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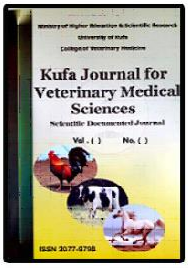

Role of N-acetyl cysteine and gallic acid or their combination in Some criteria related to hepatic damage in sodium fluoride treated rats (Part -II)

[1]Haneen B. Jaddoua [2]Khalisa K. Khudair

[1]Researcher , [2] Department of Physiology, Biochemistry and Pharmacology / College of Veterinary Medicine / University of Baghdad, Iraq.

Corresponding author : khalessa.k@covm.uobaghdad.edu.iq Mobile no.

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Abstract

This study was designed to investigate the ameliorative role of gallic acid (GA) and N-acetylcysteine (NAC) in reducing deleterious effect of sodium fluoride (NaF) such as, oxidative stress and hepatic dysfunction in adult male rats. Thirty adult male rats were randomly and equally divided into five groups, they were handled daily for 60 days, as follows: Control group (C), received tap water only, Sodium fluoride group (T1), received 100ppm of NaF in drinking tap water, gallic acid group (T2), rats in this group were injected intraperitoneal (i/p) 150 mg/kg/day/ of GA, N-acetylcysteine group (T3), animals in this group were administrated orally 25 mg/kg/day/of NAC, while the combination of GA and NAC were given to NaF treated group(T4) in the same previous mentioned doses and method of administration. Fasting blood sample were collected at the beginning and the end of the experiment and serum were collected for estimation of hepatic enzymes concentration and antioxidant status. After animal scarifying, samples from hepatic tissue were taken for measuring hepatic reduced glutathione (GSH)and malondialdehyde (MDA) concentration. The results showed that adminstration of NaF (T1group) caused hepatic damage manifested functionally by**:** significant increase in serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) concentrations, a case of oxidative stress as explained by depression in (GSH) and elevation in MDA concentration in serum and hepatic tissue. The current result also recorded that i/p injection of GA oral administration of NAC alone or in combination with NaF caused amelioration of all previously estimated parameters.

Keywords: Gallic acid, N-acetylcysteine, sodium fluoride, antioxidant.

دور أن-اسيتيل سستين وحمض الكاليك أو مزيجهما في بعض المعايير المتعلقةبأضرار الكبد في الجرذان المعاملة بفلوريد الصوديوم (الجزء -2)

م[1]حنين بشير جـــوع و[2]خالصـة كاظم خضير

[1]باحثة ، [2] فرع الفسلجة ، الكيمياء الحياتية والأدوية /كلية الطب البيطري/ جامعة بغداد –العراق

الخلاصة

صممت هذه الدراسة للتحقيق في الدور التحسيني لحمض الكاليك (GA) وان استيل سستين N-acetylcysteine (NAC) في تقليل التأثير الضار لفلوريد الصوديوم (NaF) مثل الإجهاد التأكسدي والخلل الكبدي في ذكور الجرذان البالغة. تم تقسيم ثلاثين من ذكور الجرذان البالغة بشكل عشوائي وبالتساوي إلى خمس مجموعات ، تم التعامل معها يوميًا لمدة 60 يومًا ، على النحو التالي: المجموعة السيطره (C)، والتي أعطيت مياه الشرب فقط ، مجموعة فلوريد الصوديوم (T1) ، اعطيت 100جزء بالمليون من فلوريد الصوديوم في مياه الشرب ، مجموعة حمض الكاليك (T2) ، تم حقن الجرذان في البريتون 150 ملغم / كغم / يوم / من GA ، مجموعة استيل سستين (T3) ، تم أعطاء حيوانات هذه المجموعة 25 مجم / كغم / يوم / من NAC فمويا ، بينما تم إعطاء

