ROLE OF ETHANOLIC ALLIUM SATIVUM EXTRACT IN PERFLUOROOCTANOIC ACID-INDUCED HEPATOTOXICITY IN ADULT ALBINO RATS

Samar Sakr [1], Hend S. Eisa [1], Marwa Ahmed Abass [1], Shaimaa A. Abdelrhman [2], Eman El-Sayed Khayal[1]

[1]Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Zagazig University, Egypt
[2]Department of Medical Histology and Cell Biology, Faculty of Medicine, Zagazig University, Egypt

ABSTRACT

Background: Perfluorooctanoic acid (PFOA) is a human-made compound characterized by its persistence and bioaccumulation in humans, raising great health issues. Allium sativum (Garlic) is a popular plant in Mediterranean culture exhibiting beneficial antioxidant and anti-inflammatory effects. **Aim of the work**: was to study the hepatotoxic impact of PFOA and the probable mitigative role of Allium sativum against PFOA-induced hepatotoxicity. **Material and Methods:** 50 Albino rats (adult male) were used and divided into 5 equal groups: Control, Vehicle (distilled water), Allium sativum ethanolic extract (300 mg per kg), PFOA (25 mg per kg), and PFOA + Allium sativum. All groups were administered orally for eight weeks. At the end of the experiment, samples of blood were obtained to detect serum alanine transaminase (ALT), aspartate transaminase (AST), and lactate dehydrogenase (LDH). Liver samples were tested for malondialdehyde (MDA), heme oxygenase-1 (HO-1), superoxide dismutase (SOD), catalase (CAT), and inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) levels. Expressions of nuclear factor erythroid 2-related factor (Nrf2), Kelch-like ECH-associated protein1 (Keap1), and peroxisome proliferator-activated receptor α (PPAR α) genes were determined. Histopathological and immunohistochemical studies were also conducted. **Results:** PFOA altered liver enzymes, reduced antioxidants, increased MDA and NF-κB levels, downregulated Nrf2 and PPAR α, and upregulated Keap1 gene expressions. Histopathological examinations revealed liver damage besides strong caspase 3 immunoreaction. Allium sativum co-treatment lowered liver enzymes, reduced oxidative stress and inflammation, regulated Nrf2-Keap1/PPAR α pathways, and improved histological alterations. **Conclusion:** Allium sativum protects against PFOA-induced hepatotoxicity mostly via regulating Nrf2-Keap1/PPAR α pathway.

Keywords: *PFOA, Allium sativum, Liver, Nrf2, PPAR α.*

Corresponding author: *Dr. Samar Sakr*
Email: Samar.samy2000@yahoo.com
ORCID: 0000-0001-7107-7265

INTRODUCTION

Among the 4700 Per- and poly-fluoroalkyl substances (PFAS)-related chemicals, PFOA is frequently detected in both groundwater and surface water, exhibiting concentrations that vary from picograms per liter to milligrams per liter ***(Razak et al., 2023)***.

Perfluorooctanoic acid is a human-made fluorinated surfactant comprising a terminal carboxylate group and a seven-carbon perfluorinated alkyl chain. The strong carbon-fluorine bond is responsible for its chemical stability and resistance to degradation. This results in its accumulation in the environment and the human body, raising important public health issues**.** PFOA is frequently utilized for the coating of commercial products such as clothing, water-repellent textiles, carpets, food packaging, nonstick cookware, footwear, and in firefighting foam because of its chemical stain, grease, and water-resistant properties ***(Eggert et al., 2019)***.

Food ingestion, water drinking, and inhalation of dust are the major ways humans are exposed to PFOA. Following exposure, PFOA is absorbed and dispersed throughout the body, with the largest concentrations occurring in the liver, kidneys, heart, and blood ***(Tian et al., 2021)***.

The human metabolism of PFOA is extremely little or not at all. Smaller levels of PFOA are

expelled in feces and breast milk, but the majority is removed through urine. The human body eliminates PFOA at a half-life of 2.1-8.5 years ***(Langenbach and Wilson, 2021)***.

Exposure to PFOA has been previously claimed to be associated with hepatotoxicity, immunotoxicity, and disruptions in lipid and glucose metabolism ***(Rosen et al., 2017)***. Particularly in the liver, PFOA increases inflammation and oxidative stress, which results in structural damage and liver malfunction ***(Wang et al., 2020)***.

The molecular mechanisms for PFOA-mediated toxicity have been thoroughly investigated, even though they remain unclear. Multiple mechanisms are thought to be involved including oxidative stress, interference with lipid metabolism and hormone control, and disruption of cellular signaling pathways ***(Li et al., 2017)***. The pathway of Nrf2 was the focus of an investigation as a fundamental molecular mechanism of PFOA-induced toxicity ***(Tang et al., 2018)***.

Natural antioxidants have garnered attention for their potential to mitigate toxicant-induced oxidative stress. Allium sativum is a popular plant in Mediterranean culture. Whether raw or cooked, it is frequently ingested in various dosages and forms including fresh plant, garlic oil, powder, and pills ***(Ezz El Arab et al., 2021)***. Garlic possesses various bio-abilities such as antioxidation, anti-inflammation, cardio-protection, anti-cancer, and anti-bacterial activities ***(Luo et al., 2021)***. Garlic is also rich in a range of bioactive compounds, particularly the beneficial organosulfur compounds. This indicates that incorporating antioxidant plant extracts like garlic extract into the diet may offer greater benefits than single, pure antioxidants ***(Khatua et al., 2017)***.

In the liver, garlic has been shown to positively impact enzymes like alanine transaminase and gamma-glutamyl transferase, as well as fatty liver. It exerts its modulatory effect on the liver via different pathways. The anti-inflammatory function is mostly achieved by downregulating the pathways of NF-κB and Jun N-terminal kinase while exerting its antioxidant effect via upregulating the Nrf2 pathway. Induction of the Nrf2 pathway is responsible for the production of detoxifying enzymes and glutathione (GSH) ***(Pourreza et al., 2022)***. Furthermore, garlic supplementation has been demonstrated to enhance defense against oxidative injury in non-alcoholic fatty liver disease by activating the PPAR α pathway ***(Fajrani et al., 2020)***.

THE AIM OF THE WORK

This experiment intended to study the hepatotoxic impact of PFOA and the probable mitigative role of Allium sativum ethanolic extract against PFOA-induced hepatotoxicity.

MATERIAL AND METHODS

Chemical compounds

1) Perfluorooctanoic acid: appeared as white flakes and was brought from Sigma-Aldrich with the CAS number 335-67-1.

2) Allium sativum: commonly known as garlic, was locally cultivated in Zagazig, Sharqia, Egypt. Specimens were identified as belonging to the Liliaceae family by the Plant Protection Research Institute in Sharqia **(Figure 1)**.

Figure (1): Pictures showing fresh garlic and peeled garlic during the preparation of Allium sativum extract.

The garlic cloves underwent processing, which included peeling, mashing, and drying at a 25°C controlled temperature. The dried garlic was pulverized, followed by six extractions using ethanol (95%) at 60°C with a Soxhlet apparatus. The ethanol extract was further concentrated by vacuum evaporator at a temperature of 50°C (Yamato Rotatory Evaporator 300, Japan) over 6 h. The final extract was preserved at -20°C for subsequent analysis, as described by ***El-Sebaey et al. (2019)***.

The garlic extract underwent analysis using gas chromatography-mass spectrometry (GC-MS) in the Research lab at the Department of Forensic Medicine and Clinical Toxicology, Medicine Faculty, Zagazig University. The separation by chromatography was conducted with a column of TR. 35 MS, 30 m in length, 0.25 mm in internal diameter, and 0.25 µm in film thickness. Helium was the carrier gas at 1mL per min flow rate and a programmed temperature of 50 to 280°C. A diluted 1 µL sample was introduced in a spitless mode (50–600 atomic mass scan). Chemical constituents of garlic extract were identified by comparing both the times of retention as well as the mass spectra to the mass spectral database of the National Institute of Standards and Technology (NIST) released in 2005 ***(Abd El-Kareem et al., 2016)***.

Animals

The experiment included fifty albino rats (adult male;180 to 200 grams). Rats were acquired and housed at the Pet House of Veterinary Medicine, Zagazig University. The experiment aligned with the National Research Council's and the ARRIVE recommendations. The work got the approval of the authorized Committee at Zagazig University *(ZU IACUC/3/F/312/2022).*

Before initiating the work, rats underwent a fourteen-day acclimatization to adapt to their new environment. During this time, their general health was monitored, and any unwell animals were excluded. The rats were boarded in chemical-free plastic cages under standard conditions (22±2°C, 12 h light-dark cycle, and 50±5% humidity). A balanced food of bread and barley, designed to meet all nutritional requirements, was available freely. Clean drinking water was supplied in sanitized containers, ensuring that animals remained healthy throughout the preparation period.

Experimental design

Rats were allocated at random and equally divided into five groups. They received treatments according to the protocol outlined in **Table (1)**.

All treatments were administered orally once daily for eight weeks. The dosages of Allium sativum and PFOA were daily prepared and maintained according to rats' body changes to ensure accuracy.

Rats were sedated with pentobarbital (60 mg/kg) twenty-four hours following the final dose. After that, samples of blood were taken from the retro-orbital plexus utilizing the microcapillary glass tubes per ***Johnson's (2007)*** methodology. About 5 mL of blood samples were drawn, placed in sterile tubes devoid of anticoagulants, and left to coagulate at 25°C for 30 min. Thereafter, samples were centrifuged over 15 min. and 3000 revolutions per minute to separate the sera. Before being utilized for the AST, ALT, and LDH analyses, the acquired serum samples were stored at -20°C.

