Ameliorative Effects of Rhamnus Fruit (*Ziziphus spina-christi* L.) and Zinc on Sodium Fluoride-Induced Oxidative Stress in Rats.

[1] Nanees Y.E., Awad, [2] Rania Shams El deen Fakher Eldeen.

[1]Home Economics Dept., Faculty of Specific Education, Mansoura University, Egypt

[2]Home Economics Department, Faculty of Specific Education, Tanta University .Egypt.

Abstract

Background: Fluoride is widely distributed in nature in many forms and its compounds are being used extensively. Increased oxidative stress is proposed to mediate the toxic effects of fluoride on soft tissues. Anti-inflammatory and antioxidant properties have been describedrom species of the Rhamnaceae family. **Aim of the study:** Accordingly, this research was conducted to investigate the possible protective effects of rhamnus fruit *Ziziphus spina-christi* L. (Powder and extract), zinc and their co-treatment against sodium fluoride (NaF)-induced oxidative stress in male albino rats. **Materials and methods:** Thirty five rats were divided into 7 equal groups. Group 1 served as negative control group fed on the basal diet. Group 2: positive control group was given NaF (10 mg/kg) orally once daily for 2 weeks. Group 3: zinc 20mg/Kg b.wt/ rats. Group 4: fed on the formulated diet with 5% rhamnus fruit powder. Group 5: given orally rhamnus extract at dose of 5 ml. Group 6: 5% rhamnus fruit powder +zinc and Group 7: 5 ml of rhamnus fruit extract +zinc. At the end of the experiment, all animals were sacrificed and blood samples were obtained for assessment of serum total cholesterol, HDL-C, LDL-C, VLDL-C and triglycerides levels in addition to liver and kidney functions. Oxidative indices including total antioxidant status (TAS), total oxidant status (TOS), kidney TAS and TOS. As well as, kidneys histopathological changes were assessed. **Results:** NaF intoxicated groups showed significant alterations of biochemical indices with significantly decreased in TAS and TOS levels. **Conclusions**: The obtained results showed that NaF intoxication caused hepatic and renal damage by increasing oxidative stress and suggested a possible protective effect of rhamnus fruits and zinc administration against fluoride-induced oxidative stress.

Keywords: Na Fluoride, Rhamnus, Zinc, Oxidative Stress, TAS, TOS.

Introduction

Fluoride (F–) is an essential trace element that, in low concentrations, has been proven to be beneficial for teeth and bone development **(Pendrys, 2001)**. It is widely distributed in the environment in different forms and its compounds are extensively used. Typically, water consumption is the largest contributor to daily F intake either due to runoff of F– containing rocks and soils into groundwater or artificial fluoridation of drinking water in some areas **(ATSDR, 2003)**. The permissible amount of fluoride in drinking water is 0.5 - 1.5 mg/L **(Cotruvo, 2017 and Fallahzadeh *et al.,* 2018)**. Furthermore, F– anions are incorporated in various insecticides, Teflon-lined cookware **(NRC, 2006**), air (due to gaseous industrial waste) **(Nabavi *et al.,* 2012)** dietary supplements and in drugs designed to reduce dental decay **(Fallahzadeh *et al.,* 2018)**. the consumption of fluoride became uncontrolled and unpredictable often exceeding its therapeutic window **(Natalia and Gennadii, 2012)**. The excessive consumption of fluoride results in fluorosis as a serious health problem (**Madhusudhan *et al.,* 2010)** which linked to reduce antioxidant defense and increases oxidative stress of brain, liver, kidney and spinal cord **(Strunecka and Strunecky, 2020** and **Wang and Li, 2002).)**, a slow degenerative diseases, affecting teeth and bone tissues **(Sarkar *et al.,* 2014)**, as well as inducing neurological defect **(Malin and Christine, 2015 and Kumar *et al.,* 2020)**. Numerous investigations have established that the toxicity of fluoride as fluoride intoxication leads to the down-regulation of antioxidant enzymes **(Vani and Reddy, 2000)**, an increase in relative oxygen species (ROS), and oxidative stress **(Ghosh *et al.,* 2002)**. The pro-oxidant/ antioxidant imbalance caused by fluoride intoxication may lead to multi-organ dysfunctions **(Chlubek, 2003)**. Excessive ROS production and/or diminished antioxidant defenses have been implicated in cancer, diabetes, and cardiovascular diseases **(Fatehi-Hassanabad *et al.,* 2010; Montezano and Touyz, 2012** and **Storz, 2006).** NaF administration increased levels of lipid peroxidation and reduced SOD and catalase activities. Furthermore, glutathione levels in erythrocytes diminished after NaF exposure, suggesting an induction of oxidative stress **(Nabavi *et al.,* 2013).** Moreover, **Al-Sabaawy and Al-Kaisie (2020)** reported that NaF may reduce the efficiency of male reproductive system and reduce the levels of sexual hormones in rats. However, zinc antagonizes oxidative stress, apoptosis and cell cycle changes induced by excess fluoride **(Yu *et al.,* 2006)**.

Rhamnus (*Ziziphus spina-christi L.)* belongs to the Rhamnaceae family that produces small orange-yellow fruits, tasted like a mixture of dates and apples and was usually eaten fresh or dried **(Bukar *et al.,* 2015)**. It is grows

wild in Egypt especially, in Sinai. Usually in Arabic the fruits have the name of the tree, but in the case of Z. spina-christi, the tree is called siddir and the fruit nabag indicating the specific importance of this plant to local people (**Michel *et al.,* 2011** and **Saied *et al.,* 2008)**. It is shown to have antiviral, antifungal, antibacterial, laxative, purgative and depurative activities and used in the Egyptian folk medicine for treatment of several diseases including gastrointestinal tract ailments, diabetes and diarrhea **(Amin and Ghoneim, 2009; Michel *et al.,* 2011** and **Mubaraki *et al.,* 2017)**. Recently, hypoglycemic, hypotensive, hepatoprotective, anti-inflammatory, antioxidant, free radical scavenging, antibactericidal, antimutagenic as well as antiproliferative, pro-apoptotic activity in human cancer cell lines and antigenoapoptosis-inducing properties have been described for species of Rhamnaceae family **(Almeer *et al.,* 2018 ; Campbell *et al.,* 2019; Comlekcioglu *et al.,* 2017; Chen *et al.,* 2018; Dkhil *et al.,* 2018; Guizani *et al.,* 2013; Hemeg *et al* 2020** and **Jafarian *et al.,* 2014**).

There is an increasing interest in the natural antioxidants contained in medicinal and dietary plants, which are candidates for the prevention of oxidative damages. The genus *Zizyphus* (Rhamnaceae) is characterized from a phytochemical point of view by the abundance of phenolic substances, especially flavonoids, anthraquinones and tannins **(Shahat *et al.,* 2001 and Tripathi *et al.,* 2001),** which are described by numerous authors as antioxidant molecules (**Kim *et al.,* 2020; Moreira *et al.,* 2018; Park *et al.,* 2004** and **Vaya *et al.,* 2003)**. The active constituents of *Ziziphus spina-christi* includes triterpenoid sapogenins, geranyl acetate, sterols, saponins, methyl hexadecanoate, peptide, cyclopeptide alkaloids, methyl octadecanoate, tannines, and flavonoids (such as rutin and quercetin derivatives) **(Almeer *et al.,* 2018; Jafarian *et al.,* 2014** and **Kadioglu *et al.,* 2016).** Administration of *Ziziphus spina-christi* resulted in a greater reduction of inflammatory colonic injury, restored the balance between the oxidants and antioxidants and effectively modulated the mRNA expression of redox-sensitive transcription factors; therefore, it could be considered as an alternative and/or additive therapeutic approach for the management of inflammatory disease **(Almeer *et al.,* 2018)**. **Tessema and Molla (2021)** revealed that the methanolic extract of crude rhamnus leaves can help the healing of wounds as evidenced by an increase in wound contraction rate and tensile strength, decrease in Epithelialization period. **Ghaffari *et al.,* (2021)** concluded that the mechanism of action has occurred through the Bax-independent apoptotic pathway in breast cancer MCF-7 cells and inhibited cells proliferation after exposed to Ziziphus spina-christi leaf extracts. **Tacherfiout *et al.,* (2018)** suggested that rhamnus leaves are rich in

flavonoids and flavonoid derivatives with an anti-hyperlipidemic effect in vivo and in hepatic cells**.**

The aim of this study was to characterize the phenolic compounds in rhamnus fruit (*Ziziphus spina Christi*) by HPLC, and explore the effect of rhamnus fruit (powder and extract), zinc and their co-treatment against sodium fluoride (NaF)-induced oxidative stress in male albino rats.

MATERIALS AND METHODS

Materials:

Rhamnus fruits: Rhamnus fruits were obtained from the local market in Cairo city, Egypt.

Basal diet: Casein, vitamins, minerals and cellulose were obtained from El-Gomhariya Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt. While starch and corn oil were obtained from local market.

Zinc: Octozinic capsules produced by October pharma S.A.E and contain 110 zinc sulphate heptahydrate. The human therapeutic dose of zinc sulphate heptahydrate was converted to rat dose according to **Paget and Barnes, (1964)** that was 20 mg/Kg body weight, dissolved in distilled water and given to rats by oral intubations.

Sodium fluoride: Sodium fluoride was purchased from Sigma Aldrich Chemical Co.

Rats: Thirty-five mature male albino rats of Sprague - Dawley strain weighing 110±5 g. at age of 9-12 weeks were obtained from Laboratory of Animal Colony, Helwan, Egypt.

Methods:

Fresh rhamnus fruits were washed and cleaned with water and dehydrated into air circulated oven at 45°C for 24 hrs. Then, dried fruit was crushed to powder. Part of the rhamnus dried powder was added to the diet at a level of 5% of the diet. The other part of dried powder was used for the preparation of ethanolic extract, where, 100 g of rhamnus fruits powdered was soaked in 500 ml of 80% ethanol with frequent agitation. Clarification was then carried out using vacuum filtration through filter paper whatman number 2. The resultant extract was concentrated to dryness using a rotary evaporator under reduced pressure at a temperature of 40°C.

Phytochemical analysis of Rhamnus fruits: Types and concentrations of polyphenolic compounds were estimated as recommended by **(Goupy *et al.*, 1999)**. At Laboratory of Food Technology Research Institute, Agriculture Research Center, Egypt.