مزيج GA و NAC للمجموعة المعالجة بفلوريد الصوديوم (مجموعه T4) بنفس الجرع وطريق الإعطاء المذكورة سابقًا. جمعت عينة الدم (بعد تجويع الحيوانات) في بداية التجربة ونهايتها، كما تم جمع مصل الدم لتقدير تركيز الإنزيمات الكبدية وحالة مضادات الاكسده. بعد التضحية بالجرذان ، تم أخذ عينات نسيجيه من الكبد لقياس تركيز الجلوتاثيون الكبدي الكلوتاثايون (GSH) و المالوندالديهايد (MDA) . اظهرت النتائج أن إعطاءفلوريد الصوديوم (T1group)تسبب في التلف الكبدي المتمثل وظيفيًابحدوث زيادة معنوية في تركيز خميره الأمين الناقل للالانين (ALT)وتركيز الفوسفاتيز القلوي (ALP) ،و حالة من الإجهاد التأكسدي كما هو موضح بالانخفاض في الكلوتاثايون والارتفاع في المالوندالديهايد في أنسجة الكبد والمصل. سجلت النتيجة الحالية أيضًا أن حقن حمض الكاليك في الغشاء البريتوني والتجريع الفوي لـ أن استيل سستين بصورة منفرده أو سويا للحيوانات المعرضة لفلوريد الصوديوم أدى الى تحسين جميع المعايير المذكورة سابقًا.

الكلمات الدالة: حمض الكاليك ، N-acetylcysteine ، فلوريد الصوديوم ، مضادات الأكسدة.

Introduction

Sodium fluoride is an organic salt of fluoride that distributes very extensively in the natural environment, and is widely used among industry, agriculture as well as medicine (1). Moderate levels of fluorine or fluoride ingestion can decrease the incidence of dental caries (2) and promote the development of bones (3). However, fluoride chronic ingestion at high doses is toxic (4) and caused adverse effects on human health and animals. Fluoride toxicity targets to not only bone and teeth, but also soft tissues (5). Previous studies have proved that fluorine can induce genotoxicity, cytotoxicity, immunotoxicity, oxidative damage and lesions in different organs (6-8). As an oxidant, fluoride is known to be an inhibitor of the antioxidant enzymes, which in turn promote the accumulation of ROS, systematically observed in different organs (8-14).

Gallic acid, is a polyphenolic compound (15) has gradually won a considerable amount of attention because it is ubiquitous in fruits, vegetables, and herbal medicines, such as grapes (16), gallnuts (17), pomegranates (18), tea leaves (19) and mango peel (20). Gallic acid and its derivatives have demonstrated a large number of applications in pharmaceutical, cosmetic, food, printing, and dyeing and food industries (21-23). There are also various kinds of studies on their biological and pharmacological activities, including antioxidant (24,25), antimicrobial (26,27), anticancer (28), anti-inflammatory (29,30), cardioprotective (31), gastro and immune protective (32,33) neuroprotective (34), and metabolic disease prevention activities (35).

N-acetylcysteine is a thiol compound with chemical formula (C5H9NO3S). Being a thiol(R-SH), NAC may be oxidized by various radicals and serves as a nucleophile (36). NAC stimulates glutathione-S-transferase activity and promotes detoxification by providing sulfhydryl groups and acting as a scavenger of free radicals through direct interaction with reactive oxygen species(ROS) (37).NAC has been used in a number of therapies, including paracetamol poisoning, mucolytics, and combating the toxicity of different compounds that can induce the production of free radicals (38,39), which is closely associated with oxidative stress, inflammation, and metabolic disturbance (40). Also, in previous study in vivo showed that NAC raised the antioxidant capacity of hepatic tissue in rats administered alcohol (41). In addition to its antioxidant and anti-inflammation properties, there is a growing interest in the beneficial effects of NAC for treating metabolic disease (42). Recently, NAC is used as synergistic therapy for prevention and treatment of COVID-19 (43).

