Ameliorative role of Omega-3 fatty acids and Vitamin E against Carbamazepine induced Hepatoxicity in Rats

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Abstract:

Background: Carbamazepine has been utilized to treat epilepsy and neuralgic conditions. Drug-induced liver injury triggered by Carbamazepine. Drug-induced liver injury from Carbamazepine consequently leads to liver failure. Existing research found that Omega-3 fatty acids and vitamin E known for their cytoprotective properties, and anti-inflammatory characteristics but their protective role against Carbamazepine induced liver injury yield inconclusive results.

Objective: To investigate closely omega-3 and vitamin E potential protective effect against carbamazepine hepatoxicity in rats.

Materials & Methods: Group 1: control received distilled water alone; Group 2: Treated with carbamazepine; Group 3: Treated with carbamazepine then followed by omega-3; Group 4: Treated with carbamazepine then followed by vitamin E; Group 5: Treated with carbamazepine then followed by a combination of omega-3 plus vitamin E experimental period lasted for fourteen days, at the end of the study all rats anesthetized. The liver tissue was dissected from each rat, part of liver tissue was used to evaluate Nuclear Factor Erythroid 2-Related Factor 2 gene expression and histological analysis using H&E stain was performed on the other part of liver tissue after formalin fixation (10%).

Results: The Nuclear Factor Erythroid 2-Related Factor 2 gene expression showed significant increase in treated groups 3,4,5 versus Carbamazepine group which indicate protective cellular response to restore redox balance. Histopathological evaluations were conducted that liver tissue architecture and cellular integrity showed normal liver appearance, the hepatocytes and sinusoids looked normal in Group 3,4,5 in comparison to Carbamazepine group that showed severe congestion of sinusoids, and signs of liver damage indicate that liver tissue changes had occurred.

Conclusion: Omega -3 and vitamin E alleviated Carbamazepine hepatoxicity through elevation antioxidant Nuclear Factor Erythroid 2-Related Factor 2 gene expression and maintenance integrity of liver tissue.

Keywords: Drug induced liver injury, Carbamazepine, omega-3, vitamin E, *Nrf2*, and Histopathology.

الدور المُحسن لأحماض أوميغا3- الدهنية وفيتامين هـ ضد تلف الكبد الناجم عن الكاربامازيبين لدى الجرذان

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الخلاصة:

الخلفية: الكاربامازبين هو دواء للجيل الاول من مضادات الصرع ويستخدم لعلاج الصرع والحالات العصبية وقد وجد ان الاعتلال الكبدي الناتج عن الكاربامازبين يؤدي الى الفشل الكبدي الحاد. اشارت الابحاث والدراسات السابقة ان اوميغا3- وفيتامين هـ معروفة بخصائصها المضادة للالتهابات وحماية الخلايا. اظهر دواء الاوميغا3- وفيتامين هـ في تضرر الكبد الناتج عن الكاربامازبين نتائج متضادة والدلائل غير محسومة.

الهدف من الدراسة: هدفت الدراسة اعلاه الى الكشف عن التأثير الوقائي للاوميغا 3- وفيتامين هـ على الاعتلال الكبدي الناجم عن الكاربامازبين في الجرذان .

المواد وطرق العمل: في هذه الدراسة تم استخدام ثلاثين جرذ، قسمت الجرذان الى خمس مجاميع، ست جرذان في كل مجموعه. المجموعة الاولى: مجموعة السيطرة تلقت الماء المقطر فقط يوميا عن طريق الفم. المجموعة الثانية: عولجت بالكاربامازبين , المجموعة الثالثة عولجت بالكاربامازبين بعدها عولجت ب اوميغا-3بعد مرور ساعة من التجريع ب كاربامازبين. المجموعة الرابعة: عولجت ب كاربامازبين بعدهـــا عولجت ب فيتامين هـ بعد مرور ساعـــــة. المجموعـــــــة الخامسة: عولجت ب كاربامازبين يتبعها مزيج من اوميغا 3- وفيتامين هـ عن طريق الفم، بعد مرور ساعة. أجريت التجربة لمدة اربعة عشر يوم بعدها تم قتل الجرذان واستخدم نسيج الكبد المتجانس لتقييم التعبير الجيني لعامل النسخ المرتبط بكريات الدم الحمراء اما الجزء الاخر من نسيج الكبد نم حفظه في 10% فورمالين لأغراض الدراسة النسيجية باستخدام صبغة الهيماتوكسلين - أيوسين.

النتائج: أظهر التعبير الجيني لعامل النسخ المرتبط بكريات الدم الحمراء ارتفاعاً معنويا في المجاميع المعالجة 3,4,5 مقارنة بمجموعة الكاربامازبين مما يشير إلى استجابة خلوية وقائية تهدف إلى استعادة الاتزان التأكسدي- الاختزالي. بالنسبة للتقييم النسيجي، ان بنية نسيج الكبد والخلايا بدت وكأنها طبيعيـــة وكذلك الجيوب الكبدية في المجاميع 3،4،5 التي تم إعطائها الاوميغا-3 وفيتامين هـ مقارنة مع المجموعة المستحثة بالكاربامازبين والتي اظهرت احتقان شديد للجيوب الكبدية وتلف شديد في انسجة الكبد وهذا دليل على حدوث تغييرات موجبة في النسيج

الاستنتاج: اكدت النتائج التأثير الوقائي ل اوميغا3- وفيتامين هـ للتخفيف من التأثير السمي للكبد والناتج من استخدام الكاربامازبين من خلال زيادة التعبيرالجيني لمضاد الاكسدة لعامل النسخ المرتبط بكريات الدم الحمراء والحفاظ على سلامة انسجة الكبد.

