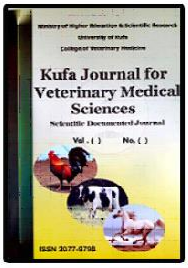
Kufa journal for Veterinary Medical Sciences Vol.(12) No.(1)

Kufa journal for Veterinary Medical Sciences

Kj.vs@uokufa .edu.iq

www.uokufa .edu.iq\journals\index.php\kjvs



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.

Received date:6 Jun 2021 Accepted:(477) 13Jul 2021 page: (23-36) Published:30 July 2021

DOI: https://doi.org/10.36326/kjvs/2021/v12i13222

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)

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

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

الخلاصة

صمم لـ سة للتحقيق في لـ لتحسيني لحم لكالي مثـ (GA) ستي سستي N-acetylcysteine (NAC) في تقلي لتأثي لضا لفلـ ي لصـ ي : يمًا ، على (NaF) لإجها لتأكس لخلـ لكبـ في كـ لجـ لبالغة. تـ تقسي ثلاثي مـ لنحـ لتالي60 كـ لجـ لبالغة بشكـ عشـ ئي بالتسا لى خمـ مجمعا ، تـ لتعامـ معها يميًا لمـ

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).

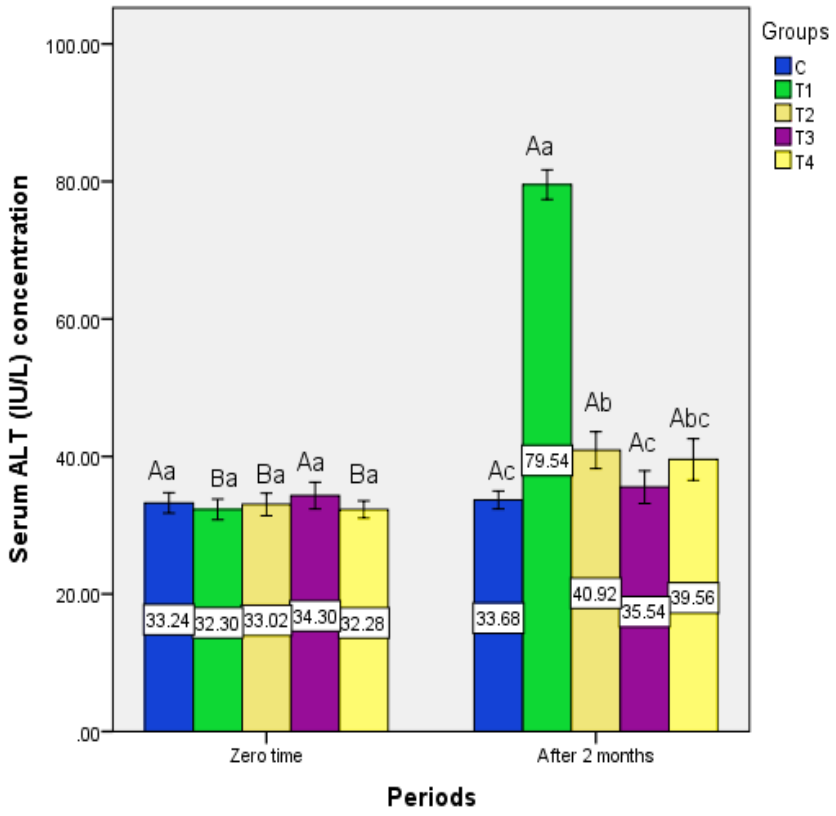
Materials and Methods

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

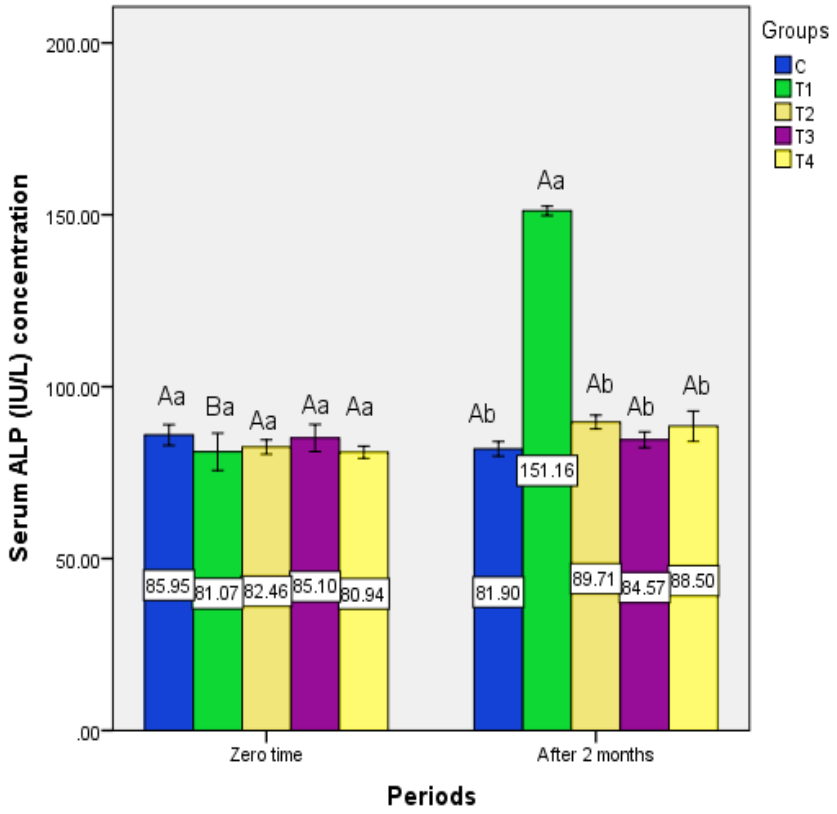
Significant (p<0.05) elevation in serum ALT concentration was observed in groups T1 after two months of the experiment comparing to the value in other treated groups. Where oral intubation of GA, NAC (T2 andT3) or their combination (T4) caused significant (p <0.05) decrease in enzyme concentration comparing 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.

