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Effects of Beta aminobutyric acid (BABA) on the gene expression profiles of rats with CCl4-induced Hepatic injury

Ansam Awad Ali[1], Mohammed Abbas Jasim[2] *
[1,2]Biology Department, College of Education for Women, Al-Ramadi 30001, Al-Anbar, Iraq
*Corresponding Author: mohammed.a.jasim@uoanbar.edu.iq.

Abstract:
Background: The Liver plays a crucial role in regulating metabolic function and various physiological processes, it participates in the detoxification of medicines and xenobiotics, resulting in heightened susceptibility to the toxicity of these substances. The present study investigated the hepatotoxic effects of oxidative stress by analyzing the expression of the mir210, mir-34a, and mir146a genes in Thirty-four male Sprague–Dawley rats aged 8–10 weeks carbon tetrachloride-induced hepatotoxicity. Hepatic damage was stimulated in the groups (T1, T2, and T3) using CCL4. Beta aminobutyric acid (BABA) was added for five weeks at two different concentrations. T4, was treated with only CCL4 twice a week for five weeks, T5, which was divided into groups A, B, and C, was given BABA at different concentrations (100, 150, 200). The mean levels of the MIR34 34, MIR210, mIR146a, CD4 and CD 8 genes were evaluated.
Results: MIR34, MIR210 and MIR146a presented high mean levels in the T4 group (8.76, 2.67, 1.41), whereas MIR34 presented low levels in the control group (1.28±0.972), MIR210 in the T2 group (0.53) and MIR146a in the T5C group (0.56). CDs 4 and 8 presented high mean levels in the T4 group (11.53, 613.25), whereas CD 8 presented low levels in the control group (342.0), and CD 4 presented high mean levels in the T3 group (2.13). The results of MIR34 gene expression folding were (6.433, 5.282, 5.269, 8.364, 5.365, 2.990, 2.033, and 1.000) in the studied groups (T1, T2, T3, T4, T5A, T5B, T5C, and T6), the results of MIR34 gene expression according to the fold change were (1.613, 0.530, 0.951, 2.513, 1.634, 1.273, 0.549, and 1.004) in the studied groups (T1, T3, T4, T5A, T5B, T5C, and T6), and the results of MIR146a gene expression according to the fold change were (0.992, 0.419, 0.812, 1.807, 0.633, 0.638, 0.694, and 1.004) in the studied groups (T1, T5C, T4, T5A, T5B, T5C, and T6).
Conclusions: Compared with the other groups, the group injected with CCL4 presented high means of gene expression and greater fold changes in gene expression, which indicates that CCL4 causes severe damage to the rats.

Keywords
Hepatic injury, Liver, CCl4, CD4, CD8, Gene Expression, BABA, qPCR, Rats.

BACKGROUND

The Liver plays a crucial role in regulating metabolic function and various physiological processes (1). Furthermore, it participates in the detoxification of some medicines and xenobiotics, resulting in heightened susceptibility to the toxicity of these substances [2]. Oxidative stress is associated with inflammation, cancer, and multiorgan damage [3]. Exposure to harmful substances can induce hepatocyte damage via the metabolic activation of reactive oxygen species (ROS) [5, 6]. Antioxidant enzymes function as free radical scavengers and serve as the primary defense against reactive oxygen species (ROS), including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and dietary antioxidants [7].

Carbon tetrachloride (CCl4) is a chemical contaminant that adversely affects the kidneys, Hepatic, blood, and heart by increasing lipid peroxidation and producing free radicals. CCl4, a significant hepatotoxin, is frequently employed to create experimental animal models that replicate human hepatotoxicity (2). The cytochrome P450 enzymes in the Hepatic catalyze the conversion of CCl4 into the trichloromethyl radical ($CCl3\bullet$), which rapidly interacts with oxygen to produce the highly reactive trichloromethyl peroxy radical ($CCl3OO\bullet$). Both radicals produce lipid peroxidation and oxidative stress (OS) via covalent attachment to cellular proteins, potentially resulting in Hepatic damage(3). CCl4 has the potential to increase the levels of inflammatory markers throughout the body, exacerbate disease development and contribute to Hepatic fibrosis, complicating hepatic treatment (4).

β-Amino butyric acid (BABA) is a nonprotein amino acid that plays an important role in several vital processes. Although it does not enter into the synthesis of proteins, it possesses biological and physiological properties that make it an important subject of study in multiple fields, such as medicine and nutrition. Research has shown that BABA has a beneficial function in accelerating wound healing and reducing inflammation. In addition, BABA stimulates B cells to generate Igg and IgM antibodies, which in turn activate the complement system and facilitate communication between innate and acquired immunity (5, 6).

cDNA microarray technology serves as a potent instrument for discovering and characterizing alterations in gene expression linked to toxicity. Microarrays estimate gene expression levels by assessing the hybridization of mRNA to various genes fixed on nylon filters or glass slides(7). This approach has been effectively employed to examine modified gene expression in several biological processes, including inflammatory diseases and cancer. Moreover, microarrays can yield highly sensitive and useful indicators of toxicity and elucidate mechanisms of action by analyzing gene expression patterns induced by toxicants (8). Microarray technology has been extensively utilized by researchers worldwide to investigate the impact of CCl4 on gene expression. Research has been conducted using cultured cell lines or animal models, specifically mice or rats(9).