الكلمات المفتاحية: اصابة الكبد الناجمة عن الادوية، كاربامازبين, اوميغا3- وفيتامين هـ، *Nrf2* وعلم الامراض التشخيص النسيجي

Introduction

Carbamazepine was synthesized in (1953) by Swiss chemist Walter Schindler to treat trigeminal neuralgia. CBZ had been endorsed for use as an antiepileptic drug in UK and Switzerland in 1963. Carbamazepine structure ($C_{15}H_{12}N_2O$), is typically a white crystalline type of powder that has poor aqueous solubility. CBZ is lipophilic in nature and can cross-over the brain barrier and exerts their effects on the central nervous system. Carbamazepine is employed in managing seizure disorders and treated neuropathic pain and psychiatric disorder (1,2). Carbamazepine ~~was~~ recommended to be taken with meals, it

was undergone extensive metabolism by multiple pathways, with CBZ epoxide–diol pathway being the primary way. This pathway involves enzymes that convert Carbamazepine to active metabolite Carbamazepine 10,11 epoxide (3). CBZ metabolized to form radicals and releases reactive oxygen species (ROS) lead to production of protein adducts, carbamazepine -2,3 epoxide which is intermediate metabolite of CBZ was found to be hepatotoxic and lead to mitochondrial dysfunction (4). It was a drug known to be involved in causing Drug-Induced Liver Injury (DILI). Drug-Induced Liver Injury represents a health problem, tied with substantial rates of morbidity and mortality. DILI first described by Popper and Schaffner in1965. DILI typically occurs when the drug metabolism is modified and leads to liver injury attributed to inflammation, oxidative stress, necrosis and organ dysfunction (5). DILI is a significant serious concern faced by clinicians, medical practice, and pharmaceutical companies. It is considered a substantial challenge due to its high frequency and potential liver damage severity (6). DILI is categorized into two primary types: intrinsic and idiosyncratic. Intrinsic DILI is known as a dose dependent liver damage results from drug itself. On the other hand, idiosyncratic DILI (iDILI) is known as unpredictable, not strongly dose-related, and its onset is influenced by individual susceptibility (7). DILI is recognized as one major cause leading to acute liver failure worldwide (8). DILI approximately accounts for (20–40%) of the liver transplantation cases in United States (9). Numerous drugs can be hepatotoxic like herbal and dietary supplements (10). DILI cases resulting from antibiotics uses is declining, while treatment with antineoplastic agents is increasing (11). The mortality rate of DILI may reach 50٪ (12) and might developed into liver fibrosis, liver failure and loss of life (13). DILI treatment includes ceasing the medicine because it is great strategy to avoid liver injury, and it must be immediately done to prevent the disease from turning into cirrhosis and acute liver failure. Corticosteroids are considered a standard therapeutic method for treating immune mediated DILI by decreasing inflammation (14). There are 3 types of omega-3 obtained from different types of foods resources and supplements. First type Alpha-Linolenic Acid (ALA), the second type Eicosapentaenoic Acid (EPA) and the third type Docosahexaenoic Acid (DHA). It possesses protective activities against rheumatoid arthritis, eczema, heart disorders, stroke and cancer (15). Omega-3 fatty acids had demonstrated protective effect on liver, heart, and kidney tissue (16,17,18). Studies on animal and human research stated that omega 3 fatty acids might reduce liver fat (19, 20). Vitamin E was discovered in green leafy vegetables in 1922, and it composed of two major groups, including tocopherols and tocotrienols (21). High diet with vitamin E intakes has beneficial effects on microbiome were associated with high bacterial diversity (22). Vitamin E deficiency causes placentation failure, neuromuscular impairments, retinopathy, reduced immunity (23). Vitamin E protect the body against oxidative stress which is named reactive oxygen species and provide protection against oxidative damage, lipid peroxidation and reduce liver toxicity (24). A previous study stated that omega-3 and vitamin E showed protective effect against Carbamazepine hepatoxicity in rats by conducting many vital tests the liver enzyme functions found normal, glutathione was elevated, while malondialdehyde was declined, inflammatory cytokines were decreased, and cytokeratin 18 gene expression was decline (25).The nuclear factor erythroid 2 related factor 2 (Nrf2) plays a crucial protective role within the cells such as defense against oxidative stress, detoxification, and promoting cell proliferation. During drug induce injury

consequently leads to excessive production of reactive metabolite ~~that~~ leading to hepatoxicity. Under normal condition Nrf2 binds to Keap1 and found inactive in the cytoplasm. Upon oxidative or electrophilic stress, Nrf2 dissociates from Keap1, accumulates and moves to nucleus and bind to antioxidant response elements (ARE) to produce cytoprotective gene (26). This study was designed to evaluate omega-3 fatty acid and Vitamin E both separately and in combination protective effect against Carbamazepine hepatoxicity in rats. The evaluation had involved assessing *NRF-2* gene expression and examining histopathological alteration of liver tissue through Hematoxylin & Eosin stains.

Materials &Methods

CBZ induced hepatotoxicity

Twelve albino Westar rats with weight 180-200 gram under normal condition dividing into four groups each groups contain 3 rats, Groups1 received distilled water, Groups 2 received CBZ 50 mg /kg/day, Groups 3 received CBZ 100 mg/kg/day, Groups 4 received 150 mg/kg/day. all were given orally for 14 days. Blood sample were collected and serum were separated by centrifugation at 3000 rpm for 15 min, liver enzymes like ALT.AST and ALP were measured using enzymatic colorimetric methods.

Carbamazepine dose and Administration (Pilot Study)

In this pilot study Carbamazepine at dose 50 mg/kg/day for 14 days showed minimal toxicity on liver enzymes function. While 150 mg/kg per day lead to death of one of the rats with severe liver enzymes elevation. Both doses 100 mg and 150mg presented severe toxicity and elevation of liver enzymes. without significant differences between them regarding to ALT and ALP. So, 100 mg/kg per day was chosen for the induction of liver toxicity and it was associated with previous study (27).

Experimental Study

Ethical approval was obtained from the research committee, College of Pharmacy, Mustansiriyah University, research number 52 and approval number 52, the date 1/10/2024. Adult male albino rats Wistar weighing 180-200 gm, aged 16–18 weeks from animal house were included ~~used~~ in this study. Thirty rats were divided into 5 groups (6 rats for each). Group 1: control received distilled water alone; Group 2: Treated with carbamazepine (100 mg/kg/day, orally); Group 3: Treated with carbamazepine (100 mg/kg/day, orally) then followed by omega-3 (400 mg/kg/day, orally) one hour later; Group 4: Treated with carbamazepine (100 mg/kg/day, orally) then followed by vitamin E (400 IU/kg/day, orally) one hour later ; Group 5: Treated with carbamazepine (100 mg/kg/day, orally) then followed by a combination of omega-3 (400 mg/kg/day) plus vitamin E (400 IU/kg/day) one hour later. The experiments were last done for 14 days (25).

Sample Collections

After fourteen days, the rats were dissected to collect liver ~~tissue.~~ Collected liver was then rinsed with normal saline, 50mg of ~~liver~~ the tissue was placed in Eppendorf with one ml TRizol and stored at -20°C for gene expression. The rest of liver tissue from each rat was washed by tap water then stored in 10% formalin for 24-48 hours for histopathology study (18).