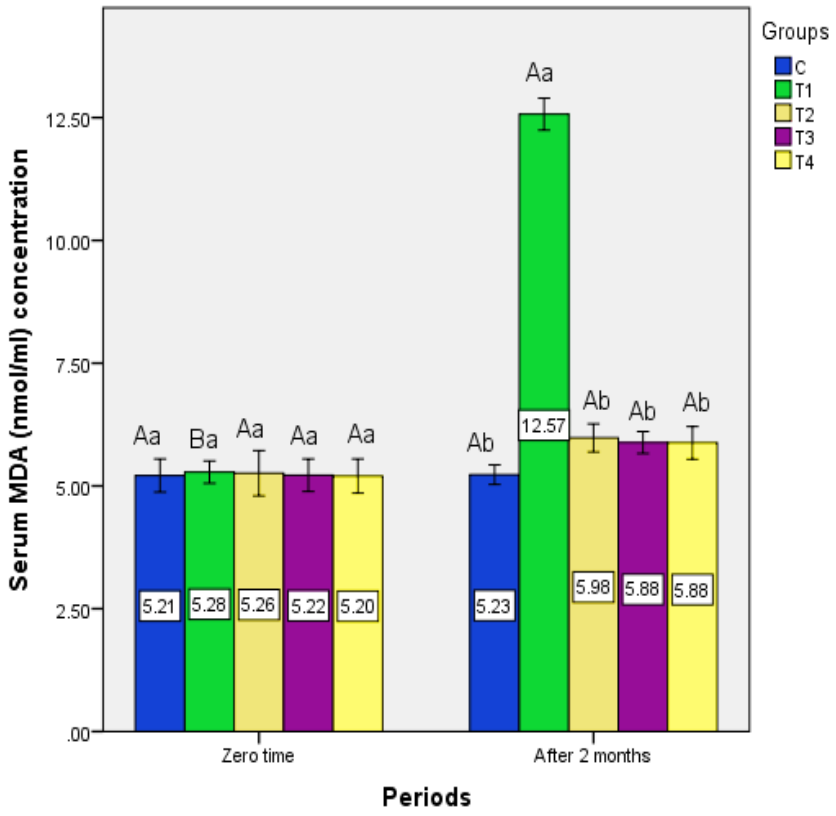
Significant ($p<0.05$) decrease in serum ALP concentration was observed in T2, T3 and T4 treated groups at the end of the experiment comparing to the value of NaF (T1) group at the end of experiment. The result also showed significant ($p<0.05$) increase in serum ALP concentration in T1 at the end of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

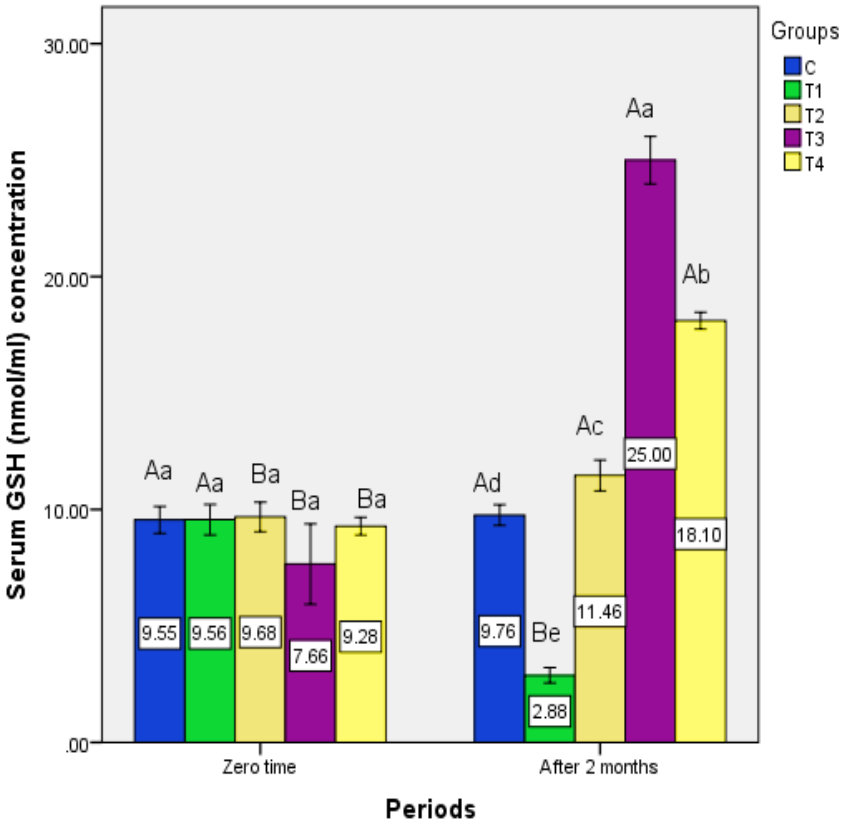
Values are expressed as mean ± SE. n= 6 / each group. C: Control Group, T1: Rats Received 100ppm Sodium fluoride in drinking water for two months. T2: Rats were injected i/p 150 mg/kg B.W Gallic acid for 60 days. T3: Rats orally administrated 25 mg/kg B.W N-acetylcysteine for 60 days. T4: Rats Received 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.