Following their euthanasia, the rats' livers were isolated and inspected for any obvious macroscopic anomalies. After that, each liver tissue was split into two equal parts. For histological examination, a piece was kept in 10% neutral buffered formalin. As for a later assessment of hepatic tissue characteristics, the remaining fraction was quickly frozen in liquid nitrogen, blotted dry, washed with ice-cold phosphate-buffered saline (PBS), and stored at -80°C.

Serum liver enzymes assay

AST, ALT, and LDH serum levels were assessed using Biodiagnostic kits from Egypt. AST and ALT serum levels were evaluated following the ***Koller and Kaplan (1984)*** method, with enzyme activities expressed as units per liter (U/L). While LDH activity was determined based on the ***Kaplan (1984)*** approach and expressed in U/L.

Liver tissue homogenate assays

The concentration of the HO-1 enzyme was evaluated spectrophotometrically at a 450 nm wavelength using MBS764989 ELISA kits of USA, with results expressed as ng/mg tissue. SOD activity was evaluated using Biodiagnostic kits based on ***Weydert and Cullen's (2010)*** method and had been given in U/mg of tissue. Assessment of CAT was undertaken using Aebi's (1984) method and Biodiagnostic kits, and results were expressed in U/mg tissue. The MDA level was analyzed by Biodiagnostic kits from Egypt, following the methodology of ***Todorova et al. (2005)*** and resultants were provided in nmol/gm of tissue.

Hepatic levels of NF-κB were spectrophotometrically measured utilizing an ELISA test kit (MBS453975) at 450 nm, and levels were provided in ng/mg of liver tissue.

Real-time quantitative PCR

According to the approach of ***Khamis et al. (2021)***, total RNA from tissue samples was extracted by Trizol (Invitrogen; manufactured by Thermo Fisher Scientific, Inc). The cDNA Reverse Transcription Kit of Applied BiosystemsTM, USA, was used to create cDNA.

Primers were made using the sequences specified in **table (2**) and following the supplier's guidelines. The Rotor-Gene of Q 2plex Real-Time PCR System obtained from Qiagen, Germany, as well as the TOPreal™ qPCR 2X PreMIX (P725 or P750) (Enzynomics, Korea) were applied to conduct real-time RT-PCR under the supplier's guidelines. A melting curve study was conducted following PCR amplification. Also, the housekeeping gene B-actin's messenger RNA levels were used for normalizing the target gene expression levels in liver samples. The 2-ΔΔCT method was used to determine the data, which had been presented in terms of fold changes in comparison to control ***(Livak and Schmittgen, 2001)***.

Table (1): Study design and experimental protocol.

Group	Treatment	Dosage
Control	Regular diet and tap water	None.
Vehicle	Distilled water.	1 mL/rat
Allium sativum extract	Allium sativum extract.	300 mg/kg of Allium sativum extract diluted in 1 mL of distilled water ***(El-Sebaey et al., 2019).***
PFOA	Perfluorooctanoic acid	25 mg/kg of PFOA diluted in 1 mL of distilled water, representing 1/20 of LD_{50} (430-680 mg/kg body weight) ***(Kennedy et al., 2004)***.
PFOA + Allium sativum extract	Both PFOA and Allium sativum extract	PFOA: 25 mg/kg diluted in 1 mL of distilled water. Allium sativum extract: 300 mg/kg diluted in 1 mL of distilled water (extract given 1 hour before PFOA).

Table (2): Sequence of used primers.

Gene	Forward Primer	Reverse Primer	Accession No.	size
Nrf2	GGTTGCCCACATTCCCAAAC	CAGGGCAAGCGACTGAAATG	NM_001399173.1	124
Keap 1	CTGTGACACTTCTCCTGGGG	GAGAAGCAGGAACCAGGCAT	NM_057152.2	159
PPAR α	GTCCTCTGGTTGTCCCCTTG	GTCAGTTCACAGGGAAGGCA	NM_013196.2	176
B-actin	AACCTTCTTGCAGCTCCTCC	CCATACCCACCATCACACCC	NM_031144.3	193

Histopathological examinations

- Examination of liver tissues with a light microscope:

The harvested livers were preserved in paraffin blocks and cut into slices that were 5 µm in thickness after being fixed in formalin saline (10%). The obtained sections were subsequently stained with hematoxylin and eosin (H&E) ***(Kiernan, 2001)***.

- Examination of liver Caspases 3 immunohistochemistry and morphometric study

Rat's liver sections were subjected to immunohistochemical reactions using anti-caspase 3 antibodies. The immunohistochemistry analysis was carried out according to ***Ramos-Vara (2005)***. The mean percentage area for the immunoreaction due to anti-caspase 3 antibodies was determined within 10 readings from 5 non-overlapping sections from each rat of all the experimental groups by ImageJ software, of K 1.45 version, at 400x magnification power ***(Baak and Oort, 2012)***.

Statistical analysis

An IBM PC microprocessor running GraphPad Prism version 10 and Statistical Package for Social Science (SPSS) software version 26 were applied for the analysis of data. The one-way analysis of variance (ANOVA) was employed for comparison among groups of more than two followed by post hoc Fisher's least significant difference (LSD) test to evaluate the difference between groups. A p-value of >0.05 was deemed non-significant, a p-value of <0.05 was perceived as significant, and a p-value of <0.001 was considered highly significant ***(Kirkwood and Sterne, 2003)***.

RESULTS

The GC/MS results

The results obtained from GC/MS revealed several components, as shown in **table (3)** and **Figure (2**). The biggest proportion of the relative area is found in 2-ethyl hexanoic acid, n-hexadecenoic acid, hexadecenoic acid methyl ester, oleic acid, diisooctyl phthalate, octadecanoic acid, trisulfide di-2-propenyl, tetradecanoic acid, palmitic acid, methyl stearate, phthalic acid, octanoic acid, dodecanoic acid, and Panaxydol.

Serum liver enzymes

ANOVA test analysis showed significant differences between different studied groups in our study ($p<0.001$). By using the post hoc LSD test, the serum ALT, AST, and LDH didn't demonstrate any significant change between the control, vehicle, and extract of Allium sativum groups ($p>0.05$) **(Table 3)**. Significant increases in the mean values of AST, ALT, and LDH were observed in the PFOA group ($p<0.001$) and the PFOA + Allium sativum extract group ($p<0.001$ for AST and LDH; $p<0.05$ for ALT) compared to control, vehicle, and extracted Allium sativum groups. Meanwhile, Allium sativum extract co-administration with PFOA has succeeded in significantly lowering the parameters ($p<0.001$) versus the group of PFOA **(Table 4)**.

Oxidative indices and inflammatory biomarker

Highly significant differences were noticed between variable groups upon using the ANOVA test ($p<0.001$). Analysis of oxidative and inflammatory markers in hepatic samples indicated no statistically significant differences among the groups of control, vehicle, and Allium sativum by post hoc LSD test ($p>0.05$). Significant declines in the mean values of each of HO-1, SOD, and CAT in the group pf PFOA in relation to control, vehicle, and Allium sativum groups ($p<0.001$) were detected. However, PFOA along with Allium sativum caused a significant rise in HO-1 ($p<0.05$), CAT ($p<0.05$), and SOD ($p<0.001$) in contrast to the PFOA group. Nevertheless, versus the control, vehicle, and Allium sativum these biomarkers were still significantly lesser ($p<0.05$) (**Table 5**).

Regarding the MDA levels, **table (5)** revealed higher MDA values in the group administered PFOA versus control, vehicle, and Allium sativum groups ($p<0.001$). Meanwhile, MDA levels showed a noticeable decline in the combined group versus the PFOA group ($p<0.001$) but were still higher than other groups ($p<0.05$). As for the NF-κB levels, they were elevated markedly in the PFOA group in contrast to the remaining groups ($p<0.001$), however, treating rats with both Allium sativum extract and PFOA had participated efficiently in reducing NF-κB levels ($p<0.001$) **(Table 5)**.

Hepatic Nrf2, Keap1, and PPAR α gene expressions

By using the ANOVA test, a highly significant difference was noticed between different groups ($p<0.001$). Comparison of Nrf2, Keap1, and PPAR α gene expressions in liver tissues by post hoc LSD test revealed no significant differences between the control, vehicle, and extracted Allium sativum groups. In the group of PFOA, Nrf2 and PPAR α expressions were significantly lesser, while Keap1 was significantly greater than the groups of control, vehicle, and Allium sativum extract groups ($p<0.001$) **(Figure 3)**. Additionally, the group of combined PFOA and Allium sativum treatments exhibited lower expressions of Nrf2 and PPAR α with higher levels of expressed Keap1 gene as compared to the control ($p<0.001$ for Nrf2, $p< 0.05$ for PPAR α, $p<0.001$ for Keap 1), vehicle ($p<0.05$ for Nrf2, $p< 0.001$ for PPAR α, $p<0.05$ for Keap 1), and Allium sativum extract groups($p<0.001$), however, these values were significantly high concerning Nrf2 and PPAR α ($p<0.001$) and significantly low concerning Keap1 ($p<0.001$) in comparison with PFOA-treated group **(Figure 3)**.

Correlations between serum parameters, oxidative indices, inflammatory biomarker, and gene expressions

A positive correlation has been found between the mean values of all ALT, AST, and LDH on one hand and MDA and inflammatory NF-κB values ($p<0.001$) on the other hand.

Conversely, the SOD, CAT, and HO-1 values correlate negatively with AST, ALT, and LDH levels ($p<0.001$).