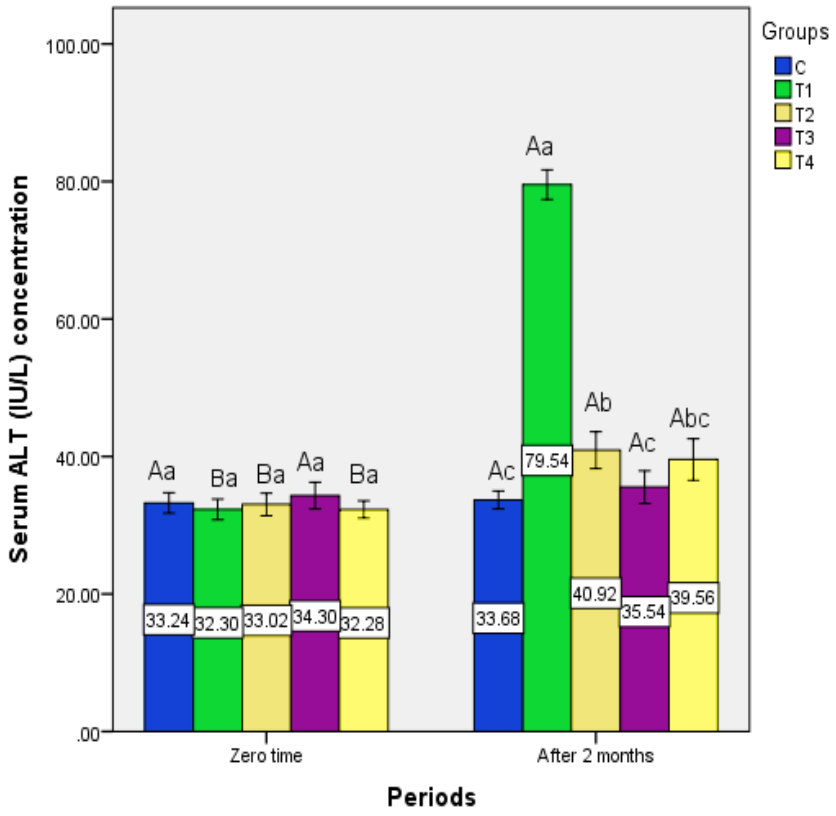
Materials and Methods

Thirty adult male rats were randomly divided into five equal groups and were treated daily for 60 days as follows: Group C: Rats of this group were allowed to ad libitum supply of drinking water (control group). Group T1: Rats of this group were allowed to ad libitum supply of drinking water containing 100 ppm of NaF (T1 group). Animals in proceeding groups were given in addition to NaF in drinking water the following: intraperitoneal (i/p) injection of 150 mg/kg/day/ of GA(T2group), oral administration 25 mg/kg/day/of NAC (T3 group), while the combination of GA and NAC were given to animal in group(T4). In the same previous mentioned doses and method of administration. Fasting blood (for 8-12 hrs.) samples were collected (by cardiac puncture

technique) at different interval 0 and 60 days of the experiment, centrifuged at 2500rpm for 15 minutes, and then serum samples were liquated and frozen at -20 °C until analysis of serum hepatic function: Alanine transaminase (ALT), Alkaline phosphatase (ALP) were measured enzymatic kit (BioMeriux, Spain) according to (44). Serum and hepatic GSH and MDA were measured using ELISA kits (My BioSource, USA) according to (45). Statistical analysis of data was performed on the basis of One and Two-Way Analysis of Variance (ANOVA) using a significant level of (P<0.05). Specific group differences were determined using least significant differences (LSD) as described by (46).