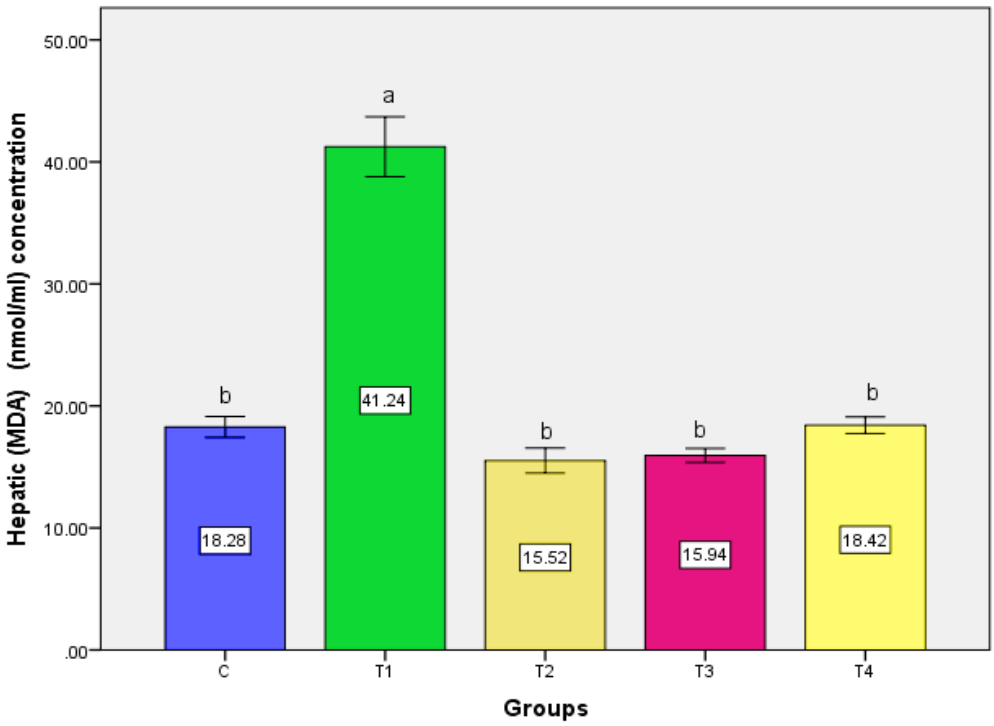
Values are expressed as mean ± SE. n= 6 / each group. C: Control Group, T1: Rats Received 100ppm Sodium fluoride in drinking water for two months. T2: Rats were injected i/p 150 mg/kg B.W Gallic acid for 60 days. T3: Rats orally administrated 25 mg/kg B.W N-acetylcysteine for 60 days. T4: Rats Received 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.



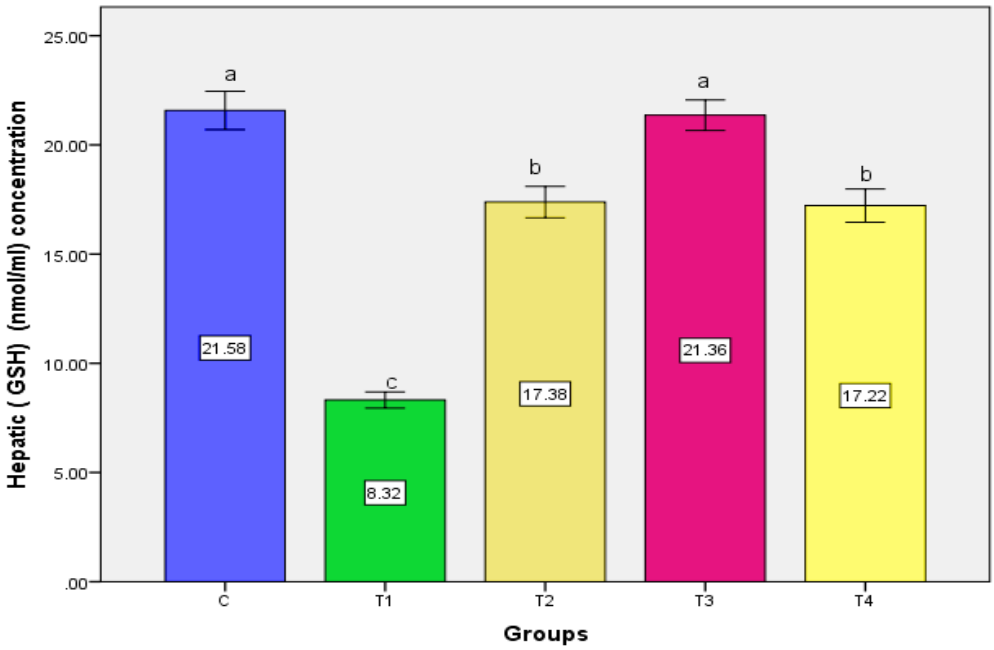
Histogram, 4: Effect of sodium fluoride, gallic acid, n-acetylcysteine and their combination on Serum glutathione (GSH) concentration (nmol/ml) in adult male rats.