Moreover, liver enzymes correlated negatively with Nrf2 and PPAR α gene expression in the liver ($p<0.001$), while the mean values of these enzymes were in positive correlation with Keap1 expression values ($p<0.001$) **(Table 6)**.

Table (3): Major compounds of Allium sativum extract by GC/MS.

RT	Compound Name	Area %	MF	Molecular Formula	Molecular Weight
4.19	d-Glycero-d-ido-heptose	1.06	683	C7H14O7	210
9.57	5-O-Methyl-d-gluconic acid dimethylamide	3.30	654	C9H19NO6	237
12.15	Pentadecanoic acid	0.65	654	C15H30O2	242
12.16	Undecanoic acid	0.65	657	C11H22O2	186
13.97	2-Ethyl Hexanoic acid	12.05	911	C8H16O2	144
14.87	Octanoic acid	1.62	944	C8H16O2	144
16.59	9- Hexadecenoic acid	0.81	727	C16H30O2	254
16.59	1- Dodecanol, 3,7,11-trimethyl	0.81	750	C15H32O	228
17.30	Nonanoic acid	0.91	796	C9H18O2	158
17.72	Trisulfide, di-2-propenyl	2.38	911	C6H10S3	178
19.78	Decanoic acid	0.43	856	C13H26O4	282
23.25	Phenol, 2,6-bis(1,1-dimethylethyl)	0.50	805	C14H22O	206
24.62	Dodecanoic acid	1.86	895	C12H24O2	200
26.24	Panaxydol	0.47	720	C17H24O2	260
26.71	Falcarinol	0.67	734	C17H24O	244
29.04	Tetradecanoic acid	1.91	892	C14H28O2	228
29.98	17-Octadecynoic acid	0.51	788	C18H32O2	280
30.22	HI-Oleic safflower oil	0.68	742	C21H22O11	450
30.37	Retinal	0.74	801	C20H28O	284
31.15	Phthalic acid	2.59	865	C23H36O4	376
32.19	Hexadecanoic acid, methyl ester	4.27	922	C17H34O2	270
33.23	n-Hexadecanoic acid	10.98	924	C16H32O2	256
35.58	1-Octadecenoic acid	3.23	894	C19H36O2	296
36.03	Methyl stearate	2.16	847	C19H38O2	298
36.55	Oleic Acid	10.5	861	C18H34O2	282
36.92	Octadecanoic acid	3.40	857	C18H36O2	284
38.16	Palmitic acid	1.51	762	C32H64O3	496
41.23	Docosanoic acid, 1,2,3-propanetriyl Ester	1.68	788	C69H134O6	1058
43.23	Diisooctyl phthalate	6.46	940	C24H38O4	390
45.28	Isochiapin B	.71	841	C19H22O6	346

Rt: retention time MF: Matching factor %: percent.

Table (4): Statistical comparison between different groups regarding mean values of AST, ALT, and LDH in serum as per ANOVA and post hoc LSD tests.

Group / Parameter	Control group N=10	Vehicle group N=10	Allium sativum extract group N=10	PFOA group N=10	PFOA+ Allium sativum extract group N=10	F	p-value
	Mean± SD						
AST(U/L)	23.6±1.71	23.9±2.13	23.7±2.26	79.4±2.01 **a b c**	39.7±2.98 **a b c d**	1138.94	**0.000 ****
ALT(U/L)	26.4±2.22	25.6±1.9	25.9±2.73	67.2±15.5**a b c**	38.1±7.74 **d e f g**	50.520	**0.000 ****
LDH (U/L)	219.5± 6.92	216.7±10.2	219.2±6.44	491±21.2 **a b c**	252.5±26.43 **a b c d**	528.247	**0.000 ****

*Results are displayed as mean± SD. (SD: standard deviation). **P-value <0.001: indicates high significance by ANOVA test.*
Number of rats in each group=10 rats. AST: serum aspartate transaminase ALT: alanine transaminase LDH: lactate dehydrogenase
By LSD: a: p<0.001 versus the control; b: p<0.001 versus vehicle group; c: p<0.001 versus Allium sativum extract group; d: p<0.001 versus PFOA group; e: p<0.05 versus the control; f: p<0.05 versus vehicle group; g: p<0.05 versus Allium sativum extract group.

Table (5): Statistical comparison between different groups regarding mean values of oxidative and inflammatory biomarkers in hepatic tissues among groups as per ANOVA and post hoc LSD tests.

Group / Parameter	Control group N=10	Vehicle group N=10	Allium sativum extract group N=10	PFOA group N=10	PFOA+ Allium sativum extract group N=10	F	p-value
	Mean± SD						
Hepatic HO-1(ng/mg)	0.83±0.05	0.83±0.05	0.85±0.06	0.73±0.02 **a b c**	0.78±0.022 **e f g h**	13.41	**0.000****
Hepatic SOD(u/mg)	59.8±5.051	60.3±1.70	60.5±3.10	32.5±1.96 **a b c**	52.4±3.31 **a b c d**	136.47	**0.000****
Hepatic CAT(u/g)	5.711±0.34	5.64±0.37	5.86±0.29	2.71±0.23 **a b c**	3.11±0.195 **a b c h**	278.10	**0.000****
Hepatic MDA (nmol/g)	0.892±0.0	0.856±0.05	0.88±0.02	2.25±0.12 **a b c**	0.978±0.046 **b d e g**	958.88	**0.000****
Hepatic NF-κB (ng/gm)	21.3±2.36	20.3±1.64	21.3±2.31	61.4±1.71 **a b c**	30.9±3.107 **a b c d**	585.82	**0.000****

*Results are displayed as mean± SD. (SD: standard deviation). ** P-value < 0.001: indicates high significance by ANOVA test Number of rats in each group=10 rats. HO-1: heme oxygenase-1 SOD: superoxide dismutase CAT: catalase MDA: malondialdehyde NF- κB: nuclear factor kappa-light-chain-enhancer of activated B cells*
By LSD: a: p<0.001 versus the control; b: p<0.001 versus vehicle group; c: p<0.001 versus Allium sativum extract group; d: p<0.001 versus PFOA group; e: p<0.05 versus the control; f: p<0.05 versus vehicle group; g: p<0.05 versus Allium sativum extract group; h: p<0.05 versus PFOA group.

Table (6): Correlation coefficient between the mean values of liver enzymes (AST, ALT, LDH) on one side and oxidative indices, inflammatory marker, and gene expression results on the other side in hepatic tissue homogenates.

	AST(U/L)		ALT(U/L)		LDH(U/L)	
	R	P	R	P	R	P
Hepatic HO-1 (ng/mg)	-0.72	<0.0001	-0.67	<0.0001	-0.67	**<0.0001**
Hepatic MDA (nmol/g)	0.96	<0.0001	0.95	<0.0001	0.98	**<0.0001**
Hepatic CAT(u/g)	-0.85	<0.0001	-0.81	<0.0001	-0.74	**<0.0001**
Hepatic SOD(u/mg)	-0.95	<0.0001	-0.94	<0.0001	-0.95	**<0.0001**
Hepatic NF-κB (ng /gm)	0.99	<0.0001	0.97	<0.0001	0.97	**<0.0001**
Hepatic Nrf2	-0.84	<0.0001	-0.84	<0.0001	-0.82	**<0.0001**
Hepatic Keap1	0.84	<0.0001	0.82	<0.0001	0.74	**<0.0001**
Hepatic PPAR α	-0.95	<0.0001	-0.94	<0.0001	-0.94	**<0.0001**

Results are displayed as mean± SD. (SD: standard deviation). P-value < 0.001: indicates high significance
HO-1: heme oxygenase-1 MDA: malondialdehyde CAT: catalase SOD: superoxide dismutase NF- κB: nuclear factor kappa-light-chain-enhancer of activated B cells AST: serum aspartate transaminase ALT: alanine transaminase LDH: lactate dehydrogenase Nrf2: nuclear factor erythroid 2-related factor Keap 1: Kelch-like ECH-associated protein 1 PPAR α: peroxisome proliferator-activated receptor α

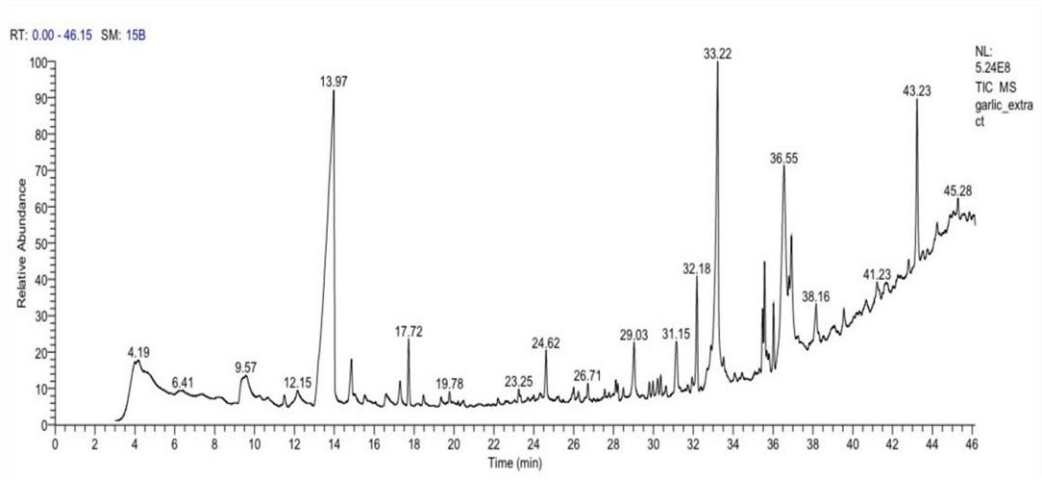

Figure (2): Allium sativum ethanolic extract using GC/MS chromatogram.