Results

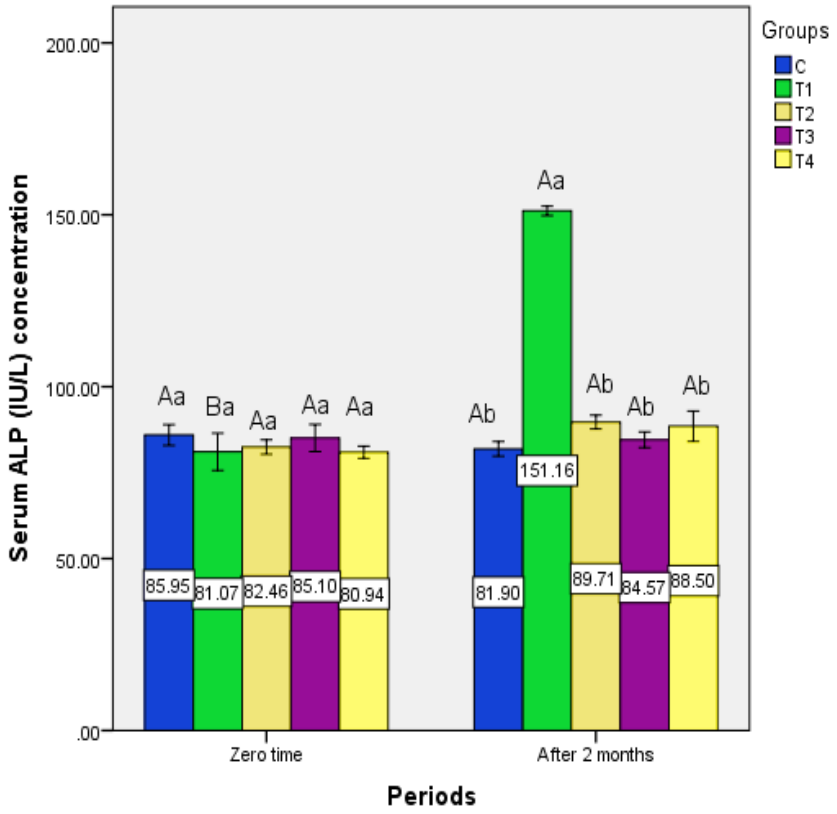
Significant (p<0.05) elevation in serum ALT concentration was observed in groups T1 after two months of the experiment comparing to the value in other treated groups. Where oral intubation of GA, NAC (T2 andT3) or their combination (T4) caused significant (p <0.05) decrease in enzyme concentration comparing to the NaF treated group. Prevalence of NAC treatment (T3) or combination of NAC and GA (T4) on reducing serum ALP activity were observed comparing to (T2) group. (Histogram, 1).



Histogram, 1: Effect of sodium fluoride, gallic acid, n-acetylcysteine and their combination on Serum alanine-aminotransferase (ALT) concentration (U/L) in adult male rats.

Values are expressed as mean ± SE. n= 6 / each group. C: Control Group, T1: Rats Received 100ppm Sodium fluoride in drinking water for two months. T2: Rats were injected i/p 150 mg/kg B.W Gallic acid for 60 days. T3: Rats orally administrated 25 mg/kg B.W N-acetylcysteine for 60 days. T4: Rats Received 100ppm Sodium fluoride, 150 mg/kg B.W Gallic acid and 25 mg/kg B.W N-acetylcysteine. Means with a different small letter in the same column are significantly different ($P \leq 0.05$) between group. Means with a different capital letter in the same row are significantly different ($P \leq 0.05$) within group.

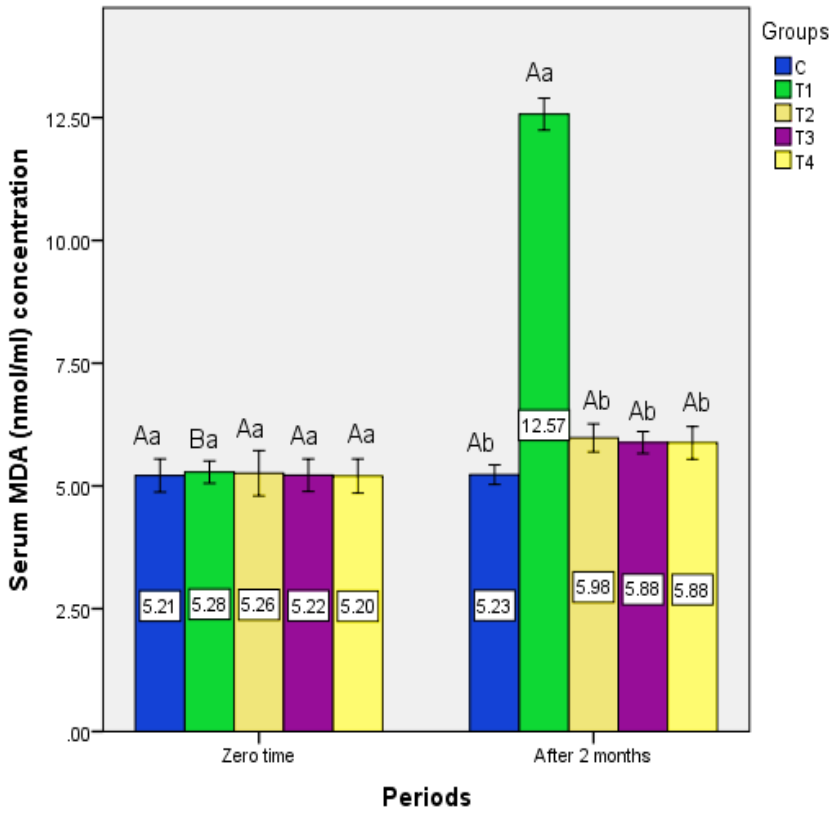
Significant ($p<0.05$) decrease in serum ALP concentration was observed in T2, T3 and T4 treated groups at the end of the experiment comparing to the value of NaF (T1) group at the end of experiment. The result also showed significant ($p<0.05$) increase in serum ALP concentration in T1 at the end of the experimental period as compared to the value at zero time (histogram, 2).