miR-34a is a direct target gene of p53, and one of its targets is sirtuin 1 (SIRT1), which inhibits p53-dependent apoptosis by deacetylating all principal p53 acetylation sites [21, 22]. The miR-34a/SIRT1/p53 signaling pathway establishes a positive feedback loop in which p53 stimulates miR-34a, and miR-34a subsequently activates p53 by inhibiting SIRT1, significantly influencing cell proliferation and death [22]. The miR-34a/SIRT1/p53 signaling pathway has been shown to be active in NAFLD and has been implicated in hepatocyte apoptosis [16]. The role of the miR-34a/SIRT1/p53 signaling pathway in Hepatic fibrosis remains ambiguous, particularly regarding its impact on hepatocytes and hepatic stellate cells (HSCs), given that miR-34a is also elevated under these conditions. This work aimed to examine the roles of *the MIR210, mir-34a*, and MIR146a genes in CCl_4-induced Hepatic fibrosis in rats.

METHODS

Chemicals

Beta aminobutyric acid (BABA) (Germany) and CCl4 (UK) were used in the study.

Laboratory analyses

Blood sample collection

This experiment was carried out with 34 male Sprague Dawley rats purchased from the animal facility at the Iraqi Center for Medical Cancer Research at Al-Mustansiriya University, Baghdad, at the age of 8–10 weeks. All the rats were in good health, with weights ranging from 140 to 160 grams, and they were placed in the animal laboratory of the College of Education for Women, University of Anbar. Laboratory animals were placed inside plastic cages with covers. Special metal mesh for raising the rats. The laboratory conditions were suitable for living, the temperature was set at 25 °C, and the duration of ventilation and lighting was adjusted day/night. The animals remained for 12 days before the start of the experiment, which was conducted during the month of June, and the animals were fed a special diet. After ensuring that the animals were anesthetized, blood samples were obtained from the abdominal aorta and stored in sterile tubes containing an anticoagulant (K3-EDTA) to examine hematological parameters.

Distribution of animal groups

The experiment was carried out by dividing the animals into six groups (T1, T2, T3, T4, T5A, T5B, T5C, and T6), and each group contained five animals. Hepatic damage was stimulated in the groups (T1, T2, and T3) in a synergistic manner with the damage-inducing substance CCl_4. BABA at different concentrations (100, 150, 200) was added for five weeks at two different concentrations. The fourth group, T4, was treated with only CCl_4 twice a week for five weeks. For the fifth group, T5, which was divided into groups A, B, and C, was given only the amino acid BABA at different concentrations (100, 150, 200). Nine animals were placed in this group, with three animals in each group, while the sixth group, T6, was maintained as a control, as it was given only a physiological solution. It was used as a control for Hepatic damage. The groups were divided as follows.

The first group, T1, was injected with CCl_4 at a concentration of 0.8 mg/kg to stimulate Hepatic damage, and BABA was injected at a concentration of 100 mg/kg. The weights of the animals in this group were 140 grams per animal.

The second group, T2, was injected with CCl4 at a concentration of 0.8 g/kg and BABA at a concentration of 150 mg/kg; the weights of the animals in this group were 140 grams.

The third group, T3, was injected with CCl4 to stimulate Hepatic damage at a concentration of 0.8 g/kg and BABA at a concentration of 150 mg/kg, as the weights of this group were 140 grams for each animal.

Fourth group, T4. This group was injected only with CCl4 to stimulate Hepatic damage at a concentration of 0.8 mg/kg, as the weights of this group were 150 grams per animal.

The fifth group, T5, was the negative control group, which was divided into three groups with different concentrations of the amino acid BABA, as follows:

The fifth group, T5A. This group was injected with BABA at a concentration of 100 mg/kg, and the weight of each animal in this group was 140 grams.

The fifth group, T5B. This group was injected with BABA at a concentration of 150 mg/kg, as the weights of each animal in this group were 145 grams.

The fifth group, T5C, was injected with BABA at a concentration of 200 mg/kg, as the weights of this group were 150 grams for each animal.

Sixth group, T6. This group was left to the positive control, where normal saline was injected with two injections per week (1 mL for each animal), and the weights of this group were 160 for each animal.

RNA extraction

Total RNA was extracted from the blood samples of both patients and healthy controls. Blood samples were obtained in EDTA tubes. A volume of 250 µl of blood from the EDTA tubes was combined with 750 µl of triazole in an Eppendorf tube. RNA was isolated via the TransZol Up Plus RNA Kit.