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.

References

1. Jha, S.K., Mishra, V.K., Sharma, D.K., Damodaran, T. (2011). Fluoride in the environment and its metabolism in humans. Rev Environ Contam Toxicol., 211:121–42. doi: 10.1007/978-1-4419-8011-3_4.
2. Wierichs, R.J., Stausberg, S., Lausch, J., Meyer-Lueckel, H. and Esteves-Oliveira, M. (2018). Caries-Preventive Effect of NaF, NaF plus TCP, NaF plus CPP-ACP, and SDF Varnishes on Sound Dentin and Artificial Dentin Caries in vitro. Caries Res., 52(3):199-211. doi: 10.1159/000484483. Epub 2018 Jan 17. PMID: 29339648.
3. Liu, Z., Zhang, M., Shen, Z., Ke, J., Zhang, D. and Yin, F. (2020). Efficacy and safety of 18 antiosteoporotic drugs in the treatment of patients with osteoporosis caused by glucocorticoid: A network meta-analysis of randomized controlled trials. PLoS ONE, 15(12): e0243851.
4. Maheshwari, M.R.C. (2006). Fluoride in drinking water and its removal. J Hazard Mater., 137:456–63. doi: 10.1016/j.jhazmat.2006.02.024.
5. Shivarajashankara, Y.M., Rao, S.M., Rao, S.H., Shivashankara, A.R. and Bhat, P.G. (2002). Histological changes in the brain of young fluoride-intoxicated rats. Fluoride, 35:12–21.
6. Khudiar, K.K., Aldabaj, A.M.A. (2015). Effect of grape seed oil on hepatic function in adult

male rabbits treated with sodium fluoride (Part-II). Adv. Anim. Vet. Sci., 3(10): 550-558.

7. Kuang, P., Deng, H., Cui, H., Chen, L., Guo, H., Fang, J., Zuo, Z., Deng, J., Wang, X. and Zhao, L. (2016). Suppressive effects of sodium fluoride on cultured splenic lymphocyte proliferation in mice. Oncotarget, 7:61905–15. doi: 10.18632/oncotarget.11308
8. 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., Apr 21;8(31):50430-50446. doi: 10.18632/oncotarget.17365. PMID: 28881573; PMCID: PMC5584147.
9. Oyagbemi, A., Omobowale, T., Asenuga, E., Adejumobi, O., Jibade, T., Ige, T., Ogunpolu, B., Adedapo, A. and Yakubu, M. (2016). Sodium fluoride induces hypertension and cardiac complications through generation of reactive oxygen species and activation of nuclear factor kappa beta. Environmental toxicology, 32.10.1002/tox.22306.
10. Zhou, B.H., Zhao, J., Liu, J., Zhang, J.L., Li, J. and Wang, H.W. (2015). Fluoride-induced oxidative stress is involved in the morphological damage and dysfunction of liver in female mice. Chemosphere, 139:504–11. doi: 10.1016/j.chemosphere.2015.08.030
11. Lu, Y., Luo, Q., Cui, H., Deng, H., Kuang, P., Liu, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., and Zhao, L. (2017). Sodium fluoride causes oxidative stress and apoptosis in the mouse liver. Aging, 9(6): 1623–1639. https://doi.org/10.18632/aging.101257
12. Malin, A.J., Lesseur, C., Busgang, S.A., Curtin, P., Wright, R.O. and Sanders, A.P. (2019). Fluoride exposure and kidney and liver function among adolescents in the United States: NHANES, 2013-2016. Environ. Int., Nov;132:105012. doi: 10.1016/j.envint.2019.105012.