Histogram, 2: Effect of sodium fluoride, gallic acid, n-acetylcysteine and their combination on Serum alkaline phosphatase (ALP) concentration (U/L) in adult male rats.

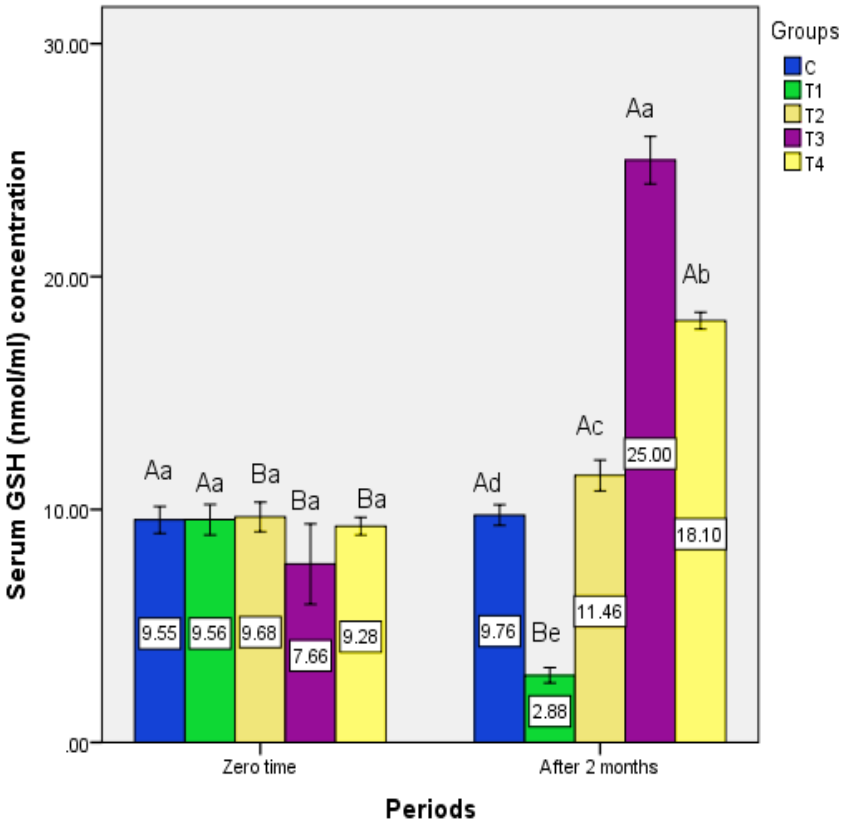
Values are expressed as mean ± SE. n= 6 / each group. C: Control Group, T1: Rats Received 100ppm Sodium fluoride in drinking water for two months. T2: Rats were injected i/p 150 mg/kg B.W Gallic acid for 60 days. T3: Rats orally administrated 25 mg/kg B.W N-acetylcysteine for 60 days. T4: Rats Received 100ppm Sodium fluoride, 150 mg/kg B.W Gallic acid and 25 mg/kg B.W N-acetylcysteine. Means with a different small letter in the same column are significantly different ($P \leq 0.05$) between group. Means with a different capital letter in the same row are significantly different ($P \leq 0.05$) within group.

The result illustrates the mean values of serum GSH and MDA concentration in control and treated groups along experimental period. The result showed significant ($P \leq 0.05$) elevation in serum GSH concentration and decrease in serum MDA in T2, T3 and T4group comparing to NaF treated group at the end of the experiment. The result also showed highest significant elevation in serum GSH concentration were observed in NAC T3 treated group comparing to other treated groups at the end of the experiment (histogram, 3-4).