Experimental design:

The rats were housed in stainless steel cages with wire mesh bottoms and maintained in temperature and humidity control with 12 hrs light / dark cycle. All rats were allowed to freely access drinking of water and basal diet for seven days adjustment to the laboratory environment. The basal diet comprised of casein (200g/kg), cornstarch (497g/kg), sucrose (100g/kg), cellulose (30 g/kg) ,corn oil (50g/kg), mineral mixture (100g/kg) , vitamin mixture (20g/kg) and DL-methionine (3g/kg) according to **NRC, (1995).** The rats were randomly divided into 7 groups (each of 5 rats) as follows:

Group 1: normal control rats fed on the basal diet.
Group 2: positive control rats were given NaF (10 mg/kg b.wt) orally once daily for 2 weeks as described by **Blaszczyk, *et al.,* (2011)**
Group 3: Naf + fed on the basal diet and given zinc 20 mg/Kg b.wt/rats orally.
Group 4: Naf + fed on formulated diet with 5% rhamnus fruits powder
Group 5: Naf + 5 ml of rhamnus fruits extract
Group 6: Naf + 5% *rhamnus* fruits powder +zinc
Group 7: Naf + 5 ml of rhamnus fruits extract +zinc

The net food intake and gained body weight were used for the calculation of Food and protein efficiency ratio (FER&PER) according to **(Chapman *et al.,* 1950)**.

Tissue preparation:

The kidney was removed, washed and perfused with normal saline to remove residual blood. Kidney tissues were homogenized (model TH 220, OMNI, Warrenton, VA, USA) 1:10 (w/v) in ice-cold 140 mM potassium chloride at pH 7.4. The homogenates tissues were centrifuged at 3000 rpm for 10 min at 4 ^{0}C, and the supernatants were removed and stored at -80 ^{0}C until analysis of oxidative stress parameter are performed.

Biochemical analysis:

At the end of the experimental period (6 weeks), rats were sacrificed after overnight fasting under ether anesthesia. Blood samples were collected from hepatic portal vein in a clean dry centrifuge tube. Then blood samples were left to clot at room temperature for 15 minutes, and centrifuged at 3000 rpm for 20 minutes for serum separate. Serum was carefully separated and transferred into clean quite fit plastic tubes and kept frozen at - 20°C until the time of biochemical analysis.

Determination of serum lipids:

Serum levels of triglycerides were determined according to the method of **Fossati and Prencipe, (1982).** Total cholesterol was determined by

colorimetric method according to **Allian *et al.*, (1974)**. High density lipoproteins cholesterol (HDL-c) were determined according to the method of **Gordon and Amer, (1977)**. Very low density lipoproteins cholesterol (VLDL-c) and low density lipoproteins cholesterol (LDL-c) were determined according to the method of **Lee and Nieman, (1996).**

Determination of Total antioxidant status and total oxidation status:

Total antioxidant status (TAS) was measured using a commercially available kits from Rel Assay Diagnostics (Gaziantep, Turkey). The method was based on the reduction of colored 2.20-azino-bis(3-ethylbenzotiazoline-6-sulfonic acid) (ABTS) radical to a colorless reduced form by antioxidants present in the sample. Absorbance was measured spectrophotometrically at a wavelength of 660 nm. The method was calibrated using the vitamin E analog trolox, and data were expressed as mmol Trolox equivalent (eq.) per liter (mmol Trolox eq./L) (**Erel, 2004**).

Total antioxidant status (TOS) was measured using a commercially available kit from Rel Assay Diagnostics **(Erel, 2005)**. The method was based on the principle that the oxidants in the sample oxidized ferrous ions, previously bounded to a chelator to ferric ions. In the acidic medium of the assay, these ferric ions formed a colored complex with a chromogen. The color intensity was measured spectrophotometrically at a wavelength of 530 nm. This assay was calibrated with hydrogen peroxide (H_2O_2), and the results were expressed as lmol H_2O_2 eq./L.

Determination of liver and kidney functions:

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated according to **Reitman and Frankel, (1957**), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were determined according to **Bergmeyer and Horder, (1980)** and **Vassault, (1983),** respectively. Total bilirubin was measured according to the method of **Reitman and Frankel, (1957).** Serum creatinine and Uric acid were determined according to the methods described by **Bartles *et al.*, (1972** and **Haisman and Muller (1977**), respectively.

Histopathological examination:

The kidney was subjected to histological examination according to **Frankel and Reitman (1963).**

Statistical analysis:

The obtained data were statistically analyzed using computerized SPSS. Effects of different treatments were analyzed by one way ANOVA (Analysis of

variance) test using Duncan's multiple range test and $p<0.05$ was used to indicate significance between different groups **(Snedecor and Cochran, 1967).**

RESULTS AND DISCUSSION

Polyphenol fractions of Rhamnus fruit (*Ziziphusspina Christi*):

The phytochemical study of rhamnus fruit showed the presence of various qualitative and quantities of total polyphenolic compounds Table 1. As it can be seen, rhamnus fruit contained higher amounts of chlorogenic acid, nimbolide, vanillic acid, ferulic acid, phenolic acids, *p*-coumaric acid, pyrocatechol, 2′, 3′-dehydrosalannol, quercetin-3-rhamnoside, caffeic acid, gallic acid, rutin and epoxy-azadiradione, respectively. These results were consistent with **Marzouk *et al.,* (1999)** who reported that flavonol glycosides represent an important part in the polyphenolic compounds contained in rhamnus fruits. **Cuoco *et al.,* (2014)** revealed that the flavonol compounds present in green species of rhamnus fruits were quercetin, kaempferol, isorhamnetin, rhamnetin, rhamnocitrin and rhamnazin. **Rocchetti, *et al.,* (2019)** showed that mature ramnus fruits were rich source of flavonols (glycosidic forms of quercetin and kaempferol). The fresh nabak fruit *(Ziziphus spina-christi)* contained phenolic as 1644 mg GAE/100 g **(Guizani *et al.,* 2013).** Its active constituents include flavonoids such as rutin and quercetin derivatives **(Jafarian *et al.,* 2014)**. Moreover**, Almeer *et al.,* (2018)** characterized polyphenolic compounds of *Ziziphus spina-christi* fruit extract as catechin, gallic acid, ellagic acid, chlorogenic acid, rutin, isoquercitrin, quercetin, and kaempferol. Those active compounds have many biological activities: for example Chlorogenic acid can mitigate oxidative and infammatory stresses **(Dkhil *et al.,* 2018** and **Liang and Kitts 2015)**, quercetin, apigenin, and kaempferol are potent antioxidants **(Al-Olayan *et al.,* 2014)**. Numerous studies have confirmed the abundant of kaempferol and Quercetin in Rhamnaceae family **(Boussahel *et al.,* 2013; Chaouche *et al.,* 2020; Moussi *et al.,* 2015** and **Zeouk, *et al*., 2020)**. The interesting antioxidant potency of this species has been also demonstrated **(Ammar *et al.,* 2018** and **Bhouri *et al.,* 2011). Moussi *et al.,* (2015)** identified phenolic compounds of leaves extract of *Rhamnus alaternus* L were rutin, quercetin-3-rhamnoside, kaempferol, *p*-coumaric acid, ferulic acid, gallic acid, luteolin and anthraquinones. These phytochemical families have been demonstrated to play a role in antioxidant mechanism of action due to their molecular structures **(Ammar *et al.* 2018; Huang and Frankel, 1997 and Montoro *et al.,* 2005)**. Flavonoids as putative examples were considered as good electron and hydrogen donors; this character brings to the end of radical chain through converting free radicals to more stable compounds **(Kelly, 2010). Bhouri *et al.,* (2012)** isolated two antioxidant flavonoids namely Kaempferol 3-

O-beta isorhamninoside and Rhamnocitrin 3-O-beta isorhamninoside which had capacities to transfer electron leading to an attack against free radicals and then combating cellular damage.

Table (1): Types and amount of polyphenol fractions of rhamnus fruit *(Ziziphus spina Christi)*

Phenolic Compounds	Total Phenols (ppm)
Caffeic acid	453.14
Chlorogenic acid	2519.57
Ferulic acid	808.26
p-coumaric acid	624.80
Gallic acid	445.23
Vanillic acid	1215.51
Pyrocatechol	599.16
Pyrogallol	18.45
quercetin-3-rhamnoside	504.50
Rutin	358.23
Epoxy-azadiradione	113.40
Nimbolide	2514.15
Myricetin	29.00
Quercetin	96.30
Kaempferol	57.40
phenolic acids	682.35
2′, 3′-dehydrosalannol	510.37

The effect of rhamnus fruit (powder and extract), zinc and their co-treatment on body weight, body weight gain, food intake and feed efficiency ratio (FER).

Effect of rhamnus fruits (powder and extract), zinc and their co-treatment against sodium fluoride (NaF)-induced oxidative stress on body weight, body weight gain, food intake, feed efficiency ratio (FER) and protein efficiency ratio (PER) are presented in Table 2. The initial body weights of rats were similar in all groups and all of them gave positive body weight gain at the end of the experiment. Meanwhile, NaF-treated group recorded the lowest body weight gain and food intake as compared with all groups. It was noticed that the treated rats with NaF+Zn+ rhamnus fruit (powder and extract were the best mitigating ability against NaF toxicity; although, all of rhamnus fruits (powder and extract) and zinc showed a positive and protective effect on NaF toxicity. These results are in accordance with **Nageshwar *et al.*, (2017)** and **Kumar *et al.*, (2020)** who reported that the body weight of rats treating with NaF significantly decreased, compared with the negative control group. The decreased body weight might be

due to reduced food intake or disturbed protein and energy metabolism after fluoride ingestion **(Chinoy *et al.*, 1991). Yossef *et al.*, (2011)** concluded that *Ziziphus spina-christi* fruit significantly increased the body weight and weight gain, which decreased by carbon tetrachloride (CCL4). In contrast, **Lopes *et al.*, (2020)** reported that during the experimental period, the fluoride exposure did not impair the body weight gain and showed no difference in the beginning, middle, and end of exposure protocol ($p = 0.05$).

Table (2): Effect of Rhamnus fruit (powder and extract), zinc and their co-treatment on Body weight, body weight gain, food intake and feed efficiency ratio (FER) of the study groups.

Variables / Groups	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	Food Intake (g/d))	FER	PER
G(1): Negative control (N-)	110.55 ± 3.67[a]	203.47 ± 13.01[a]	92.92 ± 11.33[a]	16.65 ± 2.11[a]	0.093 ± 0.001[a]	0.046 ± 0.03[a]
G(2): positive control (N+)	110.41 ± 3.50[a]	154.71 ± 12.13[b]	44.30 ± 7.71[b]	14.20 ± 2.17[a]	0.051 ± 0.002[b]	0.025 ± 0.01[b]
G(3): zinc 20 mg/Kg b.w./rats	112.14 ± 3.41[a]	190.11 ± 12.38[a]	80.97 ± 10.14[a]	15.95 ± 1.99[a]	0.084 ± 1.002[a]	0.042 ± 0.02[a]
G(4): 5% Rhamnus powder	109.14 ± 2.45 a	189.71 ± 11.22 a	80.57 ± 8.17 a	15.90 ± 2.11 a	0.084 ± 0.003 a	0.042 ± 0.03 a
G (5): 5 ml Rhamnus extract	113.33 ± 2.99 a	199.41 ± 13.78 a	91.08 ± 10.22 a	16.35 ± 2.91 a	0.092 ± 0.001 a	0.046 ± 0.02 a
G (6): 5% Rhamnus powder +zinc	110.22 ± 3.11 a	201.14 ± 12.35 a	90.92 ± 11.11 a	16.55 ± 2.18 a	0.091 ± 0.001 a	0.045 ± 0.04 a
G (7): 5 ml Rhamnus extract +zinc	110.34 ± 3.14 a	205.11 ± 14.41 a	94.77 ± 11.21 a	16.75 ± 2.81 a	0.094 ± 0.003 a	0.047 ± 0.03 a

Mean values± SD in each column having different superscript (a, b,) are significant.