NRF2 Gene Expression by RT-PCR

RNA was extracted by tranZol up plus RNA reagent made in (China). Then RNA was converted to complementary DNA by EasyScript® First Strand cDNA Synthesis Mix kit (China). Primers sequences were used for target and endogenous genes (*HKG2*) as

follow.

- *NRF2: F: CACATCCAGACAGACACCAGT; R: CTACAAATGGGAATGTCTCTGC*
- *HKG2: F: GACAGCCGCATCTTCTTGTG; R: GATGGCATGGACTGTGGTCA*

Conversion of RNA to c DNA this procedure was done according to manufacturing kit procedure, about 20 µl final volume of reaction was needed including many components. All primers were lyophilized and included target genes and endogenous genes they were dissolved in free ddH20 to get final concentration 100 pmol/µl as stock solution. Then prepare 10 pmol/µl. After that, the final mix reaction in volume 25 µl was prepared for each sample including all components which are listed in Table (1) to amplified target gene by RT-PCR regarding to the program in Table (2). Finally, target gene expressions were evaluated by using relative quantitative method and calculated as Fold change ($2^{-\Delta\Delta Ct}$).

Table 1: Reaction components for each sample

Compound	Volume
qPCR Master Mix (sypergreen)	8 µl
Primer forward	1 µl
Primer reverse	1 µl
DNA	8 µl
dd H2O	7 µl
Total volume	25 µl

The reaction mixture was incubated in the SaCycler_96 Real Time PCR SYSTEM under the following program

Table 2: RT-PCR amplification program

Steps	Temperature	Time	Cycle
Enzyme activation	94 °C	30 second	1
Denaturation	94 °C	5 second	45
Annealing and Extension	60 °C	35 second	45
High resolution melting	90 °C	15 second	100

Histopathological Study

The liver tissue was fixed in 10% formalin and dehydrated through graded ethanol, cleared in xylene, and embedded in paraffin wax. Sections the livers of approximately 5 µm thickness were cut using a microtome, mounted on glass slides, and stained with hematoxylin and eosin (H&E). Histopathological alterations were evaluated under a light microscope by a specialized histopathologist (28,29,30).

Statistical Analysis

Data was analyzed by using SPSS, version 2019. One-way ANOVA, LSD post hoc analysis and Chi- square test was carried out. p- value < 0.05 would be considered significant, while $p < 0.01$ would be considered as high significant.

Results

NRF- 2 Gene Expression

The Fold change of nuclear factor erythroid 2-related factor 2 (*Nrf-2*) target gene and housekeeping gene (*HKG2)*, had been determined. Amplification plots represent fluorescent signals of each sample across the PCR cycles were generated, In G2 the fold was lower than control (0.898 ±0.12) while the expression showed significant increased expression in all treated groups 3,4,5 with *P*-value ≤ 0.01 **, as shown in Table (3) &Figure (1).

Table 3: Comparison between different groups in *NRF2* fold change

NRF2 relative *index*	G1	G2	G3	G4	G5
Mean ± SD	1.049 ±0.07 c	0.898 ±0.12 c	3.507 ±0.24 b	3.993 ±0.19 b	6.569 ±0.34 a
L.S.D. (P-value)	1.065 ** (0.0001)				
Data were presented as mean ± SE, mean with different lowercase letters (a, b, c) denote differ statistically, LSD analysis was carried out to compare mean among groups, *P* value ≤ 0.05 * represent significant difference and *P*-value ≤ 0.01 ** represent high significant difference.					

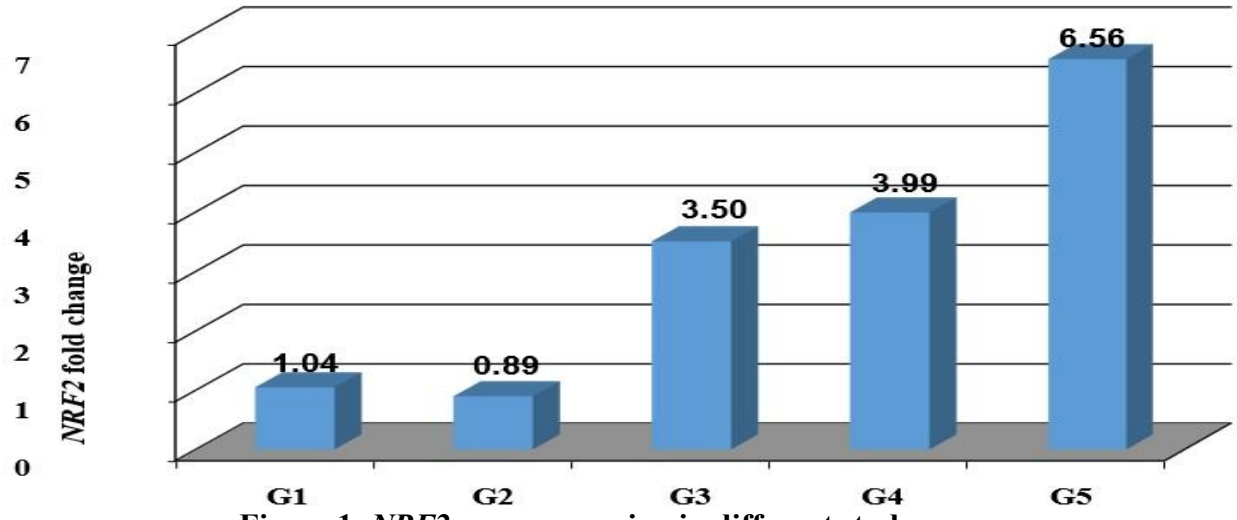

Figure 1: *NRF2* gene expression in different study groups

Histopathological Changes in All Study Groups

G1 (Control Group): Liver showed normal appearance of central veins with normal arrangement of the hepatic cords, normal appearance of sinusoids and hepatocytes, cytoarchitecture of the portal triad exhibited normal portal vein, bile duct and arteriole, as shown in Figure 2 (A, B, C, D).