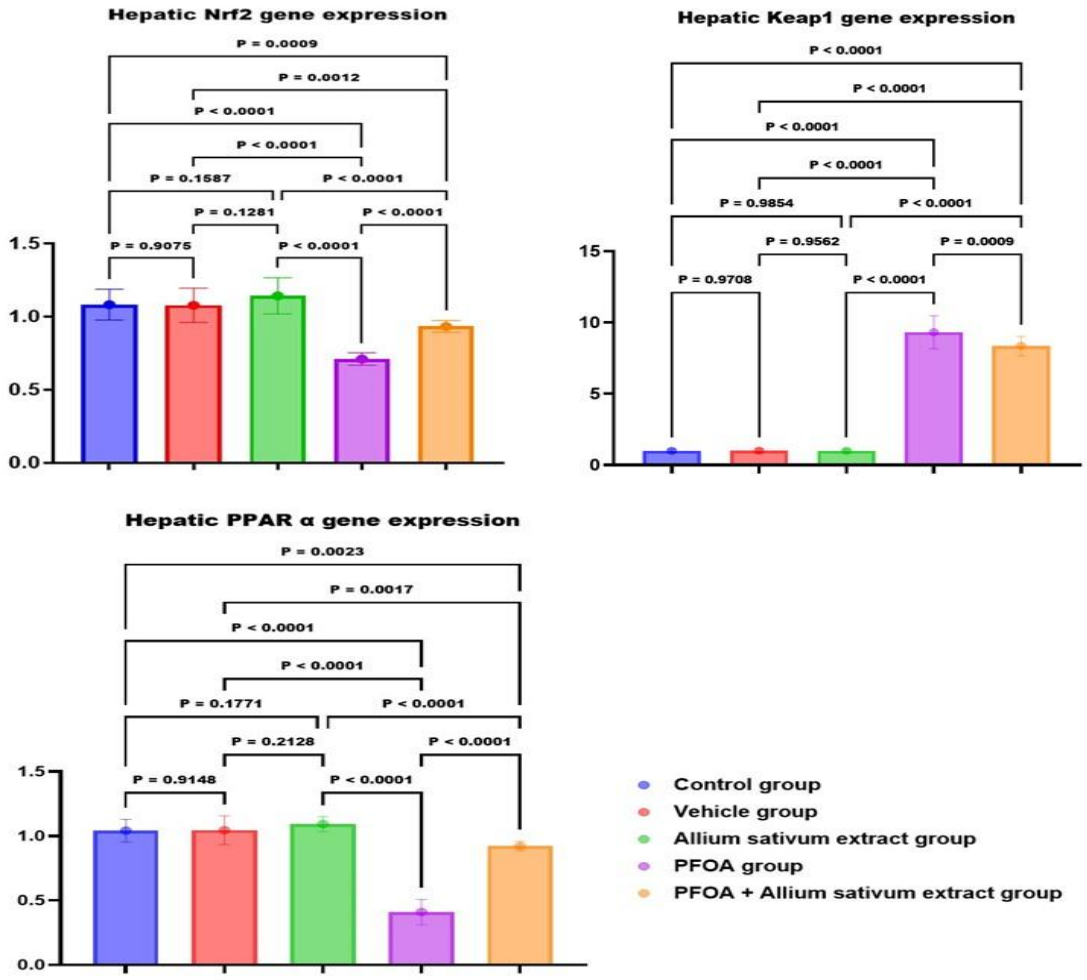

Figure (3): Statistical comparison between different groups regarding the mean values of hepatic Nrf2, Keap1, and PPAR α gene expressions as per ANOVA and post hoc LSD tests.
Results are displayed as mean± standard deviation (SD). LSD: least significant test
P-value<0.001: indicates high significance, P-value<0.05: indicates significance
Nrf2: nuclear factor erythroid 2-related factor, Keap 1: Kelch-like ECH-associated protein 1
PPAR α: peroxisome proliferator-activated receptor α.

Results of Histopathological examinations

Regarding H&E staining and immunohistochemical analysis of hepatic tissues, no histologically observed variations by light microscope examination were detected between the control and vehicle groups, therefore to compare the outcomes of the other groups, images from the control one were used.

- Light microscopic results:

The liver sections of both control and extracted Allium sativum groups stained with hematoxylin and eosin, respectively, revealed a polygonal traditional hepatic lobule with a central vein and a portal region at its periphery. From the central vein, which was lined with intact endothelium, the hepatocytes were distributed in cords. Hepatocytes had polygonal shapes, acidophilic cytoplasm, and vesicular nuclei positioned in the center. Two nuclei were present in several of them. Endothelium-lined blood sinusoids divided the hepatocyte cords (**Figure 4A and B**).

Liver sections from the PFOA group showed disorganized hepatic lobules. Some hepatocytes appeared with dark apoptotic nuclei and fatty infiltration. Inflammatory cellular infiltration was obvious at the portal area. Congested blood sinusoids were also seen **(Figure 4C)**.

Hepatocytes were polygonal with centrally positioned vesicular nuclei and acidophilic cytoplasm, while liver slices from the PFOA + Allium sativum extract group showed an improved hepatic lobule shape with intact endothelium lining the central vein. They

were organized in cords with blood sinusoids between them. Only vacuolated cytoplasm was visible in a few cells **(Figure 4D)**.

-Results of immunohistochemistry and morphometry

Sections for anti-caspase 3 immunostaining presented a weak immune reaction in hepatocytes of each control group **(Figure 5A)**, and Allium sativum extract group **(Figure 5B)**.

PFOA administration resulted in cellular apoptosis as detected by a strong brown nuclear reaction in various hepatocytes of the same group **(Figure 5C)**.

A few hepatocytes' nuclei exhibited mild reactivity when Allium sativum extract and PFOA were administered **(Figure 5D)**. The groups differed significantly ($p<0.001$) in the percentages of the areas representing the hepatic immunoreaction induced by caspase 3 antibodies in liver tissues. In the PFOA group, the anti-caspase 3 antibodies-mediated reaction was noticed to be significantly higher than in the control and Allium sativum extract groups. Contrary, a significantly lower reaction was found in the combined group **(Figure 5E)**.

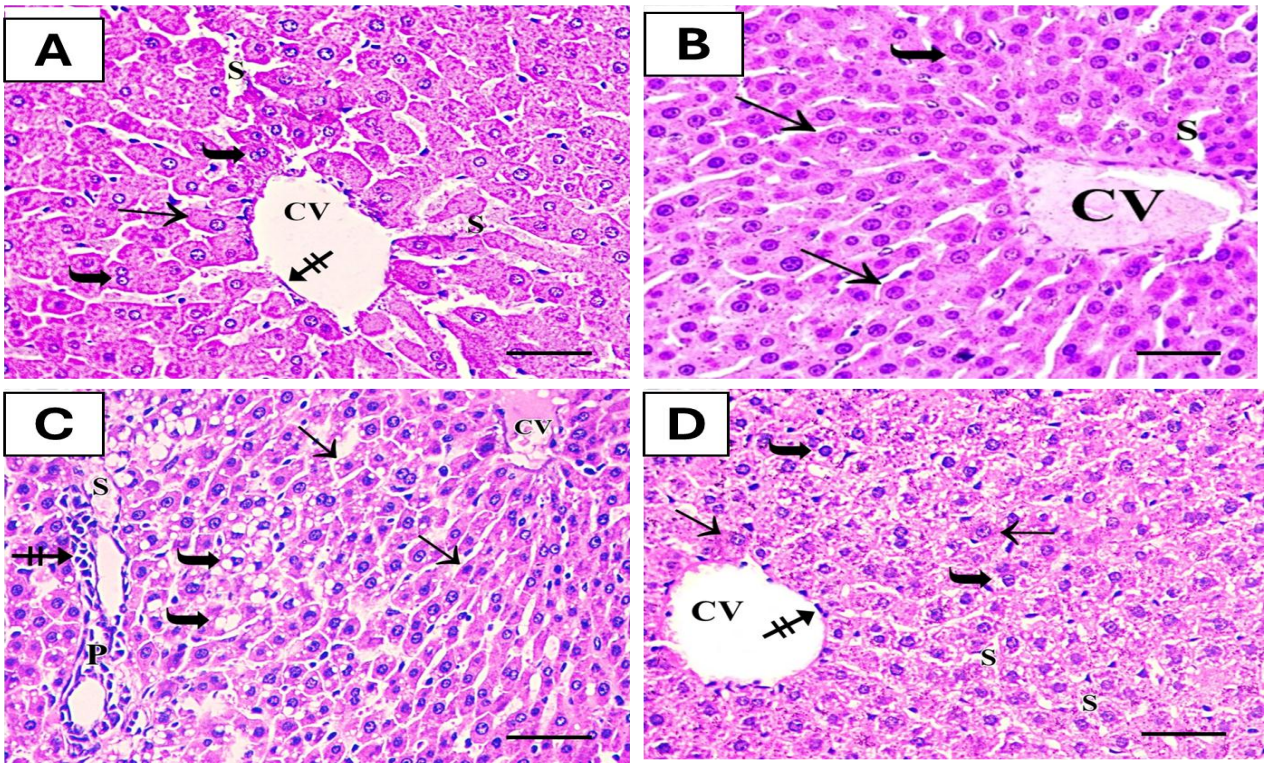

Figure (4)**: A photomicrograph showing H&E-stained sections of the liver in; **A:** The control showing central vein (CV) with intact endothelial (crossed arrow) lining. Polygonal hepatocytes that have central vesicular nuclei (arrow) as well as acidophilic cytoplasm. Some hepatocytes are binucleated (curved arrow). They are well arranged in regular cords and normally separated by the blood sinusoids (S). **B:** Allium sativum extract group shows polygonal hepatocytes that radiate from the central vein (CV) and are also normally separated by blood sinusoids (S). They have central vesicular nuclei (arrow) as well as acidophilic cytoplasm, some of them are binucleated (curved arrow). **C:** The PFOA group shows a disorganized hepatic lobule. Some hepatocytes with dark apoptotic nuclei (arrow), and fatty infiltration (curved arrow). Inflammatory cellular infiltration (crossed arrow) is obvious at the portal area (P). Congested blood sinusoids can be seen (S). **D:** The Allium sativum and PFOA co-treated group demonstrates a normal appearance of the hepatic lobule. The central vein shows an intact endothelial lining (crossed arrow). The hepatocytes are polygonal with central vesicular nuclei (arrow) along with acidophilic cytoplasm. Some cells show vacuolated cytoplasm (curved arrow). They are well arranged in regular cords and are separated by blood sinusoids (S) **(H&E X 400, Scale bar: 30 µm).

