



Hepatoprotective Role of Gallic Acid on Sodium Fluoride-Induced Liver Injury in Rats

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ABSTRACT

This study was undertaken to evaluate the protective effect of Gallic acid (GA) on oxidative damages in liver of rats intoxicated by sodium fluoride. Rats were randomly divided into three groups of six animals each: group (C): served as a control; group (NaF): treated for three weeks with sodium fluoride (100 ppm in drinking water) and group (NaF+GA) : treated daily and for three weeks also by both of NaF and GA (20mg/Kg orally). It was found that NaF induced liver damages as evidenced by the elevation of plasma amino transferases (ALT, AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) activities associated with a decrease in total protein, albumin and bilirubin levels. Hepatotoxicity was objectified by the significant increase of malondialdehyde (MDA) level and a decrease of antioxidant enzyme activities such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and reduced glutathione (GSH) in liver of NaF treated rats. However, the co-administration of gallic acid caused an amelioration of the previous parameters. This study clearly showed that gallic acid has protective role against the oxidative damage in sodium fluoride intoxicated rats.

Keywords: Gallic acid, Hepatotoxicity, Oxidative stress, Rat, Sodium fluoride.

INTRODUCTION

Sodium Fluoride (NaF) is used in various pesticide formulations, including insecticides and wood preservatives. It can be deposited into soil from several anthropogenic sources, both directly through phosphate fertilizers or indirectly through atmospheric pollution from industrial activities, burning of fossil fuels and from environmental pollutants such as pesticides. In the last years, interest in inorganic fluoride undesirable effects has resurfaced.¹ many studies demonstrated that inorganic fluoride even at low doses can interact with a wide range of cellular processes such as gene expression, cell cycle, proliferation and migration, metabolism, apoptosis, necrosis and oxidative stress.²

Numerous investigations indicate that reactive oxygen species (ROS) are implicated in the development and/or progression of cancer and possibly involved in the etiology of many other human diseases.³ Among its adverse biochemical effects, fluoride causes increased lipid peroxidation in the blood of humans and in the blood and tissues of experimental animals. Over the last decade, numerous antioxidants plants have been discovered.⁴ Several studies reveal that the protective effects of plants can be due to the presence of flavonoids, anthocyanins and phenolic compounds.⁵ Gallic acid (3,4,5-trihydroxybenzoic acid) is a natural product (phenolic acid) from plants which is found particularly abundant in grapes, different berries, fruits as well as tea leaves.⁶ It has been reported that gallic acid have a potent free radical scavenging and antioxidant actions, that's why it received much attention in the last years.⁷ The aim of our study is the investigation of hepatoprotective role

and antioxidant capacity of Gallic acid on sodium fluoride-induced liver injury in rats

MATERIALS AND METHODS

Chemicals

Sodium Fluoride (NaF) and Gallic acid (GA) were purchased from Sigma Chemical Co (St Louis, USA) and all other chemicals used in the experiment were of analytical grade.

Animals and experimental design

Eighteen healthy adult male Wistar rats weighing around 238±2g were obtained from Pasteur Institute (Algiers, Algeria). Animals were acclimated under the same laboratory conditions of photoperiod (12h light:12h dark) with a minimum relative humidity of 40% and room temperature of 23±2°C. Food (standard diet, supplied by the ONAB, Algiers, Algeria) and water were supplied *ad libitum*. After two weeks, rats were randomly divided into three groups of six animals each as follow:

- Group (C): served as a control.
- Group (NaF): treated daily for three weeks with sodium fluoride at a dose of 100ppm in drinking water.
- Group (NaF+GA): was treated daily and for three weeks by both of NaF plus GA (20mg/Kg orally).

Body weight, food and water consumption were monitored during the treatment. The experimental procedures were carried out according to the National Institute of Health Guide- lines for Animal Care and approved by the Ethics Committee of our Institution.



Quantities of NaF ingested by each rat were calculated from daily consumption.

Blood collection

At the end of the experiment, total body weights were recorded and animals were sacrificed by cervical decapitation without anesthesia to avoid animal stress. Blood samples were collected in heparin tubes. These ones were centrifuged at 3000g for 10 min (to separate plasma from the erythrocytes) and the resultant supernatant plasma was used for biochemical analysis of total proteins, albumin, bilirubin levels and enzymes activities (AST, ALT, ALP and LDH).