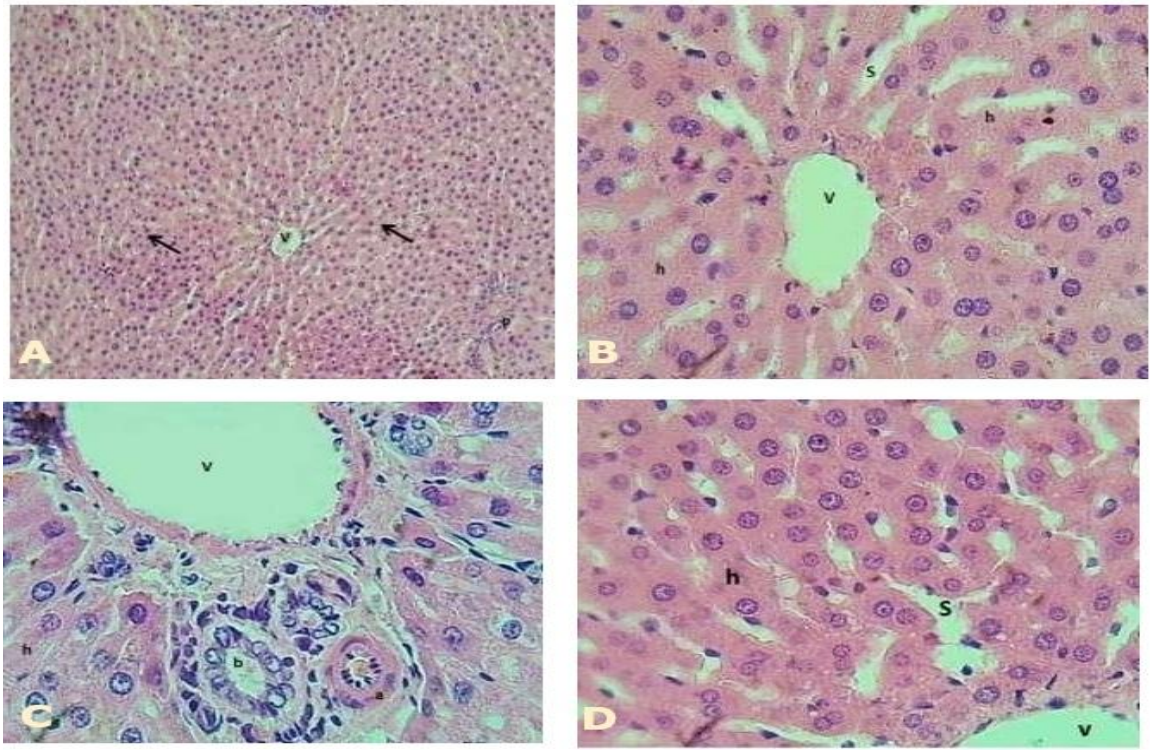

Figure 2: Section of liver (G1). A: it exhibited normal central vein (V) & normally arranged hepatic cords and sinusoids (arrows), portal area (p) .HE 100x. B: liver showed normal central vein (V) & normally arranged hepatocytes (h) and sinusoids (S) HE 400x. C: section of portal triad shows: normal portal vein (V) and normal hepatocytes (h) and bile duct (b) & arteriole (a). 400x. D: liver showed normal central vein (v) normal hepatocytes (h) and sinusoids (S),HE 400x.

G2 (Induction Group with Carbamazepine): liver exhibited moderate congestion of central veins and portal veins with many hemorrhagic foci that revealed mild aggregation of mononuclear leukocytes with severe sinusoidal congestion and mild periportal fibrosis, as illustrated in Figure 3 (E, F, G, H, I).

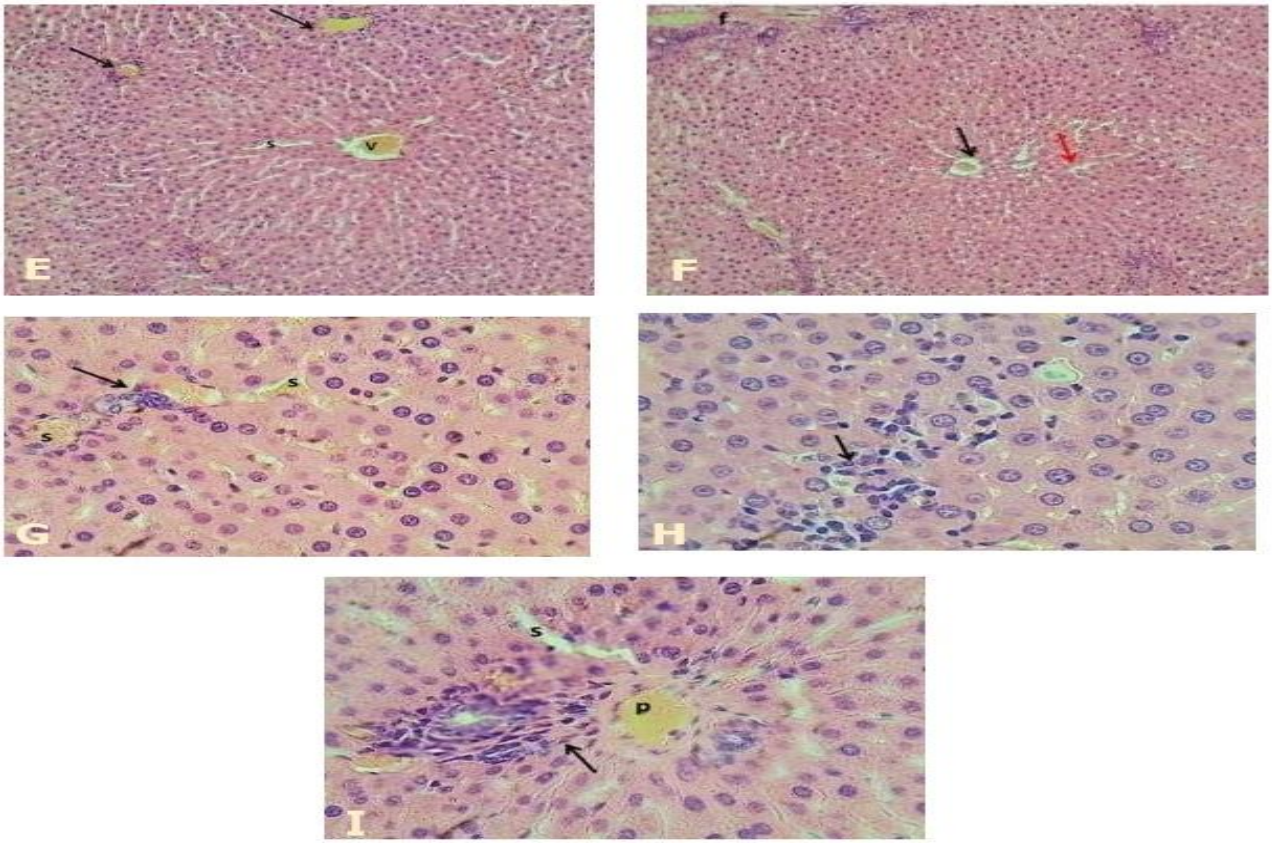

Figure 3: Section of liver (G2), E: liver exhibited congestion of central vein (v) with many hemorrhagic foci and aggregation of leukocytes (arrows) & severe sinusoidal congestion (S). HE 100x. F: exhibited severe congestion of central vein (Black arrow) with sinusoidal congestion (red arrow) & periportal fibrosis (f). HE 100x. G: showed hemorrhagic focus with aggregation of leukocytes (arrows) & sinusoidal congestion (S). 400x. H: showed focal aggregation of leukocytes (Arrow) & sinusoidal congestion. H&E.400x. I: showed congestion of portal vein (p) with mild periportal fibroplasia (Arrows) & sinusoidal congestion (S),HE 400x.