13. Ajoke, S., Ayodeji, O., Leken, I., Yetunde, U., Abiodun, I.-A., Aminu, I., Yetunde, A., Monsur, S., Raheem, A., Iyabo, A. and Salihu, A. (2020). Exogenous Melatonin Ameliorates Pontine Histoarchitecture and Associated Oxidative Damage in Sodium Fluoride Induced Toxicity. Nepal Journal of Neuroscience, 17(2): 4-10. https://doi.org/10.3126/njn.v17i2.30110.
14. Srivastava, S. and Flora, S.J.S. (2020). Fluoride in drinking water and skeletal fluorosis: A review of the global impact. Curr Environ Health Rep., 7:140–146.
15. National Center for Biotechnology Information (2020). PubChem Database. Gallic Acid, CID=370. Available online at: https://pubchem.ncbi.nlm.nih. gov/compound/Gallic-acid
16. Scoccia, J., Perretti, M.D., Monzón, D.M., Crisóstomo, F.P., Martín, V.S. and Carrillo, R. (2016). Sustainable oxidations with air mediated by gallic acid: potential applicability in the reutilization of grape pomace. Green Chem., 18:2647–50. doi: 10.1039/C5GC02966J
17. Liu, S., Li, S., Lin, G., Markkinen, N., Yang, H., Zhu, B. and Zhang, B. (2019). Anthocyanin copigmentation and color attributes of bog bilberry syrup wine during bottle aging: effect of tannic acid and gallic acid extracted from Chinese gallnut. J Food Process Pres., 43:8, e14041. doi: 10.1111/jfpp.14041.
18. Zhang, J., Li, B., Yue, H., Wang, J. and Zheng, Y. (2018). Highly selective and efficient imprinted polymers based on carboxyl-functionalized magnetic nanoparticles for the extraction of gallic acid from pomegranate rind. J Sep Sci., 41:540–7. doi: 10.1002/jssc.201700822
19. Jiang, H., Yu, F., Qin, L., Zhang, N., Cao, Q., Schwab, W., Li, D. and Song, C. (2019). Dynamic change in amino acids, catechins, alkaloids, and gallic acid in six types of tea processed from the same batch of fresh tea (Camellia sinensis L.) leaves. J Food Compos Anal., 77:28–38. doi: 10.1016/j.jfca.2019.01.005
20. Velderrain-Rodríguez, G. R., Torres-Moreno, H., Villegas-Ochoa, M. A., Ayala-Zavala, J. F., Robles-Zepeda, R. E., Wall-Medrano, A., and González-Aguilar, G. A. (2018). Gallic Acid Content and an Antioxidant Mechanism Are Responsible for the Antiproliferative Activity of 'Ataulfo' Mango Peel on LS180 Cells. Molecules, (Basel, Switzerland), 23(3):695. https://doi.org/10.3390/molecules23030695 .
21. Su, T. R., Lin, J. J., Tsai, C. C., Huang, T. K., Yang, Z. Y., Wu, M. O., Zheng, Y. Q., Su, C. C. and Wu, Y. J. (2013). Inhibition of melanogenesis by gallic acid: possible involvement of the PI3K/Akt, MEK/ERK and Wnt/β-catenin signaling pathways in B16F10 cells. International journal of molecular sciences, 14(10): 20443–20458. https://doi.org/10.3390/ijms141020443
22. Alfei, S., Oliveri, P. and Malegori, C. (2019). Assessment of the efficiency of a nanospherical gallic acid dendrimer for long-term preservation of essential oils: an integrated chemometric-assisted FTIR study. Chemistryselect, 4:8891–901. doi: 10.1002/slct.201902339