Histogram, 3: Effect of sodium fluoride, gallic acid, n-acetylcysteine and their combination on Serum malondialdehyde (MDA) concentration (nmol/ml) in adult male rats.

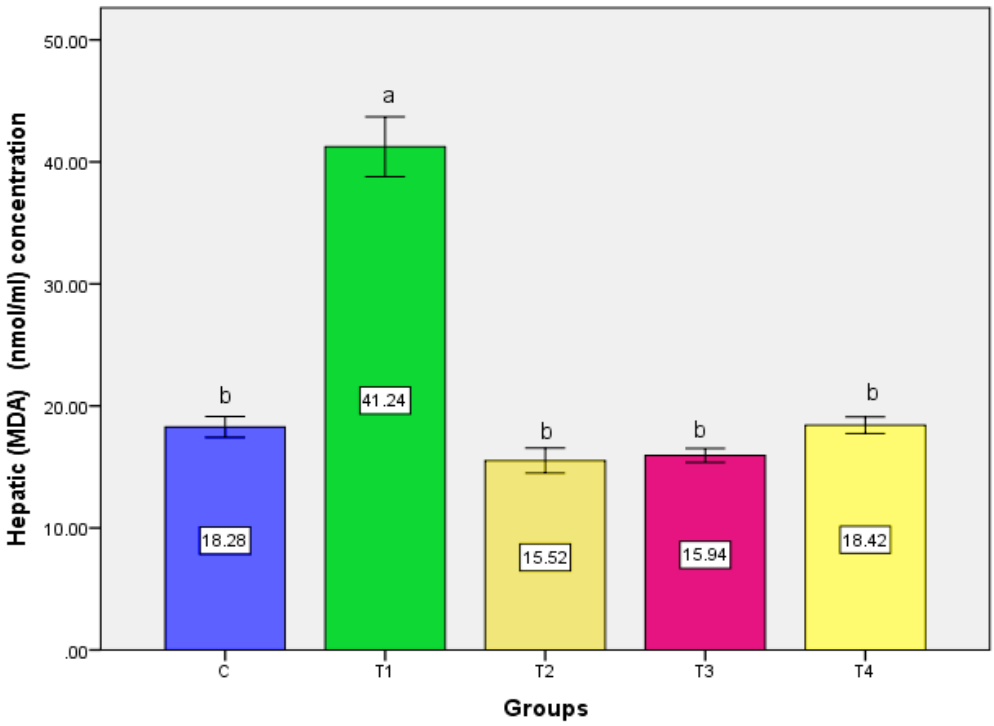
Values are expressed as mean ± SE. n= 6 / each group. C: Control Group, T1: Rats Received 100ppm Sodium fluoride in drinking water for two months. T2: Rats were injected i/p 150 mg/kg B.W Gallic acid for 60 days. T3: Rats orally administrated 25 mg/kg B.W N-acetylcysteine for 60 days. T4: Rats Received 100ppm Sodium fluoride, 150 mg/kg B.W Gallic acid and 25 mg/kg B.W N-acetylcysteine. Means with a different small letter in the same column are significantly different ($P \leq 0.05$) between group. Means with a different capital letter in the same row are significantly different ($P \leq 0.05$) within group.