Tissue preparation

Livers were immediately removed, washed in 0.9% NaCl solution and weighed, in order to obtain the organ weight ratio. The relative weight of organs (%) was calculated as g/100g body weight. Then about 1g of liver was homogenized in 2 ml of buffer solution of Tris Buffered Saline 1:2 (w/v; 1g tissue with 2ml TBS, pH 7.4). Homogenates were centrifuged at 9000g for 15min at 4°C, and the resultant supernatant was used for the determination of malondialdehyde, reduced glutathione and protein levels in one hand, and for measuring the activity of GPx, SOD and CAT in the other hand.

Determination of plasma biochemical markers

Plasma bilirubin, albumin and total protein levels were determined by the colorimetric methods using kits from Spinreact (Bil T-100 1044, Alb-1001020-1001023; Tprot-1001291. The transaminases (alanine transaminase: ALT and aspartate transaminase: AST), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activities were assayed using commercial kits from Spinreact (Spain, Ref: ASt-1001 160-1001 161, ALT-1001 170-1002 171, ALP-1001 130-1001 131, LDH-1001 260).

Determination of malondialdehyde (MDA) level

The lipid peroxidation level in liver homogenate was measured as malondialdehyde (MDA) which is the last product of lipid peroxidation, and reacts with thiobarbituric acid (TBA) as a TBA reactive substance (TBARS) to produce a red colored complex with a peak absorbance at 530nm according to Buege and Aust.⁸ Brief, 357µl of supernatant were homogenized by sonication with 150µl of PBS, 375µl of TCA-BHT (trichloroacetic acid-butylhydroxytoluene) in order to precipitate proteins, and then centrifuged (1000g, 10min). After that, 400µl of supernatant were mixed with 80µl of HCl (0.6M) and 320µl of TBA dissolved in Tris, and then the mixture was heated at 80°C for 10min. The absorbance of the resultant supernatant was obtained at 530nm. The amount of MDA was calculated using a molar extinction coefficient of 1.56×10^5 M/cm.

Determination of reduced glutathione (GSH) content

Liver GSH content was estimated using a colorimetric technique, as mentioned by Jollow *et al*⁹, based on the

development of a yellow color when DTNB [(5,5 dithiobis-(2-nitrobenzoic acid)] is added to compounds containing sulfhydryl groups. In brief, 0.8ml of supernatant was added to 0.3 ml of 0.25% sulfosalicylic acid, then tubes were centrifuged at 2500g for 15min. 0.5ml of supernatant was mixed with 0.025ml of 0.01M DTNB and 1ml phosphate buffer (0.1M, pH 7.4). The absorbance at 412 nm was recorded. Finally, total GSH content was expressed as nmol GSH/mg protein.

Determination of glutathione peroxidase activity

Glutathione peroxidase (GPx) (E.C.1.11.1.9) activity was determined according to the method Flohe and gunzler¹⁰. Supernatant obtained after centrifuging 5% liver homogenate at 1500g for 10min followed by 10.000g for 30min at 4°C was used for GPx assay. Reaction mixture of 1ml was prepared containing 0.3ml of phosphate buffer (0.1M, pH 7.4), 0.2ml of GSH (2mM), 0.1ml of sodium azide (10mM), 0.1ml of H₂O₂ (1mM) and 0.3ml of supernatant. After incubation at 37°C for 15 min reaction was terminated by addition of 0.5ml of 5% TCA. Tubes were centrifuged at 1500g for 5min and supernatant was collected. 0.2ml of phosphate buffer (0.1M, pH7.4) and 0.7ml of DTNB (0.4mg/ml) were added to 0.1ml of reaction supernatant. After mixing, absorbance was recorded at 420nm.

Determination of catalase activity

The catalase (E.C.1.11.1.6) activity was measured according to Aebi.¹¹ This assay is based on the ability of the enzyme to induce the disappearance of hydrogen peroxide. The reaction mixture consist of 780µl phosphate buffer (pH 7.5), 200 µl of hydrogen peroxide (500mM) and 20µl supernatant in a final volume of 1ml. Absorbance was recorded at 240 nm every 15 second for 1min. The enzyme activity was calculated by using an extinction coefficient of 0.043mM cm⁻¹.