Table (1): Components of the RNA extraction kit

Components	Size
TransZol Up	100 ml
Clean Buffer 9 (CB9)	110 ml
Wash Buffer 9 (WB9)	24 ml
RNase-free Water	40 ml
RNase-free Tube (1.5 ml)	100
RNA Spin Column Wish Collection Tubes	100

Primers

Primer preparation

The primers were made by dissolving the lyophilized primers in nuclease-free water according to the manufacturer's instructions, and a stock solution with a concentration of 100 µM was prepared and stored at -20 °C. Each 10 µL primer sample was diluted from the stock solution in 90 µL of nuclease-free water, resulting in a working solution with a concentration of 10 µM, which was preserved at -20 °C until use. Table 2 presents the primers used in the present study**.**

Table 2: The primers designed for this study

Genes	Primer Sequence	Product Size(bp)
MIR210 ID:100314053	(F) GCTCTTGTTGAGGGGAGTGG (R) GGATTTGGGGATGGGTGGTT	471
MIR-34 (102) ID:100314015	(F) TGGCAGTGTCTTAGCTGGTT (R) AACGTGCAGCACTTCTAGGG	81
MIR146a ID:100314241	(F) TGTCCTCGAGTTCCCGGTAT (R) GAAACAACACTGCCTGAGCG	398

Synthesis of cDNA from mRNA

The reverse transcription of total RNA into complementary DNA (cDNA) was carried out via the Thermo Fisher Scientific One-Step gDNA Removal and cDNA Synthesis SuperMix Kit. Following the manufacturer's guidelines, the reaction volume was 20 µL, and 20 µL of total RNA was used for the conversion process.

Table (3): Reaction components for converting RNA into cDNA

Components	Volume
Total RNA/mRNA	0.1 ng-5 ug/10 pg-500 ng
Anchored Oligo(dT)18 Primer (0.5 µg/ul)	1 µl
Or Random Primer(0.1 µg/ul)	1 µl
2 x ES Reaction Mix	10 µl
EasyScript RT/RI Enzyme Mix	1 µl
gDNA Remover	1 µl
RNase-free Water	To 20 ul

Gene expression of *MIR34, MIR210, miR146*, CD4 and CD8 by quantitative real-time PCR (qRT–PCR)

The total RNA was reverse transcribed into complementary DNA (cDNA) via the Thermo Fisher Scientific Kit (US) in a reaction volume of 20 µl, following the manufacturer's guidelines. Quantitative real-time PCR (qRT–PCR) was carried out via the QIAGEN Rotor Gene Q Real-time PCR System (Germany). Each qRT–PCR involved 2 µl of cDNA, 1 µl of both the forward and reverse primers (with a concentration of 10 µM), as listed in **Table 3,** and 10 µl of the Thermo Fisher Scientific Kit, USA. The thermal profile consisted of an initial step at 94 °C for 5 minutes (one cycle), followed by 40 cycles involving denaturation at 94 °C for 5 minutes; annealing at 58 °C for *MIR210, mir-34*, and *MIR146a* for 15 seconds; and extension at 72 °C for 20 seconds. The final dissociation stage spanned from 55 to 95 °C, with each degree lasting 5 seconds. The specificity of the amplified product was confirmed through melting curve analyses. To evaluate the relative expression of the genes in the samples from the study groups, the expression levels were normalized to those of the reference **gene.** Compared with the healthy controls, the data are presented as the fold change in gene expression within the study groups. This allowed normalization of the expression levels against the reference **gene (U6).**

DNA was extracted from the blood samples of both patients and healthy controls. The samples were collected in EDTA tubes. DNA isolation was performed via the "EasyPure Blood Genomic DNA Kit."

Gene expression calculation

The fold variations in the quantitative expression of the mature RNAs were determined via the relative cycle threshold (2-ΔΔCt) approach, which was first described by Livak and Schmittgen (2001). It is the ratio of the relative gene expression between the control group and the test group. Double delta Ct (threshold cycle) analysis was used to assess the expression of genes, including the housekeeping reference genes. The calculations were as follows: By using real-time cycler software, the threshold cycle (CT) was calculated for each sample. The samples were duplicated, and the average results were computed. The Ct values for the target genes evaluated in both patients and controls are reported. The ΔCt, or difference in CT values, which is also referred to as the "normalized raw data," was determined by subtracting the specified normalization factor from the Ct value of each target gene and the housekeeping gene.

ΔCt (control)=CT (gene)-CT (HKG)

ΔCt (patient)=CT (gene)-CT(HKG) ΔΔCt = ΔCt(patient)- ΔCt (control)

RESULTS

Mean expression of the *MIR34, MIR210* and *MIR146a* genes in the studied rat groups

The results revealed significant differences (P ≤0.05) in the expression of the studied genes (*MIR34, MIR210* and *MIR146a*) in injured rats. The mean levels of *MIR34, MIR210* and *MIR146a* were high in the T4 group (8.76±13.12, 2.67±0.93, and 1.41±0.423), whereas the mean level of MIR34 was low in the control group (1.28±0.972), the mean level of MIR210 in the T2 group (0.53±0.04) and the mean level of MIR146a in the T5C group (0.56±0.051), as shown in Table (4).

Table (4): Means of the MIR34, MIR210 and MIR146a genes in the studied rat groups

Groups	*MIR34*	*MIR210*	*MIR146a*
T1	5.67 ±1.95b	1.70±0.64 a	0.83 ±0.83 a
T2	4.51±2.72 b	0.53±0.04 c	0.61 ±0.084 c
T3	5.30±2.84 b	1.21±0.33 b	0.77±0.215 b
T4	8.76±13.12 a	2.67±0.93a	1.41±0.423a
T5A	4.30±1.76 b	1.74±0.78 a	0.76±0.152 b
T5B	3.17±1.38 b	1.86±0.51 a	0.73±0.120 b
T5C	2.60±2.10 b	0.65±0.47 c	0.56±0.051 c
T6 (control)	1.28±0.972 b	1.05±0.36 b	1.05±0.428 b
P value	**0.001****	**0.01****	**0.005****

Means of CD 4 and CD 8 genes in the studied rat groups

The results revealed significant differences (P ≤0.05) in the expression of the studied genes (MIR34, MIR210 and MIR146a) in injured rats. CDs 4 and 8 presented high mean levels in the T4 group (11.53±1.20, 613.25±132.01), whereas CD 8 presented low levels in the control group (342.0±65.61), and CD 4 presented high mean levels in the T3 group (2.13±0.36b), as shown in Table (5) and Figures (6) and (7).