G3 (Carbamazepine + Omega 3): Liver exhibited mild congestion of the portal veins with few hemorrhagic foci and sinusoidal congestion and the hepatocytes showed normal appearance, the portal triad showed congestion of portal veins and obvious hyperplasia of cholangiocytes. Few figures showed general swelling of hepatocytes, as shown in Figure 4 (J,K,L)

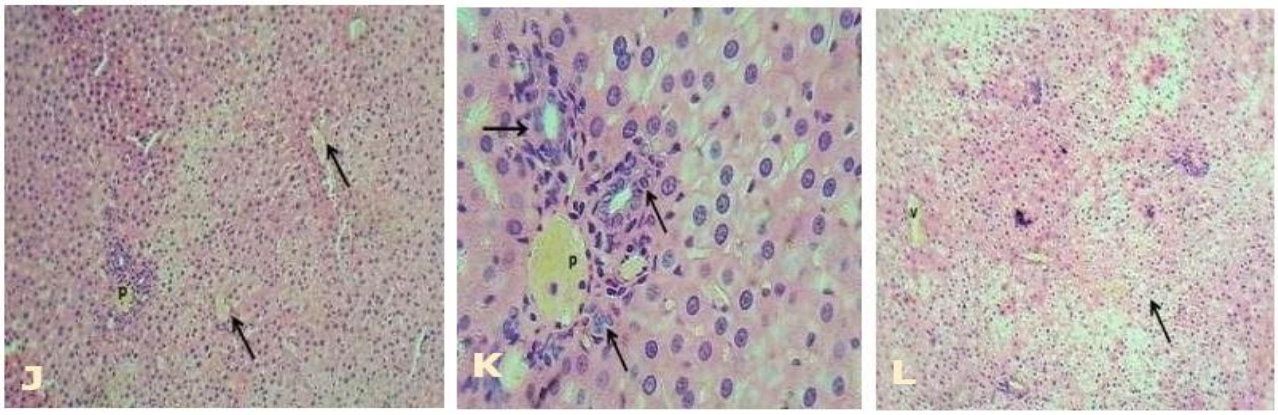

Figure 4: Section of liver (G3) J: congestion of portal vein (p) with many hemorrhagic foci (Arrows) & sinusoidal congestion. HE 100x. K: showed congestion of portal vein (p) with obvious hyperplasia of cholangiocytes (Arrows) with sinusoidal congestion.HE 400x. L: showed congestion of central vein (v) with generalized swelling of hepatocytes (arrow), HE 400x.

G4 (Carbamazepine+ Vit E): The liver exhibited mild congestion of the portal veins with normal arrangement and appearance of the hepatocyte's normal appearance sinusoids. Few figures showed sinusoidal congestion, of portal vein and obvious hyperplasia of cholangiocytes, as shown in Figure 5 (M,N,O,P).

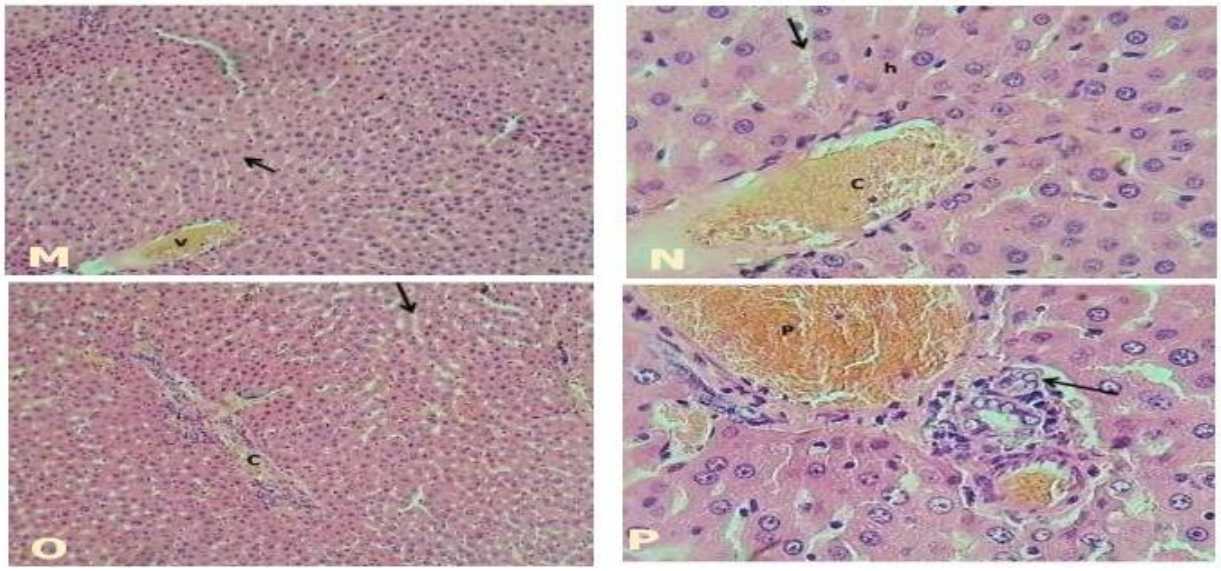

Figure 5: Section of liver (G4) M: showed mild congestion of central vein (v) with normal hepatocytes (arrow). HE 100x. N: showed mild congestion of central vein (C) with normal hepatocytes (h) & sinusoid (Arrow). HE 100x. O: congestion of portal vein (C) & sinusoidal congestion (arrow).HE 100x. P: congestion of portal vein (P), hyperplasia of cholangiocytes & sinusoidal congestion,HE 400x.