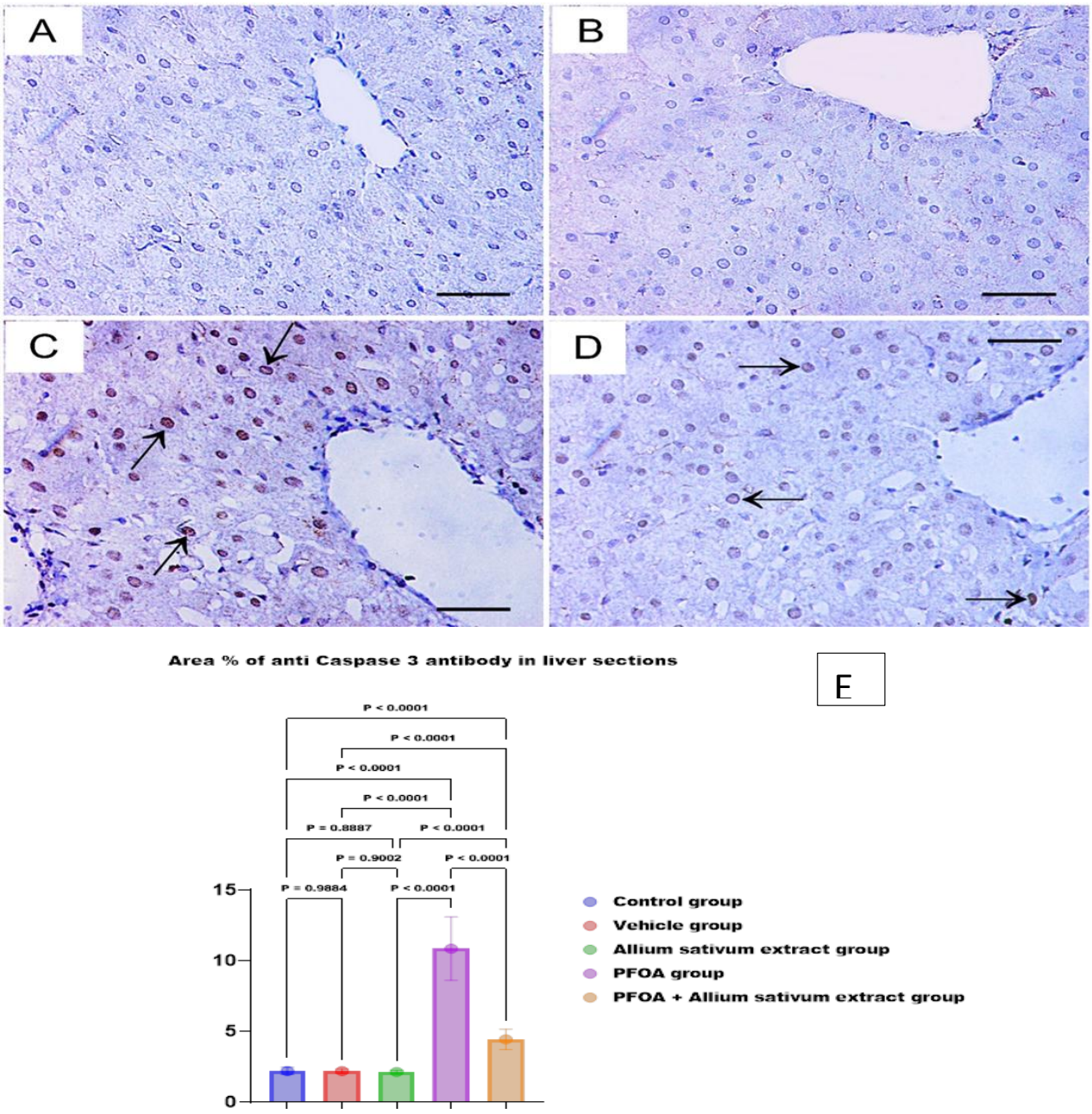

Figure (5): *A Photomicrograph showing the Caspase3 immune-stained liver sections showing* ***A:*** *A weak expression in hepatocytes in the control.* ***B:*** *A weak expression in hepatocytes of the Allium sativum extract group.* ***C:*** *A strong positive nuclear reaction in various hepatocytes (arrow) of the PFOA group.* ***D:*** *A weak nuclear reaction (arrow) in a few hepatocytes. (Avidin biotin Peroxidase system x 400, Scale bar: 30 µm) in the combined group.* ***E:*** *A histogram of Caspase3 immuno-reaction area percentage in liver sections. P<0.05 indicates significance, and p<0.001 indicates high significance.*

DISCUSSION

Perfluorooctanoic acid has currently emerged as a critical environmental contaminant due to its broad industrial usage and environmental persistence. Its probable adverse effects on human health, particularly its hepatotoxicity, have become a growing concern ***(Liu et al., 2023)***. Garlic is one of the well-known natural remedies whose health-promoting qualities have long been known. Research indicates that garlic offers considerable benefits for liver health, ascribed to its anti-inflammatory as well as antioxidant properties***(Shang et al., 2019)***.

In this experiment, we explored the hepatotoxic effect of PFOA and the probable mitigative role of Allium sativum ethanolic extract against PFOA-induced hepatotoxicity.

Elevated levels of both ALT and AST indicate liver impairment due to various causes including toxins-induced hepatic injury ***(McGill, 2016; Panjaitan, 2021)***. Also, LDH, the enzyme involved in glycolysis, is released into the bloodstream upon cellular damage and serves as a biomarker for tissue injury ***(Khan et al., 2020)***.

The study demonstrated a significant elevation in AST and ALT values in the PFOA group, reflecting liver damage. These results align with ***Owumi et al. (2021)*** and ***Naderi et al. (2023)*** who have informed elevated liver enzymes in PFOA-exposed rodents. Also, PFOA exposure significantly increased LDH levels, which was found to be consistent with ***Obiako et al. (2024)***.

Garlic's hepatoprotective properties have been repeatedly highlighted in earlier research. Garlic extracts have successfully reduced AST and ALT levels in mice subjected to carbon tetrachloride ***(Almatroodi et al., 2020)***, and in rats administered acetaminophen ***(Khaki et al., 2021)***, and dexamethasone ***(Alkot et al., 2022)***. Also, garlic exhibited a mitigating effect on fluoride-induced LDH elevation in male albino rats ***(Abdel-Baky and Abdel-Rahman, 2020)***.

To evaluate the underlying mechanism contributing to PFOA-induced hepatotoxicity; the oxidative stress-related biomarkers including HO-1, SOD, CAT, and MDA were investigated. HO-1 is an important enzyme counteracting both oxidation and inflammatory response by breaking heme down into biliverdin forming a strong antioxidant ***(Consoli et al., 2021)***. The antioxidant SOD and CAT enzymes protect cells against oxidative damage by neutralizing superoxide radicals and hydrogen peroxide ***(Jomova et* al., *2024)***. MDA is a widely used marker for the peroxidation of lipids and oxidative injury ***(Cordiano et al., 2023)***.

Results revealed a significant rise in hepatic MDA levels and impaired antioxidant defense as reflected by the reduced HO-1, CAT, and SOD enzymes in the PFOA group. Increased MDA was previously indicated in both the serum and liver of rats ***(Owumi et al., 2021)*** and in the liver of mice ***(Endirlik et al., 2022)*** treated with PFOA. Similarly, ***Ma et al. (2021)*** and ***Endirlik et al. (2022)*** have indicated decreased SOD and CAT in the hepatocytes in the hepatocytes of mice administered PFOA. Conversely, the co-administration of Allium sativum extract could significantly reduce MDA levels and increase HO-1, SOD, and CAT values in the hepatic tissues of the combined group. ***Khushboo et al. (2023), Sheweita et al. (2023)***, and ***Yahya et al. (2023)*** have similarly demonstrated garlic's ability to contradict oxidative stress in hepatic tissues of reserpine, cadmium, and cisplatin-treated rats, respectively as indicated by diminished MDA and increased antioxidant enzymes (SOD and CAT). ***Funes et al. (2020)*** claimed that organosulfur constituents in garlic induce HO-1 gene expression in HepG2 cells. Also, the extract of aged garlic has been shown to stimulate the expression of multiple antioxidants, including HO-1 as well as the modifier subunit of glutamate-cysteine ligase. This effect primarily occurs through Nrf2 pathway activation, leading to antioxidant protection in human endothelial cells ***(Liu et al., 2018)***.