Table (5): Means of the CD4 and CD8 genes in the studied rat groups

Groups	CD4	CD 8
T1	2.19 ±0.37 b	384.60±95.38 b
T2	2.18±0.53 b	391.72±56.83 b
T3	2.13±0.36b	560.28±122.26 a
T4	11.53±1.20 a	613.25±132.01 a
T5A	2.86±1.72 b	473.46±92.80 a
T5B	3.34±1.87 b	418.46±90.43 b
T5C	3.28±1.68b	365.96±61.39 c
T6 (control)	2.64±1.09 b	342.0±65.61 c
P value	**0.001****	**0.01****

Gene expression of *MIR34*

Table (6) shows the means of the Ct values of the studied groups. The mean Ct values of the T1, T2, T3, T4, T5A, T5B, T5C, and T6 groups were 29.82, 29.71, 29.92, 28.40, 29.57, 30.46, and 30.98, respectively. The mean Ct values of U6 (housekeeping gene) in the T1, T2, T3, T4, T5A, T5B, T5C, and T6 groups were 14.42, 14.03, 14.23, 15.54, 13.91, 13.96, 13.92, and 13.97, respectively. The ΔCt values in the T1, T2, T3, T4, T5A, T5B, T5C, and T6 groups were 15.40, 15.68, 15.69, 12.86, 15.66, 16.50, 17.06, and 18.09, respectively. The fold change in MIR34 gene expression was (6.433, 5.282, 5.269, 17.364, 5.365, 2.990, 2.033, and 1.000) in the studied groups (T1, T2, T3, T4, T5A, T5B, T5C, and T6), indicating the upregulation of gene expression in Hepatic-injured rats compared with that in the control group (Figure (1)).

Table 6: Fold expression of the MIR34 gene according to the $2^{-\Delta Ct}$ method

Groups	*Means Ct of MIR34*	*Means Ct of U6*	*ΔCt (Means Ct of MIR34)*	$2^{\Delta Ct}$	*experimental group/Control group*	*Fold of gene expression*
T1	29.82	14.42	15.40	0.0000232	0.0000232/0.0000036	6.433
T2	29.71	14.03	15.68	0.0000190	0.0000190/0.0000036	5.282
T3	29.92	14.23	15.69	0.0000190	0.0000190/0.0000036	5.269

T4	28.40	15.54	12.86	0.0000345	0.0000345/0.0000036	17.364
T5A	29.57	13.91	15.66	0.0000193	0.0000193/0.0000036	5.365
T5B	30.46	13.96	16.50	0.0000108	0.0000108/0.0000036	2.990
T5C	30.98	13.92	17.06	0.0000073	0.0000073/0.0000036	2.033
Control	32.06	13.97	18.09	0.0000036	0.0000036/0.0000036	1.000