Effect of Rhamnus fruit (powder and extract), zinc and their co-treatment on lipid profile:

Table (3) shows the changes in serum lipid profiles as a result of different treatment. It could be observed that NaF- treated group represented significant increases in serum TC, TG, LDL-C and VLDL-C as compared with negative control and other treatment groups. On the other hand NaF- treated group showed significant decreases in serum HDL-C as compared with all treated groups with fruits and Zn. The increment in serum TC, TG, LDL-C and VLDL-

C were ameliorated in all groups that received rhamnus fruits (powder and extract) and zinc. However, G 3 of treated rats with zinc (20 mg/Kg b.w.) showed the lowest effect in the lipid improvement against NaF toxicity. From these data, it is clear that G6 and G7 had the greatest effect in the decrement of TC, TG, LDL-C and VLDL-C and the increment of HDL-C as compared with positive control G2 and in some parameters they had no significant changes as compared with the negative control G1.

In the current study, sodium fluoride administration led to significant increases in serum cholesterol and triglycerides, compared with the negative control groups, these NaF -alteration in lipid profile are in agreement with the obtained results by **Al-Harbi *et al.,*(2014)**, **Khudair and Aldabaj (2014)** and **Abou Anza and Salah Eldin (2015)**. Conflicting results were also obtained by **Kanbur *et al.* (2009)** who reported lowering in plasma cholesterol and TG levels following NaF administration. Enzymes inhibited by fluoride (triglyceride lipase, unspecific esterase and pyrophosphates) were suggested to be responsible for the rise in serum triglycerides and cholesterol. Moreover, fluoride was found to cause hypercholesterolemia due to lowering of insulin level **(Garcia-Montalvo *et al.,* 2009)**. Also, NaF intoxication increased lipid peroxidation and loss of membrane integrity might be important in altered lipid metabolism and closely associated with the observed hyperlipidemia **(Abdel-Wahab, 2013)**. **Yossef *et al.,* (2011)** concluded that *Ziziphus spina-christi* fruit extract restores significantly normal levels of serum cholesterol, triglyceride, LDL and VLDL as compared to the elevated level induced by CCl_4. **Tacherfiout *et al.,* (2018)** found that oral treatment with 200 mg/kg b.wt and 400 mg/kg b.wt of rhamnus leaves extract decreased serum triacylglycerols by 70% and 42%, and serum total cholesterol by 60% and 40%, respectively, relative to the hyperlipidemic control group. Flavonoids derivatives from R. *alaternus* leaves showed a similar positive impact on murine preadipocyte 3T3-L1 cellular model. The hypolipidemic activity of rhamnus fruit is likely to be due to its flavonoids content. Flavonoids or flavonoid-rich extracts have been reported to lower serum lipids in diverse animal models of hyperlipidemia, through a variety of mechanisms. These include: down-regulating the production of intestinal-associated lipoprotein apoB48 **(Ma *et al.,* 2015)**, inhibiting the activity of hepatic HMG-CoA reductase **(Khamis *et al.,* 2017** and **Kuang *et al.,* 2017)**, inhibiting hepatic lipogenesis through suppressed expression of SREBP1 and fatty acid synthase (**Bao *et al.,* 2016** and **Kuang *et al.,* 2017)** and stimulating hepatic fatty acid oxidation **(Chang *et al.,* 2011 and Mulvihill *et al.,* 2011).**

Table (3): Effect of Rhamnus (powder and extract), zinc and their co-treatment on Lipid profile.

Variables / Groups	Total Cholestrol TC (mg/dl)	Triglycerides TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
G(1): Negative control (N-)	85.37± 4.16 d	67.13± 5.97 d	39.50± 2.29a	29.24± 9.99e	13.51± 0.59d
G(2): positive control (N+)	118.23± 9.71 a	106.55± 10.66 [a]	25.23± 2.37c	78.87± 6.55 [a]	21.47± 2.13a
G(3): zinc 20 mg/Kg b.w./rats	110.46± 8.26 b [**]	89.79± 6.14 b	31.17± 3.76b	60.14± 5.76b	17.85± 0.63b
G(4): 5% Rhamnus powder	98.75± 7.44 c	78.67± 5.22bc	32.53± 3.02b	48.57± 3.34c	15.79± 1.04bc
G (5): 5 ml Rhamnus extract	93.27± 6.89 c	72.48± 5.42cd	37.70± 2.07a	39.91± 3.66d	15.17± 0.28cd
G (6): 5% Rhamnus powder +zinc	91.27± 6.89 c	70.48± 5.42cd	37.99± 2.07a	37.51± 3.66d	14.91± 0.28cd
G (7): 5 ml Rhamnus extract +zinc	90.66± 6.89 c	69.18± 5.42cd	38.10± 2.07a	35.21± 3.66d	14.17± 0.28cd

Mean values± SD in each column having different superscript (a, b,) are significant.

Effect of Rhamnus fruit (powder and extract), zinc and their co-treatment on liver and kidneys functions:

Table (4) shows a comparison between negative control group, NaF-treated group, NaF+Zn-treated group, NaF+ rhamnus fruits (powder and extract)-treated groups and NaF+Zn+ rhamnus fruits (powder and extract)-treated groups, as regards of liver and kidney parameters. Significant increases were noticed in NaF-treated group in compare to the negative control group and all treatment groups as regards of all experimental parameters except, total bilirubin it showed a significant decrease. The most significant amelioration was observed in the NaF+zn+ rhamnus fruits (powder and extract)-treated groups (G6 and G7) at the level of serum biomarkers related to hepatic dysfunction (AST, ALT, ALP and lactate dehydrogenase activities and total bilirubin level) and kidney function (creatinine and uric acid levels) suggesting a potential protective role of rhamnus fruit against NaF-induced hepatic damage. These results are in parallel with those obtained by **Abou Anza and Salah Eldin (2015)** and **Pratt and Kaplan, (2005)** who showed that oral administration of NaF induced a significant increase in serum liver enzymes (AST, ALT, ALP and total bilirubin and kidney enzymes; creatinine and Urea **(Iheka *et al.,* 2015).** These indices usually reflect hepatocyte integrity and cholestasis and their elevation indicates hepatocellular damage. **AL-Harbi *et al.,* 2014; Atmaca *et al.,* 2014** demonstrated that the induction of both pathomorphological and metabolic changes in the liver by exposure to fluoride**.**

As a site of active metabolism, the liver can be especially susceptible to fluoride toxicity **(Shashi and Thapar, 2001)**. NaF-induced cytotoxicity and necrotic death of hepatocytes can be related to toxic fluoride effects ultimately leading to cell death. These cellular events include an induction of inflammatory reactions, inhibition of protein synthesis and cell cycle progression, oxidative stress, and DNA damage. The molecular mechanisms underlying fluoride-induced apoptosis include the stimulation of G protein dependent signaling systems, oxidative stress, ATP depletion, activation of the cell surface death receptors, disruption of outer mitochondria membrane, alterations in the ratio of anti-apoptotic-apoptotic Bcl-2 proteins, upregulation of p53 expression, expression of apoptosis-related genes, endoplasmic reticulum stress and disturbances in protein synthesis **(Ghosh *et al.,* 2008** and **Agalakova and Gusev, 2013)**. *Ziziphus spina-christi* fruit extract restores normal levels of ALT, AST and ALP in serum reduced the CCL4-induced levels of ALT and AST **(Shen *et al.* 2009** and **Yossef *et al.,* 2011).** It can be concluded that NaF induced a hepatic damage, which represented in elevating markedly activities of ALT and AST in serum. **Guizani *et al.,* (2013)** stated that the treatment of rats with 175mg GAE/kg b. wt (per week) *Ziziphus spina-christi* fruit extract for 16 weeks produced no functional disturbances in liver and kidney and no haematological changes were detected as well as *Ziziphus spina-christi* fruit significantly ameliorated the Azoxymethane -induced Oxidative Stress.

Absorbed fluoride is carried by the blood, causes metabolic disturbances in the body **(Sahay, 1986)**. The major rout for the removal of fluoride from the body is by the kidney. Kidney is the primary target for fluoride toxicity **(Inkiewicz and Krechniak 2003)**. Disturbances in kidney function influenced by fluoride have been reported by numerous authors (**Birkner *et al.,* 2006; Grucka-Mamczar *et al.,* 2003 and Sashi *et al.,* 2002)**. High concentrations of fluoride usually lead to kidney damage included tubular degeneration, inflammation and fibrosis (**Dote *et al.,* 2000).** Concurrently, fluoride caused degeneration and necrosis of the tubular cells, renal tubular hyaline casts and glomeruli swelling (**Luo *et al.,* 2017)** found that fluoride in excess of 12 mg/kg are induced renal oxidative damage, which was characterized by the alteration of renal function parameters including elevated contents of serum creatinine, serum uric acid, blood urea nitrogen, and the activities of urinary N-acetyl-b-D-glucosaminidase, renal lactate dehydrogenase (LDH), and reduced activities of sodium-potassium adenosine triphosphatase (Na+/K+-ATPase) and acid phosphatase (ACP) in the kidney.

Table (4): Effect of Rhamnus (powder and extract), zinc and their co-treatment on liver and kidney functions

Variables / Groups	AST (μ /ml)	ALT (μ /ml)	ALP (μ /ml)	LDH (Umg/dl)	Total bilirubin (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
G(1): Negative control (N-)	41.17 ± 5.81 [b]	13.35 ± 1.12 [b]	30.17 ± 5.66 [b]	354 ± 28.11e	39.50± 3.29a	0.77 ± 0.01 [b]	1.83 0.26 [c]
G(2): positive control (N+)	72.39± 9.61 [a**]	28.55± 3.35 [a**]	50.38± 5.81 [a**]	857.56± 37.55a**	28.23± 2.37c**	1.95 ±0.11 [a**]	4.41 ±1.01 [a***]
G(3):zinc 20 mg/Kg b.w./rats	49.37 ± 6.01 [b]	15.71 ± 1.81 [b]	37.80 ± 4.11 [b]	450.14± 24.76b	31.17± 3.76b	0.99 ± 0.02 [b]	2.11 ± 0.81 [b]
G(4):5% Rhamnus powder	51.14 ± 8.10 [b]	16.28 ± 2.01 [b]	38.73 ± 4.37 [b]	423.57± 23.34c	33.53± 3.02b	0.88 ± 0.12 [b]	2.41 ± 0.77 [b]
G(5):5ml Rhamnus extract	48.21 ± 6.15 [b]	18.13 ± 3.51 [b]	38.34 ± 5.01 [b]	406.91± 21.66d	35.70± 2.07a	0.75 ± 0.13 [b]	2.17 ± 0.67 [b]
G(6):5% Rhamnus powder +zinc	40.21 ± 4.13 [b]	14.11 ± 3.65 [b]	32.11 ± 3.11 [b]	402.10± 22.66d	37.10± 2.03a	0.70 ± 0.15 [b]	1.74 ± 0.74 [c]
G(7):5ml Rhamnus extract +zinc	43.19 ± 4.61 [b]	15.31 ± 3.66 [b]	35.30 ± 2.99 [b]	396.20± 22.66d	38.35± 2.01a	0.98 ± 0.18 [b]	2.25 ± 0.16 [b]

Mean values± SD in each column having different superscript (a, b,) are significant.
AST: aspartate transferase ALT: alanine aminotransferase ALP: alkaline phosphatase
LDH: lactate dehydrogenase

Effect of Rhamnus (powder and extract), zinc and their co-treatment on oxidative status levels:

In the present study, exposure to sodium fluoride increased total oxidant status (TOS) antioxidant enzyme and decreased total antioxidant status (TAS) as shown in Table 5, suggesting an impaired function of antioxidant defense system. However, supplementation of NaF intoxicated groups with rhamnus fruits (powder and extract), zinc and their co-treatment restored antioxidative homeostasis. This was evidenced by increased assayed markers of the endogenous antioxidant system (TAS) with concomitant decrease of markers of oxidative stress mediated damage (TOS).