23. Fereidoonfar, H., Salehi-Arjmand, H., Khadivi, A., Akramian, M. and Safdari, L. (2019). Chemical variation and antioxidant capacity of sumac (Rhus coriaria L.). Ind Crop Prod., 139:111518. doi: 10.1016/j.indcrop.2019.111518
24. Gao, J., Hu, J., Hu, D. and Yang, X. (2019). A Role of Gallic Acid in Oxidative Damage Diseases: A Comprehensive Review. Natural Product Communications, 14:8. doi.org/10.1177/1934578X19874174
25. Wang, Y., Xie, M., Ma, G., Fang, Y., Yang, W., Ma, N., Fang, D., Hu, Q. and Pei, F. (2019a). The Antioxidant and Antimicrobial Activities of Different Phenolic Acids Grafted onto Chitosan. Carbohydrate Polymers, 225:115238. 10.1016/j.carbpol.2019.115238.
26. Sorrentino, E., Succi, M., Tipaldi, L., Pannella, G., Maiuro, L., Sturchio, M., Coppola, R. and Tremonte, P. (2018). Antimicrobial activity of gallic acid against food-related Pseudomonas strains and its use as biocontrol tool to improve the shelf life of fresh black truffles. Int J Food Microbiol., Feb 2;266:183-189. doi: 10.1016/j.ijfoodmicro.2017.11.026. PMID: 29227905.
27. Wang, Q., Leong, W.F., Elias, R.J., Tikekar, R.V. (2019b). UV-C irradiated gallic acid exhibits enhanced antimicrobial activity via generation of reactive oxidative species and quinone Food Chem., 287:303–12. doi: 10.1016/j.foodchem.2019.02.041
28. Zhang, T., Ma, L., Wu, P., Li, W., Li, T., Gu, R., Dan, X., Li, Z., Fan, X. and Xiao, Z. (2019). Gallic acid has anticancer activity and enhances the anticancer effects of cisplatin in non‑small cell lung cancer A549 cells via the JAK/STAT3 signaling pathway. Oncol Rep., Mar;41(3):1779-1788. doi: 10.3892/or.2019.6976. Epub 2019 Jan 22. PMID: 30747218.
29. Hyun, K.H., Gil, K.C., Kim, S.G., Park, S-Y., Hwang, K.W. (2019). Delphinidin chloride and its hydrolytic metabolite gallic acid promote differentiation of regulatory T cells and have an anti-inflammatory effect on the allograft model. J Food Sci., 84(4):920-930.
30. BenSaad, L.A., Kim, K.H., Quah, C.C., Kim, W.R., Shahimi, M. (2017). Antiinflammatory potential of ellagic acid, gallic acid and punicalagin A&B isolated from Punica granatum. BMC Complem Altern Med., 17:47. doi: 10.1186/s12906-017-1555-0
31. El-Hussainy, E.M.A., Hussein, A.M., Abdel-Aziz, A. and El-Mehasseb, I. (2016). Effects of aluminum oxide (Al2O3) nanoparticles on ECG, myocardial inflammatory cytokines, redox state, and connexin 43 and lipid profile in rats: possible cardioprotective effect of gallic acid. Can J Physiol Pharm., 94:868– 78. doi: 10.1139/cjpp-2015-0446.
32. Pandurangan, A.K., Mohebali, N., Esa, N.M., Looi, C.Y., Ismail, S. and Saadatdoust, Z. (2015). Gallic acid suppresses inflammation in dextran sodium sulfate-induced colitis in mice: possible mechanisms. Int Immunopharmacol., 28:1034–43. doi: 10.1016/j.intimp.2015.08.019.
33. Yang, K., Zhang, L., Liao, P., Xiao, Z., Zhang, F., Sindaye, D., Xin, Z., Tan, C., Deng, J., Yin, Y. and Deng, B. (2020). Impact of Gallic Acid on Gut Health: Focus on the Gut Microbiome, Immune Response, and Mechanisms of Action. Frontiers in immunology, 11:580208. https://doi.org/10.3389/fimmu.2020.580208
34. Maya, S., Prakash, T. and Madhu, K. (2018). Assessment of neuroprotective effects of gallic acid against glutamate-induced neurotoxicity in primary rat cortex neuronal culture. Neurochem Int., 121:50–8. doi: 10.1016/j.neuint.2018.10.011.
35. Abdel-Moneim, A., Abd El-Twab, S.M., Yousef, A.I., Reheim, E.S.A. and Ashour, M.B. (2018). Modulation of hyperglycemia and dyslipidemia in experimental type 2 diabetes by gallic acid and p-coumaric acid: the role of adipocytokines and PPAR gamma. Biomed Pharmacother, 105:1091– 7. doi: 10.1016/j.biopha.2018.06.096
36. Samuni, Y., Goldstein, S., Dean, O.M. and Berk, M. (2013). The chemistry and biological activities of N-acetylcysteine. Biochim Biophys Acta., 1830:4117–4129. doi: 10.1016/j.bbagen.2013.04.016.
37. Radomska-Leśniewska, D.M. and Skopiński, P. (2012). N-acetylcysteine as an anti-oxidant and anti-inflammatory drug and its some clinical applications. Cent J Immunol., 37:57–66.
38. Pannu, N., Manns, B., Lee, H. and Tonelli, M. (2004). Systematic review of the impact of N-acetylcysteine on contrast nephropathy. Kidney Int., 65:1366–1374. doi: 10.1111/j.1523-1755.2004.00516. x.
39. Zhang, L. and Liu, Y. (2020). Potential interventions for novel coronavirus in China: a systematic review. J Med Virol.,92(5):479–490. http://doi.org/10.1002/jmv.25707.
40. Raffaele, M., Barbagallo, I., Licari, M., Carota, G., Sferrazzo, G., Spampinato, M., Sorrenti, V., Vanella, L. (2018). N-Acetylcysteine (NAC) Ameliorates Lipid-Related Metabolic Dysfunction in Bone Marrow Stromal Cells-Derived Adipocytes, Evidence-Based