G5 (Carbamazepine + Omega 3+ Vit E): The liver showed mild congestion of the portal veins with normal arrangement and appearance of the hepatocyte's normal appearance sinusoids, as illustrate in Figure 6 (Q, R, S).

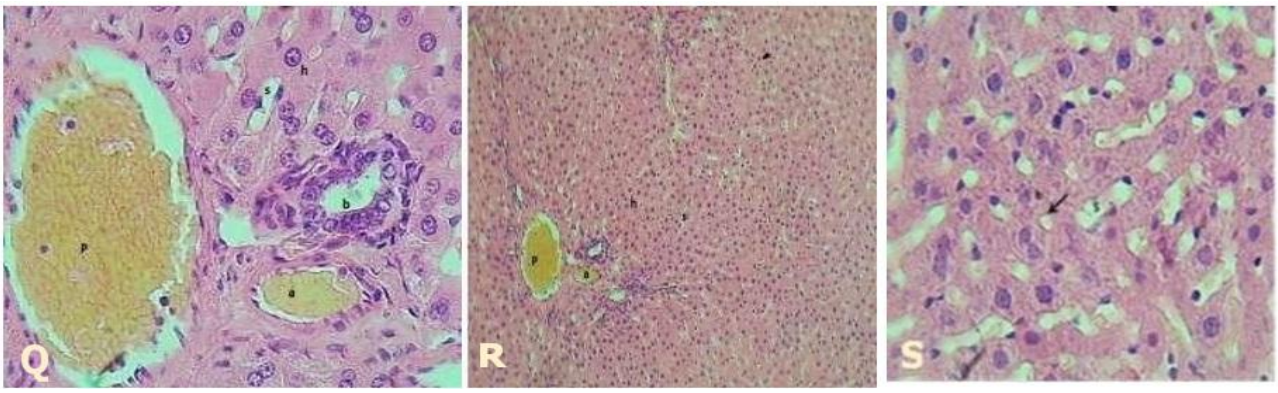

Figure 6: Sections of liver (G5) Q: congestion of portal vein (p) & arteriole (a), normal sinusoids & hepatocytes. HE 100x.
R: congestion of portal vein (p), normal sinusoids (s), hepatocytes (h), bile duct (b) & arteriole (a). HE 400x. S: show normal sinusoids (s), hepatocytes (h), bile canaliculi (arrow),

Table (4) summarized the changes of all pathological parameters in liver tissue. The modification and reversible changes to the normal in tissue had appeared clearly in the groups received Omega- 3 plus Vit-E combination in versus to the group induced by CBZ.

Table 4: Pathological parameters assessing liver injury

. Parameter	cor e	Group 1	roup 2	Group 3	Group 4	Group 5	Chi-squar e of score	P-valu e of score	Chi-square of paramete r	P-value of paramete r
inflammator y infiltration and aggregation	0	5	0	4	5	6	5.500	0.70 3	17.72	0.0234 *
		83.30%	0.00%	66.70%	83.30%	100.00 %				
	1	1	5	2	1	0	8.220	0.41 2		
		16.70%	83.30 %	33.30%	16.70%	0.00%				
	2	0	1	0	0	0	4.000	0.85 5		
		0.00%	16.70 %	0.00%	0.00%	0.00%				
	3	0	0	0	0	0	0.000	1.00 0 NS		
		0.00%	0.00%	0.00%	0.00%	0.00%				
Blood vessels dilation and	0	5	0	1	1	5	9.670	0.65 0		
		83.33%	0.00%	16.67%	16.67%	83.33%				
	1	1	0	5	5	1	9.670	0.65 0		
		16.67%	0.00%	83.33%	83.33%	16.67%				
	2	0	1	0	0	0		0.97		

congestion		0.00%	16.67 %	0.00%	0.00%	0.00%	4.000	5	43.34	0.000001 ***
	3	0	5	0	0	0	20.000	0.06 6		
		0.00%	83.33 %	0.00%	0.00%	0.00%				
Hemorrhage	0	6	1	1	3	6	7.412	0.49 2	23.957	0.0023 **
		100.00 %	16.70 %	16.70%	50.00%	100.00 %				
	1	0	3	5	3	0	8.545	0.38 2		
		0.00%	50.00 %	83.30%	50.00%	0.00%				
	2	0	2	0	0	0	8.000	0.43 3		
		0.00%	33.30 %	0.00%	0.00%	0.00%				
	3	0	0	0	0	0	0.000	1.00 0 NS		
		0.00%	0.00%	0.00%	0.00%	0.00%				
neratio n	0	5	1	1	5	5	5.647	0.68 7	19.465	0.0126 *
		83.30%	16.70 %	16.70%	83.30%	83.30%				
	1	1	3	5	1	1	5.818	0.66 7		
		16.70%	50.00 %	83.30%	16.70%	16.70%				
	2	0	2	0	0	0	8.000	0.43 3		
		0.00%	33.30 %	0.00%	0.00%	0.00%				
	3	0	0	0	0	0	0.000	1.00 0 NS		
		0.00%	0.00%	0.00%	0.00%	0.00%				
Necrosis	0	6	0	4	6	6	6.282	0.90 1	38.182	0.0001 ***
		100.00 %	0.00%	66.70%	100.00 %	100.00 %				
	1	0	0	2	0	0	8.000	0.78 8		
		0.00%	0.00%	33.30%	0.00%	0.00%				
	2	0	5	0	0	0	20.00 0	0.06 5		
		0.00%	83.30 %	0.00%	0.00%	0.00%				
	3	0	1	0	0	0	0.000	1.00 0 NS		
		0.00%	16.70 %	0.00%	0.00%	0.00%				
eri portal fibrosis	0	6	0	1	6	6	9.6.84	0.28 8	28.684	0.0004 ***
		100.00 %	0.00%	16.70%	100.00 %	100.00 %				
	1	0	5	5	0	0	15.00 0	0.05 9		
		0.00%	83.30 %	83.30%	0.00%	0.00%				
	2	0	1	0	0	0	4.000	0.85 7		
		0.00%	16.70 %	0.00%	0.00%	0.00%				
	3	0	0	0	0	0				

		0.00%	0.00%	0.00%	0.00%	0.00%	0.000	1.000 NS		
Cellular hyperplasia	0	6	5	0	1	6	9.222	0.056	23.056	0.0001***
		100.00%	83.30%	0.00%	16.70%	100.00%				
	1	0	1	6	5	0	13.833	0.008		
		0.00%	16.70%	100.00%	83.30%	0.00%				
	2	0	0	0	0	0	0.000	1.000 NS		
		0.00%	0.00%	0.00%	0.00%	0.00%				
	3	0	0	0	0	0	0.000	1.000 NS		
		0.00%	0.00%	0.00%	0.00%	0.00%				

Note: 0, none, 1 mild, 2 moderate, 3 severe (31).