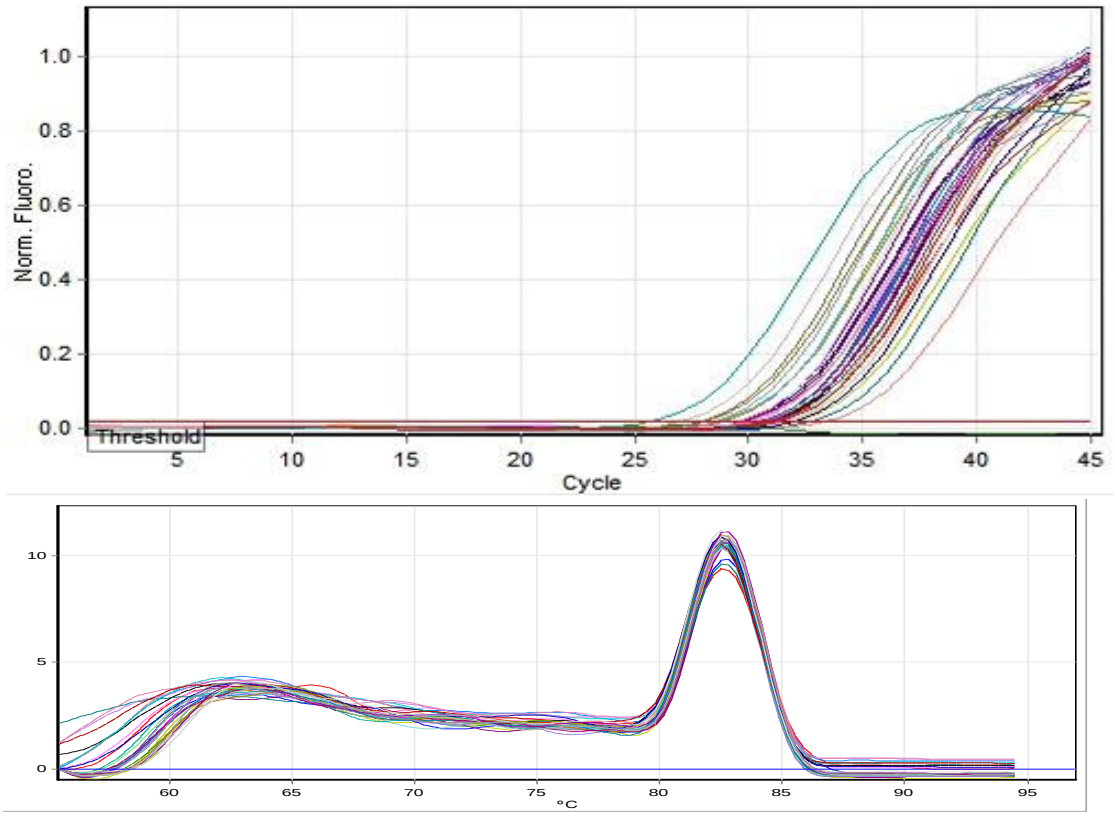

Figure (1): The results of gene expression (A) amplification of MIR34 via real-time qPCR (B) Dissociation of MIR34 gene curves via the use of the qPCR melting temperature

Gene expression of *MIR210*

Table (7) shows the means of the Ct values of the studied groups, and the mean Ct values of the T1, T2, T3, T4, T5A, T5B, T5C, and T6 groups were 27.27, 28.54, 27.90, 27.80, 26.79, 27.20, 28.37, and 27.56, respectively. The mean Ct values of U6 (housekeeping gene) in the T1, T2, T3, T4, T5A, T5B, T5C, and T6 groups were 14.36, 14.03, 14.23, 15.54, 13.91, 13.96, 13.92, and 13.97, respectively. The ΔCt values in the (T1, T2, T3, T4, T5A, T5B, T5C, and T6) groups were 12.90, 14.51, 13.66, 12.26, 12.88, 13.24, 14.46, and 13.59, respectively. The MIR34 gene expression according to the fold change was 1.613, 0.530, 0.951, 2.513, 1.634, 1.273, 0.549, and 1.004 in the studied groups (T1, T2, T3, T4, T5A, T5B, T5C, and T6, respectively). Figure (2).

Table 7: Fold expression of the MIR34 gene according to the $2^{-\Delta Ct}$ method

groups	*Means Ct of MIR210*	*Means Ct of U6*	*ΔCt (Means Ct of MIR210)*	$2^{\Delta Ct}$	*experimental group/Control group*	*Fold of gene expression*

T1	27.27	14.36	12.90	0.0001307	0.0001307/0.0000813	1.613
T2	28.54	14.03	14.51	0.0000429	0.0000429/0.0000813	0.530
T3	27.90	14.23	13.66	0.0000770	0.0000770/0.0000813	0.951
T4	27.80	15.54	12.26	0.0002035	0.0002035/0.0000813	2.513
T5A	26.79	13.91	12.88	0.0001324	0.0001324/0.0000813	1.634
T5B	27.20	13.96	13.24	0.0001031	0.0001031/0.0000813	1.273
T5C	28.37	13.92	14.46	0.0000445	0.0000445/0.0000813	0.549
Control	27.56	13.97	13.59	0.0000813	/0.0000813/0.0000813	1.004