To reveal the inflammatory effect of PFOA, NF-κB levels were investigated. NF-κB is the transcriptional factor regulating the inflammatory response and is thought to be activated by stressors like PFOA ***(Alharbi et al., 2021)***. Results referred to increased NF-κB levels in the hepatic tissues of rats administered PFOA. ***Liu et al. (2023)*** have also highlighted the inflammatory role mediated by NF-κB in PFOA-treated male, black-spotted frogs. Interestingly, Allium sativum extract could efficiently decline NF-κB levels in the PFOA + Allium sativum group as compared with the PFOA group. ***Gong et al. (2024)*** suggested that sulfur compounds in garlic can directly reduce NF-κB activation and combat inflammation in mice exposed to carbon tetrachloride.

Nrf2 is the chief transcription gene regulating the cellular defense against oxidative stress. Under non-stressful situations, Nrf2 is attached to Keap1 in the cytoplasm, causing its degradation. However, under oxidative circumstances, Nrf2 is liberated from Keap1 into nucleus and stimulates the defensive antioxidant response element-driven genes'

transcription, including HO-1 and other antioxidant enzymes and GSH, which helps protect cells from oxidative damage ***(Wang et al., 2021)***. PPARα is a nuclear receptor that acts as a transcriptional modulator incorporated in the metabolic process of lipids and energy production in the liver ***(Berthier et al., 2021)***. Interestingly, PPARα interacts with the Nrf2 pathway and exerts a major effect in maintaining the antioxidant defense, reducing ROS production, and counteracting inflammation ***(Xi et al., 2020)***. PPARα activation prevents the expression of proinflammatory cytokines like IL6 IL1, and TNFα through negative crosstalk with NF-κB ***(Berthier et al., 2021)***.

The findings of the transcription of genes in our work indicated a significant decline in the expression of Nrf2 and PPARα besides increased Keap1 in the PFOA group, suggesting the detrimental effect of PFOA on Nrf2-Keap1/ PPARα pathways. ***Murase et al. (2023)*** implied that PFOA can enhance the stability of Keap1, thereby inhibiting Nrf2 activation and diminishing the cellular antioxidant response in human hepatocyte models, 24 hours post-exposure. Additionally, ***Ma et al. (2024)*** revealed that exposure to PFOA caused a reduction in Nrf2, an elevation in Keap1, and an increase in oxidative stress within mouse kidneys. The observed decrease in PPAR α levels in the PFOA-treated group aligns with previous research of ***Nanji et al. (2004)***.

Garlic extract could re-regulate Nrf2, Keap1, and PPARα gene expression in the existing study. The regulatory role of Allium sativum on the antioxidant Nrf2-Keap1 pathway matches with ***Serafini et al. (2020)*** and ***Ming et al. (2023)***. Moreover, the up-regulatory effect of garlic on PPARα copes with ***Lai et al. (2014)***, who explained the alleviative influence of garlic on obesity-induced fatty liver by the organosulfur-induced upregulation of PPAR α.

Results of GC-MS in our study demonstrated the presence of valuable constituents in the ethanolic extract of Allium sativum, that exhibit both antioxidant and anti-inflammatory properties.

The polyunsaturated fatty acid, hexadecenoic acid (palmitoleic acid), is one of the major constituents of Allium sativum that has a strong antioxidant capability ***(Bermúdez et al., 2022)***. The monounsaturated fatty acid, oleic acid, is another antioxidant that neutralizes free radicals and slows lipid peroxidation ***(Wei et al., 2016)***. Dodecanoic acid, a medium-chain fatty acid, possesses effective antimicrobial, antioxidant, and anti-inflammatory properties ***(Ameena et al., 2024)***. Trisulfide, di-2-propenyl is a sulfur-containing organosulfur compound that exerts a unique role in cell defense through Nrf2 pathway activation exerting both antioxidant and anti-inflammatory responses ***(Novakovic et al., 2024)***. Panaxydol is a bioactive polyacetylene primarily stimulating the Nrf2 pathway and thus enhancing HO-1 and SOD gene expression. Additionally, it downregulates NF-κB expression and reduces the inflammatory cytokines ***(Truong and Jeong, 2022)***. Gluconic acid, tetradecanoic acid, methyl stearate, palmitic acid, and octanoic acid are additional constituents that contribute to Allium sativum's antioxidant capacity ***(Furdak et al., 2023; Soliman et al., 2023)***.

Results of the correlation between the disturbed liver enzymes and the remaining parameters in this work were in support of the oxidative and inflammatory mechanisms exerted by PFOA and its dysregulatory effect on Nrf2-Keap1/PPAR α pathways.

The histopathological examination of the PFOA group demonstrated disorganized hepatic lobules, apoptotic nuclei, fatty infiltration, inflammatory cell infiltration in the portal area, and congested blood sinusoids. These changes are in line with ***Owumi et al. (2021)*** and ***Endirlik et al. (2022)***. The structural protection mediated by garlic was reflected by the nearly normal hepatic lobular structure, intact central vein endothelium, and well-organized hepatocytes. Similar observations were recorded by ***Saved et al. (2014)*** and ***Osman et al. (2022)*** in different toxicological models.

Additionally, our study observed a strong immune reaction for caspase 3 in the hepatic tissues of the PFOA-treated rats.

This observation matches with ***Wang et al. (2022)*** in human liver cells and ***Han et al. (2018)*** in rat liver, and is particularly

attributed to oxidative stress. Allium sativum co-treatment resulted in weak caspase 3 immunostaining in a few hepatocytes. Similarly, ***Reda et al. (2017)*** and ***Yu et al. (2021)*** indicated the beneficial anti-apoptotic effect of garlic due to enhancing antioxidant mechanisms.

CONCLUSION

Perfluorooctanoic acid exerted detrimental effects on the liver of albino rats as reflected by the altered liver enzymes, disturbed oxidative and inflammatory indices, and dysregulated Nrf2-Keap1/PPAR α pathways. Co-administration of ethanolic extract of Allium sativum could efficiently ameliorate liver enzymes, redox mismatch, and inflammatory response besides regulating Nrf2-Keap1/PPAR α pathways. Accordingly, Allium sativum may exhibit promise as a liver protector against PFOA-induced hepatotoxicity, mostly via regulating Nrf2-Keap1/ PPAR α pathways.

REFERENCES

1. **Abd El-Kareem, M. S.; Rabbih, M. A. E. F.; Selim, E. T. M. et al. (2016):** Application of GC/EIMS in combination with semi-empirical calculations for identification and investigation of some volatile components in basil essential oil. *Int. J. Anal. Mass Spect. Chromat.,4(1):14-25.*
2. **Abdel-Baky, E. S. and Abdel-Rahman, O. N. (2020):** Cardioprotective effects of garlic (Allium Sativum) in sodium fluoride-treated rats. *J. Basic App. Zool.,81:1-7.*
3. **Aebi, H. (1984):** Catalase in vitro. In Packer, L., (ed.), *Methods in Enzymology, Acad. Press, 105, pp. 121-126.*
4. **Alharbi, K. S.; Fuloria, N. K.; Fuloria, S. et al. (2021):** Nuclear factor-kappa B and its role in inflammatory lung disease. *Chem. Biol. Interact.,345:109568.*
5. **Alkot, A. M. F.; Naeem, S. A.; El Sayed, S. S. et al. (2022):** Study of the protective effect of garlic water extract on dexamethasone-induced hepatotoxicity and insulin resistance in adult male albino rats. *Int. J. Med. Arts, 4(8): 2606-2618.*
6. **Almatroodi, S. A.; Anwar, S.; Almatroudi, A. et al. (2020):** Hepatoprotective effects of garlic extract against carbon tetrachloride (CCl4)-induced liver injury via modulation of antioxidant, anti-inflammatory activities and hepatocyte architecture. *App. Sci., 10(18): 6200.*
7. **Ameena, M.; Arumugham, M.; Ramalingam, K. and Shanmugam, R. (2024):** Biomedical Applications of Lauric Acid: A Narrative Review. *Cureus, 16(6): e62770.*
8. **Baak, J. P. and Oort, J. A. (2012):** A Manual of Morphometry in Diagnostic Pathology. Springer Science & Business Media.
9. **Bermúdez, M. A.; Pereira, L.; Fraile, C. et al. (2022):** Roles of palmitoleic acid and its positional isomers, hypogeic and sapienic acids, inflammation, metabolic diseases and cancer. *Cells, 11(14): 2146.*
10. **Berthier, A.; Johanns, M.; Zummo, F. P. et al. (2021):** PPARs in liver physiology. *BBA Molec. Basis Dis., 1;1867(5):166097.*
11. **Consoli, V.; Sorrenti, V.; Grosso, S. et al. (2021):** Heme oxygenase-1 signaling and redox homeostasis in physiopathological conditions. *Biomolec., 11(4): 589.*
12. **Cordiano, R.; Di Gioacchino, M.; Mangifesta, R. et al. (2023):** Malondialdehyde as a potential oxidative stress marker for allergy-oriented diseases: An update. *Molec.,28(16): 5979.*
13. **Eggert, A.; Cisneros-Montalvo, S.; Anandan., S. et al. (2019):** The effects of perfluorooctanoic acid (PFOA) on fetal and adult rat testis. *Rep. Toxicol.,90: 68-76.*
14. **El-Sebaey, A. M.; Abdelhamid, F. M. and Abdalla, O. A. (2019):** Protective effects of garlic extract against hematological alterations, immunosuppression, hepatic oxidative stress, and renal damage induced by cyclophosphamide in rats. *Environ. Sci. Poll. Res.,26(15): 15559-15572.*
15. **Endirlik, B. Ü.; Eken, A.; Canpınar, H. et al. (2022):** Perfluorooctanoic acid affects mouse brain and liver tissue through oxidative stress. *Arh. Hig. Rada Toksikol.73(2): 148-156.*
16. **Ezz El Arab, A.; Abbas, O. A. and Abdelrahman, M. T. (2022):** Effect of different garlic preparations on testosterone, thyroid hormones, and some serum trace elements in rats. *Biol. Trace Elem. Res., 200(3): 1274-1286.*
17. **Fajrani, A. M.; Sulchan, M. and Muis S. F. (2020):** Effect of black garlic on visceral fat, oxidative stress and insulin resistance in nonalcoholic fatty liver disease rats. *Nut. Food Sci., 51(7):1084–1095.*
18. **Funes, S. C.; Rios, M.; Fernández-Fierro, A. et al. (2020):** Naturally derived heme-oxygenase 1 inducers and their therapeutic application to immune-mediated diseases. *Front. Immunol., 11:1467.*