Determination of superoxide dismutase activity

The method of the determination of SOD (E.C.1.15.11) activity by the NBT test is a method of photo reduction of riboflavin/methionine complex that generates superoxide anion. The oxidation of the NBT by the anion superoxide O₂⁻ is used as the basis for the presence of SOD detection. In aerobic environments the riboflavin, methionine, and NBT mixture gives a blue color. The presence of SOD inhibited the oxidation of the NBT. SOD activity was estimated by Asada *et al*.¹² procedure. Briefly, 5µl of the supernatant was combined with 1ml of EDTA/methionine (0.3mM), 1890 ml phosphate buffer (pH 7.8) 85 µl of 2.6mM NBT and 22 µl of riboflavin (0.26mM) was added as the last and switching on the light. The reaction changes in absorbance at 560nm were recorded after 20min.

Protein estimation

The protein contents of various samples were determined according to the method of Bradford¹³ by using bovine serum albumin as a standard.



Statistical analysis

Data were expressed as means \pm SD. Data comparisons were carried out by using Student's t-test to compare means between the different treated groups. Differences were considered statistically significant at $p \leq 0.05$.

RESULTS

Effects of treatments on body and relative liver weights

The variations in body and relative liver weights of animals subjected to different treatments were shown in Table 1. No death was observed in any group during the experimental period, it was observed that the body weight have increased progressively throughout the study in all the groups without statistical difference between them. However water intake was lower in NaF-treated group as compared to control group. A significant increase of NaF-treated group in relative liver weights was also recorded (+17.4%) and in NaF+GA treated group (5%) as compared to the control.

Table 1: Effects of sodium fluoride (NaF) and gallic acid (GA) with NaF on body and relative liver weights and on water consumption and NaF ingested of rats after 3-week treatment.

Parameters studied	Control	NaF	NaF + GA
Initial body weight (g)	236.33 \pm 5.13	237.666 \pm 3.13	240.5 \pm 2.18
Final body weight (g)	311.16 \pm 5.46	349 \pm 3.58	322 \pm 2.56
Relative liver weight (g/100gbw)	2.63 \pm 0.17	3.087 \pm 0.46*	2.749 \pm 0.25
Water consumption (ml/day/rat)	28.63 \pm 0.89	23.41 \pm 0.331*	19.47 \pm 0.32**#
Quantities of NaF ingested (mg/day/rat)	-	0.234 \pm 0.03	0.298 \pm 0.02

Values are given as mean \pm SD for groups of 6 animals each. Significant difference: All treated groups compared to the controls one (* $p \leq 0.05$, ** $p \leq 0.001$). NaF+GA group compared to the NaF treated one (# $p \leq 0.001$).

Effects of treatments on plasma biochemical parameters

Exposure to NaF caused a significant increase in the activities of AST, ALT, ALP and LDH compared to the control (Table 2). The administration of GA with NaF caused an important decrease in the activities of these enzymes as compared to the NaF-treated group. Compared to the control, total protein, albumin and bilirubin levels in NaF-treated animals were decreased, but the treatment by NaF plus GA has produced a recovery in the above mentioned biochemical variables.

Effects of treatments on hepatic oxidative stress parameters

Treatment with NaF caused significant reduction of GSH level (-74.84%) (Figure 1), and in the activities of GPx (-58.24%), CAT (-18.8%) and SOD (-30.44%). It caused also a

significant increase in MDA levels (+108.2%) (Figure 2). However, the co-administration of NaF+GA produced recovery in the above mentioned oxidative stress parameters (Table 3).

Table 2: Effects of sodium fluoride (NaF) and gallic acid (GA) with NaF on plasma biochemical changes of rats after 3-week treatment.

Parameters studied	Control	NaF	NaF+GA
AST (U/L)	152.711 \pm 4.72	261.818 \pm 2.26**	173.32 \pm 6.35#
ALT (U/L)	162.428 \pm 3.18	281.3 \pm 1.56***	219.25 \pm 1.05***#
ALP (U/L)	113.253 \pm 2.10	277.56 \pm 1.74***	87.66 \pm 0.48***#
LDH (U/L)	704.83 \pm 5.14	1118.5 \pm 5.27***	807.83 \pm 7.78***#
T BIL (mg/l)	1.6 \pm 0.18	0.975 \pm 0.16**	1.74 \pm 0.14##
ALB (g/dl)	43.1 \pm 3.2	32.96 \pm 3.27**	40.58 \pm 3.69###
T prot (g/dl)	92.7 \pm 4.2	73.33 \pm 5.58**	81.41 \pm 2.42**#

Values are given as mean \pm SD for groups of 6 animals each. Significant difference: All treated groups compared to the controls one (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$). NaF+GA- group compared to the NaF treated one (# $p \leq 0.05$, ## $p \leq 0.01$, ### $p \leq 0.001$).