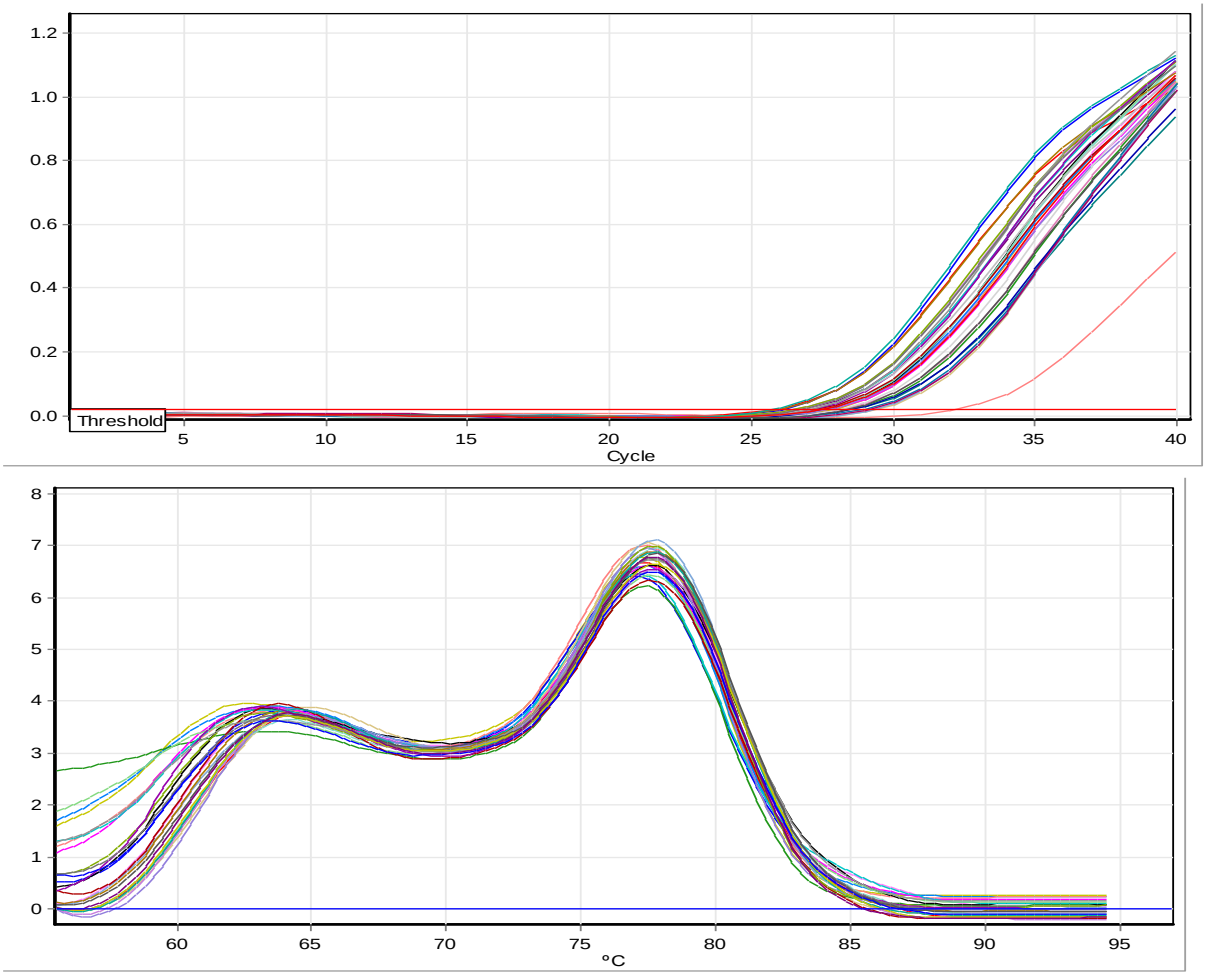

Figure (2): The results of gene expression (A) amplification of MIR210 via real-time qPCR (B) Dissociation of MIR210 gene curves via the use of the qPCR melting temperature

Gene expression of *MIR146a*

Table (8) shows the means of the Ct values of the studied groups, and the mean Ct values of the T1, T2, T3, T4, T5A, T5B, T5C, and T6 groups were 24.68, 25.59, 24.84, 24.99, 24.88, 24.91, 24.75, and 24.27, respectively. The mean Ct values of U6 (housekeeping gene) in the T1, T2, T3, T4, T5A, T5B, T5C, and T6 groups were 14.36, 14.03, 14.23, 15.54, 13.91, 13.96, 13.92, and 13.97, respectively. The ΔCt values in the T1, T2, T3, T4, T5A, T5B, T5C, and T6 groups were 10.32, 11.56, 10.61, 9.45, 10.97, 10.95, 10.83, and 10.30, respectively.

The mip146a gene expression according to the fold change was 0.992, 0.419, 0.812, 1.807, 0.633, 0.638, 0.694, and 1.004 in the studied groups (T1, T2, T3, T4, T5A, T5B, T5C, and T6, respectively). Figure (3).

Table 8: Fold expression of the MIR146a gene according to the $2^{-\Delta Ct}$ method

groups	*Means Ct of MIR146a*	*Means Ct of U6*	*ΔCt (Means Ct of MIR146a)*	$2^{\Delta Ct}$	*experimental group/Control group*	*Fold of gene expression*
T1	24.68	14.36	10.32	0.0007834	0.0007834/0.0007932	0.992
T2	25.59	14.03	11.56	0.0003306	0.0003306/0.0007932	0.419
T3	24.84	14.23	10.61	0.0006416	0.0006416/0.0007932	0.812
T4	24.99	15.54	9.45	0.0014273	0.0014273/0.0007932	1.807
T5A	24.88	13.91	10.97	0.0004997	0.0004997/0.0007932	0.633
T5B	24.91	13.96	10.95	0.0005043	0.0005043/0.0007932	0.638
T5C	24.75	13.92	10.83	0.0005481	0.0005481/0.0007932	0.694
Control	24.27	13.97	10.30	0.0007932	/0.0007932/0.0007932	1.004