19. **Furdak, P.; Pieńkowska, N.; Kapusta, I. et al. (2023):** Comparison of antioxidant and antiproliferative effects of various forms of garlic and ramsons. *Molec., 28(18): 6512.*
20. **Gong, Q.; Wang, X.; Liu, Y. et al. (2024):** Potential hepatoprotective effects of allicin on carbon tetrachloride-induced acute liver injury in mice by inhibiting oxidative stress, inflammation, and apoptosis. *Toxics, 12(5): 328.*
21. **Han, R.; Hu, M.; Zhong, Q. et al. (2018):** Perfluorooctane Sulphonate induces oxidative hepatic damage via mitochondria-dependent and NF-κB/TNF-a-mediated pathway. *Chemosph., 191:1056-1064.*
22. **Johnson, M. D. (2007):** The Rat. In Animal Models of Toxicology, Gad, S.C. (ed.),2nd ed., CRC press, Tylor and Francis Group, LLC, *Boca, Raton, London, New York, Ch.,3, pp. 187188.*
23. **Jomova, K.; Alomar, S. Y.; Alwasel, S. H. et al. (2024):** Several lines of antioxidant defense against oxidative stress: Antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. *Arch. Toxicol., 98(5):1323-1367.*
24. **Kaplan, A. and Pesce A. (1984):** Lactate dehydrogenase. *Clin. Chem. J., 1124:117.*
25. **Khaki, A.; Heidari, M. and Khaki, A. A. (2021):** The effects of allium sativum (garlic) extract on acetaminophen-induced hepatotoxicity in male rats. *World J. Men's Health, 39(3): 483-490.*
26. **Khamis, T.; Abdelalim, A. F.; Saeed, A. A. et al. (2021):** Breast milk mscs upregulated B-cells PDX1, Ngn3, and PCNA expression via remodeling ER stress/inflammatory/apoptotic signaling pathways in type 1 diabetic rats. *Eur. J. Pharmacol., 905: 174188.*
27. **Khan, A. A.; Allemailem, K. S.; Alhumaydhi, F. A. et al. (2020):** The biochemical and clinical perspectives of lactate dehydrogenase: an enzyme of active metabolism. *Endocr. Metab. Imm. Disor. Drug Targets, 20(6): 855-868.*
28. **Khatua, T. N.; Borkar, R. M.; Mohammed, S. A. et al. (2017):** Novel sulfur metabolites of garlic attenuate cardiac hypertrophy and remodeling through induction of Na+/K+-ATPase expression. *Front. Pharmacol., 8: 18.*
29. **Khushboo, Gupta, V. K. and Sharma, B. (2023):** Hepatoprotective effect of ethanolic extract of garlic against reserpine induced toxicity in wistar rats. *Ind. J. Clin. Biochem., 38(2): 251-261.*
30. **Kiernan, J. A. (2001):** Classification and naming of dyes, stains and fluorochromes. *Biotech. Histochem., 76(5-6): 261-278.*
31. **Kirkwood, B. R. and Sterne, J. A. C. (2003):** Essential Medical Statistics, 2nd ed. Blackwell Publishing. *Oxford, UK. pp. 78-165.*
32. **Koller, A., and Kaplan, A. (1984):** AST and ALT. *Clin. Chem. J., 418: 1316-1324.*
33. **Lai, Y. S.; Chen, W. C. and Ho, C. T. (2014):** Garlic essential oil protects against obesity-triggered nonalcoholic fatty liver disease through modulation of lipid metabolism and oxidative stress. *J. Agri. Food Chem., 62(25): 5897-5906.*
34. **Langenbach, B. and Wilson, M. (2021):** Per- and polyfluoroalkyl substances (PFAS): Significance and considerations within the regulatory framework of the USA. *Int. J. Environ. Res. Public Health, 18(21): 11142.*
35. **Li, K.; Gao, P.; Xiang, P. et al. (2017):** Molecular mechanisms of PFOA-induced toxicity in animals and humans: implications for health risks. *Environ. Int., 99: 43-54.*
36. **Liu, J., Guo W.; Yang M. L. et al. (2018):** Investigation of the dynamic changes in the chemical constituents of Chinese "laba" garlic during traditional processing. *Rsc. Adv., 8:41872–41883.*
37. **Liu, Z.; Lin, H.; Zheng, Y. et al. (2023):** Perfluorooctanoic acid and perfluorooctanesulfonic acid induce immunotoxicity through the NF-Kb pathway in black-spotted frog (Rana nigromaculata). *Chemosph., 313: 137622.*
38. **Livak, K. J. and Schmittgen, T. D. (2001):** Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *Methods, 25(4): 402-408.*
39. **Luo, J. F.; Dong, Y.; Chen, J. Y. et al. (2021):** The effect and underlying mechanisms of garlic extract against cognitive impairment and Alzheimer's disease: A systematic review and meta-analysis of experimental animal studies. *J. Ethnopharmacol., 280: 114423.*

40. **Ma, S.; Sun, Y.; Zheng, X. et al. (2021):** Gastrodin attenuates perfluorooctanoic acid-induced liver injury by regulating gut microbiota composition in mice. *Bioengin., 12(2): 11546-11556.*
41. **Ma, X.; Ren, X.; Zhang, X. et al. (2024):** Rutin ameliorates PFOA induced renal damage by reducing oxidative stress and improving lipid metabolism. *Nut. Biochem. J., 123: 109501.*
42. **McGill, M. R. (2016):** The past and present of serum aminotransferases and the future of liver injury biomarkers. *EXCLI J., 15: 817-828.*
43. **Ming, S. H. I.; Hao, L.; Yuan, Y. et al. (2023):** Inhibitory effect of garlic powder on mptp-induced dopaminergic neurodegeneration by activation of Nrf2-HO1 and mitochondrial dynamics. DOI:10.21203/ rs.3.rs-3110931/v1.
44. **Murase, W.; Kubota, A.; Ikeda-Araki, A. et al. (2023):** Effects of perfluorooctanoic acid (PFOA) on gene expression profiles via nuclear receptors in heparg cells: Comparative study with in vitro transactivation assays. *Toxicol., 494: 153577.*
45. **Naderi, M.; Seyedabadi, M.; Amiri, F. T. et al. (2023):** Rutin mitigates perfluorooctanoic acid-induced liver injury via modulation of oxidative stress, apoptosis, and inflammation. *Iran. J. Basic Med. Sci., 26(11): 1291-1297.*
46. **Nanji, A. A.; Dannenberg, A. J.; Jokelainen, K. et al. (2004):** Alcoholic liver injury in the rat is associated with reduced expression of peroxisome proliferator-α (PPARα)-regulated genes and is ameliorated by PPARα activation. *J. Pharmacol. Exp. Ther., 310(1): 417-424.*
47. **Novakovic, J.; Muric, M.; Bradic, J. et al. (2024):** Diallyl trisulfide and cardiovascular health: Evidence and potential molecular mechanisms. *Int. J. Molec. Sci., 25(18): 9831.*
48. **Obiako, P. C.; Ayisire, S. O. and Sayes, C. M. (2024):** Impact of perfluorooctanoic acid (PFOA) and perfluorobutanoic acid (PFBA) on oxidative stress and metabolic biomarkers in human neuronal cells (SH-SY5Y). *Environ. Int., 190: 108864.*
49. **Osman, H. E. H.; El-Kenawy, A. E. M.; Elsahra, D. G. et al. (2022):** Ameliorative effect of garlic oil extract after hepatotoxicity aggravated by subchronic effect ethephon in white albino rat. *Egy. J. Histol., 45(3): 825-834.*
50. **Owumi, S.; Bello, T. and Oyelere, A. K. (2021):** N-Acetyl cysteine abates hepatorenal toxicities induced by perfluorooctanoic acid exposure in male rats. *Environ. Toxicol. Pharmacol.,86: 103667.*
51. **Panjaitan, R. S. (2021):** The effect of the shift work system on function enzymes which are characterized by increased levels of SGOT and SGPT in the blood of workers at the aluminium smelting plant, PT Inalum (Persero) Kuala Tanjung in 2017 *(Doctoral Dissertation, Universitas Medan Area).*
52. **Pourreza, S.; Azar, P. S.; Sanaie, S. et al. (2022):** Therapeutic effects and mechanisms of action of garlic (allium sativum) on nonalcoholic fatty liver disease: A comprehensive systematic literature review. *Evidence-based Complement. Altern. Med., 2022:6960211.*
53. **Ramos-Vara, J. A. (2005):** Technical aspects of immunohistochemistry. *Vet. Pathol., 42(4): 405-426.*
54. **Razak, M. R.; Aris, A. Z.; Zainuddin, A. H. et al. (2023):** Acute toxicity and risk assessment of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) in tropical cladocerans Moina micrura. *Chemosph.,313: 137377.*
55. **Reda, M.; Abdel Fattah, L.; Helmi, D. et al. (2017):** Histological study on the effect of garlic extracts on induced wound in male rat: possible role of endogenous stem cells. *J. Med. Histol. (2): 119-133.*
56. **Rosen, M. B.; Schmid, J. E.; Corton, J. C. et al. (2017):** Gene expression profiling in wild-type and PPARα-null mice exposed to PFOA. *Toxicol., 283(1): 100–111.*
57. **Saved, H. S. E. D.; Abdel Nasser, A. S. and Fatma A, E. (2014):** Pharmacological and antioxidant actions of garlic and/or onion in non-alcoholic fatty liver disease [NAFLD] in rats. *J. Egy. Soc. Parasitol., 44(2): 295-308.*
58. **Serafini, M. M.; Catanzaro, M.; Fagiani, F. et al. (2020):** Modulation of keap1/Nrf2/ARE signaling pathway by curcuma-and garlic-derived hybrids. *Front. Pharmacol., 10: 1597.*
59. **Shang, A.; Cao, S. Y.; Xu, X. Y. et al. (2019):** Bioactive compounds and biological functions of garlic (allium sativum l). *Foods, 8(7): 246.*
60. **Sheweita, S. A.; Rafea, A. A. E. and Elbana, S. G. (2023):** The deleterious effects of cadmium on oxidative stress markers, drug-metabolizing, and antioxidant enzyme

