Coadministration of ascorbic acid and metformin ameliorates valproic acid -Induced hepatotoxicity in male rats

Zainab Khalid Ali [1], Noor M. Al-Dabooni[2], Shamam Kanaan M Abdlkareem[3]

1) Department of Pharmacy, Al-Hikma University College, Baghdad, Iraq. **zainab.ali@hiuc.edu.iq**

2) Department of Pharmacy, Al-Hikma University College, Baghdad, Iraq.
noor.mohammed@hiuc.edu.iq

3) Department of Pharmacy, Al-Hikma University College, Baghdad,
Iraq.shamam.kanan@hiuc.edu.iq

Abstract
Valproic acid is used to treat neurological disorders such as epilepsy, bipolar disorder and migraine headaches. It has been demonstrated that its usage can have bad consequences, such as hepatotoxicity. Vitamin C and metformin have anti-inflammatory and antioxidant effects. Thus, this study aimed to investigate the potential hepatoprotective effects of vitamin C and metformin pretreatment alone and in combination against hepatotoxicity of valproic acid. Male rates were randomly divided into five groups: the group I, which received only normal saline; the group II (400 mg/kg/day); the group III) pretreated with ascorbic acid (200 mg/kg/day orally) for 30 days; the group IV, pretreated with metformin (250 mg/kg/day orally); and the group V, which received ascorbic acid (200 mg/kg) and metformin (250 mg/kg). Valproic acid was administered (400 mg/kg, intraperitoneally) starting from the 22nd day of the experiment for 8 days by the intraperitoneal route to GII, GIII, GIV, and GV to induce hepatotoxicity. Serum samples were collected by cardiac puncture to measure liver enzyme levels. Liver tissue samples were collected to study glutathione, malondialdehyde, tumor necrosis factor alpha, and nuclear factor kappa B levels. The results found Valproic acid significantly increased (P-value < 0.001) the serum levels of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase as compared to the control group. It also significantly increased (P-value < 0.001) malondialdehyde levels, as well as significantly reduced (P-value ≤ 0.001) glutathione. Concurrently, valproic acid significantly elevated (P-value ≤ 0.001) tumor necrosis factor alpha and nuclear factor Kappa B levels. Pretreatment with a combination of metformin at 250 mg/day and vitamin C at 200 mg/day significantly reduced (P-value < 0.05) oxidative stress and inflammation effects of valproic acid on liver
Keywords: metformin, ascorbic acid, valproic acid, anti-inflammatory, antioxidant, hepatotoxicity

1- Introduction

Valproic acid is an antiepileptic drug used to treat epilepsy, migraines, and bipolar disorders [(1)]. Despite its effectiveness, a hepatotoxicity problem was recorded following valproic acid use [(2)]. The main causes of it were excessive production of reactive oxygen species and elevated levels of inflammatory mediators such as tumor necrosis factor-alpha and nuclear factor kappa Mechanisms for promoting redox homeostasis should be

maintained to overcome oxidative stress [(3)]. Metformin and ascorbic acid have been extensively explored for their efficient anti-inflammatory and antioxidant effects [(4)]. Metformin activated AMPK in hepatocytes by altering the ATP/AMP balance and blocking complex I [(5)]. Activated AMPK can suppress the inflammatory process, involve mTOR-related TNF-α and NF_KB activation [(5)], and have antioxidative effects by inhibition of the NAD(P)H/PKC pathways [(6)]. Ascorbic acid interacts with glutathione and thioredoxin, it can activate antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase [(7)]. Moreover, ascorbic acid stimulate the activity of (Nrf2,Ref-1.AP-1) which enables expression of genes encoding antioxidant proteins[(8).] Additionally ascorbic acid effects on NFKB/TNF α pathway[(9).] However, its role in prevention inflammation and oxidative stress, important in ameliorating hepatotoxicity.

2. Materials and methods

2.1. Chemicals and reagents

Distilled water (PDPL:India), metformin (pioneer pharmaceutical company, Ascorbic acid powder (NOW foods), and valproic acid (Sigma-Aldrich St Louts, MO, USA).

2.2. Experimental animals

Thirty male albino rats with a weight range of 110-140 grams were used in this experiment. These rats were brought and acclimatized from the animal house of the College of Pharmacy of Al-Mustansiriyah University for 10 days. They were housed in research plastic cages and provided pellets and water.