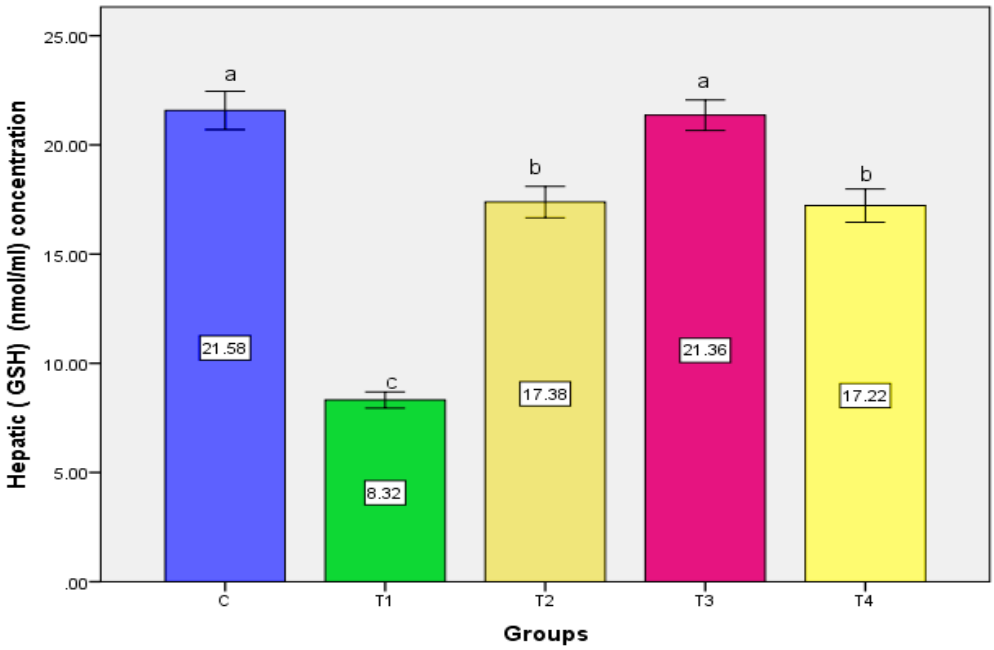
Histogram, 4: Effect of sodium fluoride, gallic acid, n-acetylcysteine and their combination on Serum glutathione (GSH) concentration (nmol/ml) in adult male rats.

Values are expressed as mean ± SE. n= 6 / each group. C: Control Group, T1: Rats Received 100ppm Sodium fluoride in drinking water for two months. T2: Rats were injected i/p 150 mg/kg B.W Gallic acid for 60 days. T3: Rats orally administrated 25 mg/kg B.W N-acetylcysteine for 60 days. T4: Rats Received 100ppm Sodium fluoride, 150 mg/kg B.W Gallic acid and 25 mg/kg B.W N-acetylcysteine. Means with a different small letter in the same column are significantly different ($P \leq 0.05$) between group. Means with a different capital letter in the same row are significantly different ($P \leq 0.05$) within group.

Significant elevation in hepatic MDA concentration were observed after NaF treated T1 group comparing to the values in the control and other treated group at the end of the experiment. The results also showed significant elevation in hepatic GSH concentration in NAC treated group comparing to control and other treated groups. Besides, significant elevation in this parameter were observed in T2 and T4 comparing to T1group (histogram, 5 and 6).



Histogram, 5: Effect of sodium fluoride, gallic acid, n-acetylcysteine and their combination on Hepatic malondialdehyde (MDA) concentration (nmol/ml) in adult male rats. Values are expressed as mean ± SE. n= 6 / each group. C: Control Group, T1: Rats Received 100ppm Sodium fluoride in drinking water for two months. T2: Rats were injected i/p 150 mg/kg B.W Gallic acid for 60 days. T3: Rats orally administrated 25 mg/kg B.W N-acetylcysteine for 60 days. T4: Rats Received 100ppm Sodium fluoride, 150 mg/kg B.W Gallic acid and 25 mg/kg B.W N-acetylcysteine. Means with a different small letter in the same column are significantly different ($P \leq 0.05$) between group. Means with a different capital letter in the same row are significantly different ($P \leq 0.05$) within group.



Histogram, 6: Effect of sodium fluoride, gallic acid, n-acetylcysteine and their combination on Hepatic glutathione (GSH) concentration (nmol/ml) in adult male rats. Values are expressed as mean ± SE. n= 6 / each group. C: Control Group, T1: Rats Received 100ppm Sodium fluoride in drinking water for two months. T2: Rats were injected i/p 150 mg/kg B.W Gallic acid for 60 days. T3: Rats orally administrated 25 mg/kg B.W N-acetylcysteine for 60 days. T4: Rats Received 100ppm Sodium fluoride, 150 mg/kg B.W Gallic acid and 25 mg/kg B.W N-acetylcysteine. Means with a different small letter in the same column are significantly different ($P \leq 0.05$) between group. Means with a different capital letter in the same row are significantly different ($P \leq 0.05$) within group.