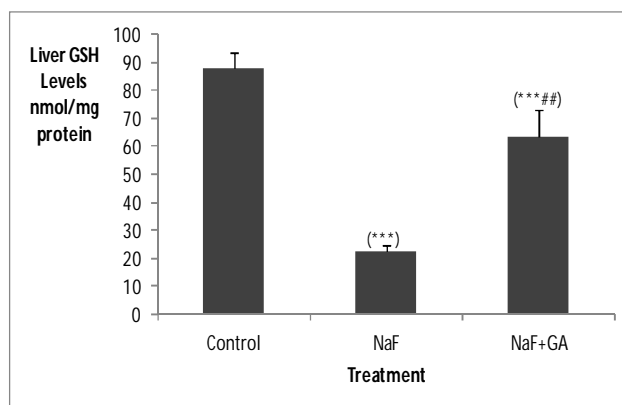


Figure 1: Effects of sodium fluoride (NaF) and gallic acid (GA) with NaF on liver reduced glutathione (nmol/mg protein) levels of rats after 3-week treatment. Significant differences: *Compared to control (** $p \leq 0.001$). #Compared to NaF (# $p \leq 0.01$).

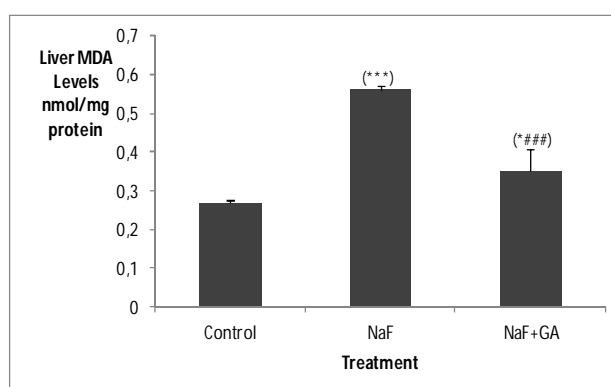


Figure 2: Effects of sodium fluoride (NaF) and gallic acid (GA) with NaF on liver malondialdehyde (nmol/mg protein) levels of rats after 3-week treatment. Significant differences: *Compared to control (* $p \leq 0.05$, *** $p \leq 0.001$). #Compared to NaF (### $p \leq 0.001$).

Table 3: Effects of sodium fluoride (NaF) and gallic acid (GA) with NaF on liver antioxidant enzyme activities of rats after 3-week treatment

Parameters studied	Control	NaF	NaF + GA
Glutathione peroxidase ^a	0.170±0.005	0.071±0.002 ^{***}	0.127±0.003 ^{****##}
Superoxide dismutase ^b	0.818±0.09	0.57±0.02 ^{**}	0.743±0.03 ^{###}
Catalase ^c	7.055±0.09	5.728±0.13 ^{***}	7.403±0.36 ^{###}

^a Glutathione peroxidase: nmoles of GSH/min/mg protein, ^b Superoxide dismutase: units represents the amount of enzyme that inhibits the oxidation of NBT by 50% mg de protein; ^cCatalase: μ moles H₂O₂ degraded/min/mg protein; Values are given as mean \pm SD for groups of 6 animals each. Significant difference: All treated groups compared to the controls one (**p \leq 0.01, ***p \leq 0.001); NaF+GA group compared to the NaF treated one (###p \leq 0.001).

DISCUSSION

Reduction in body weight is used as an indicator for the deterioration of rat general health status. The results presented in this paper indicated reduction in body weight in fluoride-treated rats. This is in agreement with earlier reports that fluoride ingestion leads to retarded animal growth and decline in organo-somatic index as a consequence of excessive breakdown of tissue proteins.¹⁴ In addition, we did not observe any appreciable change in the diet consumption of the rats following fluoride intoxication, suggesting that the poor body weight gain may be due to the overall increased degeneration of lipids and proteins as a result of the direct effects of fluoride.