2.3. Treatments and animals grouping

This research employed a sample randomized approach; these rats were divided randomly into five groups of six rats each. Group I (negative control) was orally administered distilled water 1 ml/kg/day for 30 days, and Group II (positive control) rats were intraperitoneally administered 400 mg of valproic acid per kg, starting from the 22nd day of the study for eight days to induce hepatotoxicity. Rats in groups III to V were orally treated with 200 mg/kg/day of ascorbic acid, 250 mg/kg/day of metformin, and their combination of ascorbic acid and metformin separately for thirty days and valproic acid (400 mg/kg) starting from the 22nd day for 8 days by the intraperitoneal route for groups III, IV, and V separately. The doses of metformin and ascorbic acid were chosen according to the human equation dose, whereas dose determination for valproic acid depended on previous studies [(10).]

2.4. Collection of serum and liver tissues

On day thirty-one, all rats were anesthetized and injected with 50 mg/kg ketamine and 5 mg/kg xylazine. The blood was obtained from the heart's right ventricle, placed in gel tubes, and centrifuged at 2500 rpm for 15 minutes for serum separation. The serum was collected in an Eppendorf for the estimation of liver enzymes. Whereas tissue slices from the liver were collected and homogenized for estimating malondialdehyde and glutathione levels, tumor necrosis factor alpha, and nuclear factor kappa B in liver tissue.

2.5. Determination of liver function

Aminotransferase (AST) (sigma-alorich), alanine aminotransferase, and alkaline phosphatase (ALP) (sigma-alorich) levels in serum samples were measured by colorimetric assay kits according to the manufacturer's guide.

2.6. Determination of oxidative stress and inflammatory levels in liver tissue homogenate

The levels of oxidative stress (reduced glutathione and malondialdehyde) and proinflammatory cytokines (TNF-α and NF_KB) in liver tissue homogenate done based on sandwich ELISA technique (My BioSource) according to manufacturing procedure.

2.7. Statistical analysis

Data analysis was accomplished via use of Statistical Packages for Social Sciences (SPSS) software, version (16). The descriptive statistics were reported as mean ± standard error of mean (SEM). One-way Analysis of Variance tests (ANOVA) was used to verify the significance of the difference between the five studied groups, followed by the Tukey test. *P-value* is considered nonsignificant if it is *P- value* > 0.05 and significant if it is *P*-value < 0.05

3. Results

3.1. Effect administration ascorbic acid, metformin, and their combination on serum liver enzymes of valproic acid- hepatotoxic rats

Results in GII found significant increases (p-value > 0.001) in serum levels of ALT, AST, and ALP to 203, 275, and 227 nmol/ml, respectively, in comparison to GI (control group). In contrast, rats in GIII showed a significant decline in the serum levels of the three enzymes when compared to GII (*p*-value < 0.001), but it remained significantly higher when compared with GI (*p*-value < 0.001). Animals in group IV showed a significant decrease in serum levels of ALT, AST, and ALP when compared to GII (*P*-value < 0.001) and non-significant differences in ALT level when compared to GI (*P*-value > 0.05), while AST level remained significantly different when compared to GI (*P*-value < 0.05). In contrast, ALP levels showed significant differences when compared to G1 (*P*-value < 0.05). Serum liver enzymes of rats in GV showed a highly significant decrease (*p*-value < 0.001) when compared to GII and a non-significant difference (*p*-value > 0.05) when compared to GI.

groups	N	ALT (nmol/ml)	AST (nmol/ml)	ALP (nmol/ml)
G1	6	60 ± 0.9 cd	74 ± 0.08 d	106 ± 0.6 d
GII	6	203 ± 0.7 a	275 ± 0.4 a	227 ± 1 a
GIII	6	151 ± 0.8 b	205 ± 0.7 b	136 ± 1 b
GIV	6	63 ± 0.3 c	81 ± 0.3 c	115 ± 1 c
GV	6	60 ± 1 d	75 ± 0.3 d	108 ± 1 d

Table 1: serum levels of lever enzyme (ALT, AST, and ALP)

Each value is given as the mean± SEM. The statistical analysis was done by using one-way ANOVA followed by the Tukey test GI: negative control group; GII: positive control group (400mg/kg V.A), GIII: ascorbic acid 200mg/kg +VA; GIV: 250mg/kg metformin + V.A; GV: 250mg/kg metformin + 200 mg/kg ascorbic acid + V.A. Dissimilar letters (a, b, c, and d), in same column indicate a significant difference in levels of liver enzymes between groups