Complementary and Alternative Medicine, Article ID 5310961, 9 pages, https://doi.org/10.1155/2018/5310961.

41. Wang, F.G. and Xi, J.J. (2009). The influence of acetylcysteine on cytokines of alcoholic liver injury in rat. Chin J Public Health, 25:336–337.
42. Kaga, A.K., Barbanera, P.O., do Carmo, N.O.L., de Oliveira Rosa, L.R. and Fernandes, A.A.H. (2018). Effect of N-Acetylcysteine on Dyslipidemia and Carbohydrate Metabolism in STZ-Induced Diabetic Rats. International journal of vascular medicine, 2018:6428630. https://doi.org/10.1155/2018/6428630
43. Poe, F. and Com, J. (2020). N-acetyl cysteine: Apotential theraprutic agentsfor SAR-COV-2. Medical Hypotheses, Oct;143:109862. doi: 10.1016/j.mehy.2020.109862. PMID: 32504923; PMCID: PMC7261085.
44. Friedewald, W.T., Levy, R.I. and Fredrickson, D.S. (1972). Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. Clin Chem., Jun;18(6):499-502. PMID: 4337382.
45. Burtis, C.A. and Ashwood, E.R. (1999). Tietz Textbook of Clinical Chemistry, Third edition. Clinical Chemistry, 45 (6): 913–914.
46. Snedecor, G.W. and Cochran, W.G. (1973). Statistical Methods. sixth ed.the Iowa state university press, 238-248.
47. Mohammad, A.I. and Al-Okaily, B.B. (2017). Effect of sodium fluoride on liver functions of rats and amelioration by CoQ10. J. of Entomology and Zoology studies, 5(5): 887-893.
48. Yildirim, S., Ekin, S., Huyut, Z., Oto, G., Comba, A., Uyar, H., Sengul, E. and Cinarf, D.A. (2018). Effect of chronic exposure to sodium fluoride and 7,12-dimethylbenz[a]anthracene on some blood parameters and hepatic, renal, and cardiac histopathology in rats. Fluoride, 51(3):278–290.
49. Abdel-Baky, E. and Abdel-Rahman, N.O. (2020). Cardioprotective effects of the garlic (Allium sativum) in sodium fluoride-treated rats. JoBAZ., 81: 7. https://doi.org/10.1186/s41936-020-0140-0
50. Yilmaza, B.O. and Erkana, M. (2015). Effect of vitamin C on sodium fluoride -induced oxidative damage in Sertoli cells. Fluoride, 48(3):241-251.
51. Abdel-Wahab, W.M. (2013). Protective effect of thymoquinone on sodium fluoride-induced hepatotoxicity and oxidative stress in rats. The Journal of Basic and Applied Zoology, 66:263-270. https://doi.org/10.1016/j.jobaz.2013.04.002
52. Gaur, N., Kumawat, G., Karnawat, R., Sharma, I. K. and Verma P. S. (2018). Evaluation of chelating tendenc of gallic acid as adetoxifying agent: an innovative approach. International J. of recent scientific research, 9(1): 23470-23474.
53. Esmaeilzadeh, M., Heidarian, E., Shaghaghi, M., Roshanmehr, H., Najafi, M., Moradi, A. and Nouri, A. (2020). Gallic acid mitigates diclofenac-induced liver toxicity by modulating oxidative stress and suppressing IL-1β gene expression in male rats. Pharmaceutical biology, 58(1): 590–596. https://doi.org/10.1080/13880209.2020.1777169
54. Zekair, B.S. and Khudair, K.K. (2020). Effect of gallic acid on aluminum chloride induced liver toxicity and DNA fragmentation in rats. Online Journal of Veterinary Research, 24 (4):198-210.
55. Ojeaburu, S. and Oriakhi, K. (2021). Hepatoprotective, antioxidant and, anti-inflammatory potentials of gallic acid in carbon tetrachloride-induced hepatic damage in Wistar rats. Toxicology Reports, 8. 10.1016/j.toxrep. 01.001.
56. Canbek, M., Bayramoglu, G., Senturk, H., Oztopcu Vatan, A., Uyanoglu, M. and Ceyhan, E. Ozen, A, Durmus, B., Kartkaya, K. and Kanbak, G. (2014). The examination of protective effects of gallic acid against damage of oxidative stress during induced-experimental renal ischemia-reperfusion in experiment. Bratisl Lek Listy.,115:557–562.
57. Anjum, K.M., Mughal, M.S., Sayyed, U., Yaqub, A., Khalique, A. and Rashid, M.A. et al. (2014). Influence of increasing fluoride dose rates on selected liver and kidney enzymes profile in domestic chicken (Gallus domesticus). J. of Animal Plant Science, 24(1):77-80.
58. Karimi-Khouzani, O., Heidarian, E. and Amini, S.A. (2017). Anti-inflammatory and ameliorative effects of gallic acid on fluoxetine-induced oxidative stress and liver damage in rats. Pharmacol Rep., 69(4), 830-35.
59. Aglan, H.A., Ahmed, H.H., El-Toumy, S.A. and Mahmoud, N. S.(2017). Gallic acid against hepatocellular carcinoma: An integrated scheme of the potential mechanisms of action from in vivo study. Tumour Biol., 39(6):1010428317699127.
60. Safaei, F., Mehrzadi, S., Haghighian, H. Kh., Hosseinzadeh, A., Nesari, A., Dolatshahi, M., Esmaeilizadeh ,M.and Goudarzi, M. (2018). Protective effects of gallic acid against methotrexate-induced toxicity in rats. Acta Chir Belg., 118(3):152-160.
61. Mada, S.B., Abarshi, M.M., Garba, A., Sharehu, K.L., Elaigwu, O.P. and Umar, M.J. Musa1, B., Mohammed, H. A. and Garba, I. (2019). Hypolipidemic effect of N-acetylcysteine against

dexamethasone-induced hyperlipidemia in rats. Calabar J. Health Sci., 3(2):59-67.