Discussion

In the present study (published data), liver injury consequences lead to release of liver enzymes in blood stream. Group 2 showed marked elevation of liver enzymes (ALT and ALP) whereas Group 3, Group 4, Group 5 that administered omega-3 fatty acids and vitamin E showed reduction after two weeks. Group 5, that was treated by in combination of omega-3 and Vit E had exhibited potential protective effect. Also antioxidant GSH levels were significantly increased while significant reduction in MDA levels in the groups treated with alone or combination of omega-3 and vitamin E groups compared to group received CBZ (25).

Carbamazepine (CBZ) successfully induced hepatic injury in the experimental model as demonstrated suppression of Nuclear factor erythroids-2 related factors-2 expression in CBZ-treated groups , the

Nuclear Factor erythroid factor 2 cause reduction in oxidative stress, suppresses pro inflammation pathways signals, protects from necrosis and contributes to liver injury repair by activation of ARE-responsive genes like hemeoxygenase-1 and glutathione-S-transferases (32).

The results from gene expression of this factor in Group 2 showed decrease of *NRF2* expression , and Group 3 and Group 5 combination shows significant increase of *NRF2* which aligned with omega-3 (EPA,DHA), had considerable impacts on activity of *Nrf2*, Omega-3 and their by-products may regulate carbohydrate like protein (Keap1) ,regulate *Nrf2* activation under some condition upon oxidative stress, *Nrf2* would be phosphorylated causing dissociation from Keap1 and translocation to nucleus and enhance antioxidant that cause reduce oxidative stress and cell injury (33).

The result of Group 4 and Group 5 when administered Vit E showed a significant increase of *NRF2* gene expression which aligned with the study that showed Vit E supplementation may reduce the liver's burden, mitigate hepatic damage, and enhance liver function in rats exposed to damage by encouraging the expression of *Nrf2* pathway to reduce oxidative damage (34).

In current histopathology study, Group 2 outcome was consistent with previous studies, they stated that treatment of CBZ caused severe liver damage including hepatic tissue inflammation, degeneration, vacuolated hepatocytes , hemorrhage foci, aggregation of leukocyte , sinusoidal congestion and showing hepatic damage.(27,35) In the present study the results in Group3 treated with omega -3 showed mild portal vein congestion with few hemorrhagic foci, were consistent with El -

Mowafy et al. Omega-3 treatment exhibited minimal portal inflammation and some degenerative changes (27). In recent data, it was indicated that omega-3 had protective effect against liver, heart and kidney toxicity (17,18, 19). This study was consistent with previous study; it showed treatment with different doses of vitamin E had provided protection against liver damage and all hepatocytes appeared healthy (36). In the present histopathological finding in Group 4 had mild congestion of the central vein with normally hepatocyte and preserved sinusoidal architecture indicate antioxidant effect with hepatoprotection. Group5, omega- 3 and vitamin E combination, showed mild portal veins congestion with normal arrangement of hepatocytes and normal appearance of sinusoids. Histopathology findings of Group 5 aligned with the existing study indicating that combined administration of omega-3 and Vit E exerts ameliorating effects (37).

Conclusion

Carbamazepine induced marked hepatic injury, demonstrated by severe histopathological alterations and down regulation of *Nrf2*, indicating impaired antioxidant defense. Omega-3 and Vitamin E improved liver structure and partially restored *Nrf2* activity, while their combined administration produced normal histology and the greatest *Nrf2* up regulation. These findings confirm that activation of the *Nrf2* pathway is central to protection against CBZ-induced hepatotoxicity. The combination of (Omega3 and Vit E) antioxidant therapy maintained the integrity and architecture of hepatic tissues in rats.

References

1. Schwarz A, Strakos C and Weihrich R. A Brief Review on Carbamazepine – History, Pharmacological Properties and Environmental Impact. Insights in Chemistry and Biochemistry. 2021;1(4):1-4.
2. Stevens R, Konsil J, Dechasathian S, David Mason TH, Stevens RE. Reanalysis of carbamazepine and carbamazepine- epoxide pharmacokinetics after multiple dosing of extended release formulation Reanalysis of Carbamazepine and Carbamazepine-Epoxide Pharmacokinetics after Multiple Dosing of Extended Release Formulations . J Pharm Pharm Sci . 2002;5(2):169-75
3. Yin X, Cicali B, Rodriguez-Vera L, Lukacova V, Cristofoletti R, Schmidt S. Applying Physiologically Based Pharmacokinetic Modeling to Interpret Carbamazepine's Nonlinear Pharmacokinetics and Its Induction Potential on Cytochrome P450 3A4 and Cytochrome P450 2C9 Enzymes. Pharmaceutics. 2024;16(6): 737.
4. Thorn CF, Leckband SG, Kelsoe J, Steven Leeder J, Müller DJ, Klein TE, et al. PharmGKB summary: Carbamazepine pathway. Pharmacogenet Genomics. 2011;21(12):906–10.
5. Allison R, Guraka A, Shawa IT, Tripathi G, Moritz W, Kermanizadeh A. Drug induced liver injury–a 2023 update. Journal of Toxicology and Environmental Health - Part B. 2023;26(8):442-467.
6. Korver S, Bowen J, Pearson K, Gonzalez RJ, French N, Park K, et al. The application of cytokeratin-18 as a biomarker for drug-induced liver injury. Archives of Toxicology. 2021; 95:3435–3448.
7. Maris BR, Grama A, Pop TL. Drug-Induced Liver Injury—Pharmacological Spectrum Among Children. International Journal of Molecular Sciences. International Journal of Molecular. Sciences. 2025;26: 2006.
8. Björnsson ES. The Epidemiology of Newly Recognized Causes of Drug- Induced Liver Injury: An Update. Pharmaceuticals (Basel). 2024;17(4):520.
9. Mohammed YH, Hassan AF. Evaluation the anti-inflammatory effect of Omega 369 against