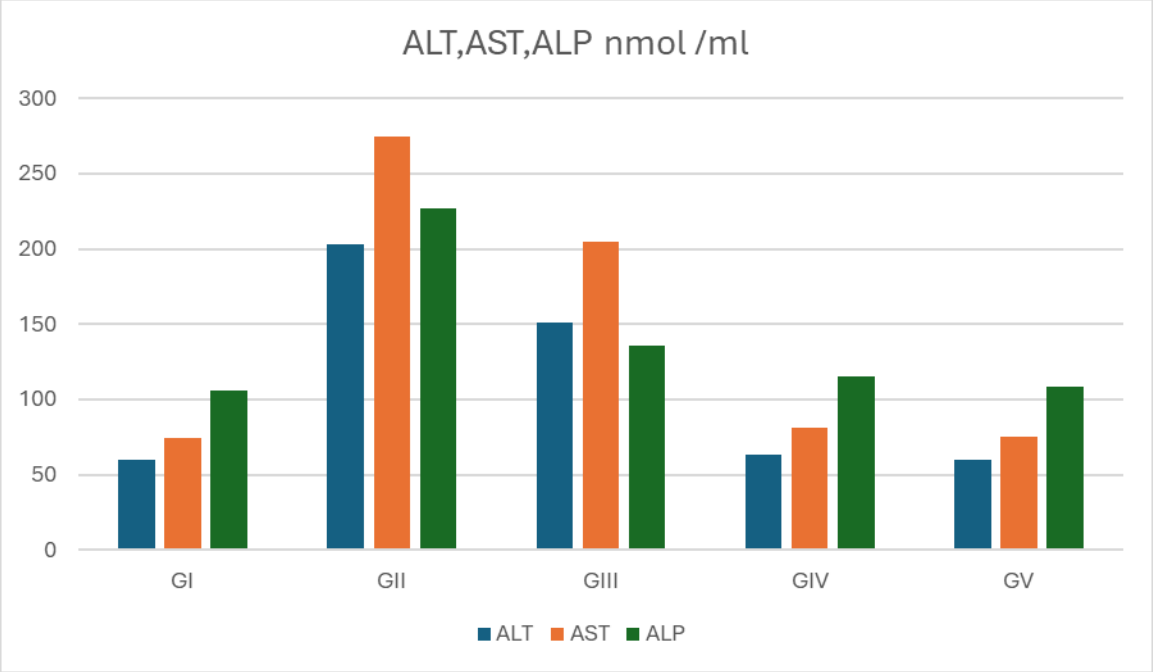


Figure 1: Change in levels of liver enzymes (ALT, AST, ALP) in all groups. The results represented as mean ± SEM. The statistical analysis was done by using one-way ANOVA followed by the Tukey test. GI: negative control group; GII: positive control group (400mg/kg V.A); GIII: 200mg/kg ascorbic acid + V.A; GIV: 250mg/kg metformin + V.A; GV: 250mg/kg metformin + ascorbic acid+ V.A.

3.2. Antioxidative effects of administration ascorbic acid, metformin, and their combination on valproic acid induced hepatotoxicity in male rats

Glutathione (GSH) levels in GII showed a significant decreased (p-value < 0.05) to (6.1± 0.5 μg /ml), and a highly significant elevated (p-value < 0.05) in lipid peroxidation (MDA) level to (5.1±0.3 nmol/ ml) when compared to GI. While the results showed levels of GSH were significantly increased in GIII to (13.6 ± 0. 2 μg /ml) (*P*-value < 0.001), GIV to (15 ± 0.7 μg /ml)(*P*-value < 0.001), and GV to(17.8 ± 1.1 μg /ml) (*P*-value < 0.001), especially in GV when compared with GII and nonsignificant difference as compared to GI, in contrast GIII,GIV, and GV showed a significantly decreased (*P*-value < 0.001) in MDA levels to (1.6 ± 0.3 nmol/ml), (1.2 ± 0.2 nmol/ml), and (1.1 ± 0.2 nmol/ml) respectively when compared with GII .as well as GIV and GV were non-significant difference when compared with GI (*P*-value = 0.44, 0.99) but GIII remained significant when matched to G1 (*P*-value = 0.022).

Table (2): Change malondialdehyde (GSH and MDA) levels in all groups.