Numerous studies linked increased oxidative stress to F exposure **(Grucka-Mamczar *et al.*, 2009; Nabavi *et al.*, 2013).** The measure of total antioxidant capacity (TAC) generally considers the cumulative action of all the antioxidants present in plasma and body fluids, thus provides an integrated parameter rather than the simple sum of measurable antioxidants **(Ghiselli *et al.*, 2000)**. NaF intoxicated groups showed significant alterations of hematological and biochemical indices with significantly depleted superoxide

dismutase enzymes (SOD), decreased TAC and concomitant increase in TBARS and AOPP **(Abou Anza and Salah Eldin, 2015).** Similar results have been reported by **Sarkar *et al.,* (2014)** they showed that the combined effect of reductions in antioxidant enzyme activity plus high levels of lipid peroxidation is associated with deleterious oxidative changes due to the accumulation of toxic products in F-treated rats. Therefore, enhancing endogenous antioxidant status by administrating exogenous compounds can provide an effective strategy to prevent and reverse NaF-induced toxicity **(Wessam, 2013). Lopes *et al.,* 2020** found that fluoride exposure decreased antioxidant capacity against peroxyl radicals (ACAP) levels at 10 mg F/L and 50 mg F/L groups compared to the control group.

Oral administration of 200 mg/kg b.w. of Z. spina-christi leaf extract either plain in STZ-diabetic rats for 28 days resulted in significant reduction in blood glucose level together with significant rise in serum insulin, C-peptide levels and TAC **(Michel *et al.,* 2011)**. **Farag *et al.,* (2015)** concluded that kidney and liver injury due to cyclosporine can be significantly decreased by thymoquinone which resets the oxidant /antioxidant balance of the affected organ through scavenging the free radicals. Currently, the use of phytochemicals as a therapy in diseases related to oxidative stress has gained immense interest for their ability to quench free radicals and their capability to protect body tissues against oxidative stress **(Nabavi *et al.,* 2012)**. **Guizani *et al.,* (2013)** stated that the mean TAC values among the control and *Ziziphus spina-christi* fruit groups were observed insignificant differences. *Ziziphus spina-christi* fruit-administration abrogated the Azoxymethane-induced TAC impairment. **Amin and Ghoneim (2009)** and **Yossef *et al.,* (2011)** demonstrated that treatment of *Ziziphus spina-christi* fruit effectively protected against carbon tetrachloride-induced liver damage by restoring the normal levels of lipid peroxidation and retaining the activities of endogenous anti-oxidants. Moreover, **Mubaraki *et al.,* (2017)** found that *Ziziphus spina-christi* extract treatment markedly reinstated the levels of oxidative markers and enhanced antioxidant enzyme activities in mice with cerebral malaria. **Dkhil *et al.,* (2018)** indicated that *Ziziphus spina-christi* fruit extract significantly and dose-dependently inhibited sepsis induced liver and spleen injury. These results suggest that it could provide a therapeutic agent for sepsis by inducing antinflammatory and antioxidant effects. High total polyphenols and flavonoids content of the R. *alaternus* extracts may be corroborated with the antioxidant and antigenotoxic activities **(Ammar *et al.,* 2007).** The antilipid peroxidation activity of various extracts from R. *alaternus*, produced using the Soxhlet extraction method, was estimated by calculating the values of malondialdehyde (MDA) in cultured K562 human chronic myelogenous leukemia cells **(Ammar *et al.,* 2011).** Rhamnus *alaternus* extracts containing total oligomer flavonoids (TOF)

and ethyl acetate (EA) inhibited lipid peroxidation at a concentration comprised within 200–800 µg/mL, the best activity being observed at the highest concentration (800 µg/mL). In this study, the IC50 values of TOF and EA extracts were determined at 196 and 265 µg/mL, respectively. In comparison, a value of 17 µg/mL was obtained for vitamin C, used as reference substance. Some flavonoids with antioxidant activity are described for Rhamnus species, like rutin, quercetin, kaempferol and rhamnocitrin **(Bhouri *et al.*, 2011** and **Moussi *et al.*, 2015)**. The ability to inhibit or prevent oxidative damage can be associated with the treatment and prevention diseases, especially those who own physiopathology associated with oxidative stress. Extracts of two *Rhamnaceae* family*: Ziziphus jujuba Mill* and *Rhamnus alaternus* L have antioxidant properties at different concentrations, with better activity for *R. alaternus* L leaves **Chaouche *et al.*, (2020)**. **Chen *et al.*, (2020)** confirmed flavonoids and their glycosides were the major ingredients of *R. prinoides* and potentially responsible for its strong antioxidant and anti-inflammatory activities.

Table (5): Effect of Rhamnus (powder and extract), zinc and their co-treatment on oxidative status levels.

Groups / Variables	G (1): Negative control (N-)	G(2): positive control (N+)	G(3): zinc 20 mg/Kg b.w./rats	G(4): 5% Rhamnus powder	G (5):5 ml Rhamnus extract	G (6): 5% Rhamnus powder +zinc	G (7): 5 ml Rhamnus extract +zinc
TAS (mmol Trolox eq./L)	1.39± 0.13a	0.82± 0.08 c	1.05± 0.05 b	0.94± 0.03 b	1.46± 0.04 a	1.11 ± 4.13 ^{b}	1.29 ± 4.61 ^{b}
TOS (lmol H2O2 eq./L)	4.14± 0.39d	9.17± 0.38 a	7.10± 0.21 b	6.50± 0.40 b	5.53± 0.40b	4.90± 0.25c	4.53± 0.41c

Mean values± SD in each column having different super script (a, b, c, d ,e) are significant
TAS: Total antioxidant status TOS: Total oxidant status

Effect of Rhamnus (powder and extract), zinc and their co-treatment on oxidative status of the kidneys

Table 6 shows comparison of negative control group, NaF-treated group, NaF+Zn-treated group, NaF+ rhamnus fruit (powder and extract)-treated groups and NaF+Zn+ rhamnus fruit (powder and extract)-treated groups, regarding to oxidative status of the kidneys. Significant decreases were noticed in NaF-treated group in comparison to the negative control group and all other treated groups with regard to total antioxidant status (TAS). The most significant amelioration was observed on the NaF+zn+ rhamnus fruit (powder and extract) -treated groups (G6 and G7). However, significant increases were noticed in

NaF-treated group in comparison to the negative control group and all other treated groups with regard to total oxidant status (TOS). It could be noticed that there were no significant differences between negative control and NaF+Zn-treated group, NaF+ rhamnus fruit (powder and extract) -treated groups and NaF+Zn+ rhamnus fruit (powder and extract)-treated groups. The observed NaF -induced TOS increment and TAS impairment represents an evidence of kidney oxidative stress. These results are consistent with the previous studies conducted that showed NaF at a dose of 50 mg/l increased excretion of fluoride in urine, promoted the activity of urine gamma-glutamyl transpeptidase (gamma-GT), inhibit the activities of serum glutathione peroxidase (GPX) and kidney superoxide dismutase (SOD), reduce kidney glutathione (GSH) content, and increased kidney malondialdehyde (MDA) **(Yu *et al.,* 2006)**. NaF at a dose of 50 mg/l also induced rat renal apoptosis, reduced the cell number of G2/M phases in the cell cycle, and decreased DNA relative content significantly. Selenium and zinc inhibited the effects of NaF on oxidative stress and apoptosis, promoted the cell number of G2/M phases in the cell cycle) اين (المرجع. **Yu *et al.,* (2002)** suggested that NaF could induce apoptosis and change the cell cycle in rat renal cells and Se and Zn could antagonize apoptosis and the changes of cell cycle induced by NaF.

Table (6): Effect of Rhamnus (powder and extract), zinc and their co-treatment on oxidative status of the kidneys

Groups / Variables	G(1): Negative control (N-)	G(2): positive control (N+)	G(3): zinc 20 mg/Kg b.w./rats	G(4): 5% Rhamnus powder	G (5):5 ml Rhamnus extract	G (6): 5% Rhamnus powder +zinc	G (7): 5 ml Rhamnus extract +zinc
kidney-TAS (mmol Trolox eq./L)	1.99± 0.23a	0.95± 0.11b	1.03± 0.09 ab	1.45± 0.09a	1.66± 0.10 a	1.81 ± 4.13 ^{b}	1.90 ± 4.61 ^{b}
kidney-TOS (lmol H2O2 eq./L)	5.66± 0.39b	8.37± 0.38a	5.62± 0.21 b	7.82± 0.40 b	6.68± 0.40b	6.03± 0.40b	5.98± 0.41b

Mean values± SD in each column having different superscript (a, b,) are significant
TAS: Total antioxidant status
TOS: Total oxidant status

Histopathological of the kidneys:-

The histological alterations, including hypertrophy of glomerular tuft and thickening of parietal layer of Bowman's capsule of NaF-treated rats, whereas rhamnus (powder and extract), zinc and their co-treatment rats showed ameliorative effects and controlled the histological alterations. Kidneys have a

prominent role in fluoride metabolism, where, 50–80% of fluoride is removed via urinary excretion **(Xiong *et al.,* 2007).** Not surprisingly, the kidney is one of the major organs affected by fluoride intoxication, and numerous studies have established a close correlation between fluoride intake and renal injury. Hence, chronically intoxicated rats with sodium fluoride (NaF) have displayed histological renal changes, interstitial edema, tubular destruction, and glomerular and medullary hyperemia. Hand in hand with the typical kidney pathology, fluoride-intoxicated rats showed an increased rate of reactive oxygen species (ROS) generation and lipid peroxidation **(Kobayashi *et al.,* 2009).** Moreover, the histopathological changes in kidneys of chronic fluoride intoxication rats were mainly in the form of vacuolization and necrosis of tubules, atrophy of glomeruli, interstitial oedema, and interstitial nephritis. Investigators have explored the mechanism of renal lesion induced by excessive fluoride, consequently, collected numerous biological evidences, including oxidative stress **(Xu *et al.,* 2005)**, apoptosis **(Xu *et al.,*2002)**, and signal transduction **(Murao *et al.,* 2000)**. In addition to, degeneration and necrosis of the tubular cells, glomeruli swelling as well as the renal tubular hyaline casts were observed in the experimental groups **(Luo *et al.,* 2017)**. Also, these histopathological lesions induced by fluoride are changed in a dose- and time-dependent manner. In high doses of NaF, the cytoachitecture of the kidneys exhibited increasing in the amounts of cloudy swellings, degeneration of tubular epithelia, tissue necrosis, extensive vacuolization in renal tubules, hypertrophy and atrophy of glomeruli, exudation, interstitial oedema, and interstitial nephritis. These changes in the kidneys result in impaired renal function in chronic fluoride intoxication **(Shashi *et al.,* 2020)**.