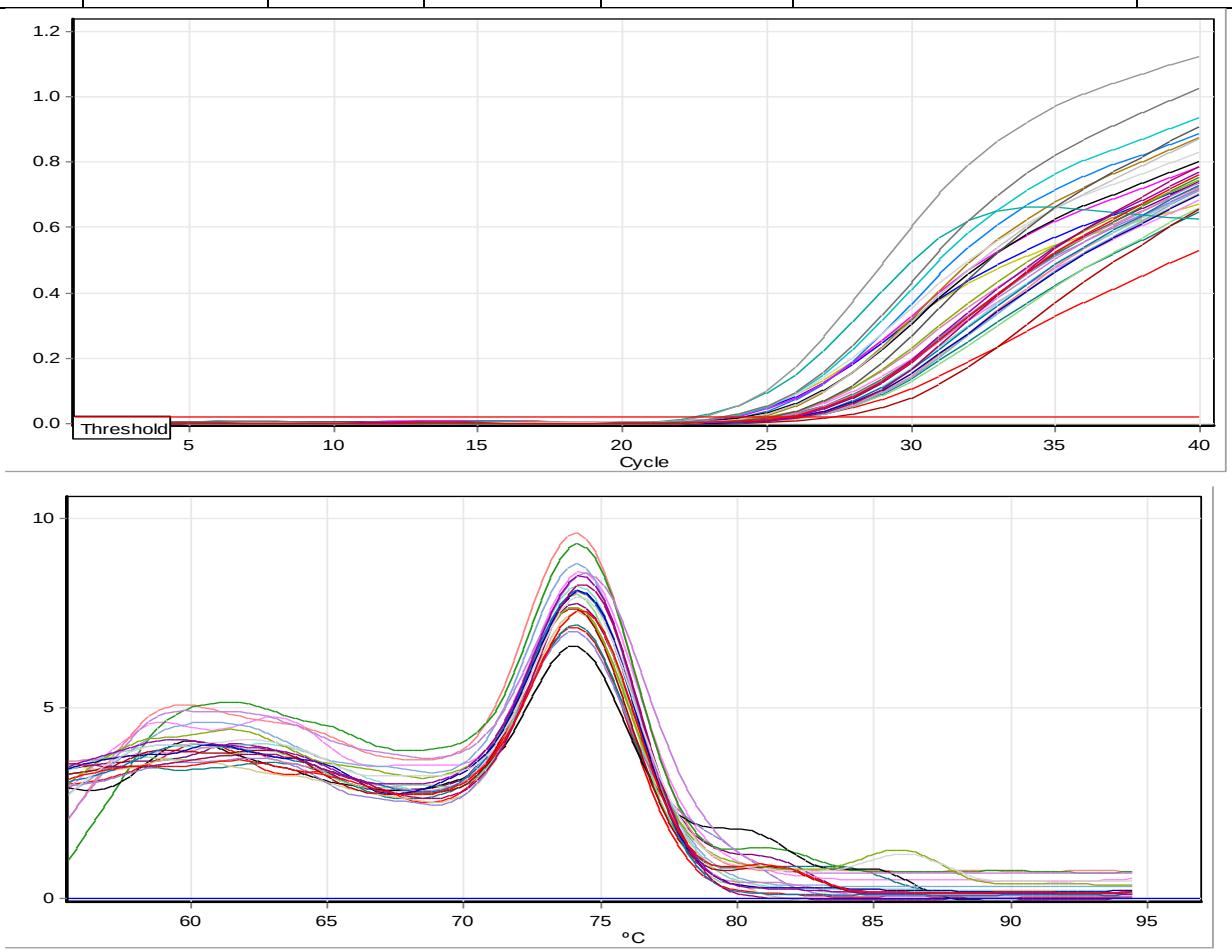

Figure (3): The results of gene expression (A) amplification of MIR146a via real-time qPCR (B) Dissociation of MIR146a gene curves via the use of the qPCR melting temperature

Correlations between the studied genes in Hepatic-injured rats

Table (9) shows positive correlations between MIR34 and MIR210 (0.581*), MIR34 and **MIR146a** (0.583**), MIR34 and **CD4 (0.824**),** and MIR34 and **CD8** (0.532**). Additionally, positive correlations were detected between MIR210 and MIR146a (0.510**), MIR210 with CD 4 (0.520**), MIR146a with CD 4 (0.500**) and CD 4 with CD 8 (0.457**).

Table (9): Correlations of genes associated with Hepatic injury in rats

Correlations						
		MIR34	**MIR210**	**MIR146a**	**CD4**	**CD8**
MIR34	**Pearson Correlation**	-				
MIR210	**Pearson Correlation**	.581**	-			
	Sig. (2-tailed)	<.001				
MIR146a	**Pearson Correlation**	.583**	.510**	-		
	Sig. (2-tailed)	<.001	.002			
CD4	**Pearson Correlation**	.824**	.520**	.500**	-	
	Sig. (2-tailed)	<.001	.002	.003		
CD8	**Pearson Correlation**	.532**	.249	.256	.457**	-
	Sig. (2-tailed)	.001	.162	.151	.007	
**. Correlation is significant at the 0.01 level (2-tailed).						

DISCUSSION

The present study revealed increased means of MIR34, MIR210 and MIR146a (microRNA genes) and their gene expression in the group that was injected with only CCl4 to stimulate Hepatic damage and in the group that was injected with low amounts of BABA amino acids compared with the control group, which indicates that CCl4 and BABA affect gene levels in Hepatic-injured rats. Compared with high concentrations, low concentrations of BABA cause greater gene expression, possibly because high concentrations cause Hepatic tissue damage, whereas low concentrations are safer and promote gene expression.

This study is in agreement with the study conducted by Mazani et al.(10), in which the expression levels of genome-wide microRNAs in the Hepatics of mice treated with carbon tetrachloride (CCl4) were examined. A total of eight miRNAs were found to be differentially expressed in the fibrotic Hepatics of the mice treated with CCl4 compared with those of the control mice (with a difference of more than twofold, with a significance level of less than 0.05). It has been discovered that microRNA-34a, also known as miR-34a, plays a role in fibrotic disorders caused by CCl4(11). In addition to its involvement in apoptosis, autophagy, and cellular senescence, microRNA-34a is also responsible for regulating the TGF-β1/Smad signaling pathway. Furthermore, it negatively influences the expression of many target genes, which in turn influences the accumulation of the extracellular matrix and affects the process of fibrosis (11).