	Number of animals	GSH (*µg* /ml)	MDA (nmol/ml)
GI	**6**	**16.7 ± 0.7 ab**	**0.78 ± 0.02 c**
GII	**6**	**6.1 ± 0.5 c**	**5.1±0.3 a**
GIII	**6**	**13.6 ± 0.2 b**	**1.6 ± 0.3 b**
GIV	**6**	**15 ± 0.7 ab**	**1.2 ± 0.2 bc**
GV	**6**	**17.8 ± 1.1 ab**	**1.1 ± 0.2 bc**

Each value is given as the mean± SEM. The statistical analysis was done by using one-way ANOVA followed by the Tukey test. G1: negative control group; GII: positive control group (400mg/kg V.A); GIII: 200mg/kg ascorbic acid+ V.A; GIV: 250mg/kg metformin + V.A; GV: 200mg/kg ascorbic acid +250mg/kg metformin + V.A. Dissimilar letters (a, b, and c) indicate a significant difference in MDA and GSH a levels between groups. Two letters (bc) indicate a nonsignificant difference to b and c.

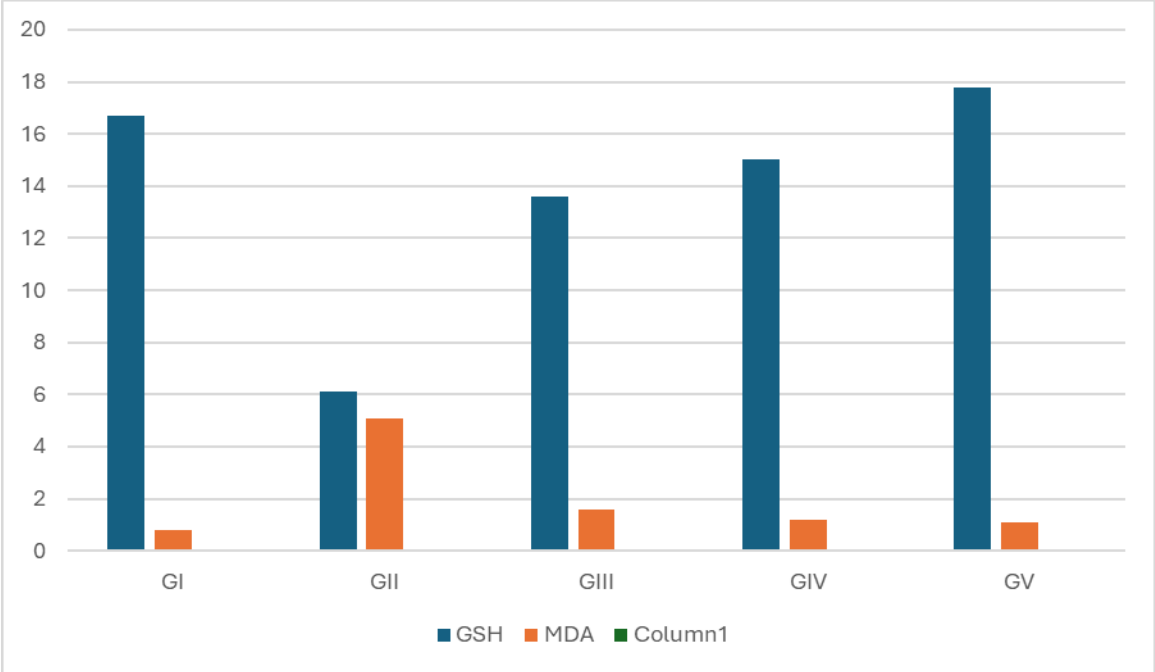


Figure : Change malondialdehyde (GSH and MDA) levels in all groups. The results represented as mean ± SEM. The statistical analysis was done by using one-way ANOVA followed by the Tukey test. GI: negative control group; G2: positive control group (400mg/kg V.A); GIII: 200 mg/ kg ascorbic acid + V.A; GIV: 250mg/kg metformin + V.A; GV: 200 mg/kg ascorbic acid +250mg/kg metformin + V.A. Dissimilar letters (a, b, and c) indicate a significant difference in MDA and GSH levels between groups.