acetaminophen-induced hepatotoxicity in mice. Pharmacia. 2023;70(3):419–24.
10. Li X, Tang J, Mao Y. Incidence and risk factors of drug-induced liver injury. Liver international. 2022;42(9):1999-2014
11. Kobayashi T, Iwaki M, Nogami A, Yoneda M. Epidemiology and Management of Drug-induced Liver Injury: Importance of the Updated RUCAM. Journal of Clinical and Translational Hepatology. 2023;11(5):1239–1245
12. Babich M, De R, Guerra L, Daniel T, Adewuyi O. Drug-Induced Liver Injury (DILI): A Practical Review. PRACTICAL GASTROENTEROLOGY .2024.
13. Zhou Y, Wang J, Zhang D, Liu J, Wu Q, Chen J, et al. Mechanism of drug- induced liver injury and hepatoprotective effects of natural drugs. Chinese Medicine. 2021;16:135.
14. Keshari AC, Thitame SN, Aher AA, Keshari UC. Drug-Induced Liver Injury: Mechanisms, Diagnosis, and Management: A Review. J Pharm Bioallied Sci .2025;17(Suppl 1): S55–8.
15. Patted PG, Masareddy RS, Patil AS, Kanabargi RR, Bhat CT. Omega-3 fatty acids: a comprehensive scientific review of their sources, functions and health benefits. Future J Pharm Sci. 2024;10:94.
16. Vell MS, Creasy KT, Scorletti E, Seeling KS, Hehl L, Rendel MD, et al. Omega-3 intake is associated with liver disease protection. Front Public Health. 2023; 11:1192099.
17. Hassani HK, Hummadi YA, Al-Mugdadi HSF. Protective Effect of Omega-3 and the Potential of Toll-like Receptor Gene Expression in Rats with Doxorubicin-induced Cardiac Toxicity. Tropical Journal of Natural Product Research. 2023;7(2):2358–61.
18. Al-Dabbagh ASM, Mshemish BAR, Al-Mugdadi SFH. Investigating the potential protective effects of omega-3 against doxorubicin-induced renal injury in rats through modification of antioxidant markers. Immunopathologia Persa. 2024 :e41707
19. Parker HM, Cohn JS, O'connor HT, Garg ML, Caterson ID, George J, et al. Effect of fish oil supplementation on hepatic and visceral fat in overweight men: A randomized controlled trial. Nutrients.2019;11(2):475.
20. Al-Mugdadi SFH, Elewi IH, Shafeeq ZF, Abd Ali MM, Hammadi SQ, Abd-Alraheem MS. Effect of Omega-3 Fatty Acids on the Lipid Profile in Rats induced-acute cardiotoxicity by doxorubicin. Journal of Neonatal Surgery. 2025; 14 (1s):1227-1234.
21. Mohd Zaffarin AS, Ng SF, Ng MH, Hassan H, Alias E. Pharmacology and pharmacokinetics of Vitamin E: Nano formulations to enhance bioavailability. International Journal of Nanomedicine. 2020; 15:9961–9974 .
22. Traber MG. Current evidence of the role of vitamin E in prolonging a healthy life. Redox Experimental Medicine. 2023:1-36.
23. Traber MG. Vitamin E. Advances in Nutrition. 2021;12 (Issue 3):1047–1048.
24. Okolonkwo et al.. "Evaluating the Impact of Vitamin E Treatment on Paraquat Induced Toxicity in Liver". Current Research in Interdisciplinary Studies. 2023; 2(2): 6 – 11.
25. Al Attar F M N, Numan I T, Al-Mugdadi, S. F. H.. Assessment of the protective effects of omega-3 fatty acid and vitamin E against carbamazepine hepatotoxicity in rats by alteration of liver enzymes, antioxidant and inflammatory markers. Pharmaceutical Sciences. 2025; 31(4): 491–496.
26. Zhou, J, Zheng, Q & Chen, Z. The Nrf2 Pathway in Liver Diseases. Frontiers in cell and developmental biology. 2022; 10: 826204.
27. El-Mowafy AM, Abdel-Aziz AA, Abdel-Dayem MA. Novel protection by Omega-3-FAs (DHA or EPA) against carbamazepine's liver-injury: differential suppression of oxidative- stress and inflammatory markers, and the influence on carbamazepine-clearance. Clinical Nutrition Open Science. 2022;42: 14–26.

28. Suvarna KS, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques E-Book. 8th Edition. Elsevier health sciences; 2018.
29. Rahman MA, Sultana N, Ayman U, Bhakta S, Afrose M, Afrin M, et al. Alcoholic fixation over formalin fixation: A new, safer option for morphologic and molecular analysis of tissues. Saudi J Biol Sci. 2022;29(1):175–82.
30. Rubbia-Brandt L., Audard V, Sartoretti P, Roth AD, Brezault C, et.al. Histological features and severity of oxaliplatin-induced liver injury and clinical associations. Journal of Clinical Oncology. 2004; *22*(6):1158–1169.
31. Chowdhury AB, Mehta KJ. Liver biopsy for assessment of chronic liver diseases: a synopsis. Clinical and Experimental Medicine. Springer Science and Business Media Deutschland GmbH; 2023;23(2) : 273–285**.**
32. Jadeja RN, Upadhyay KK, Devkar RV, Khurana S. Naturally Occurring Nrf2 Activators: Potential in Treatment of Liver Injury. Oxid Med Cell Longev. 2016; 2016:3453926.
33. Saleh DO, Mahmoud SS, Hassan A , Sanad EF. Doxorubicin-induced hepatic toxicity in rats: Mechanistic protective role of Omega-3 fatty acids through Nrf2/HO-1 activation and PI3K/Akt/GSK-3β axis modulation. Saudi Journal of Biological Sciences. 2022; 29(7): 103308.
34. Fang J, Yin H, Yang Z, Tan M, Wang F, Chen K , Zuo Z, et.al. Vitamin E protects against cadmium-induced sub-chronic liver injury associated with the inhibition of oxidative stress and activation of Nrf2 pathway. Ecotoxicology and environmental safety. 2021; 208: 111610.
35. Higuchi S, Yano A, Takai S, Tsuneyama K, Fukami T, Nakajima M, et al. Metabolic activation and inflammation reactions involved in carbamazepine- induced liver injury. Toxicological Sciences. 2012;130(1):4–16.
36. Ibrahim MA, Ibrahim HM, Mohamed AA, Tammam HG. Vitamin E supplementation ameliorates the hepatotoxicity induced by Tramadol: toxicological, histological and immunohistochemical study. Toxicol Mech Methods. 2020;30(3):177–88.
37. Lotfi A, Rafie SM, Hesaraki S, Amini K. Protective Effects of Coadministration of the Vitamin E and Omega-3 on Doxorubicin-Induced Hepatotoxicity in Mice. International journal of cancer Managment.2022;15(10): e121766.