62. Kakaei, F., Fasihi, M., Hashemzadeh, S., Zarrintan, S., Beheshtirouy, S., Asvadi-Kermani, T., Tarvirdizadeh, K., Rezaei, S. and Sanei, B. (2020). Effect of N-acetylcysteine on liver and kidney function tests after surgical bypass in obstructive jaundice: A randomized controlled trial. Asian J. Surg., Jan;43(1):322-329. doi: 10.1016/j.asjsur.2019.05.009. PMID: 31280997.
63. Saleem, T. H., Abo El-Maali, N., Hassan, M. H., Mohamed, N. A., Mostafa, N., Abdel-Kahaar, E., and Tammam, A. S. (2018). Comparative Protective Effects of N-Acetylcysteine, N-Acetyl Methionine, and N-Acetyl Glucosamine against Paracetamol and Phenacetin Therapeutic Doses-Induced Hepatotoxicity in Rats. International journal of hepatology, 7603437. https://doi.org/10.1155/2018/7603437
64. De Andrade, K.Q., Moura, F.A., dos Santos, J.M., de Araujo, O.R.P., Santos, J.C.D. and Goulart, M.O.F. (2015). Oxidative stress and inflammation in hepatic diseases: therapeutic possibilities of N-Acetylcysteine. International Journal of Molecular Sciences, 16(12):30269-30308.
65. Dhouib, E.I., Annabi, A., Lasram, M., Gharbi, N. and El-Fazâa, S. (2015). Anti-inflammatory Effects of N-acetylcysteine against Carbosulfan-induced Hepatic Impairment in Male Rats. Recent advances in biology and medicine, 1 (2015): 29–40.DOI: 10.18639/RABM.2015.01.156935.
66. Sharma D., Singh A., Verma K., Paliwal S., Sharma S. and Dwivedi J. (2017). Fluoride: A review of pre-clinical and clinical studies. Environ Toxicol Pharmacol., 56:297-313.
67. Ribeiro, D.A., Yujra, V.Q., da Silva, V.H.P., Claudio, S.R., Estadella, D., de Barros, V. M. and Oshima, C.T.F. (2017). Putative mechanisms of genotoxicity induced by fluoride: a comprehensive review. Environ Sci Pollut Res Int., Jun;24(18):15254-15259. doi: 10.1007/s11356-017-9105-3. Epub 2017 May 5. PMID: 28477256.
68. Mitta, R., Kandula, R.R., Pulala, R.Y. and Korlakunta, N. J. (2018). Alleviatory effects of hydroalcoholic extract of *Brassica oleracea* var. *botrytis* leaves against sodium fluoride induced hepatotoxicity and oxidative stress on male Wistar rats. Indian Journal of Biochemistry and Biophysics, 55:191-197.
69. Oyeyemi, O.O., Moyinoluwa, O.A., Asenuga, E.R., Omobowale, T.O., Ajayi O. and Oyagbemi, A.A. (2018). Antioxidant and anti-apoptotic effect of gallic acid on doxorubicin-induced testicular and epididymal toxicity. FASEB j., 32(1).
70. Akbari G. (2020). Molecular mechanisms underlying gallic acid effects against cardiovascular diseases: An update review. Avicenna journal of phytomedicine, 10(1): 11–23.
71. Ahmadvand, H., Nouryazdan, N., Nasri, M., Adibhesami, G. and Babaeenezhad, E. (2020). Renoprotective Effects of Gallic Acid Against Gentamicin Nephrotoxicity Through Amelioration of Oxidative Stress in Rats. Brazilian Archives of Biology and Technology, 63: e20200131. Epub October 26. https://dx.doi.org/10.1590/1678-4324-2020200131.
72. Dludla, Ph.V., Nkambule, B.B., Jack B., Mkandla, Z., Mutize, T., Silvestri, S., Orlando, P., Tiano, L., Lou, w. J. and Mazibuko-Mbeje, S. (2019). Inflammation and Oxidative Stress in an obese state and the protective effects of gallic acid. Nutrients, 11(1):23.
73. Reckziegel, P., Dias, V.T., Benvegnú, D.M., Boufleur, N., Barcelos, R.C.S., Segat, H.J., Pase, C.S., Dos Santos, C.M.M., Flores, É.M.M. and Bürger, M.E. (2016). Antioxidant protection of gallic acid against toxicity induced by Pb in blood, liver and kidney of rats. Toxicol Rep., Feb 22(3):351-356. doi: 10.1016/j.toxrep.2016.02.005.
74. Karimi-Khouzani, O., Sharifi, A. and Jafari, A. (2018). A Systematic review of the potential gallic acid effective in liver oxidative stress in Rats., J. of medical research, 4 (1): 5-12.
75. Eskiocak, S., Altaner, S., Bayir, S. and Cakir, E. (2008). The effect of N-acetylcysteine on brain tissue of rats fed with high-cholesterol diet. Turk J Biochem.,33:58-63.
76. Rushworth, G.F. and Megson, I.L. (2014). Existing and potential therapeutic uses for N-acetylcysteine: The need for conversion to intracellular glutathione for antioxidant benefits Pharmacol Ther.,141:150–159. doi: 10.1016/j. pharmthera. 09.006.
77. Vanella, L., Volti, I. G. L., Distefano, A. Raffaele, M, Zingales, V, Avola, R, Tibullo, D. and Barbagallo, I. (2017). A new antioxidant formulation reduces the apoptotic and damaging effect of cigarette smoke extract on human bronchial epithelial cells, European Review for Medical and Pharmacological Sciences, 21(23): 5478–5484.
78. Balansky, R., Ganchev, G., Iltcheva, M., Steele, V.E. and De Flora, S.D. (2010). Prevention of cigarette smoke-induced lung tumors in mice by

budesonide, phenethyl isothiocyanate, and N-acetylcysteine. Int J Cancer., 126:1047–1054.

79. Tardiolo, G., Bramanti, P. and Mazzon, E. (2018). Overview on the Effects of N-Acetylcysteine in Neurodegenerative Diseases. Molecules, 23:3305. doi: 10.3390/molecules23123305.
80. Teskey, G., Cao, R., Islamoglu, H., Medina, A., Prasad, C., Prasad, R., Sathananthan, A., Fraix, M., Subbian, S., Zhong, L. and Venketaraman, V. (2018). The Synergistic Effects of the Glutathione Precursor, NAC and First-Line Antibiotics in the Granulomatous Response Against Mycobacterium tuberculosis. Frontiers in immunology,9:2069. https://doi.org/10.3389/fimmu.2018.02069