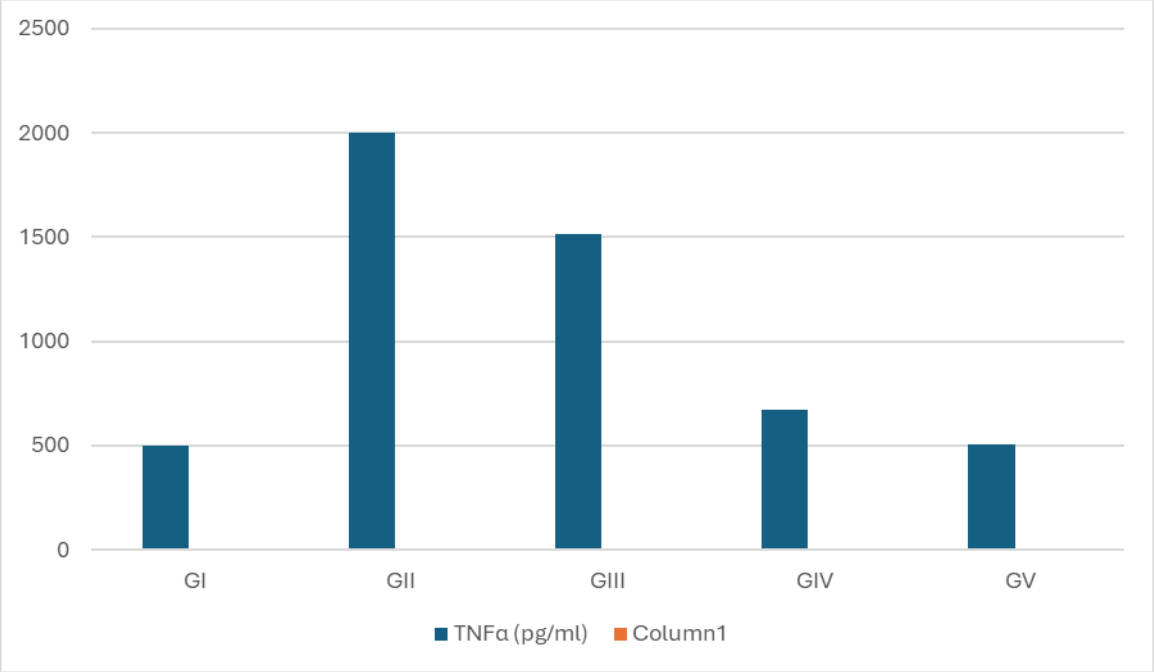
3.3. *anti-inflammatory effects of administration ascorbic acid, metformin, and their combination on valproic acid induced hepatotoxicity in rats*

The TNF-α levels were significantly increased (*p*-value < 0.001) in GII (2003 ±105 pg/ml) when compared to G1 (500 ± 25 pg/ml). Results in GIII showed a significantly decreased TNF-α level (1516 ± 144 pg/ml) (*P*-value < 0.001) when compared to GII, but it remained significantly higher when compared to G1 (*P*-value < 0.001), in contrast, the results in GIV and GV were significantly decreased (673 ± 62pg/ml) (*P*-value < 0.001), (507 ± 22 pg/ml) (*p*-value < 0.001) respectively when compared to GII and became non-significant when compared to GI (*P*-value = 0.66, 1) . The level of NF-$_k$B in GII was a highly significant increase (*P*-value < 0.001) to **(**3.6 ±0.17 ng/ml) when compared to GI (1.3 ± 0.06 ng/ml). while the results in GIII were significantly lower (2.2 ± 0.3ng/ml) (*p*-value = 0.001) when compared to GII (3.5 ± 0.1 ng/ml), but it remained significantly high when matched to G1 (*P*-value = 0.026). In contrast, the level of NF-$_k$B in GIV and GV were significantly decreased to (2± 0.2 ng/ml) and (1.7**± 0.1** ng/ml) respectively when compared with GII (*P*-value < 0.001) and non-significantly when compared to GI (*P*-value = 0.12, 0.61).

Table 4: Tumor necrosis factor alpha (TNF-α) levels and nuclear factor kappa B (NF-$_k$B) in all groups

	N	TNFα (pg/ml)	NF-$_k$B(ng/ml)
GI	**6**	**500 ± 25 c**	**1.3 ± 0.06 c**
GII	**6**	**2003 ±105 a**	**3.6 ±0.1 a**
GIII	**6**	**1516± 144 b**	**2.2± 0.3 b**
GIV	**6**	**673± 62 c**	**2± 0.2 bc**
GV	**6**	**507± 22 c**	**1.7 ± 0.1 c**

Each value is given as the mean± SEM. The statistical analysis was done by using one-way ANOVA followed by the Tukey test. N: number of animals; GI: negative control group; GII: positive control group (400mg/kg V.A); GIII: 200mg/kg ascorbic acid+ V.A; GIV: 250mg/kg metformin + V.A; GV: 200mg/kg ascorbic acid +250 mg/kg metformin + V.A. Dissimilar letters (a, b, and c) indicate a significant difference in TNF-α and NF-$_k$B levels between groups.