Discussion

Our outcome demonstrates significantly elevation in hepatic enzyme ALT and ALP after administration of NaF, reflecting liver damage in rats. These results are corroborated by (6,47, 48, 49). Change in liver enzyme activity after NaF exposure indicate a case of oxidative stress (50) that lead to disruption in membrane integrity, loss of membrane fluidity and membrane permeability (51). NaF caused oxidative damage by rising ROS, cytotoxicity and lipid peroxidation with increased in apoptosis and hepatic dysfunction (12,50) with leakage of these intracellular enzymes to the serum. Dyslipidemia and oxidative stress could be a mechanism for NaF hepatic damage. The result of current study showed that gallic acid could significantly decrease ALT and ALP activities, which is in accordance with results of others (52-55). Being abio chelator and due to its easy availability, GA can be used effectively in heavy metal detoxification (52).GA help partially neutralize the substance-induced toxicity in the liver and thus prevent hepatic damage (56). Besides an elevation in ROS production and decrease in antioxidant status of the liver affected mitochondria function, accompanied with release of cytochrome-c (57) and leakage of hepatocellular enzymes to the extracellular space. GA, by suppressing lipid peroxidation and retaining the cell membrane, hindered the diffusion of hepatic enzymes into the plasma (58-60). The current results showed that administration of NAC has exert beneficial effects on liver enzymes which decrease the elevation of ALT and ALP, these results are similar to other researcher results (61,62). Also, Saleem *et al.*, reported that Giving NAC to rats treated with paracetamol and phenacetin was found to have hepatoprotection that was significantly related to lowering serum ALT and AST levels (63). This is accomplished by NAC's efforts in

reducing pro-inflammatory markers such as interleukin (IL)-6 IL-1β, TNF-α, where there is an increase in intracellular response of antioxidants, especially glutathione (64) and reduction and lipid peroxidation resulting in improved liver function (61). Reserving of liver function marker toward respective control value by NAC could be indicative of regeneration of hepatoparenchymal cells, protective action on membrane fragility, stabilization of plasma membrane attributed to its antioxidant activity (65).

The present results showed that animals exposed to NaF showed elevation in MDA and depression in glutathione concentration in serum and hepatic tissue indicating a case of oxidative stress. These finding are matched with the results of previous reports (6, 13,48). Excessive amount of fluoride in the body can induce production of free radicals by attacks oxygen which disrupts the formation of antioxidants and disrupts metabolism resulting in the production of hydrogen peroxide (66), accompanied with lipid peroxidation and oxidative stress (14, 67).Fluoride has been shown to inhibit certain antioxidant enzymes, total antioxidant capacity (TAC), GSH, SOD, glutathione reductase, glutathione peroxidase, and catalase and increase intracellular levels of the superoxide radical, the substrate for peoxynitrite formation (68). Our findings showed that GA administration resulted in a decrease in MDA levels and a rise in GSH levels. These findings are consistent with previous researches (55, 69,70,71). GA can target specifically the adipose tissues and thus suppress lipogenesis, accompanied with improvement in insulin signaling, and alleviation oxidative stress (72). It seems that boosting the concentrations of non-enzymatic antioxidant system, such as GSH and reduced ROS products (MDA) (documented in this study) are the main GA- hepatoprotective mechanism (9, 73, 74).

The current result revealed that oral administration of NAC leading to significant increase GSH level and decrease MDA in serum and hepatic cells, documented its antioxidant activity (61,75,76). It stimulates glutathione-S-transferase activity and acts as a scavenger of free radicals and as an antioxidant by restoring the pool of intracellular GSH, in addition to its anti-inflammatory properties (77). To the best of our knowledge this is the first study that evaluated the NAC effects on oxidative stress. Effective treatment with NAC provides sufficient cysteine to promote detoxification and directly eliminate reactive oxygen species (78). It can also scavenge several reactive nitrogen species (RNS) that play a role in the oxidation of lipids, proteins, and DNA (79). On the contrary, high dose of NAC can act as pro-oxidant and so may lower GSH and elevated oxidized GSH if given to healthy individual (80).

Conclusion

On conclusion, the current study revealed that different criteria related to hepatic dysfunction induced by sodium fluoride can be attenuated by gallic acid and N-acetylcysteine through their antioxidant and hepatoprotective effect.

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