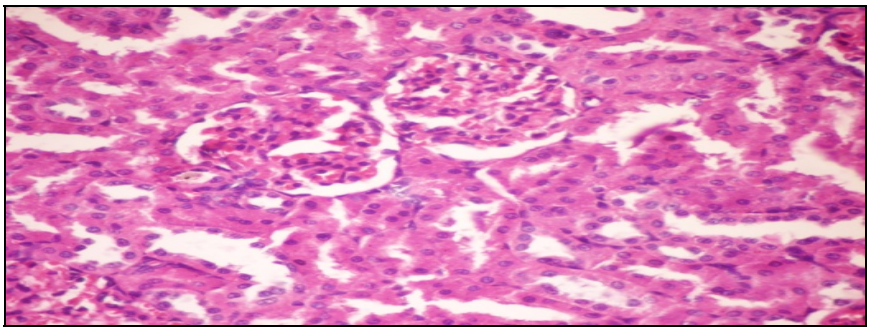

Photo (1): Kidney of rats from (normal control) healthy group showing the normal histological structure of renal parenchyma (H and E X400)

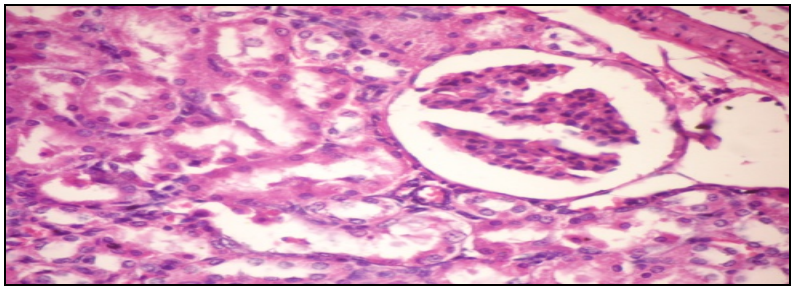

Photo (2): Kidney of rat from control (+ve) group showing hypertrophy of glomerular tuft and thickening of parietal layer of Bowman's capsule (H and E X400)

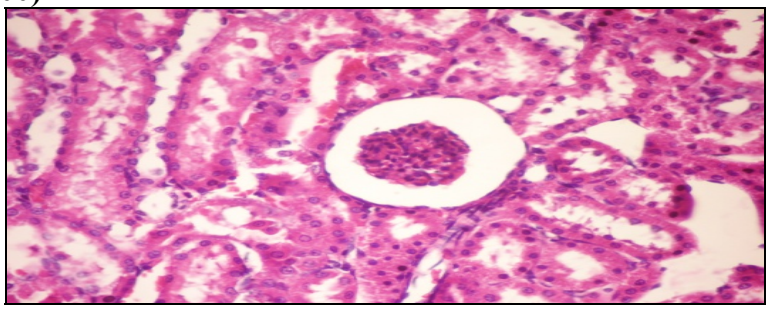

Photo (3): Kidney of rat from group 3 showing apparent normal renal parenchyma (H and E X 400).

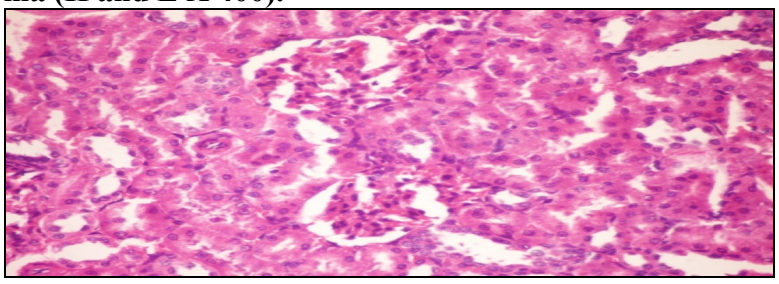

Photo (4): Kidney of rat from G4 showing congestion of renal blood vessels (H and E × 200).

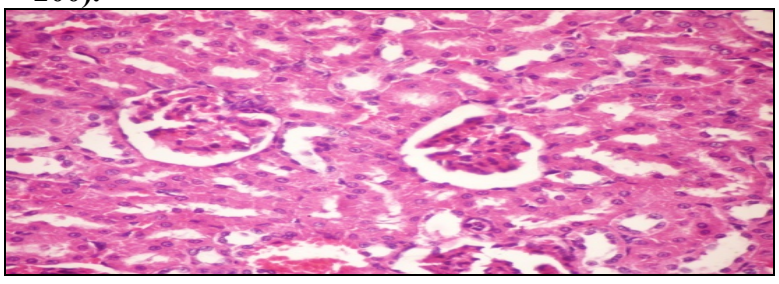

Photo (5): Kidney of rat from G5 showing revealed cystic dilatation of renal tubules with cellular cast in their lumen (H and E × 200).

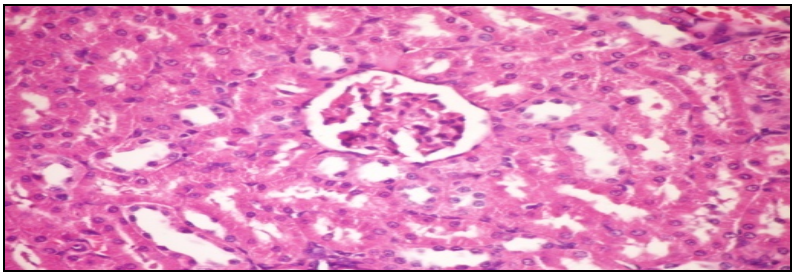

Photo (6): Kidney of rat from G6 showing cystic dilatation of renal tubules (H and E X 400).

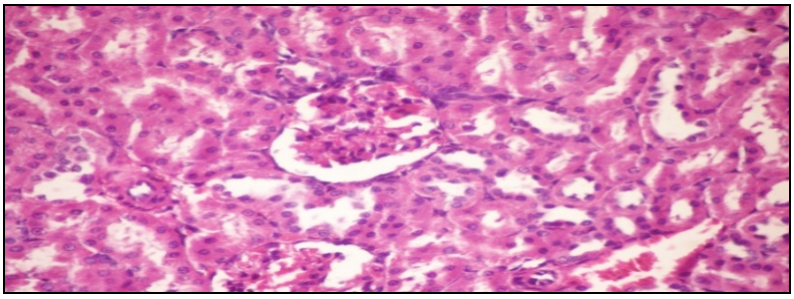

Photo (7): Kidney of rat from G7 showing no histopathological changes (H and E X400).

Conclusion: Administration of NaF caused biochemical and histopathological alternations and oxidative stress is a considered one of the main contributors to these changes. Treated NaF intoxicated rats with rhamnus fruits (powder and extract), zinc and their co-treatment caused ameliorated these effects.

Recommendations: Further studies to investigate the effect of NaF on other body organs and to explore the role of selected polyphenols compounds derivative from fruit which having antioxidant properties are recommended.

REFERENCES

Abdel-Wahab, W. (2013). Protective effects of thymoquinone on sodium fluoride–induced hepatotoxicity and oxidative stress in rats. *J Basic Appl. Zool., (66): 263-270.*

Abou Anza, R.E. and Salah Eldin, H.M.(2015). Mitigation of Fluoride Toxicity by the Use of Thymoquinone in Adult Male Albino Rat. *Ain Shams Journal of Forensic Medicine and Clinical Toxicology, 24: 1-10.*

Agalakova, N. and Gusev, G. (2013). Excessive fluoride consumption leads to accelerated death of erythrocytes and anemia in rats. *Biol Trace Elem Res., 153(1-3):340-9.*

Al-Harbi, M. Hamza, R. and Afaf, A. (2014). Hyperlipidemic and oxidative stress effects of sodium fluoride and the ameliorative role of selenium and curcumin in male mice. *Journal of Chemical and Pharmaceutical Research, 6(4):984-998.*

Allian, C. C.; Poon, L.S.; Chan, C. S. and Richmond, W. (1974). Enzymatic determination of total serum cholesterol. *Clin. Chem.; 20: 470.*

Almeer, R. S.; Mahmoud, S.M.; Amin, H. K. and Abdel Moneim, A.E. (2018). *Ziziphus spina-christi* fruit extract suppresses oxidative stress and p38 MAPK expression in ulcerative colitis in rats via induction of Nrf2 and HO-1 expression. *Food and Chemical Toxicology 115: 49–62.*

Al-Olayan, E.; El-Khadragy, M.; Metwally, D. and Abdel Moneim, A. (2014). Protective efects of pomegranate (Punica granatum) juice on testes against carbon tetrachloride intoxication in rats. *BMC Complement Altern Med., 14:164.*

Al-Sabaawy, H.B. and Al-Kaisie, B. I. (2020). Effects of Sub Lethal Concentrations of Sodium Fluoride on Sperm Activity and on the level of Sex Hormones of Adult Male Albino Rats. *The Iraqi Journal of Veterinary Medicine, 44 (2): 92–98.*

Amin, A. and Ghoneim, D. (2009) *Zizyphus spina-christi* protects against carbon tetrachloride-induced liver fibrosis in rats. *Food Chem Toxicol., 47:2111–2119. https://doi.org/10.1016/j.fct.2009.05.038*

Ammar, R. B.; Miyamoto, T. and Chekir-ghedira, L. (2018). Isolation and identification of new anthraquinones from Rhamnus alaternus L and evaluation of their free radical scavenging activity. *Nat. Prod. Res., 6419: 1–7.*

Ammar, R.B.; Bouhlel, I.; Kita Valenti, K.; Sghaier, M.B.; Kilani, S.; Mariotte, A.M.; Dijoux-Franca, M.G.; Laporte, F.; Kamel Ghedira, K. and Chekir-Ghedira, L. (2007) .Transcriptional response of genes involved in cell defense system in human cells stressed by H_2O_2 and pre-treated with (Tunisian) *Rhamnus alaternus* extracts: Combination with polyphenolic compounds and classic in vitro assays. *Chemico-Biological Interactions, 168: 171–183.*

Ammar, R.B.; Neffati, A.; Skandrani, I.; Ben Sghaier, M.; Bhouri, W.; Ghedira, K. and Chekir-Ghedira, L. (2011). Anti-lipid peroxidation and induction of apoptosis in the erythroleukaemic cell line K562 by extracts from (Tunisian) *Rhamnus alaternus* L. (Rhamnaceae). *Nat. Prod. Res., 25(11): 1047–1058.*

Atmaca, N.; Atmaca, H.; Kanici, A. and Anteplioglu, A. (2014). Protective effect of resveratrol on sodium fluoride-induced oxidativestress, hepatotoxicity and neurotoxicity in rats. *Food and Chemical Toxicology, 70: 191–197.*

ATSDR (Agency for Toxic Substances and Disease Registry) (2003). Toxicological Profile for Fluorides, Hydrogen Fluoride, and Fluorine. Public Health Service, US Department of Health and Human Services, Atlanta, Georgia.

