (Punica granatum) Juice against Nicotine Induced Toxicity in Guinea Pigs. Advances and Trends in Biotechnology and Genetics Vol, 2, 24–41.

32. Azab, A. E. and M. O. Albasha (2015). "Simultaneous administration of aqueous extract of Rosmarinus officinal with nicotine resulted in prevention of induced hepatorenal toxicity in Guinea pigs." American Journal of Bioscience and Bioengineering 3(5): 80-86.