MicroRNA-34a (miR-34a), a highly conserved member of the microRNA family, is encoded on human chromosome 1 and functions as a suppressor in human malignancies by regulating target genes. A previous study indicated that a miR-34a mimic could serve as a unique therapeutic target for Hepatic cancer, as it markedly inhibited tumor cell proliferation(12).

Amino acids such as BABA have the ability to decrease damage to Hepatic tissue and can assist in the battle against a wide variety of disorders (13). The reason for this could be related to the protective effect that beta-amino butyric acid, also known as BABA, has on the removal of toxins from the Hepatic. Additionally, it has the ability to cure Hepatic injury diseases because it helps to minimize fat peroxidation and restore the state of antioxidants to a normal state of function (14).

A study conducted by Lindqvist et al. (15) revealed that BABA intake protected against cadmium-induced lipid peroxidation and Hepatic and kidney injury in mice. Another study conducted by (16) reported that antioxidants such as BABA were effective against Hepatic toxicity or nephrotoxicity caused by CCl4 and reduced oxidative stress. BABA, also known as a cell membrane antioxidant, reduces the number of oxygen molecules that cause oxidative stress.

Compared with those in the control group, the ratios of the *mRNA* expression of *miR-210-3p* in the damaged Hepatic tissues of the rats were greater. On the other hand, retreatment resulted in a reduction in the relative gene expression ratios of miR-210-3p in the Hepatic-damaged rats. Another study (17) reported that injured Hepatic cells had elevated expression of miR-210, which was associated with increased expression of HIF-1α. It is possible to reduce the overproduction of HIF-1α and maintain Hepatic cells through the process of downregulating miR-210. Yu et al. (18) demonstrated that Hepatic disease is associated with an increase in miR210. Kim et al. (19) reported that miR-210 is a posttranscriptional regulator that disrupts BA balance and promotes cholestatic Hepatic disease in mice. Furthermore, they reported that increased miR-210 levels were found not only in cholestatic model mice but also in the Hepatics of wounded rats. Research conducted by Pan et al. (20), which demonstrated that the level of miR210 was elevated following hepatic IR injury, was published. After being subjected to hepatic IR injury, mice in which miR-210 (KO) was knocked out exhibited reduced inflammatory responses and death of these cells.

The hepatotoxicity animal model utilized in the research by Ono et al. (15) consisted of male mice that were given carbon tetrachloride (CCl4) through oral administration. They reported that the hepatotoxicity biomarkers miR-210 and miR-192 were increased in mice that were dosed with CCl4, and 42 additional extracellular vesicle (EV)-associated biomarkers were also identified. It is possible that some of these novel biomarkers could be utilized for the purpose of gaining a deeper comprehension of the mechanism of toxicity. According to the findings of Yuan et al. (21), radiation has the potential to cause histological alterations, Hepatic dysfunction, and fibrosis, all of which are accompanied by a reduction in the expression of miR-146a-5p. After the patients were treated with the miR-146a-5p agomir, their Hepatic function was restored. The results of additional in vivo investigations demonstrated that intravenous injection of miR-146a-expressing adenovirus (Ad-miR-146a) was able to successfully restore the levels of miR-146a and reduce the degree of fibrogenesis that occurred in the Hepatics of rats that had been treated with copper chloride (17). The hepatic pathological abnormalities were readily apparent; the expression of miR-146a in Hepatic tissue was dramatically elevated after being transfected with the miR-146a mimic in subgroups in the research conducted by YAN et al. (22).

Genes classified into CDs 4 or 8 typically presented elevated expression levels in the mouse retina at P0 and P8, which then declined steadily over the initial three weeks postnatally. A considerable number of genes within these CDs, which possess established activities, act as mediators of cell proliferation or are implicated in neural differentiation and migration(23). Numerous genes in CDs 4 or 8 presented characteristics pertinent to multiple facets of neuronal development in the central nervous system. According to their classification in our database, numerous genes are likely involved in the formation of the neuronal retina as well (24).

CONCLUSIONS

Compared with the other groups, the group injected with CCL4 presented high means of gene expression and greater fold changes in gene expression, which means that CCL4 caused greater damage to the rats.

List of abbreviations

Beta aminobutyric acid = BABA
Carbon tetrachloride = CCl_4
Cluster 4 = CD 4
Cluster 8 = CD 8
Ethylene diamine tetra acetic acid= EDTA
Micro ribonucleic acid = mir

Declarations

Ethics approval and consent to participate: We declares that animals received an appropriate treatment upon study which was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University of Anbar (Date.22/6/2024/ No. 93).

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Author's information (optional)
AAA is a MSc. Student in the department of biology, College of Education for Women, University of Anbar. Al-Anbar Iraq.
MAJ is an Assistant Professor and a biotechnology group leader in the department of biology, College of Education for Women, University of Anbar, Al-Anbar Iraq. A former Post-Doctoral fellow at Universite de Neuchatel, Switzerland, specialized in Biotechnology and Molecular Biology and had many contributions in different aspects of biotechnology as author, reviewer and editor in reputable scientific journals.

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