Bao, L.; Hu, L.; Zhang, Y. and Wang, Y. (2016). Hypolipidemic effects of flavonoids extracted from *Lomatogonium rotatum. Exp. Ther. Med., 11 (4): 1417–1424.*

Bartles, H.; Bohmer, M. and Heirli, C. (1972): Colorimetric kinetic method of determination of creatinine. *J. Clin. Chem. Acta., (37): 193-197.*

Bergmeyer, H. U. and Horder, M. (1980). Methods for the measurement of catalytic concentration of enzymes. *J. Clin. Chem. Clin. Biochem. 18: 521-534.*

Bhouri, B.; Ben sghaier, M.; Kilani, S. and Chekir-Ghedira, S. (2011). Evaluation of antioxidant and antigenotoxic activity of two flavonoids from *Rhamnus alaternus* L. (Rhamnaceae): Kaempferol 3-O-β-isorhamninoside and rhamnocitrin 3-O-β-isorhamninoside. *Food and chemical toxicology, 49(5):1167-1173.*

Bhouri, W.; Boubaker, J. and Kilani, S. (2012). Flavonoids from *Rhamnus alaternus* L. (*Rhamnaceae*): Kaempferol 3-O-β -isorhamninoside and rhamnocitrin 3-O-β isorhamninoside protect against DNA damage in human lymphoblastoid cell and enhance antioxidant activity. *S. Afr. J. Bot., 80: 57–62.*

Birkner, E.; Grucka-Mamczar, E.; Żwirska-Korczala, K.; Zalejska-Fiolka, J.; Stawiarska-Pięta, B.; Kasperczyk, S. and Kasperczyk, A. (2006). Influence of sodium fluoride and caffeine on the kidney function and freeradical processes in that organ in adult rats. *Biol Trace Elem Res., 109:35–47.*

Blaszczyk, I.; Birkner, E. and Kasperczyk, S. (2011). Influence of methionine on toxicity of fluoride in the liver of rats. *Biol. Trace. Elem. Res. 139:325- 331.*

Boussahel, S.; Dahamna, S.; Ruberto, G.; Siracusa, L. and Harzallah, D. (2013). Phytochemical study and antioxidant activities of leaves extracts from Rhamnus *alaternus* L. *Pharmacogn. Commun., 3: 46–53.*

Bukar, M.; Kyari, M.Z.; Gwaski, P.A.; Gudusu, M.; Kuburi, F.S. and Abadam, Y.I. (2015). Evaluation of phytochemical and potential antibacterial activity of Ziziphyus spina-christi L. against some medically important pathogenic bacteria obtained from University of Maiduguri Teaching Hospital, Maiduguri, Borno State – Nigeria. *Journal of Pharmacognosy and Phytochemistry 3:98-101*

Campbell, M.; Zhao, W.; Fathi, R.; Mihreteab, M. and Gilbert, E.S. (2019). Rhamnus *prinoides (gesho)*: A source of diverse anti-biofilm activity. *Journal of Ethnopharmacology, 241:111955.*

Chang, C.J.; Tzeng, T.-F.; Liou, S.-S.; Chang, Y.-S. and Liu, I.-M. (2011). Kaempferol regulates the lipid-profile in high-fat diet-fed rats through an increase in hepatic PPARα levels. *Planta Med., 77 (17): 1876–1882.*

Chaouche, T.M.; Haddouchi, F.; Boudjemai, O. and Ghellai, I. (2020). Antioxidant and hemolytic activity of *Ziziphus jujuba Mill* and *Rhamnus alaternus* L (*Rhamnaceae*) extracts from Algeria. *Bulletin de la Société Royale des Sciences de Liège., 89: 1 – 14.*

Chapman, D.G.; Gastilla, R. and Campbell, T.A. (1950). Evaluation of protein in food. I. A. Method for the determination of protein efficiency ratio. *Can. J. Biochem. Physio. I (37) 679-686.*

Chen, G. L.; Mutie, F. M.; Xu, Y.B.; Saleri , F. D.; Hu, G.W. and Ming-Quan Guo, M.Q. (2020). Antioxidant, Anti-inflammatory Activities and Polyphenol Profile of Rhamnus prinoides. *Pharmaceuticals, 13: 55.*

Chen, G.; Wu, J.; Li, N. and Guo, M. (2018). "Screening for antiproliferative and anti-inflammatory components from Rhamnus davurica Pall. Using bio-

affinity ultrafiltration with multiple drug targets". *Analytical and Bioanalytical Chemistry, 410 (15): 3587–3595.*

Chinoy, N. J.; Sequeria, E. and Narayana, M.V. (1991). Effects of vitamin C and calcium on the reversibility of fluoride induced alterations in spermatozoan of rabbit. *Fluoride, 24(1): 29-39.*

Chlubek, D. (2003). Fluoride and oxidative stress. *Fluoride, 36:217-228.*

Comlekcioglu, N.; Aygan, A.; Kutlu, M. and Kocabas, Y.Z. (2017). Antimicrobial activities of some natural dyes and dyed wool yarn. *Iran. J. Chem. Chem. Eng. Research Note, 36.*

Cotruvo, J. A. (2017). WHO Guidelines for Drinking Water Quality: First Addendum to the Fourth Edition. *J Am Water Works Asso.,1(109):44–51.*

Cuoco, G.; Mathe, C. and Vieillescazes, C. (2014). Liquid chromatographic analysis of flavonol compounds in green fruits of three Rhamnus species used in Stil de grain. *Microchemical Journal 115:130–137.*

Dkhil, M.A.; Al-Quraishy, S. and Moneim, A.E.A. (2018). *Ziziphus spina-christi* leaf extract pretreatment inhibits liver and spleen injury in a mouse model of sepsis via antioxidant and anti-inflammatory effects. *Inflammopharmacolog, 26: 779–791.*

Dote, T.; Kono, K.; Usuda, K.; Nishiura, H. and Tagawa, T. (2000). Acute renal damage dose response in rats to intravenous infusion of sodium fluoride. *Fluoride 33:210–217.*

Erel, O. (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin. Biochem. 37: 277–285.*

Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. *Clin. Biochem. 38: 1103–1111.*

Fallahzadeh, R. A.; Miri, M.; Taghavi, M,; Gholizadeh, A.; Anbarani, R. and Hosseini-Bandegharaei, A. (2018). Spatial variation and probabilistic risk assessment of exposure to fluoride in drinking water. *Food and Chem Toxicol., 113:314–321.*

Farag, M.; Ahmed, G.; Shehata, R. and Kazem, A. (2015): Thymoquinone improves the kidney and liver changes induced by chronic cyclosporine A treatment and acute renal ischaemia/reperfusion in rats. *Journal of Pharmacy and Pharmacology; DOI: 10.1111/jphp.12363.*

Fatehi-Hassanabad, Z.; Chan, C.B. and Furman, B.L. (2010). Reactive oxygen species and endothelial function in diabetes. *Eur J Pharmacol.,636:8-17.*

Fossati, P. and Prencipel, L. (1982). Determination of triglycerides, Bicon Diagnostics, made in Germany. *Clinical Chemistry, 28: 2077-2078.*

Frankel, S. and Reitman, S. (1963). Clinical Laboratory's Methods. *The. C.V. Mosbye Company, 1102.*

Garcia-Montalvo, E.; Reyes-Perez, H. and Del Razo, L. (2009). Fluoride exposure impairs glucose tolerance via decreased insulin expression and oxidative stress. *Toxicology, 263: 75–83.*

Ghaffari, K.; Ahmadi, R.; Behrooz Saberi, B. and Pooria Moulavi, P. (2021). Anti-proliferative effects of Ziziphus spina-christi and Phlomis russeliana leaf extracts on HEK293 and MCF-7 Cell Lines and Evaluation of Bax and Bcl-2 Genes Expression Level in MCF-7 Cells. *Asian Pac J Cancer Prev., 22(S1):81-87.*

Ghiselli, A.; Serafini, M. and Natella, F. (2000). Total antioxidant capacity as a tool to assess redox status: Critical view and experimental data. *Free Radical Biology and Medicine, 29(11): 1106–1114.*

Ghosh, D.; Das Sarkar, S.; Maity, R.; Jana, D. and Das, U.B. (2002). Testicular toxicity in sodium fluoride treated rats: association with oxidative stress. *Reprod Toxicol.,16:385-90. PMID: 12220599.*

Ghosh, J.; Das, J.; Manna, P. and Sil, P, (2008). Cytoprotective effect of arjunolic acid in response to sodium fluoride mediated oxidative stress and cell death via necrotic pathway. *Toxicol. In Vitro., 22:1918–1926.*

Gordon, T. and Amer, M. (1977). Determination of HDL. J. Med.; 62 : 707**.**

Goupy, P.; hugues, M.; Boivin, P. and Amiot, J., (1999). Phenolic compounds. Official methods(ISO). *J.Sci. Food Agric., 79:1625-1634.*

Grucka-Mamczar, E.; Birkner, E.; Polaniak, R.; Stawiarska-Pięta, B.; Cegłowska, A. and Gajda, M. (2003). Disturbances of kidney function in young rats after chronic exposure to NaF contained in drinking water. *Ann Acad Med Siles, 54–55: 9– 14.*

Guizani, N.; Waly, M.I.; Singh, V. and Rahman, M.S. (2013). Nabag (*Zizyphus spina-christi*) extract prevents aberrant crypt foci development in

colons of azoxymethane-treated rats by abrogating oxidative stress and inducing apoptosis. *Asian Pac. J. Cancer Prev. 14, 5031–5035.*

Haisman, P. and Muller, B.R. (1977). Quantitative enzymatic colorimetric determination of uric acid in serum. *Clin. Chem., 26:227.*

Hemeg , H.A.; Moussa, I.M.; Ibrahim, S.; Dawoud, T.M.; Alhaji, J.H.; Mubarak, A.S.; Kabli, S.A.; Alsubki, R.A.; Tawfik, A.M.; and Marouf, S.A. (2020). Antimicrobial effect of different herbal plant extracts against different microbial population. *Saudi Journal of Biological Sciences 27: 3221–3227.*