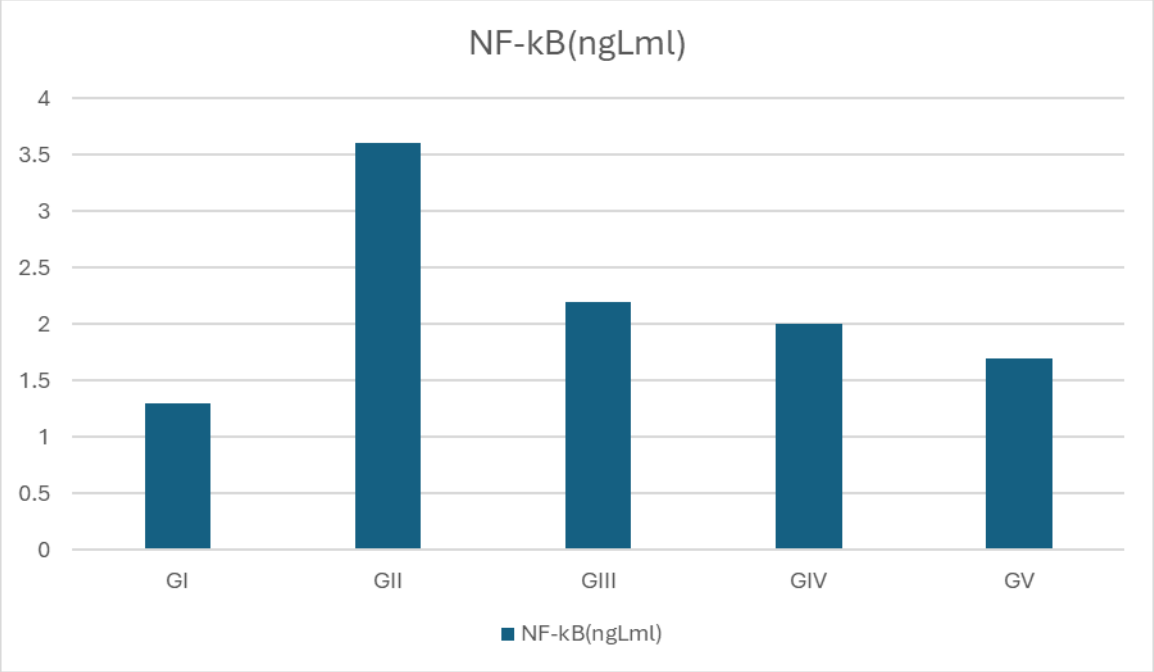


Figure 4: Change of tumor necrosis factor alpha (TNF-α) and Nuclear factor kappa B (NF-$_k$B) levels in all groups. The results represented as mean ± SEM. The statistical analysis was done by using one-way ANOVA followed by the Tukey test. GI: negative control group; GII:

positive control group (400mg/kg V.A); GIII: 200mg/kg ascorbic acid+ V.A; GIV: 250mg/k=g metformin + V.A; GV: 200mg/kg ascorbic acid+250mg/ kg metformin + V.A. Dissimilar letters (a, b, and c) indicate a significant difference in TNF-α levels between groups.

4. Discussion

In this study, Valproic acid was treated with neurological disorders [(11).] Unfortunately, a recent study has revealed that subjects at some point may suffer from liver toxicity[(12).] Previous research focused on the potential protective effects of anti-inflammatory and antioxidant agents against the hepatotoxicity of valproic acid, such as silymarin[(13)] and ellagic acid[(14).] This research estimated the possible hepatoprotective effects of ascorbic acid, metformin, and their combination in reducing valproic acid-induced inflammation and oxidative stress in the male rat's liver. In this study, the G2 showed significant elevation in ALT, AST, and ALP because of hepatocytes damaged by valproic acid and release of intracellular enzymes into the serum. These results are in accordance with previous research [(14).] Metformin (GIV) significantly decreased abnormally raised serum levels of ALT. as well as combination of metformin and ascorbic acid significantly decreased AST and ALP levels, as shown in table(1) and figure (1). These results were agreed with previous studies focused on the potential protective effect of ascorbic acid and metformin in decreased levels of liver enzymes in serum against hepatotoxicity by different agents [(15).] Since ascorbic acid and metformin had significant antioxidant and anti-inflammatory effects in prevent hepatocytes damage via stimulated AMPK by inhibiting mitochondrial complex I[(16)] and altering the ATP/AMP balance in hepatocytes. Complex I involved in the generation of ROS and inflammation [(17).] Therefore, metformin and ascorbic acid prevents hepatocyte injury and prevents the release of hepatic enzymes from the membrane of injured hepatocyte into circulation[(15).] An essential mechanism implicated in Valproic acid-induced liver injury is the promotion of inflammatory cascades. NF-$_k$B transcription factors, which bind to inflammation appear to be activated by oxidative stress[(18).] In this study, the results in GII found significantly reduced GSH and elevated MDA levels in liver tissue (p-value < 0.001) as shown in Table (3) and Figure (3) and then returned to normal levels in group IV and group V. in contrast, it remained nonsignificant differences in G III when compared to G II. These results were in line with several animal and human studies, which have found that chronic use of V.A. has been linked to hepatotoxicity by the production of reactive oxygen species (ROS) as a result of CYP2E1 metabolic activation and inducing oxidative damage by activation of lipid peroxidation, resulting in elevated MDA levels and a decrease in GSH levels [(14)(19).] Coadministration of metformin or a combination of metformin + ascorbic acid with V.A. enhanced the antioxidative stress system[(15).] Metformin and ascorbic acid have a direct scavenging action on ROS, which helps to restore the antioxidant system and decrease