activities: Role of silymarin and garlic as antioxidants. *Environ. Sci. Poll. Res., 30(52): 112490-112502.*

61. **Soliman, A. A.; Khattab, A. E.; Ali, H. E. et al. (2023):** Impacts of some garlic extracts on tomato growth and physio-bio chemistry under infection of root-knot nematode, meloidogyne incognita. *Egy. Acad. J. Biol. Sci., F. Toxicol. Pest Control, 15(1): 79-86.*
62. **Tang, J.; Jia, X.; Gao, Net al. (2018):** Role of the Nrf2-ARE pathway in perfluorooctanoic acid (PFOA)-induced hepatotoxicity in Rana Nigromaculata. *Environ. Poll., 238: 1035-1043.*
63. **Tian, D.; Geng, D.; Mehler, W. T. et al. (2021):** Removal of perfluorooctanoic acid (pfoa) from aqueous solution by amino-functionalized graphene oxide (AGO) aerogels: Influencing factors, kinetics, isotherms, and thermodynamic studies. *Sci. Total Environ., 783:147041.*
64. **Todorova, I.; Simeonova, G.; Kyuchukova, D. et al. (2005):** Reference values of oxidative stress parameters (MDA, SOD, CAT) in dogs and cats. *Comp. Clin. Pathol., 13:190–194.*
65. **Truong, V. L. and Jeong, W. S. (2022):** Red ginseng (Panax ginseng Meyer) oil: A comprehensive review of extraction technologies, chemical composition, health benefits, molecular mechanisms, and safety. *J. Ginseng Res., 46(2): 214-224.*
66. **Wang, J.; Pan, Y.; Wang, L. et al. (2020):** Perfluorooctanoic acid-induced hepatotoxicity through oxidative stress-mediated inflammation in mice. *Environ. Sci. Poll. Res., 27(5): 5302–5313.*
67. **Wang, Q.; Chen, W.; Zhang, B. et al. (2022):** Perfluorooctanoic acid induces hepatocellular endoplasmic reticulum stress and mitochondrial-mediated apoptosis in vitro via endoplasmic reticulum-mitochondria communication. *Chem. Biol. Interact., 354: 109844.*
68. **Wang, Y.; Gao, L.; Chen, J. et al. (2021):** Pharmacological modulation of nrf2/ho-1 signaling pathway as a therapeutic target of parkinson's disease. *Front. Pharmacol., 12: 757161.*
69. **Wei, C. C.; Yen, P. L.; Chang, S. T. et al. (2016):** Antioxidative activities of both oleic acid and camellia tenuifolia seed oil are regulated by the transcription factor daf-16/foxo in caenorhabditis elegans. *Pub. Lib. Sci. J., 11(6): e0157195.*
70. **Weydert, C. J. and Cullen, J. J. (2010):** Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. *Nature protocols, 5(1): 51–66.*
71. **Xi, Y.; Zhang, Y.; Zhu, S. et al. (2020):** PPAR-mediated toxicology and applied pharmacology. *Cells, 9(2): 352.*
72. **Yahya, M.; El Komy, A.; Farag, E. and Abou Elmaged, A. (2023):** Evaluation Of hepatoprotective and oxidative stress reducing activities of garlic and/or ginger against cisplatin-induced liver toxicity in rats. *Benha Vet. Med. J., 44(2): 54-58.*
73. **Yu, C. and Xiao, J. H. (2021):** The keap1-nrf2 system: a mediator between oxidative stress and aging. *Oxid. Med. Cell. Longev., 2021: 1-16.*

دور مستخلص الثوم الإيثانولي في التسمم الكبدي الناجم عن حمض البيرفلوروأوكتانويك في الجرذان البيضاء البالغة

سمر صقر[١]، هند عيسى[١]، مروة أحمد عباس[١]، شيماء عبد الرحمن[٢]، إيمان السيد خيال[١]
[١]قسم الطب الشرعي والسموم الإكلينيكية-كلية الطب جامعة الزقازيق-مصر
[٢]قسم الهيستولوجيا الطبية وبيولوجيا الخلية-كلية الطب جامعة الزقازيق-مصر

الملخص العربى

المقدمة: حمض البيرفلوروأوكتانويك هو مادة مصنعه تندرج تحت المواد الفلورية والتي تتصف باستمراريتها وتراكمها داخل جسم الانسان وهو الأمر الذي يثير مشاكل صحية.
الهدف من الدراسة: هدفت هذه التجربة إلى دراسة السمية الكبدية لحمض البيروفلوروأوكتانويك وكذا الدورالمحسن لمستخرج الثوم الايثانولي على السمية الناجمه عن حمض البيرفلوروأوكتانويك في كبد ذكور الجرذان البيضاء البالغة.
طريقة البحث: أُجريت هذه الدراسة على ٥٠ جرذ وتم تقسيمهم بالتساوي إلى ٥ مجموعات:المجموعة الضابطة: لقياس المعايير الأساسية،المجموعة الناقله :(١ مل من الماء المقطر)، مجموعة المستخلص الايثانولي للثوم: ٣٠٠ ملجم/كجم مذابًا في ١ مل ماء مقطر، مجموعة حمض البيرفلوروأوكتانويك: ٢٥ ملجم/كجم مذابًا في ١ مل ماء مقطر، ومجموعة حمض البيرفلوروأوكتانويك +المستخلص الإثانولي للثوم. تم إعطاء جميع العلاجات عن طريق الحقن الفموي يوميًا لمدة ٨ أسابيع متتالية. بعد نهاية الدراسة ، تم تخدير الجرذان ثم سحب عينات الدم الوريدية واستخلاص مصل الدم لقياس الانزيمات الكبدية الأسبارتات ترانسامينياز والألنالين ترانساميناز ولاكتات ديهيدروجيناز. وبعدها تم الذبح الرحيم للجرذان واستخلاص الكبد و استخدام جزء منه لتقييم المؤشرات الحيوية للإجهاد التأكسدي [إنزيم الهيم الأوكسيداز-١، المالونديالديهيد، الكاتلاز، سوبر أكسيد ديسميوتاز]، وعامل الالتهاب [عامل تعزيز كابا]، وتحليل التعبير الجيني [جين النيرف ٢، جين البروتين كيب ١وكذلك مستقبلات البيروكسيسوم المنشط ألف] كما تم إستخدام الجزء الآخر من أنسجة الكبد لصبغه بالهيماتوكسيلين والإيوزين واختبار المناعة الكيميائية باستخدام صبغة الأجسام المضادة ضد الكاسباز-٣
النتائج: في مجموعة حمض البيرفلوروأوكتانويك، ارتفعت مستويات انزيمات الكبد بشكل ملحوظ مقارنة مع المجموعة الضابطه وكذلك بالنسبه للمالونديالديهيد وعامل تعزيز كابا، فيما انخفضت مضادات الأكسده .كما أظهر تحليل التعبير الجيني في أنسجة الكبد إنخفاضًا ملحوظا في تعبيرالنيرف ٢ ومستقبلات البيروكسيسوم المنشط ألفا، مع زيادة في تعبير الكيب١، فضلا عن حدوث تغيرات ظاهريه في الانسجه الكبديه وتفاعل مناعي قوي ضد الكاسباز-٣.
في مجموعة حمض البيرفلوروأوكتانويك +مستخلص الثوم تحسنت انزيمات الكبد وكذلك كافة الدلالات الكيميائيه الخاصة بالاكسدة والالتهاب ولوحظ تحسن التعبيرالجيني للنيرف ٢ ومستقبلات البيروكسيسوم وانخفاض في تعبير الكيب١مقارنة بمجموعة حمض البيرفلوروأوكتانويك فضلا عن التحسن النسيجي الملحوظ في الكبد وضعف التفاعل المناعي ضد الكاسباز-٣.
الإستنتاج: وفقاً للنتائج فيمكن استنتاج الدور الوقائي لمستخلص الثوم ضد السميه الكبديه الناجمه عن حمض البيرفلوروأوكتانويك غالبا عن طريق تنظيم المسار الجيني الخاص بالنيرف ٢والكيب ١ و مستقبلات البيروكسيسوم المنشط ألفا.