Huang, S. W. and Frankel, E.N. (1997). Antioxidant activity of tea catechins in different lipid systems. *J. Agric. Food Chem., 45: 3033–3038.*

Iheka C. U.; Onyegeme-Okerenta, B. M. and Anacletus F. C. (2015). Impact of Fluoride Toxicity and Ameliorative Effects of Some Antioxidants on Selected Biochemical Indices of Male Rats. *AASCIT Journal of Health, 2(6): 87-92.*

Inkiewicz, I. and Krechniak, J. (2003). Fluoride content in soft tissues and urine of rats exposed to sodium fluoride in drinking water. *Fluoride 36:263–266.*

Jafarian, A.; Zolfaghari, B. and Shirani, K. (2014). Cytotoxicity of different extracts of arial parts of Ziziphus spina-christi on Hela and MDA-MB-468 tumor cells. *Adv. Biomed. Res., 3: 38.*

Kadioglu, O. Jacob, S.; Bohnert , S.; Na, J.; Saeed, M.E.M. Khalid, H.; Merfort, I.; Thines, E. Pommerening, T. and Efferth, T. (2016). Evaluating ancient Egyptian prescriptions today: anti-infammatory activity of Ziziphus spina-christi. *Phytomedicine, 23:293–306 (S0944-7113(16)*

Kanbur, M.; Eraslan, G.; Silici, S. and Karabacak, M. (2009): Effects of sodium fluoride exposure on some biochemical parameters in mice: Evaluation of the ameliorative effect of royal jelly applications on these parameters. *Food Chem Toxicol; 47:1184-1189.*

Kelly, F.J. (2010). Oxidative Stress : Its Role in Air Pollution and Adverse Health Effects POLLUTION STRESS : AND ITS ROLE ADVERSE IN AIR HEALTH EFFECTS. *Occup. Environ. Med., 60:, 612–616.*

Khamis, A.A.; Salama, A.F.; Kenawy, M.E. and Mohamed, T.M. (2017). Regulation of hepatic hydroxy methyl glutarate–CoA reductase for controlling hypercholesterolemia in rats, *Biomed. Pharmacother. 95: 1242–1250.*

Khudair, K. and Aldabaj, A. (2014). Effect of High Concentration of Sodium Fluoride on Serum Lipid Profile of Male Rabbits: Hypolipidemic Effect of Grape Seed Oil. *Online International Interdisciplinary Research Journal; {BiMonthly}, ISSN2249-9598, Volume-IV, Special Issue.*

Kim, H. O.; Shin, K. R.; Jang, B. and Kim, Y.C. (2020). Action mechanism of anti-wrinkle efect of Rhamnus yoshinoi methanol extract in human dermal fbroblast and keratinocyte cell lines. *Toxicol Res. 36:69–77.*

Kumar, N. K.; Nageshwar, M. and Pratap Reddy, K. (2020). Protective Effect of Curcumin on Hippocampal and Behavior Changes in Rats Exposed to Fluoride During Pre- and Post-natal Period. *Basic and Clinical Neuroscience, 11(3), 289-300.*

Kobayashi, C. A. N.; Leite, A. L.; Silva, T. L.; Santos, L. D.; Nogueira, F. C. S.; Oliveira, R. C. Palma, M. S.; Domont, G. B. and Buzalaf, M.A.R. (2009). Proteomic analysis of kidney in rats chronically exposed to fluoride. *Chem-Biol Interact., 180:305–311.*

Kuang, W.; Zhang, X. and Lan, Z. (2017). Flavonoids extracted from Linaria vulgaris protect against hyperlipidemia and hepatic steatosis induced by western-type diet in mice, *Arch. Pharm. Res.: 1–9.*

Lee, R. D. and Nieman, D. C. (1996). Nutritional assessment. *2 nd Ed., Mosby, Missoun, USA.*

Liang, N. and Kitts, D.D. (2015). Role of chlorogenic acids in controlling oxidative and infammatory stress conditions. *Nutrients, 8(1): 16 https:// doi.org/10.3390/nu8010016.*

Lopes, G.O.; Ferreira, M.K.M.; Davis, L.; Bittencourt, L.O.; Aragão, W.A.B.; Dionizio, A.; Buzalaf , M.A.R.; Crespo-Lopez, M.E.; Maia, C.S.F. and Lima, R.R.(2020). Effects of Fluoride Long-Term Exposure over the Cerebellum: Global Proteomic Profile, Oxidative Biochemistry, Cell Density, and Motor Behavior Evaluation. *Int. J. Mol. Sci., 21: 7297.*

Luo,Q.; Cui, H.; Deng, H.; Kuang, P.; Liu, H.; Lu, Y.; Fang, J.; Zuo, Z.; Deng, J.; Li, Y.; Wang, X. and Zhao, L. (2017). Histopathological findings of renal tissue induced by oxidative stress due to different concentrations of fluoride. *Oncotarget, 8(31): 50430–50446.*

Ma, Y.; Jiang, C.; Yao, N.; Li, Y.; Wang, Q.; Fang, S.; Shang, X.; Zhao, M.; Che, C. and Ni, Y. (2015). Antihyperlipidemic effect of Cyclocarya

paliurus (Batal.) Iljinskaja extract and inhibition of apolipoprotein B48 overproduction in hyperlipidemic mice, *J. Ethnopharmacol, 166: 286–296.*

Madhusudhan, N.; Basha, P. M.; Rai, P.; Ahmed, F. and Prasad, G. R. (2010). Effect of maternal fluoride exposure on developing CNS of rats: Protective role of Aloe vera, Curcuma longa and Ocimum sanctum. *Indian Journal Experimental Biology, 48(8): 830-6.*

Malin, A and Christine, T (2015). Exposure to fluoridated water and attention deficit hyperactivity disorder prevalence among children and adolescents in United States: An ecological association. *Environmental Health; 14(17): doi:10.1186/s12940-015-003-1.*

Marzouk, M.S.; El-Toumy, S.A.A.; Merfort, I. and Nawwar, M.A.M. (1999). Polyphenolic metabolites of Rhamnus *disperma*. *Phytochemistry, 52.*

Michel, C.G.; Nesseem, D.I. and Ismail, M. F. (2011). Anti-diabetic activity and stability study of the formulated leaf extract of Zizyphus spina-christi (L.) Willd with the influence of seasonal variation. *J Ethnopharmacol 133:53–62 (pii:S0378-8741(10)00639-2)*

Montezano, A. C. and Touyz, R. M. (2012). Molecular mechanisms of hypertension-reactive oxygen species and antioxidants: a basic science update for the clinician. *Can J Cardiol., 28:288-295.*

Montoro, P.; Braca, A.; Pizza, C. and Tommasi, N. (2005). Structure – antioxidant activity relationships of flavonoids isolated from different plant species. *Food Chem., 92: 349–355.*

Moreira, T. F. Deoliveira, D. M. and Arruda, M. F. (2013). "Lipid peroxidation inhibition by ethanolic extract and fractions from *Rhamnus sphaerosperma var. pubescens* (Reissek) M.C. Johnst. (*Rhamnaceae*)". *International Journal of Phytomedicine, 5 (2): 136–140.*

Moreira, T.F.; Sorbo, J.M; Souza, F.D.O.; Fernandes, B.C.; Ocampos, F.M.M.; de Oliveira, D.M.S.; Arcaro, C.A.; Assis, R.P.; Barison, A.; Migue, O.G; Baviera, A.M.; Soares, C.P. and Brunetti, I. L. (2018). Emodin, Physcion, and Crude Extract of Rhamnus sphaerosperma var. pubescens Induce Mixed Cell Death, Increase in Oxidative Stress, DNA Damage, and Inhibition of AKT in Cervical and Oral Squamous Carcinoma Cell Lines. *Oxidative Medicine and Cellular Longevity, ID 2390234, 18 pages https://doi.org/10.1155/2018/ 2390234.*

Moussi, K. Nayak, B.; Perkins, L. B.; Dahmoune, F.; Madani, K. and Chibane, M. (2015). HPLC-DAD profile of phenolic compounds and antioxidant activity of leaves extract of Rhamnus *alaternus* L. *Industrial Crops and Products, 74: 858–866.*

Mubaraki, M.A.; Hafiz, T.A.; Al-Quraishy, S. and, Dkhil, M.A. (2017) Oxidative stress and genes regulation of cerebral malaria upon Zizyphus spina-christi treatment in a murine model. *Microb Pathog 107:69–74. https://doi.org/10.1016/j.micpath.2017.03.017*

Mulvihill, E.E.; Assini, J.M.; Lee, J.K.; Allister, E.M.; Sutherland, B.G.; Koppes, J.B.; Sawyez, C.G.; Edwards, J.Y.; Telford, D.E. and Charbonneau, A. (2011). Nobiletin attenuates VLDL overproduction, dyslipidemia, and atherosclerosis in mice with diet-induced insulin resistance. *Diabetes, 60 (5): 1446–1457.*

Murao, H.; Sakagami, N.; Iguchi, T.; Murakami, T. and Suketa, Y. (2000). Sodium fluoride increase intracellular calcium in rat renal epithe-lial cell line NRK-52E. *Biol. Pharm. Bull., 23 (5): 581–584.*

Nabavi, S. M,; Nabavi, S. F.; Eslami, S. and Moghaddam, A.H. (2012). In vivo protective effects of quercetin against sodium fluoride induced oxidative stress in the hepatic tissue. *Food Chem., 132: 931-935.*

Nabavi, S.F.; Habtemariam, S.; Sureda, A.; Moghaddam, A. H.; Daglia, M. and Nabavi, S.M. (2013). In vivo protective effects of gallic acid isolated from *Peltiphyllum Peltatum* against sodium fluoride-induced oxidative stress in rat erythrocytes. Inhibition of oxidative stress by gallic acid in vivo. *Arh Hig Rada Toksikol, 64:553-559.*

Nageshwar, M.; Kirankumar, N.; Rajkiran R. B.; Chandrashakar, R. N.; and Reddy, K. P. (2017). Quercetin treatment against NaFinduced oxidative stress related neuronal and learning changes in developing rats. *Journal of King Saud University-Science, 29(2), 221- 229.*

Natalia, I. and Gennadii P. (2012). Molecular mechanisms of cytotoxicity and apoptosis induced by inorganic fluoride. *ISRN Cell Biology; Article ID 403835.*

NRC (National Research Council) (2006). Fluoride in Drinking-Water. *A scientific review of EPA's standards, Washington.*

NRC, (1995). National Research Council: Nutrient Requirements of Laboratory Animals. *Fourth Revised Edition, National Academy Press. Washington, DC: 29-30.*

Paget, G. E. and Barnes, J. M. (1964): Inter species dosages conversion scheme in evaluation of results and quantitative application in different species toxicity test. *Academic Press London and NY: 135-165.*

Park, K.Y.; Jung, G.O.; Lee, K.T.; Choi, J.; Choi, M.Y.; Kim, G.T.; Jung, H.J. and Park, H.J. (2004). Antimutagenic activity of flavonoids from the heartwood of Rhus verniciflua. *J. Ethnopharmacol, 90: 73–79.*

Pendrys D (2001). Fluoride ingestion and oral health. Nutrition; 17(11-12): 979-980.