oxidative damage [20]. Previous researchers have been focused on the antioxidant effect of metformin and ascorbic acid in lowering oxidative stress and have found metformin inhibition of mitochondrial complex I. The complex have a significant role in the formation of reactive oxygen species [21], resulting in enhanced lipid peroxidation and increased MDA levels. Therefore, inhibiting this complex by metformin due to reduced MDA level and elevated GSH level through decreased ROS and lipid peroxidation [22]. In this study, the rats treated with valproic acid were shown to have significantly stimulated NF-kB expression and increased tumor necrosis factor-alpha (TNF-α) production, as shown in Table 4 and Figure 4. These data agree with previous research outcomes, which also showed elevation in TNF-α and NF-kB levels with valproic acid [14]. The results in group IV and group V showed significantly decreased TNF-α levels. This suggests that metformin inhibits mechanisms involved in inflammation, involving mTOR-related TNF-α and decreasing NF-kB activation and phosphorylation of inhibitor of kappa B by activated AMPK [23]. These results were in line with previous studies that showed metformin inhibition of proinflammatory cytokine expression by inactivating NF-kB [24]. as well as ascorbic acid significantly reduced the expression of TNF-α and NF_kB levels through the influence of the interaction between NF_KB/TNF-α and Nrf_2/Keap 1 pathways [8]. The activation of Nrf_2 by ascorbic acid significantly reduced NF_KB through the induced expression of heme oxygenase 1, inhibiting the proinflammatory signaling by NF_{KB}[7].

5- Dedication

To the Al- Hikma University College (http://hiuc.edu.iq.) For their invaluable support and assistance in completing this research

References

1. Rosenberg G. The mechanisms of action of valproate in neuropsychiatric disorders: can we see the forest for the trees? 2007;64:14.
2. Pourahmad J, Eskandari MR, Kaghazi A, Shaki F, Shahraki J, Fard JK. A new approach on valproic acid induced hepatotoxicity: Involvement of lysosomal membrane leakiness and cellular proteolysis. Toxicology in Vitro. 2012 Jun;26(4):545–51.
3. Pourahmad J, Eskandari MR, Kaghazi A, Shaki F, Shahraki J, Fard JK. A new approach on valproic acid induced hepatotoxicity: Involvement of lysosomal membrane leakiness and cellular proteolysis. Toxicology in Vitro. 2012 Jun;26(4):545–51.
4. Araújo AA de, Pereira A de SBF, Medeiros CACX de, Brito GA de C, Leitão RF de C, Araújo L de S, et al. Effects of metformin on inflammation, oxidative stress, and

bone loss in a rat model of periodontitis. Reddy SV, editor. PLoS ONE. 2017 Aug 28;12(8):e0183506.

5. Junfei G, Shandong Y, Shan W, Wenjia S, Yuanyuan H. Metformin inhibits nuclear factor-κB activation and inflammatory cytokines expression induced by high glucose via adenosine monophosphate-activated protein kinase activation in rat glomerular. Chinese Medical Journa. 2014;127(9):1755-1760.

6. Kelly B, Tannahill GM, Murphy MP, O'Neill LAJ. Metformin Inhibits the Production of Reactive Oxygen Species from NADH:Ubiquinone Oxidoreductase to Limit Induction of Interleukin-1β (IL-1β) and Boosts Interleukin-10 (IL-10) in Lipopolysaccharide (LPS)-activated Macrophages. Journal of Biological Chemistry. 2015 Aug;290(33):20348–59.