Liver is the target organ of NaF toxicity¹⁵, and the leakage of hepatic enzymes such as ALT, AST and ALP are commonly used as an indirect biochemical index of hepatocellular damage. In the present finding, NaF intoxication caused a significant increase in the activities of ALT, AST and ALP, probably resulting from hepatocyte membrane damage. If the liver is injured, its cells spill out the enzymes into blood. These results are similar to the findings of Guo-Ving et al.¹⁵ and Shanthakumari et al.¹⁶ The rise of the enzymes activities is probably due to the enhancement of cytoplasmic and/or mitochondrial membranes permeability, it could be expected to occur associated with pathology involving damage or necrosis of hepatocytes. on the other hand, The activity of lactate dehydrogenase (LDH) was also significantly increased in rats treated with sodium fluoride. This may be attributed to a generalized increase in membrane permeability and is particularly useful in the diagnosis of muscular dystrophy.¹⁷ Yadav et al.¹⁸ have reported that the increased levels of LDH result from superoxide anions and hydroxyl radicals in the presence of transition metal ions which cause oxidative damage to the cell membrane .

In the present study, significant decrease in plasma proteins and albumin levels were recorded. The decrease in the proteins levels of fluoride-treated rats might be due to changes in protein synthesis and/or metabolism.¹⁹⁻

²⁰ Decreased plasma proteins and albumin concentrations were also found in previous studies.²¹ However, recent studies have reported that treatment with sodium fluoride could suppress Na–K-activated ATPase, an essential enzyme for the uptake of amino acids by tissues and inhibited incorporation of amino acids into protein.²² Although bilirubin levels in exposed rats to NaF were decreased as compared to control group, which is contrary to the findings of Panneerselvam et al.,²³ and Lee²⁴ observed also an hyperbilirubinemia in patients with Gilbert's disease that was depend on fluoride level. The administration of GA with NaF has protected the liver function from NaF intoxication as indicated by the significant restoration of plasma biochemical indicators such as AST, ALT, ALP and LDH activities, proteins and albumin levels.

It is well known that reactive oxygen species (ROS) are constantly generated *in vivo* for physiological purposes. Their productions are often controlled by endogenous enzymatic or non-enzymatic defense system. However, the excess ROS production beyond the ability of antioxidant system can cause oxidative damage to lipid, protein and nucleic acid resulting in oxidative stress. The obtained data revealed that the treatment with NaF increased MDA level which is an indicator of lipid peroxidation.²³ Our present results have also showed decrease of GSH levels and GPx, CAT and SOD activities. Similar observations were made previously by Joydeep et al.²⁵

Several investigations have been undertaken to elucidate the mechanism of fluoride toxicity. Firstly many studies have shown that sodium fluoride induces excessive production of oxygen free radicals and causes a decrease in the biological activities of some enzymes, such as catalase, superoxide dismutase, xanthine oxidase, and glutathione peroxidase, which play an important role in antioxidation and elimination of free radicals²⁶. Secondly, fluoride can also disturb the metabolism of proteins. It is evidently indicated that fluoride can impair the activities of a series of enzymes such as alkaline phosphatase, cholinesterase, adenylate cyclase.²⁷ Thirdly, fluoride can interfere with the metabolism of carbohydrate, lipid and nucleic acids, injure immune system, and damage the parts of the body.²⁸

The findings of this study have shown that daily administration of gallic acid improved the alteration of biochemical parameters. Furthermore gallic acid enhanced the activity of antioxidant enzymes, glutathione levels and inhibited the lipid peroxidation in liver. This data are in agreement with other results obtained in previous human experiment. Moreover, the hepatoprotective activity of gallic acid against carbon tetrachloride induced oxidative stress has also been reported previously.²⁹ Many authors have suggested that phenolic hydroxyl groups in gallic acid structure are responsible of its scavenging free radicals activity.³⁰



CONCLUSION

The results suggest that gallic acid could act as a suppressor against liver tissue damage and inhibit the progression of this organ dysfunction induced by sodium fluoride. Future studies should be undertaken to elucidate the mechanism of hepatoprotective effect of gallic acid.

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