Pratt, D. and Kaplan, M. (2005). Evaluation of liver function. In: Harrison's principles of internal medicine. *Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL (eds.), 16th ed., McGraw-Hill, New York, pp: 1711–171.*

Reitman, S. and S. Frankel, (1957). Determination of glutamate pyruvat transaminase and glutamate oxaloacetate transaminase. *Am. J. Clin. Path., 28(1): 56-63.*

Rocchetti, G.; Miras-Moreno, M.B.; Zengin, G.; Senkardes, I.; Sadeer, N. B.; Mahomoodally, M. F. and Lucini, L. (2019). UHPLC-QTOF-MS phytochemical profiling and in vitro biological properties of *Rhamnus petiolaris* (Rhamnaceae). *Industrial Crops & Products 142: 111856.*

Sahay, M. (1986). Histopathological And Cytogenetic Effect Of Aflatoxins In Mammal. *Ph.D Thesis: T. M Bhagalpur Univ. Bhagalpur.*

Saied, A.S.; Gebauer, J.; Hammer, K. and Buerkert, A. (2008). *Ziziphus spina-christi (L.)* Willd.: a multipurpose fruit tree. Genet Resour Crop Evol., 55:929–937.

Sarkar, C.; Das, N.; Pal, S. and Dinda, B. (2014). Oxidative stress induced alteration of protein and nucleic acid metabolism in fluoride intoxicated rat brain. Protection by 3α-hydroxy oleanolic acid isolated from Neonatis Wightiana. *IJPSR; 5 (7):3047-3066.*

Sashi, A.; Aihgh, J. P. and Thapar, S.P. (2002). Toxic effect of fluoride on rabbit kidney. *Fluoride 35:38–50*.

Shahat, A.A.; Pieters, L.; Apers, S.; Nazeif, N.M.; Abdel-Azim, N.S.; Bergh, D.V. and Vlienk, A.J. (2001). Chemical and biological investigations on Zizyphus spina-christi L. *Phytother. Res., 15: 593–597.*

Shashi, A. and Thapar, S. (2001). Histopathology of fluoride-induced hepatotoxicity in rabbits. *Fluoride, 34(1):34–42.*

Shashi, A.; Singh, J.P. and Thapar, S.P. (2020). Toxic effects of fluoride on rabbit kidney. *Fluoride 35(1):38-50.*

Shen , X. ; Tang ,Y. ; Yang, R.; Yu, L. ; Fang, T. and Duan , (2009). The protective effect of *Zizyphus jujube* fruit on carbon tetrachloride-induced hepatic injury in mice by anti-oxidative activities. *Journal of Ethnopharmacology, 122: 555–560.*

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods. *7th Ed., The Lowa State University Press. Ames, Lowa, U.S.A.*

Storz, P. (2006). Reactive oxygen species-mediated mitochondria-tonucleus signaling: a key to aging and radical-caused diseases. *Sci STKE., 332:re3. doi: 10.1126/stke.3322006re3.*

Strunecka, A. and Strunecky, O. (2020). Mechanisms of luoride toxicity: from enzymes to underlying integrative networks. *Appl Sci.,10(20):7100.*

Tacherfiout, M.; Petrov, P.D.; Mattonai, M.; Ribechini, E.; Joan Ribot, J.; M. Luisa Bonet, L. and Khettal, B. (2018). Antihyperlipidemic effect of a Rhamnus alaternus leaf extract in Tritoninduced hyperlipidemic rats and human HepG2 cells. *Biomedicine & Pharmacotherapy, 101: 501–509.*

Tessema, Z. and Molla, Y. (2021). Evaluation of the wound healing activity of the crude extract of root bark of Brucea antidysentrica, the leaves of Dodonaea angustifolia and Rhamnus prinoides in mice. *Heliyon 7 (1): e05901.*

Tripathi, M.; Pandey, M.B.; Jha, R.N.; Pandey, V.B.; Tripathi, P.N. and Singh, J.P. (2001). Cyclopeptide alkaloids from Zizyphus jujuba. *Fitoterapia 72, 507–510.*

Vani, M.L. and Reddy, K. P. (2000). Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice. *Fluoride, 33:17-26.*

Vassault, A. (1983). Methods of enzymatic analysis, enzymes1. *Oxidoreductases, transferases Vol. III. Verlag Chemie, Basel, 118-126.*

Vaya, J.; Mohmod, S.; Goldblum, A.; Aviram, M.; Volkavor, N.; Shaalam, A.; Musa, R. and Tamir, S. (2003). Inhibition of LDL oxidation by flavonoids

in relation to their structure and calculated enthalpy. *Phytochemistry, 62: 89–99.*

Wang, W. and Li, Y. (2002). Environmental epidemiology of fluorine and its effects on health. *Soil, water and Environmental Science, 11(4): 383-387.*

Wessam, M. (2013). Protective effect of thymoquinone on sodium fluoride-induced hepatotoxicity and oxidative stress in rats. *The Journal of Basic and Applied Zoology; 66(5):263-270.*

Xiong, X.; Liu, J. and He, W. (2007). Dose effect relationship between drinking water fluoride levels and damage to liver and kidney functions in children. *Environ Res.,103:112–116.*

Xu, H.; Sun, B. and Li, G.S. (2002). The mechanism of nephric apop-tosis induced by chronic fluorosis. *Chin. J. Endemicol., 21 (4):251–253.*

Xu, H.; Zhang, J. M.; Chang, M. and Li, G.S. (2005). Expression of Bcl-2on the oxidative stress of renal tubular cells treated by NaF. *Chin.J. Endemiol., 24 (1): 17–20.*

Yossef, H. E.; Khedr, A.A. and Mahran, M. Z. (2011). Hepatoprotective activity and antioxidant effects of El Nabka (Zizyphus spina-christi) fruits on rats hepatotoxicity induced by carbon tetrachloride. *Nature and Science, 9 (2).*

Yu, R.; Xia, T.; Wang, A. and Chen, X. (2002). Effects of selenium and zinc on rat renal apoptosis and change of cell cycle induced by fluoride. *Chinese Journal of Preventive Medicine, 36(4):219-221.*

Yu, R.A.;, Xia, T.; Wang, A.G. and Chen, X.M. (2006). Effects of selenium and zinc on renal oxidative stress and apoptosis induced by fluoride in rats. *Biomedical and EnvironmentalSciences,19(6):439-444.*

Zeouk, I.; Ouedrhiri, W.; Jiménez, I.A.; Lorenzo-Morales, J.; Bazzocchi, I.L. and Bekhti, K . (2020). Intra-combined antioxidant activity and chemical characterization of three fractions from Rhamnus alaternus extract: Mixture design. *Industrial Crops & Products, 144: 112054.*

الملخص العربى

التأثيرات التحسينية لفاكهة النبق على الإجهاد التأكسدي الناجم عن فلوريد الصوديوم في الفئران

ينتشر الفلوريد على نطاق واسع في الطبيعة بأشكال عديدة ويتم استخدام مركباته على نطاق واسع. يُقترح ان للفلورايد العديد من التأثيرات السامة في زيادة الإجهاد التأكسدي بالأنسجة الرخوة. ولقد تم وصف الخصائص المضادة للالتهابات ومضادات الأكسدة لأنواع عائلة Rhamnaceae. وفقًا لذلك ، تم إجراء هذا البحث للتحقيق في التأثيرات الوقائية المحتملة لمسحوق ومستخلص فاكهة النبق*Ziziphus spina-christi L* Rhamnus وكذلك الزنك بالإضافة الى تأثيرهم المشترك ضد الإجهاد التأكسدي الناتج عن فلوريد الصوديوم (NaF) في ذكور الجرذان البيضاء. و تم تقسيم خمسة وثلاثين فأرا إلى ٧ مجموعات متساوية. المجموعة (١) المجموعة الضابطة السالبة تتغذى على النظام الغذائي الأساسي. المجموعة (٢) المجموعة الضابطة الموجبة تتغذي علي الغذاء الاساسي كما تم إعطاءها 10 مجم / كجم من وزن الجسم فلوريد الصوديوم (NaF) عن طريق الفم مرة واحدة يوميًا لمدة أسبوعين. المجموعة الثالثة (٣): تتغذي علي الغذاء الاساسي كما تم اعطاؤها الزنك بنسبة ٢٠ مجم/ كجم من وزن الجسم عن طريق الفم. المجموعة (٤): تم إعطاءها 10 مجم / كجم من وزن الجسم فلوريد الصوديوم (NaF) و تتغذي علي الغذاء الاساسي المدعم بـ ٥٪ من مسحوق فاكهة النبق. المجموعة (٥): تم إعطاءها 10 مجم / كجم من وزن الجسم فلوريد الصوديوم (NaF) و تم اعطاؤها ٥ مل من مستخلص فاكهة النبق عن طريق الفم. المجموعة (٦): تم إعطاءها 10 مجم / كجم من وزن الجسم فلوريد الصوديوم (NaF) و تتغذي علي الغذاء الاساسي المدعم بـ ٥٪ من مسحوق فاكهة النبق ٥٪ + الزنك والمجموعة (٧):): تم إعطاءها 10 مجم / كجم من وزن الجسم فلوريد الصوديوم (NaF) و ٥ مل من مستخلص فاكهة النبق + زنك. في نهاية التجربة تم ذبح الحيوانات و الحصول على مصل الدم لتقدير الكوليسترول الكلي، HDL-C، LDL-C، VLDL-C والدهون الثلاثية (TG) ، بالإضافة إلى إنزيمات الكبد والكلى. تم أيضًا تقييم مؤشرات الأكسدة بما في ذلك حالة مضادات الأكسدة الكلية (TAS) وحالة الأكسدة الكلية (TOS) وكذلك (TAS) و (TOS) للكلى بالإضافة الى التغيرات النسيجية المرضية للكلى.

النتائج: أظهرت نتائج مجموعات NaF تغيرات كبيرة في المؤشرات البيوكيميائية مع انخفاض كبير في TAS و ارتفاع TOS. فى حين ان كلاً من النبق والزنك وخليطهم ادي إلي تحسن التغيرات الكيميائية الحيوية والنسيجية في الكلى التي سببها NaF و يرجع ذلك لخصائصه المضادة للأكسدة على.

الاستنتاجات: أظهرت النتائج التي تم الحصول عليها أن تناول NaF يتسبب في تلف كبد وكلى الفئران عن طريق زيادة الإجهاد التأكسدي. كما تشير إلى الآثار الوقائية المحتملة لثمار فاكهة النبق والزنك ضد الإجهاد التأكسدي الناجم عن الفلوريد.

الكلمات المفتاحية: فلوريد الصوديوم، النبق، الزنك، الإجهاد التأكسدي، TAS، TO S .