7. Gęgotek A, Skrzydlewska E. Antioxidative and Anti-Inflammatory Activity of Ascorbic Acid. *Antioxidants (Basel)*. 2022;11(10):1993. Published 2022 Oct 7. doi:10.3390/antiox11101993

8. Gęgotek, A., & Skrzydlewska, E. (2022). Antioxidative and Anti-Inflammatory Activity of Ascorbic Acid. *Antioxidants (Basel, Switzerland)*, *11*(10), 1993. https://doi.org/10.3390/antiox11101993

9. Ellulu, M. S., Rahmat, A., Patimah, I., Khaza'ai, H., & Abed, Y. (2015). Effect of vitamin C on inflammation and metabolic markers in hypertensive and/or diabetic obese adults: a randomized controlled trial. *Drug design, development and therapy*, *9*, 3405–3412. https://doi.org/10.2147/DDDT.S83144

10. Shakya R , Hoque MK , Sapkota AS , Gupta PK . Differential Hepatotoxic Effects of Sodium Valproate at Different Doses in Albino Rats. Kathmandu Univ Med J (KUMJ). 2018;16(61):78-82

11. Rosenberg G. The mechanisms of action of valproate in neuropsychiatric disorders: can we see the forest for the trees? 2007;64:14.

12. Meseguer ES, Elizalde MU, Borobia AM, Ramírez E. Valproic Acid-Induced Liver Injury: A Case-Control Study from a Prospective Pharmacovigilance Program in a Tertiary Hospital. JCM. 2021 Mar 10;10(6):1153.

13. 139. Aktaş İ. , Armağan İ. Investigation of the positive effects of silymarin on valproic acid-induced liver damage in rats. ADYÜ Sağlık Bilimleri Derg. 2019; 5(2): 1445-1458.

14. Abdelkader NF, Elyamany M, Gad AM, Assaf N, Fawzy HM, Elesawy WH. Ellagic acid attenuates liver toxicity induced by valproic acid in rats. Journal of Pharmacological Sciences. 2020 May;143(1):23–9.

15. Adeneye AA, Benebo AS. Oral metformin-ascorbic acid co-administration ameliorates alcohol-induced hepatotoxicity in rats. *Nig Q J Hosp Med*. 2007;17(4):155-159. doi:10.4314/nqjhm.v17i4.12698

16. Fontaine E. Metformin-Induced Mitochondrial Complex I Inhibition: Facts, Uncertainties, and Consequences. Front Endocrinol. 2018 Dec 17;9:753.

17. Noor HB, Mou NA, Salem L, Shimul MFA, Biswas S, Akther R, et al. Anti-inflammatory Property of AMP-activated Protein Kinase. AIAAMC. 2020 Feb 24;19(1):2–41.

18. Aktaş İ. , Armağan İ. Investigation of the positive effects of silymarin on valproic acid-induced liver damage in rats. ADYÜ Sağlık Bilimleri Derg. 2019; 5(2): 1445-1458.

19. Ola OS, Adewole KE. Anticlastogenic and hepatoprotective effects of Kolaviron on sodium valproate-induced oxidative toxicity in Wistar rats. Egyptian Journal of Basic and Applied Sciences. 2021 Jan 1;8(1):167–79.

20. Araújo AA de, Pereira A de SBF, Medeiros CACX de, Brito GA de C, Leitão RF de C, Araújo L de S, et al. Effects of metformin on inflammation, oxidative stress, and bone loss in a rat model of periodontitis. Reddy SV, editor. PLoS ONE. 2017 Aug 28;12(8):e0183506.

21. Borole K, Padalkar P, Swami R. Assessment of antioxidant activity of metformin in ethanol induced liver damage in Sprague Dawley rats. Int J Basic Clin Pharmacol. 2016;324–8.

22. Rizk FH, El Saadany AA, Dawood L, Elkaliny HH, Sarhan NI, Badawi R, et al. Metformin ameliorated methotrexate-induced hepatorenal toxicity in rats in addition to its antitumor activity: two birds with one stone. JIR. 2018 Nov;11:421–9.

23. Dehkordi AH, Abbaszadeh A, Mir S, Hasanvand A. Metformin and its anti-inflammatory and anti-oxidative effects; new concepts. J Renal Inj Prev. 2018 Sep 6;8(1):54–61.

24. Junfei G, Shandong Y, Shan W, Wenjia S, Yuanyuan H. Metformin inhibits nuclear factor-κB activation and inflammatory cytokines expression induced by high glucose via adenosine monophosphate-activated protein kinase activation in rat glomerular. Chinese Medical Journa. 2014;127(9):1755-1760.